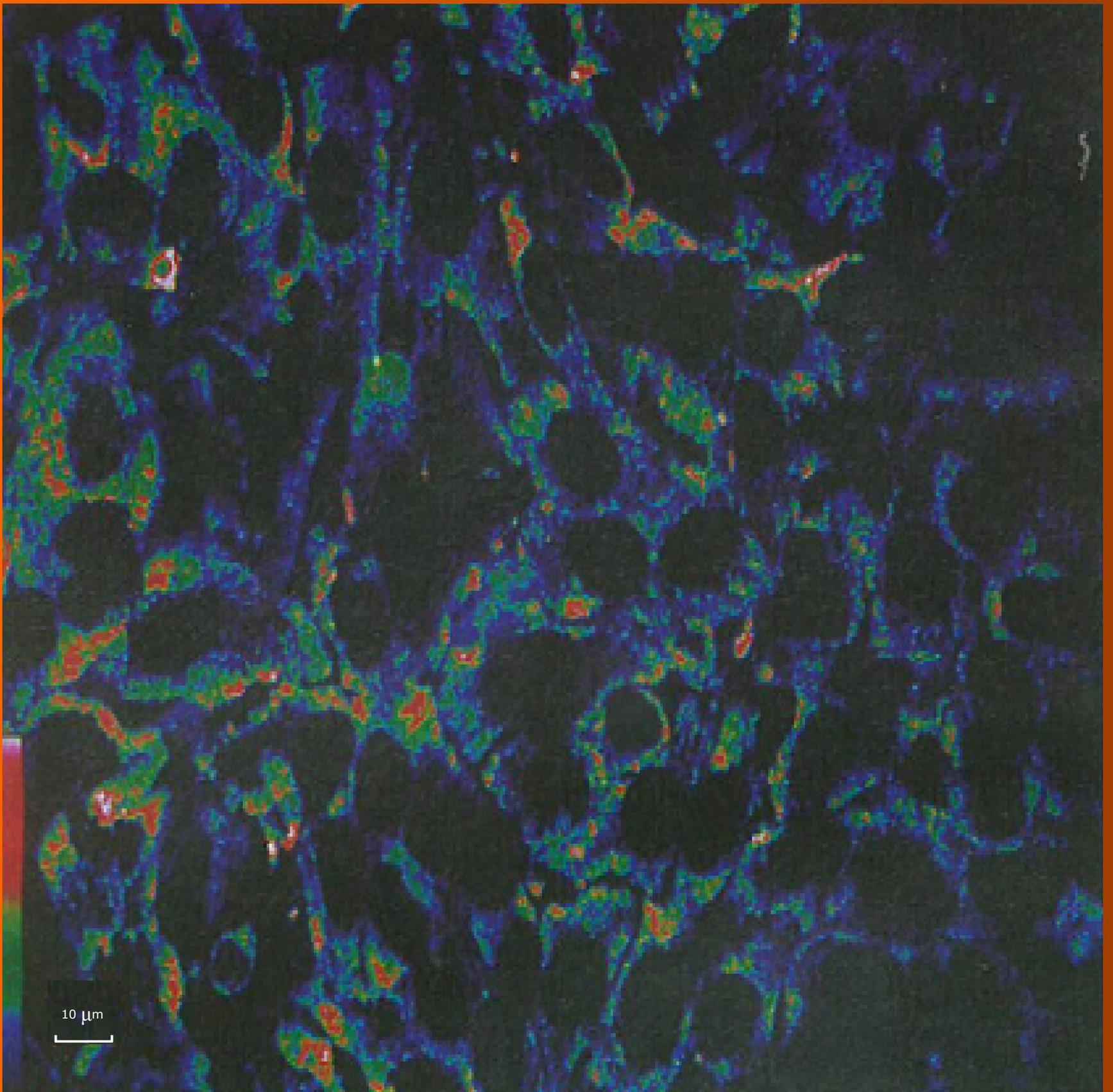


# World Journal of *Gastroenterology*

*World J Gastroenterol* 1997 March 15; 3(1): 1-60



**CONFERENCE PROCEEDINGS**

- 1 Highlights in mechanisms and therapies for gastrointestinal and hepatic diseases: 1996 Shanghai International Gastroenterology Conference  
*Wu XN*

**ORIGINAL RESEARCH**

- 3 Uptake of bacterial lipopolysaccharide and expression of tumor necrosis factor- $\alpha$ -mRNA in isolated rat intrahepatic bile duct epithelial cells  
*Chen XM, Han DW, Noguchi K, Tanikawa K*
- 6 Effects of  $\gamma$ -interferon on hepatic fibrosis of schistosoma japonicum-infected mice  
*He YW, Liu W, Zen LL, Luo DD*
- 9 Construction of retroviral vector carrying *HSV-tk* gene under control of human AFP enhancer core sequence and human pgk promotor  
*Gao J, Cao GW, Qi ZT, Qiu XF, Wu ZD, Du P, Yang WG, Cui L*
- 12 Sequencing of hepatitis C virus cDNA with polymerase chain reaction directed sequencing  
*Wei L, Wang Y, Chen HS, Tao QM*
- 16 Inhibitory effect of sulindac against chemically-induced primary colonic tumors by N-methyl-N-nitrosourea in mice  
*Wang Q, Fan LY, He J, Wang YH*
- 19 Studies on the relationship between the point mutation of ras oncogenes and the prognosis of patients with gastric cancer  
*Fang DC, Luo YH, Lu R, Liu WW*
- 22 Studies on the relationship between the point mutation of ras oncogenes and the prognosis of patients with gastric cancer  
*Fang DC, Luo YH, Lu R, Liu WW*
- 24 Surgical treatment of biliary ductal stricture complicating localized left hepatolithiasis  
*Sun WB, Han BL, Cai JX, He ZP*
- 27 Study of the influence of hiatus hernia on gastroesophageal reflux  
*Zhu HM*
- 31 Reduced secretion of epidermal growth factor in duodenal ulcer patients with Helicobacter pylori infection  
*Chen XQ, Zhang WD, Jiang B, Song YG, Reng RZ, Zhou DY*
- 35 Adherent properties of Helicobacter pylori to human epithelial cells  
*Wang ZX, Shen HF, Chen HJ*
- 38 Evaluation of a fecal occult blood test with reverse passive hemagglutination for colorectal neoplasm screening  
*Zhou L, Yu H, Zheng S*

- 41 Analysis of lactate dehydrogenase activities and isoenzyme patterns in colorectal cancer tissues  
*Zhao CH, Jiang CY, Zhang YY, Liu XX, Luo DC, Zhang XT, Lin YQ*
- 43 Clinical observation of 125I-labeled anti-alpha fetoprotein antibody radioimmunotherapy in hepatocellular carcinoma  
*Wu YD, Yang KZ, Zhou DN, Gang YQ, Song XQ, Hu XH, Huang BY*
- 47 Drinking water and liver cancer  
*Ruan CC, Chen YH, Zhang ZQ*
- 50 Co-regulative effects of the cAMP/PKA and DAG/PKC signal pathways on human gastric cancer cells during differentiation induced by traditional Chinese medicines  
*Gu SQ, Liang YY, Fan LR, Li BY, Wang DS*
- 54 Study of the regulatory effect of acupuncture on rotation-induced gastric dysrhythmia in rabbits  
*Zhang AL, Chen RX, Kang MF, Fan HL, Wang WL*

**REVIEW**

- 56 Current status of basic and clinical research in the field of gastroenterology in China  
*Wu XN*

**ABOUT COVER**

Chen XM, Han DW, Noguchi K, Tanikawa K. Uptake of bacterial lipopolysaccharide and expression of tumor necrosis factor- $\alpha$ -mRNA in isolated rat intrahepatic bile duct epithelial cells. *World J Gastroenterol* 1997; 3 (1): 3-5

**AIMS AND SCOPE**

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

**INDEXING/ABSTRACTING**

*World Journal of Gastroenterology* is now indexed in Current Contents<sup>®</sup>/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch<sup>®</sup>), Journal Citation Reports<sup>®</sup>, Index Medicus, MEDLINE, PubMed, PubMed Central.

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Wen-Xi Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ze-Mao Gong*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Gastroenterology*

**ISSN**  
ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

**LAUNCH DATE**  
October 1, 1995

**FREQUENCY**  
Quarterly

**EDITORS-IN-CHIEF**  
**Bo-Yong Pan**, President of China Speciality Council of Gastrology and China Association of Huatuo Medicine, Room 12, Building 621, the Fourth Military Medical University, Xi'an 710033, Shaanxi Province, China

**Lian-Sheng**, Member of the Speciality Committee of Digestive Diseases, Chinese Association of Combined Traditional Chinese and Western Medicine, Taiyuan Research and Treatment Centre for Digestive Diseases, Taiyuan 030001, Shanxi Province, China

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Gastroenterology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: bpgoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
June 15, 1997

**COPYRIGHT**  
© 1997 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_19970116143427.htm](http://www.wjgnet.com/bpg/g_info_19970116143427.htm)

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Highlights in mechanisms and therapies for gastrointestinal and hepatic diseases: 1996 Shanghai International Gastroenterology Conference

Xie-Ning Wu

Xie-Ning Wu, Department of Gastroenterology, Shanghai First People's Hospital, Shanghai 200080, China

Xie-Ning Wu, MD, Academic Committee Member of the Conference

Author contributions: The author solely contributed to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Xie-Ning Wu, **Professor**, Shanghai First People's Hospital, Shanghai 200080, China.  
Telephone: +86-21-63240090

Received: January 3, 1997

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: March 15, 1997

**Key words:** Gastroenterology; Congresses; Shanghai

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Wu XN. Highlights in mechanisms and therapies for gastrointestinal and hepatic diseases: 1996 Shanghai International Gastroenterology Conference. *World J Gastroenterol* 1997; 3(1): 1-2 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/1.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.1>

The 3<sup>rd</sup> session of the Shanghai International Gastroenterology Conference was held on November 28-30, 1996 in Shanghai under the auspices of president Dong XS and co-presidents Tytgat GNJ, Kurihara M and Owyang C. Top quality state of the art lectures were given by many top experts on all aspects of gastroenterology and hepatology, from the United States, United Kingdom, Australia, Canada, Netherlands, Germany, Japan and other European and Asian countries, as well as from all parts of China. Many basic and clinical research papers were presented, focusing on current concepts and recent developments in these fields. Only the most important and fascinating advances will be discussed here.

A new concept has been devised about the mechanism of acupuncture action on the gastrointestinal tract by Hunt RH (Canada), the efficacy of which is through the action of endogenous opioids and other neural pathways to regulate gastrointestinal motor and secretory function and the gut brain axis. The effect is negated by naloxone, which further supports the involvement of central opioids in this mechanism. Gastroesophageal reflux disease has been defined primarily as a motor disorder. Reflux events are associated with transient relaxation of LES, esophageal clearance abnormalities and occasional failures in gastric emptying. Refluxate

containing acid and peptic activity can lead to mucosal damage. GERD can be relapsing with recalcitrant symptoms and motor disturbance. Long-term proton pump inhibitors are necessary (Tytgat GNJ, Netherlands). Approximately 50%-60% of cases of non-ulcer dyspepsia are associated with CagA toxin-produced *H. pylori* gastritis. About 50% of patients with NUD experience pain or discomfort with balloon distention of the gastric fundus and whether or not this visceral hyperalgesia is related to psychological stress is still to be investigated. With the eradication of *H. pylori*, symptoms of NUD will improve and become asymptomatic one year later (Lam SK Hong Kong). It is known *H. pylori* infection is related to gastric cancer. Farthing MJG (London) found that *H. pylori* can cause disruption of the intercellular cytoskeleton and perturbation of the tight junction of gastric epithelial cells, leading to epithelial permeability alteration. Epithelial cells infected with *H. pylori* produced IL-8, a neutrophil chemotactic enhancing inflammatory cascade. The ammonia liberated also inhibits gastric cell growth in the S phase, thus progressing to atrophy, involving the antrum or even the corpus, then metaplasia and dysplasia occur and finally carcinoma. The inflammation and oxygen free radicals liberated lead to a hyperproliferative state and an extrinsic diet deficient in antioxidant vitamins may contribute to promoting the neoplastic process. Ming SC (Philadelphia) claims that the carcinogenesis of GI cancer primarily follows two routes: (1) **chronic inflammation**, epithelial hyperplasia, metaplasia, dysplasia, carcinoma; and (2) metaplasia, adenoma, increasing dysplasia and carcinoma. Carcinoma involves the alteration of many genes which variably regulate cell growth, cell differentiation, cell death, DNA repair and intracellular adhesion, as well as mutational changes in oncogenes, tumor suppressor genes and genes related to invasion and metastasis. Growth factors and their receptors, stromal cells and inflammatory cells also contribute to the growth of cancer. Various GI peptide hormones have been investigated regarding the growth of gastric, colonic and pancreatic cancers (Chen YF, Beijing). Bombesin has been found to exert an autocrine regulatory effect on cell growth in human gastric epithelial cells. Vasoactive intestinal peptide stimulates the growth of pancreatic and colonic cancer cell lines which express the VIP receptor and the antagonist of the latter inhibits VIP-promoted cancer cell growth. Cholecystokinin stimulates the growth of human pancreatic carcinoma cells and this effect can be inhibited by the CCK receptor antagonist, proglumide. Finally, somatostatin has been found to inhibit EGF promoted cell growth in human gastric cancer and human hepatoma cell lines, all of which express EGF receptors. The signal transduction pathways are still to be elucidated. A lysosomal cysteine protease, cathepsin B, has been implicated in the progression of human and rodent tumors and positive tumor cathepsin B staining is correlated with the depth of invasion. The clones with a high metastatic potential show a higher expression of cathepsin B than those with a low metastatic potential. The level of cathepsin B mRNA is increased in GI cancer and plays a significant role in GI cancer progression (Ren WP, Shanghai).

$\beta$ -carotene in its physiological amount of 5 mg/d, Vitamin E and selenium have been shown to lower stomach cancer incidence significantly during a 5 year period. However, large supplements of 30 mg/d with 2500 IU of vitamin A/D, owing to its nonspecific oxidation cleavage, produce metabolites as beta-apo-carotenol aldehyde and beta-apo-carotenoic acids which, in conjunction with heavy smoking or asbestos exposure, lead to lung cancer (Russel RM, Boston). Parvovirus HI has been proved to suppress tumor growth of human epithelial cells in immunocompromised recipient mice. The mechanism of parvovirus-induced oncosuppression is believed to be oncolysis. The viral protein NS-1 is likely to have a major effect, its cellular cofactors or targets undergo transformation-triggered modification and it is required for toxicity (Rommelaere J, Germany).

*H. pylori* infection leads to persistent hyperproliferation of gastric epithelial cells, causes chronic gastritis and gastric atrophy and is associated with both proliferation and apoptosis of the gastric epithelium. The decreased acid secretion might result in changes of the gastric bacterial flora and promote the nitroso compounds causing gastric bacterial flora and gastric carcinogenesis (Liu WZ, Shanghai). Kurihara M (Japan) advocates a combination of 5 DFUR, CDDP and a derivative of camptothecin, CPT-II to treat late gastric cancer, which can raise the effective rate to 43%. Preoperative chemical or chemoradiotherapy and postoperative intraperitoneal chemotherapy are currently under consideration as a new strategy for prevention of micrometastasis, so as to increase curative resection of gastric and esophageal cancer, but this is still controversial (Ajani JA). Early and radical resection of gastric cancer yielded a 5 year survival rate of 49.2% (355/803) in the period of 1984-1991 in Shanghai Rui Jin Hospital, better than 32.6% (383/1175) during 1958-1983. Cooperation between gastroenterology endoscopists and surgeons played a key role (Lin YZ, Shanghai). Folic acid supplements are recommended to decrease the recurrence rate of colorectal adenomatous polyps after removal because a diminished folate status promotes carcinogenesis, folate deficiency induces p53 specific strand breaks in colonic mucosa and DNA strand breaks and genomic DNA hypomethylation participate in the evolution of neoplastic transformation. Induction of strand breaks creates a chromosomal aberration and increases the mutation rate of critical cancer associated genes and the development of neoplastic transformation. A 5-6-fold supplementation of folic acid might give the opposite effect (Mason JB, Boston). Familial adenomatous polyposis and hereditary non polyposis colorectal cancer genes have been identified and gene testing to search for the gene carrier in affected families is now available commercially. Fecal occult blood tests and colonoscopy once every 1-2 years are recommended for all adults, starting at the age of 50 years (Burt RW, Utah).

For the treatment of inflammatory bowel disease (Singleton JW, Denver, United States), 5 ASA and glucocorticoids are still the most effective drugs to achieve remission in Crohn's disease and ulcerative colitis, with immunosuppressants such as 6 mercaptopurine, azathioprine or methotrexate resulting in remission for both diseases. Metronidazole and ciprofloxacin are also adjuncts for Crohn's disease. TNF antagonists and bradykinin antagonists are currently being explored in IBD.

A metastatic model of human HCC in nude mice has been developed, with 100% intrahepatic, lung and lymph node metastasis after 30 generations showing invasiveness of HCC related to certain oncogenes and growth factors, including p16, p53 mutation, H-ras, c-erbB-2, TNF- $\alpha$ , EGF receptor and MMP2, but not nm23-H1 and TIMP2. Antisense H-ras and anti HBx antibody plus LAK cells can

inhibit cancer growth in that model. TNF gene liposome intralesional administration also had an inhibitory effect in the HCC model in nude mice (Tang ZY, Shanghai). Treatment of chronic hepatitis B has been advanced by developing new antiviral agents, such as lamivudine and famciclovir which act as reverse transcriptase inhibitors and HBV DNA polymerase inhibitors, respectively. Both of these drugs can produce a profound decrease in serum HBV DNA levels within 12 wk of treatment but very few patients cleared HBeAg, with a current study indicating that prolonged usage is necessary for a sustained effect. Clinical trials of synthetic peptide vaccines containing cytotoxic T cell epitopes for HBeAg and DNA vaccines with recombinant HBV surface gene are underway, showing promising results (Lok ASF, Michigan). For compensated liver cirrhosis due to chronic hepatitis B, a high protein vegetarian diet combined with acyclovir and ribavirin resulted in a negative to positive nitrogen balance, with 30% HBsAg, 25% HBeAg and 33% positive HBV DNA becoming negative at the end of the treatment (Tang ZD, Shanghai). Tropical chronic pancreatitis is common in southern India, characterized by recurrent chronic abdominal pain, insulin dependent diabetes and large pancreatic calculi affecting young male adolescents. Malnutrition, protein deficiency and an impaired immune response are believed to play an etiological role. High doses of insulin, analgesics for pain and pancreatic enzymes are necessary for control and surgical decompression of a dilated pancreatic duct with removal of pancreatic stones, resulting in improvement in both endocrine and exocrine insufficiency, as well as pain relief. Sphincterotomy, fragmentation of stones with subsequent removal and pancreatic stenting have also been effective (Tandon RK, India). In acute hemorrhagic necrotizing pancreatitis, target treatment with liposomes of proglumide and emodin in a rat model showed a significant increase of autophagy and autophagic vacuoles within which organelles were seen on ultramicroscopy. Thereafter, the autophages decreased in number and size. It is believed that emodin has a stabilizing effect on the lysosomal membrane and a direct effect in the pancreas, which differs in its mechanism of action from that of somatostatin (Xu JY, Shanghai). Patients with irritable bowel syndrome have visceral hypersensitivity and the visceral perception is via activation of afferent pathways by stimulating mucosal receptors, smooth muscle mechanoreceptors and mesenteric nociceptors. Trivial physiological stimuli can induce pain and those with diarrhea and rectal urgency experience diffuse abdominal pain, whereas healthy subjects only note discomfort localized in a single quadrant. Abnormal psychological features are also found. Bulking agents should be given to those with constipation, whereas those with diarrhea can have octreotide or the 5-HT-3 receptor antagonist fedotoxine which blunts perception of visceral distention (Owyang C, Michigan). A series of western medicines, colchicine, polyunsaturated lecithin, IFN- $\gamma$ , retinoids, etc., to treat hepatic fibrosis were reviewed by Wang BE (Beijing), with a special emphasis on the use of herbal medicine compound 861. Both animal experiments and clinical B hepatitis patients showed a significant attenuation of inflammation and fibrosis, stimulation of collagen degradation, less synthesis of total and type I, III and V collagen, less mRNA expression for procollagen I, III and IV in liver tissue and also inhibition of TGF- $\beta$ , in which collagenase activity was increased and TIMP activity decreased. Diabetic gastroparesis may exhibit myelin degeneration of the vagus nerve or be associated with impairment of sympathetic nervous function. There is also decreased smooth muscle contractility with a biochemical abnormality at the smooth muscle cell level. Prokinetics such as metoclopramide, domperidone, cisapride and erythromycin have their nature and sites of action delineated (Wiley IW, Michigan).

S- Editor: Ma JY L- Editor: Ma JY E- Editor: Liu WX

## Uptake of bacterial lipopolysaccharide and expression of tumor necrosis factor- $\alpha$ -mRNA in isolated rat intrahepatic bile duct epithelial cells

Xian-Ming Chen, De-Wu Han, K Noguchi, K Tanikawa

Xian-Ming Chen, De-Wu Han, Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

K Noguchi, K Tanikawa, Second Department of Medicine, Kurume University School of Medicine, Kurume, Japan

Xian-Ming Chen, Associate Professor of pathophysiology with major specialty in hepatic pathophysiology, with 15 published papers

Presented at The Annual Meeting of American Association for the Study of Liver Diseases, Chicago, 11-15 November, 1994 and the 1st China Japan International Congress of Pathophysiology, Dalian, 10-11 October, 1995

Supported by The Natural Science Foundation for Youth of Shanxi Province, No. 95013.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Xian-Ming Chen, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China  
Telephone: +86-351-2021511-521  
Fax: +86-351-2024239

Received: January 26, 1996  
Revised: September 1, 1996  
Accepted: December 1, 1996  
Published online: March 15, 1997

### Abstract

**AIM:** To study the uptake of bacterial lipopolysaccharides (LPS) and expression of tumor necrosis factor  $\alpha$ -mRNA (TNF- $\alpha$ -mRNA) with cultured rat intrahepatic bile duct epithelial cells.

**METHODS:** By using fluorescent, immunohistochemical and in situ hybridization techniques, the uptake of Escherichia coli LPS and expression of TNF- $\alpha$ -mRNA with isolated rat intrahepatic bile duct epithelial cells were observed with confocal laser scanning microscopy.

**RESULTS:** Positive reactions to LPS were found in the cytoplasm of isolated intrahepatic bile duct epithelial cells after incubation with LPS for 15 min and the FITC fluorescent intensity against LPS was significantly higher than that of the controls ( $121.45 \mu\text{FI}/\mu\text{m}^2 \pm 15.62 \mu\text{FI}/\mu\text{m}^2$  vs  $32.12 \mu\text{FI}/\mu\text{m}^2 \pm 9.64 \mu\text{FI}/\mu\text{m}^2$ ,  $P < 0.01$ ). After incubation with LPS for 3 h, fluorescein isocyanate (FITC) fluorescent intensities of the expression of TNF- $\alpha$ -mRNA with fluorescent in situ hybridization in the cytoplasm and nuclei of the cultured bile duct epithelial cells were significantly higher than those of the controls ( $189.15 \mu\text{FI}/\mu\text{m}^2 \pm 21.33 \mu\text{FI}/\mu\text{m}^2$  vs  $10.00 \mu\text{FI}/\mu\text{m}^2 \pm 8.99 \mu\text{FI}/\mu\text{m}^2$ ,  $64.85 \mu\text{FI}/\mu\text{m}^2 \pm 14.99 \mu\text{FI}/\mu\text{m}^2$  vs  $21.20 \mu\text{FI}/\mu\text{m}^2 \pm 2.04 \mu\text{FI}/\mu\text{m}^2$ ,

respectively ( $P < 0.01$ )). The increase of FITC fluorescent intensity of TNF- $\alpha$ -mRNA expression in the cytoplasm peaked at 6 h after incubation ( $221.38 \mu\text{FI}/\mu\text{m}^2 \pm 22.99 \mu\text{FI}/\mu\text{m}^2$ ). At various time points after incubation with LPS, the increase of fluorescent intensities of TNF- $\alpha$ -mRNA in the cytoplasm were much higher than those in the nuclei ( $P < 0.01$ ).

**CONCLUSION:** LPS can act on and enter into isolated intrahepatic bile duct epithelial cells and stimulate the expression of TNF- $\alpha$ -mRNA.

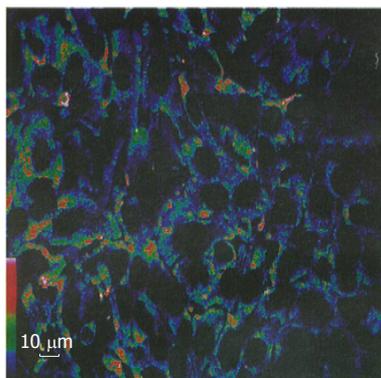
**Key words:** Lipopolysaccharides; Epithelial cell bile ducts; Tumor necrosis factor; *In situ* hybridization

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen XM, Han DW, Noguchi K, Tanikawa K. Uptake of bacterial lipopolysaccharide and expression of tumor necrosis factor- $\alpha$ -mRNA in isolated rat intrahepatic bile duct epithelial cells. *World J Gastroenterol* 1997; 3 (1): 3-5 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/3.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.3>

### INTRODUCTION

Endotoxins, mainly consisting of lipopolysaccharides (LPS) and proteins, can induce a number of reactions, both beneficial and harmful to the body<sup>[1]</sup>. It is generally known that the cells of the reticuloendothelial system, mainly the Kupffer cells of the liver, are responsible for the clearance of LPS<sup>[2]</sup>. LPS that have undergone degradation in the liver are subsequently excreted mainly into the gut through the bile ducts. Although the LPS present in the liver after the injection exhibited a lower fatty acid to carbohydrate ratio than the original LPS, lipid A was still present, covalently bound to the polysaccharide, thus showing that the overall macromolecular structure of LPS had remained unaltered<sup>[3]</sup>. Besides, the toxic activity of LPS in the bile was the same as the original LPS, indicating that LPS in the bile are biologically active<sup>[4]</sup>. The bile duct epithelial cells have been postulated to be involved in absorptive and secretory activities, including the transport of water, electrolytes, sugars, amino acids, bile acids and proteins<sup>[5-6]</sup>, and might play a role in the liver immune system, but only a little information exists about their interactions with LPS. In the present study, by using a monoclonal antibody specific to the core lipid A region of LPS and a fluorescein isocyanate (FITC) labelled oligonucleotide as a probe for TNF- $\alpha$ -mRNA, the uptake of LPS and expression of TNF- $\alpha$ -mRNA with isolated rat intrahepatic bile duct epithelial cells were observed with immunofluorescence and fluorescent in situ hybridization techniques followed by confocal laser scanning microscopy.



**Figure 1** Fluorescent reactions to lipopolysaccharides in cultured intrahepatic bile duct epithelial cells. Positive reactions were obvious in the cytoplasm. The color bar in the bottom on the left side shows the fluorescent intensities (the highest is red and the lowest green).

## MATERIALS AND METHODS

### Materials

Collagenase (Type I), trypsin, insulin, heparin and wild form LPS for *Escherichia coli* serotype 0111:B4 were purchased from Sigma Chemical Company. Monoclonal antibody against LPS specific to the core lipid A region was a gift from Dr. Noguchi. Fluorescent (FITC) labelled affinity purified goat anti mouse IgM was from O.E.M. Concepts Incorporation. Eagles' MeM was obtained from Nissui Pharmaceutical Co. William's Medium E. was from ICN Biomedicals Incorporation. Low LPS fetal bovine serum (FBS) was from Life Technologies. The endotoxin concentration in the prepared media with 10% FBS used in the experiments was as low as 0.6 ng/mL by measurement using the Endotoxin Species Test Kit (Seikagakce Kogyo Ltd.). Percoll (sterile) was purchased from Pharmacia Bioprocess Technology AE. All other reagents were of analytical grade from the usual commercial sources. Male Wistar rats under specific pathogen free conditions were used (each 150 to 200 mg). Twenty-four hours before the experiments, the rats were given kanamycin (1 mg), lactulose (100 mg), sodium picosulfate (0.125 mg) and magnesium citrate (50 mg) diluted in 2 mL sterile water orally every other hour. All the rats were deprived of food but allowed free access to sterile water.

### Isolation in culture of intrahepatic bile duct epithelial cells

The intrahepatic bile duct epithelial cells were isolated from the rat livers by means of a modified technique described by Ishii *et al.*<sup>[7]</sup>. The rats were anesthetized and their livers were perfused in situ at a flow rate of 10 mL/min with the perfused liquid (heparin 2.5  $\mu$ /mL, Ca<sup>2+</sup> and Mg<sup>2+</sup> free) followed with perfusion of the 0.05% collagenase solution for 15 min. After perfusion, the livers were then carefully removed and placed in ice coated Eagles' MEM. The intact hyperplastic bile ductular tissue was gently separated from the enzyme dissociated hepatic parenchymal and sinusoidal components. An essentially pure bile ductular tissue fraction was obtained by shaking the tissue fragments for 50 min in Hank's medium containing 0.1% collagenase and 0.25% trypsin in order to remove any residual hepatic parenchymal cells that might have remained attached. Following centrifugation at 467  $\times$  *g* for 5 min at 4 °C, the cells were then resuspended in Eagles' MEM and laid as 5 mL aliquot on top of 15 mL of 30% and 50% Percoll in Hank's medium. Following centrifugation at 1870 *g* for 30 min at 4 °C, viable intrahepatic bile duct epithelial cells were obtained and viability was determined by the methods of trypan blue dye exclusion. The cell count was made (1-3  $\times$  10<sup>5</sup> cell/mL). After incubation at 37 °C for 24 h in 35 mm plastic dishes in a 5% CO<sub>2</sub> and 40% O<sub>2</sub> atmosphere (in William's Medium E with 10% FBS), the media were changed to William's medium E (FBS free) with LPS (10 mg/L). After incubation for 15 min, the cells were fixed with 99% methanol for 10 min, washed with sterile saline and then underwent the immunohistochemistry procedure.

In a separate experiment, the isolated bile duct epithelial cells were set in glass dishes. After incubation at 37 °C for 24 h in a 5% CO<sub>2</sub> and 40% O<sub>2</sub> atmosphere (in William's Medium E with 10% FBS),

the media were changed to William's medium E (FBS free) with LPS (10 mg/L). After incubation for 3, 6 and 9 h, the cells were fixed with PHA, washed with sterile saline and then the fluorescent in situ hybridization procedure was done to determine the expression of TNF- $\alpha$ -mRNA.

### Immunofluorescence staining and confocal laser scanning microscopic observation

To localize LPS in the cells, the indirect staining procedure was followed by using the monoclonal antibody against lipid A of LPS (1:800) and FITC labelled immunoglobulin as the secondary antibody (1:30). After immunofluorescence staining, the dishes were mounted with VECTASHIELD mounting medium for fluorescence under the coverslip. With a confocal laser scanning microscope (LSM-GB 200, Olympus, Japan) and an analysis system of LSM-SB 200 Ver. 2.01 provided by Olympus Company, the fluorescent intensities of FITC in the cytoplasm of the intrahepatic bile duct epithelial cells were measured under the same conditions as the analysis system. For each dish, 20 to 30 cells were measured.

### Fluorescent in situ hybridization procedure and confocal laser scanning microscopic observation

A fluorescein-dUTP labelled oligonucleotide as a fluorescent in situ hybridization probe for TNF- $\alpha$ -mRNA (5'-GCCACGTCGTAGCCAAACCA-CCAAGTGG-3') was used in this study. After fixation with PHA, the cells were hybridized with the probe in the hybridization mixture for 20 h at 45 °C. After hybridization, the dishes were rinsed three times in phosphate buffered saline and dehydrated. FITC activities against the expression of TNF- $\alpha$ -mRNA in the cytoplasm and nuclei were observed and measured with a confocal laser scanning microscope by the same method as described above.

### Statistical analysis

All the data were expressed as  $\bar{x} \pm s$  from three to six separate experiments and were analyzed with the StatView program. Student's *t* test and ANOVA *F* test were applied where appropriate.

## RESULTS

### Uptake of LPS by cultured intrahepatic bile duct epithelial cells

Cultured intrahepatic bile duct epithelial cells showed a strong positive reaction to LPS after 15 min of incubation. Positive reactions were found in the cytoplasm but not in the nuclei (Figure 1). The fluorescent intensities in the cytoplasm of the bile duct epithelial cells were significantly higher than that of the controls (121.45  $\mu$ FI/ $\mu$ m<sup>2</sup>  $\pm$  15.62  $\mu$ FI/ $\mu$ m<sup>2</sup> vs 32.12  $\mu$ FI/ $\mu$ m<sup>2</sup>  $\pm$  9.64  $\mu$ FI/ $\mu$ m<sup>2</sup>, *P* < 0.01). No fluorescence was found in the negative controls using only the monoclonal antibody or FITC labelled secondary antibody.

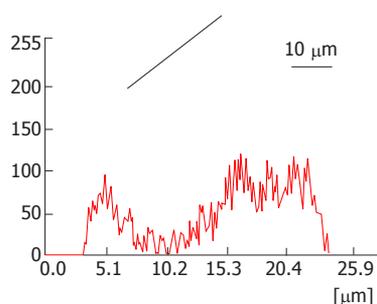
### Expression of TNF- $\alpha$ -mRNA

Positive FITC reactions to TNF- $\alpha$ -mRNA were found in the cytoplasm of the cultured intrahepatic bile duct epithelial cells after incubation with LPS for 3 h. Positive reactions were also seen in the nuclei. The positive FITC reactions became stronger in the cytoplasm after 6 h of incubation (Figure 2) and decreased after 9 h of incubation. Changes of FITC intensities in the cytoplasm and nuclei of the cultured cells are shown in Table 1.

The FITC fluorescent intensities in the cytoplasm at various time points were significantly higher than those in the nuclei.

## DISCUSSION

Endotoxins mainly consist of LPS and some proteins. It is LPS which inflicts all the ill, toxic effects from the endotoxins<sup>[1]</sup>. It is generally known that most endotoxins in the bile flow excreted from the liver remain to exhibit the overall macromolecular structure of LPS and are biologically active<sup>[3-4]</sup>. The LPS injected through the portal vein could be detected in the intrahepatic bile duct epithelial cells in rats<sup>[8]</sup>. In the present study, strong positive FITC reactions against LPS could be seen in the cytoplasm of the cultured intrahepatic bile duct epithelial cells after incubation with LPS for



**Figure 2** Fluorescent reactions to TNF- $\alpha$ -mRNA in cultured intrahepatic bile duct epithelial cells. Positive reactions were found in the cytoplasm and nuclei of the cells. The chart shows the fluorescent intensities along the line through the cells as indicated.

**Table 1** Fluorescein isocyanate fluorescent intensities of TNF- $\alpha$ -mRNA expression in isolated intrahepatic bile duct epithelial cells after incubation with lipopolysaccharides ( $\mu\text{FI}/\mu\text{m}^2$ ,  $\bar{x} \pm s$ )

	FITC fluorescent intensities	
	Cytoplasm	Nuclei
Controls	10.00 $\pm$ 8.99	2.20 $\pm$ 2.04
LPS incubations		
3 h	189.15 $\pm$ 21.33 <sup>b</sup>	64.85 $\pm$ 14.99 <sup>b</sup>
6 h	221.38 $\pm$ 22.99 <sup>bd</sup>	138.15 $\pm$ 36.54 <sup>bd</sup>
9 h	170.00 $\pm$ 15.25 <sup>b</sup>	70.42 $\pm$ 9.08 <sup>b</sup>

<sup>b</sup>Compared with the controls ( $P < 0.01$ ); <sup>d</sup>Compared with those of LPS incubations for 3 and 9 h ( $P < 0.01$ ). FITC: fluorescein isocyanate.

15 min, indicating that biologically active LPS could act on and get into the bile duct epithelial cells. Two different kinds of mechanisms, *i.e.* specific and nonspecific, are suggested to be involved in the initial interaction of LPS with the cells<sup>[9]</sup>. The specific interactions result from the binding of LPS to a specific receptor on the plasma membrane. On the other hand, nonspecific interactions result from the binding of LPS macromolecules to any membrane constituents other than the receptors. Both mechanisms are involved in the uptake process of LPS by cells<sup>[10,11]</sup>. A number of receptors in the membranes of various cells specific to different parts of LPS such as a 73 kDa membrane localized protein, CD14 and high lipoprotein receptor were recently reported<sup>[12,13]</sup>. Whether there are such receptors in the membranes of the bile duct epithelial cells and with which mechanism the biologically active LPS enter those cells are still to be clarified.

Although the bile duct epithelial cells were not considered to play a significant role in liver function, the involvement in immunological functions is being described more frequently now. The bile duct epithelial cells in the primary culture have been shown to activate T cells<sup>[14]</sup> and in cholestatic liver diseases they displayed aberrant expression of MHC class I and II antigens and of TNF- $\alpha$  receptors<sup>[15]</sup>. The expression of TNF- $\alpha$ -mRNA was detected in the intrahepatic bile duct epithelial cells of LPS perfused livers<sup>[16]</sup>. The results of the present study showed that strong expression of TNF- $\alpha$ -mRNA could be detected in the cultured bile duct epithelial cells after 3 h incubation with LPS, denoting that the bile duct epithelial cells could synthesize TNF- $\alpha$ -mRNA for the stimulation of LPS. TNF is generally considered to be an important cytokine which is associated with the

injury of hepatocytes, the production of collagen and proliferation and differentiation of the cells. It plays a critical role in provoking the development of certain liver diseases. The proliferation and differentiation of the bile duct epithelial cells is likely to be a common feature related to quite a number of liver diseases. The oval cells are postulated to originate from the differentiation of the bile duct epithelial cells<sup>[5]</sup>. *In vitro*, the bile duct epithelial cells reacted to cytokines such as TGF, EGF and TNF to proliferate<sup>[17]</sup>. With the observations in our study, the authors tended to conclude that the interaction of the biologically active LPS in the bile flow with the bile duct epithelial cells might take part in the processes of forming the pathological changes in the liver in some liver diseases.

## REFERENCES

- 1 Rietschel ET, Brade H. Bacterial endotoxins. *Sci Am* 1992; **267**: 54-61 [PMID: 1641625 DOI: 10.1038/scientificamerican0892-54]
- 2 Nakao A, Taki S, Yasui M, Kimura Y, Nonami T, Harada A, Takagi H. The fate of intravenously injected endotoxin in normal rats and in rats with liver failure. *Hepatology* 1994; **19**: 1251-1256 [PMID: 8175149 DOI: 10.1002/hep.1840190525]
- 3 Freudenberg MA, Galanos C. Alterations in rats in vivo of the chemical structure of lipopolysaccharide from *Salmonella abortus equi*. *Eur J Biochem* 1985; **152**: 353-359 [PMID: 4054113 DOI: 10.1111/j.1432-1033.1985.tb09205.x]
- 4 Freudenberg M, Galanos C. Metabolic fate of endotoxin in rat. In: Friedman H, Klein TW, Nakano M, Nowotny A. editors. *Advances in experimental medicine and biology*. Vol 256. New York: Plenum Press, 1990: 499-509 [DOI: 10.1007/978-1-4757-5140-6\_44]
- 5 Tavoloni N. The intrahepatic biliary epithelium: an area of growing interest in hepatology. *Semin Liver Dis* 1987; **7**: 280-292 [PMID: 3324347 DOI: 10.1055/s-2008-1040583]
- 6 Alpini G, Lenzi R, Sarkozi L, Tavoloni N. Biliary physiology in rats with bile ductular cell hyperplasia. Evidence for a secretory function of proliferated bile ductules. *J Clin Invest* 1988; **81**: 569-578 [PMID: 2448343 DOI: 10.1172/JCI113355]
- 7 Ishii M, Vroman B, LaRusso NF. Isolation and morphologic characterization of bile duct epithelial cells from normal rat liver. *Gastroenterology* 1989; **97**: 1236-1247 [PMID: 2792660]
- 8 Chen XM, Han DW, Noguchi K, Mimura Y, Ohishi M, Harada M. Selective uptake of two types of lipopolysaccharide (LPS) by Kupffer cells and other liver cells. *Jpn J Pathophysiol* 1995; **4**: 27
- 9 Morrison DC. Nonspecific interactions of bacterial lipopolysaccharides with membranes and membrane components. In: Berry IJ, editor. *Cellular biology of endotoxin*. New York: Elsevier, 1985: 25-30
- 10 Kriegsmann J, Gay S, Bräuer R. Endocytosis of lipopolysaccharide in mouse macrophages. *Cell Mol Biol (Noisy-le-grand)* 1993; **39**: 791-800 [PMID: 8268763]
- 11 Kriegsmann J, Bräuer R. Lipopolysaccharide (LPS) binding in subpopulations of mouse peritoneal macrophages. *Cell Mol Biol (Noisy-le-grand)* 1993; **39**: 783-789 [PMID: 7505675]
- 12 Lei MG, Stimpson SA, Morrison DC. Specific endotoxic lipopolysaccharide-binding receptors on murine splenocytes. III. Binding specificity and characterization. *J Immunol* 1991; **147**: 1925-1932 [PMID: 1716286]
- 13 Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990; **249**: 1431-1433 [PMID: 1698311 DOI: 10.1126/science.1698311]
- 14 Kirkpatrick D, Baranski B, Gourley G, Kremer B, Loew N. T cell stimulation by normal mouse biliary duct cells in culture: a new model for graft versus host disease? *Gastroenterol* 1991; **96**: A630
- 15 Giroir BP, Johnson JH, Brown T, Allen GL, Beutler B. The tissue distribution of tumor necrosis factor biosynthesis during endotoxemia. *J Clin Invest* 1992; **90**: 693-698 [PMID: 1522226 DOI: 10.1172/JCI115939]
- 16 Hoffmann R, Grewe M, Estler HC, Schulze-Specking A, Decker K. Regulation of tumor necrosis factor- $\alpha$ -mRNA synthesis and distribution of tumor necrosis factor- $\alpha$ -mRNA synthesizing cells in rat liver during experimental endotoxemia. *J Hepatol* 1994; **20**: 122-128 [PMID: 8201213 DOI: 10.1016/S0168-8278(05)80478-7]
- 17 Matsumoto K, Fujii H, Michalopoulos G, Fung JJ, Demetris AJ. Human biliary epithelial cells secrete and respond to cytokines and hepatocyte growth factors in vitro: interleukin-6, hepatocyte growth factor and epidermal growth factor promote DNA synthesis in vitro. *Hepatology* 1994; **20**: 376-382 [PMID: 8045498 DOI: 10.1002/hep.1840200217]

S- Editor: Feng CY L- Editor: Ma JY E- Editor: Liu WX

## Effects of $\gamma$ -interferon on hepatic fibrosis of schistosoma japonicum-infected mice

Yong-Wen He, Wei Liu, Ling-Lan Zen, Duan-De Luo

Yong-Wen He, Wei Liu, Ling-Lan Zen, Duan-De Luo, Department of Infectious Diseases, Union Hospital, Tongji Medical University, Wuhan 430022, Hubei Province, China

Yong-Wen He, male, born on August 1, 1950 and graduated from Tongji Medical University, now Professor of Internal Medicine, having 24 papers published

Supported by The National Natural Science Foundation of China, No. 39000095

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. He-Yong Wen, Professor, Department of Infectious Diseases, Union Hospital, Tongji Medical University, Wuhan 430022, Hubei Province, China  
Telephone: +86-27-5807711-512

Received: September 9, 1996

Revised: October 12, 1996

Accepted: January 21, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To probe the effect of  $\gamma$ -IFN on hepatic fibrosis in *schistosomiasis japonica*.

**METHODS:** The amount and distribution of  $\gamma$ -IFN and extracellular matrix in the liver of 60 *S. japonicum* infected mice and 30 healthy mice at different stages, and their dynamics in 20 infected mice after administration of recombinant  $\gamma$ -interferon were determined by immunohistochemical streptavidin biotin peroxidase complex method.

**RESULTS:** The amount of  $\gamma$ -IFN in liver peaked at the 16<sup>th</sup> week after infection (3 mice respectively reached 2+, 3+ and 4+ grade), which was higher than the levels of infected mice at the 8<sup>th</sup>-12<sup>th</sup> week ( $P < 0.01$ ), and  $\gamma$ -IFN was mostly distributed around egg granuloma. Fibronectin, laminin, type I and III collagens in liver of most infected mice reached 1+ grade and individual 2+ grade at the 8<sup>th</sup> week after infection, which were higher than those of healthy controls ( $P < 0.01$ ), and were linearly distributed around egg granuloma. With chronicity and decrease of  $\gamma$ -interferon, however, the matrix proteins and collagens gradually increased, peaked respectively at the 20<sup>th</sup> and 24<sup>th</sup> week (over 70% infected mice with 3+ to 4+ grade), became wide and thick, and deposited in band like or retiform shape around and in egg granuloma. After administration of  $\gamma$ -IFN, only 3 infected mice had 2+ grade of fibronectin at the 20<sup>th</sup> week, and 2 mice had 3+ grade of type III collagen at the 24<sup>th</sup> week, and none of them reached 4+ grade, which were significantly less than the untreated group at the same stage ( $P < 0.01-0.05$ ).

**CONCLUSION:**  $\gamma$ -interferon may play an important role in opposing the inflammatory response of egg granuloma, decreasing secretion and deposition of extracellular matrix in the liver and suppressing hepatic fibrosis.

**Key words:** Schistosomiasis; Liver cirrhosis; Interferon type II; Granuloma; Extracellular matrix

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

He YW, Liu W, Zen LL, Luo DD. Effects of  $\gamma$ -interferon on hepatic fibrosis of schistosoma japonicum-infected mice. *World J Gastroenterol* 1997; 3(1): 6-8 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/6.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.6>

### INTRODUCTION

Some cytokines play important roles during egg-induced immune response, leading to the granuloma formation and hepatic fibrosis after schistosoma infection<sup>[1,2]</sup>. For example, studies in schistosomiasis mansoni<sup>[3,4]</sup> and japonica<sup>[5]</sup> showed that  $\gamma$ -interferon ( $\gamma$ -IFN) can affect the granuloma formation and inflammatory response. However, these studies just focused on the changes of  $\gamma$ -IFN in peripheral blood or induced *in vitro*. The amount and distribution of  $\gamma$ -IFN in the liver and its relationship with hepatic extracellular matrix were not involved. Therefore, we chose a sensitive immunohistochemical technique, streptavidin biotin peroxidase complex (SABC) method to measure  $\gamma$ -IFN in the liver of *S. japonicum* infected mice, as well as laminin (LN), fibronectin (FN), types I and III collagen in the liver which were used for the markers of hepatic fibrosis, and gave exogenous  $\gamma$ -IFN to infected mice to find out the relationship between  $\gamma$ -IFN and hepatic fibrosis.

### MATERIALS AND METHODS

#### Animals and interferon treatment

One hundred and ten 8-wk-old Kun Ming mice were randomly divided into infected group, control group and  $\gamma$ -IFN injected group. Sixty mice in infected group were equally subdivided into 6 groups, of which each mouse was infected with 20 cercariae of *S. japonica*, and then were killed at the 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, 20<sup>th</sup>, 24<sup>th</sup> and 28<sup>th</sup> week after infection, respectively; 30 healthy mice in control group were subdivided into 3 groups, and were killed at the 8<sup>th</sup>, 16<sup>th</sup>, and 24<sup>th</sup> week after infection respectively; and 20 infected mice were subdivided into 2 groups, each of which was treated with murine recombinant  $\gamma$ -IFN (Gibco BRL, United States) 50000 units daily I.M. for 20 d, starting at the 16<sup>th</sup> week after infection, and then were killed at the 20<sup>th</sup> and 24<sup>th</sup> week after infection, respectively. All fresh livers were stored in liquid nitrogen.

**Table 1** Dynamics of  $\gamma$ -IFN and extracellular matrix in the liver of healthy control and infected mice

Groups (wk)	Cases	$\gamma$ -IFN				FN				LN				Type I coll.				Type III coll.				
		1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+	
Control																						
8	10	1				2				2				2						1		
16	10	2				2				1				0						2		
24	10	1				1				0				1						2		
Infected																						
8	10	9	1			9	1			10				10					9	1		
12	10	3	7			8	2			7	3			5	5				5	5		
16	10	1	3	3	3		6	4			6	1	3		8	2				6	4	
20	10	5	4	1			3	4	3		2	6	2		6	4				5	2	3
24	10	8	2				5	5			4	4	2		3	5	2			2	5	3
28	10	8				8	2			7	3				4	3	3			4	4	2

Note: The numbers under 1+, 2+, 3+ or 4+ mean the cases reaching 1+, 2+, 3+ or 4+ grade.

**Table 2** Changes of  $\gamma$ -IFN and extracellular matrix in the liver before and after  $\gamma$ -IFN treatment

Groups (wk)	Cases	$\gamma$ -IFN				FN				LN				Type I collagen				Type III collagen				
		1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+	
Non-treated																						
20	10	5	4	1			3	4	3		2	6	2		6	4				5	2	3
24	10	8	2				5	5			4	2	2		3	5	2			2	5	3
Treated																						
20	10		5	3	2	7	3				4	6			7	3				5	5	
24	10	5	5			3	6	1			4	6		1	7	2			2	6	2	

Note: The numbers under 1+, 2+, 3+ or 4+ mean the cases reaching 1+, 2+, 3+ or 4+ grade.

### Streptavidin biotin peroxidase complex assay

SABC is a modified ABC immunohistochemical technique, which means that avidin is replaced by streptavidin to reduce background stain and to increase specificity and sensitivity. Briefly, frozen sections of the liver, dried after blowing, were fixed with pure acetone for 10 min. After drying at room temperature and washing with PBS (2 min  $\times$  3 times), they were incubated with 1.5% H<sub>2</sub>O<sub>2</sub>/methyl for 10 min at room temperature, and blocked with sheep serum diluted at 1:50 in PBS for 15 min at room temperature. Then, first antibodies diluted 1:1000 in PBS, including monoclonal antibodies against murine  $\gamma$ -IFN (GIBCO BRL, United States), and antisera against FN, LN, types I and III (WTB1) collagen (Beijing Friendship Hospital), were added and incubated for 1 h at 37 °C. After 3 washings, biotin conjugated anti-IgG (Sigma, United States) diluted 1:100 in PBS was added and incubated for 20 min at 37 °C. Then, 10  $\mu$ L of solution A and 10  $\mu$ L of solution B of SABC kit, diluted in 1 mL of PBS, were added to the sections that had been washed as above, and incubated for 20 min at 37 °C. After that, 3-amino-9-ethylcarbazole (AEC) was colorized for 30 min and then washed with water. Finally, the sections were examined under light microscope after covering with glycol gelatin.

### Result judgement

The amounts of  $\gamma$ -IFN, LN, FN, types I and III collagen were divided into 4 grades, according to the shade of color, range and density of distribution. Red and sporadic stain was defined as 1+ grade, red and fine lineal shape as 2+, red and flaky or band like shape as 3+, dark red, band like or retiform shape as 4+. Statistical analysis was performed by the Chi-square test.

## RESULTS

### $\gamma$ -IFN and extracellular matrix in the liver of healthy mice

In the liver of healthy mice,  $\gamma$ -IFN was sporadically scattered near sinusoid wall, and extracellular matrix in portal tracts and sinusoid wall, whose amount was very low and seldom reached 1+ grade. Changes in the amount and distribution of  $\gamma$ -IFN and extracellular matrix at different stages (8<sup>th</sup>, 16<sup>th</sup> and 24<sup>th</sup> weeks) were insignificant (Table 1).

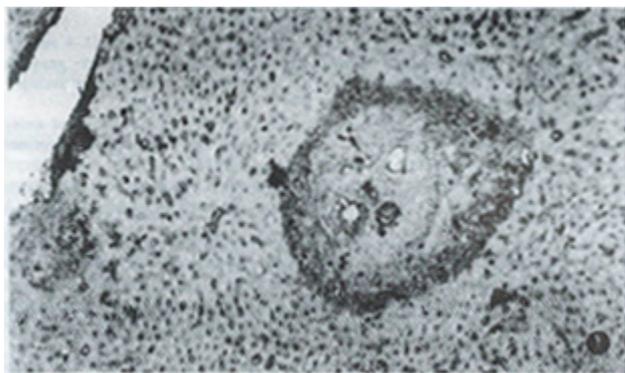
### Distribution and dynamics of $\gamma$ -IFN and extracellular matrix in the liver of infected mice

Table 1 shows the dynamics of  $\gamma$ -IFN and extracellular matrix in

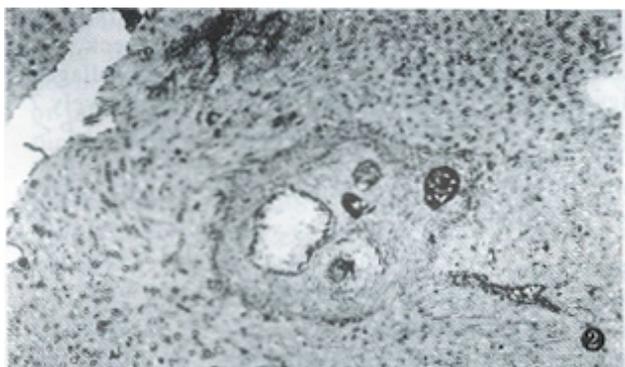
livers of infected mice.  $\gamma$ -IFN in livers of 9 infected mice reached 1+ grade and 1 mouse 2+ grade at the 8<sup>th</sup> week after infection when granuloma began to form and its inflammatory response was present, which was significantly higher than that of healthy control ( $P < 0.01$ ), and was mainly distributed around egg granuloma and a little on sinusoid wall. The level of  $\gamma$ -IFN in the liver of infected mice at the 12<sup>th</sup> week after infection was higher than that at the 8<sup>th</sup> week ( $P < 0.01$ ), and peaked at the 16<sup>th</sup> week after infection (3 mice reached 2+, 3+ and 4+ grade respectively), which was significantly higher than that at the 12<sup>th</sup> week ( $P < 0.01$ ). The more severe the inflammatory response was, the higher the amount of  $\gamma$ -IFN was.  $\gamma$ -IFN started to decrease at the 20<sup>th</sup> week when inflammatory response diminished. FN, LN, type I and III collagens in the liver of most infected mice reached 1+ grade and 2+ grade at the 8<sup>th</sup> and 12<sup>th</sup> week after infection, which were higher than those of healthy controls ( $P < 0.01$ ), and were lineally scattered in the portal tracts and around egg granuloma, but a little on the sinusoid wall. In contrast to  $\gamma$ -IFN, at the 16<sup>th</sup> week after infection when severe inflammatory response appeared and  $\gamma$ -IFN reached its peak, the amount of hepatic extracellular matrix lowered, although they continually rose. Then, the extracellular matrix increased steadily along with the chronicity of infection, the relief of inflammatory response and the reduction of the amount of  $\gamma$ -IFN. FN and LN peaked at the 20<sup>th</sup> week after infection (7 and 8 mice with 3+ grade or over); type I and III collagens peaked at the 24<sup>th</sup> week after infection (7 and 8 mice with 3+ grade or over) (Figure 1A). Among them, the level of type I collagen at the 24<sup>th</sup> week was higher than that at the 16<sup>th</sup> week ( $P < 0.05$ ). After 20<sup>th</sup> week, extracellular matrix gradually became wide and thick, and deposited in band-like or retiform shape in and around egg granuloma and portal tracts.

### Effect of $\gamma$ -IFN treatment on extracellular matrix

$\gamma$ -IFN treatment kept the hepatic  $\gamma$ -IFN at a high level in the 20<sup>th</sup> week after infection, which was significantly higher than that of the non-treated group ( $P < 0.01$ ), and close to that of non-treated group at the 16<sup>th</sup> week (Table 2). At the same time, the extracellular matrix in portal tracts, in and around granuloma diminished obviously. Only 3 mice had 2+ grade or over of FN in the 20<sup>th</sup> week, while 10 mice in the non-treated group ( $P < 0.01$ ). At the 24<sup>th</sup> week, respectively, 2 mice had 3+ grade of type I collagen (Figure 1B) and III collagen, whereas, respectively, 7 and 8 mice in non-treated group ( $P < 0.05$ ). None of mice in treated group reached 4+ grade



**Figure 1** Type I collagen in the liver at 20<sup>th</sup> week after infection before and after administration of  $\gamma$ -IFN ( $\times 100$ ). A: Red and patched or band-like type I collagen in and around egg granuloma (3 + grade) before  $\gamma$ -IFN injection; B: Decreased type I collagen in and around egg granuloma (1+ grade) after  $\gamma$ -IFN injection.



**Figure 2** Type I collagen in the liver at 20<sup>th</sup> week after infection before and after administration of  $\gamma$ -IFN ( $\times 100$ ). A: Red and patched or band-like type I collagen in and around egg granuloma (3 + grade) before  $\gamma$ -IFN injection; B: Decreased type I collagen in and around egg granuloma (1+ grade) after  $\gamma$ -IFN injection.

of extracellular matrix at the 20<sup>th</sup> or 24<sup>th</sup> week after infection.

## DISCUSSION

Present data showed that  $\gamma$ -IFN in the liver of healthy mice was very low. After *S. japonicum* infection, the amount and distribution of  $\gamma$ -IFN was associated with inflammatory response during egg granuloma formation. The more severe the inflammatory response was, the more  $\gamma$ -IFN, which may be caused by the increase of lymphocyte infiltration and  $\gamma$ -IFN release in order to oppose inflammatory response of egg granuloma.

In this study, the change of the amount of  $\gamma$ -IFN was contrary to that of extracellular matrix, *i.e.* the amounts of FN, LN, type I and III collagens were lowered while  $\gamma$ -IFN had reached its peak. However, extracellular matrix increased gradually when hepatic  $\gamma$ -IFN decreased, and collagens began to deposit in the portal tracts

in and around the egg granuloma. After a repeated injection of recombinant  $\gamma$ -IFN to the infected mice, the amount of extracellular matrix in the liver reduced obviously, as compared with the non-treated infected mice. This indicated that  $\gamma$ -IFN may inhibit the synthesis and excretion of extracellular matrix and the deposition of collagen<sup>[6]</sup>, which was mediated through the effect of  $\gamma$ -IFN on other cytokines. For example, the injection of monoclonal antibody to  $\gamma$ -IFN to the *S. mansoni* infected murine can enhance the capacity of T cell of excreting IL-2 and IL-4, which promote the formation of pulmonary granuloma. Repeated injection of enough recombinant  $\gamma$ -IFN can restrain the excretion of IL-2 and IL-4 and lighten the pulmonary granuloma formation. It is suggested that  $\gamma$ -IFN can abate the granuloma formation by inhibiting T cells from excreting IL-4 and IL-2<sup>[4]</sup>.

However, the pathogenesis of liver fibrosis is very complicated. It was reported that matrix proteins in liver and collagen may come mainly from Ito cells, secondly from hepatocyte, endothelial cell of sinusoids, and fibroblast of portal tracts<sup>[7]</sup>. Our study revealed that the distribution of  $\gamma$ -IFN in the liver of infected mice was associated with the distribution of the above cells. Therefore, in addition to its effect on above mentioned T cells, it is necessary to clarify further whether  $\gamma$ -IFN inhibit directly the synthesis and excretion of extracellular matrix from the above cells and the deposition of collagen. Besides, it is still a question why the infected mice do not go on secreting  $\gamma$ -IFN to resist the synthesis and excretion of extracellular matrix leading to hepatic fibrosis, after the granuloma-induced inflammatory response weakened.

## REFERENCES

- 1 Cheever AW, Xu Y, Macedonia JG, Cox T, Hieny S, Sher A. The role of cytokines in the pathogenesis of hepatic granulomatous disease in *Schistosoma mansoni* infected mice. *Mem Inst Oswaldo Cruz* 1992; **87** Suppl 4: 81-85 [PMID: 1343930 DOI: 10.1590/S0074-02761992000800011]
- 2 Chensue SW, Terebuh PD, Warmington KS, Hershey SD, Evanoff HL, Kunkel SL, Higashi GI. Role of IL-4 and IFN-gamma in *Schistosoma mansoni* egg-induced hypersensitivity granuloma formation. Orchestration, relative contribution, and relationship to macrophage function. *J Immunol* 1992; **148**: 900-906 [PMID: 1309844]
- 3 Henderson GS, Lu X, McCurley TL, Colley DG. In vivo molecular analysis of lymphokines involved in the murine immune response during *Schistosoma mansoni* infection. II. Quantification of IL-4 mRNA, IFN-gamma mRNA, and IL-2 mRNA levels in the granulomatous livers, mesenteric lymph nodes, and spleens during the course of modulation. *J Immunol* 1992; **148**: 2261-2269 [PMID: 1545131]
- 4 Lukacs NW, Boros DL. Lymphokine regulation of granuloma formation in murine schistosomiasis mansoni. *Clin Immunol Immunopathol* 1993; **68**: 57-63 [PMID: 8513594 DOI: 10.1006/clin.1993.1095]
- 5 Liu L, Sheng Y, Guan X, Zhang Z, Sun B. [Dynamics of IL-2 and IFN-gamma levels induced by sea or Con A in spleen cells of *Schistosoma japonicum*-infected mice]. *Zhongguo Jishengchongxue Yu Jishengchongbing Zazhi* 1995; **13**: 35-38 [PMID: 7788892]
- 6 Czaja MJ, Weiner FR, Takahashi S, Giambone MA, van der Meide PH, Schellekens H, Biempica L, Zern MA. Gamma-interferon treatment inhibits collagen deposition in murine schistosomiasis. *Hepatology* 1989; **10**: 795-800 [PMID: 2509321 DOI: 10.1002/hep.1840100508]
- 7 Schuppan D. Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. *Semin Liver Dis* 1990; **10**: 1-10 [PMID: 2186485 DOI: 10.1055/s-2008-1040452]

S- Editor: Ma JY L- Editor: Ma JY E- Editor: Liu WX

## Construction of retroviral vector carrying *HSV-tk* gene under control of human AFP enhancer core sequence and human pgk promotor

Jun Gao, Guang-Wen Cao, Zhong-Tian Qi, Xiao-Fang Qiu, Zhong-Di Wu, Ping Du, Wen-Guo Yang, Long Cui

Jun Gao, Guang-Wen Cao, Zhong-Tian Qi, Xiao-Fang Qiu, Zhong-Di Wu, Ping Du, Wen-Guo Yang, Department of Microbiology, Faculty of Basic Medical Sciences, the Second Military Medical University, Shanghai 200433, China

Long Cui, Department of General Surgery, Changhai Hospital, the Second Military Medical University, Shanghai 200433, China

Jun Gao, male, born on January 7, 1967 in Xianyang City of Shaanxi Pro vince, and graduated from the Fourth Military Medical University, is engaged in cancer gene therapy, having 3 papers published

Project supported by the National Natural Science Foundation of China (No.39500147)

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: **Dr. Jun Gao**, Department of Microbiology, Faculty of Basic Medical Sciences, the Second Military Medical University, Shanghai 200433, China  
Telephone: +86-21-65347018

Received: August 8, 1996  
Revised: September 30, 1996  
Accepted: January 1, 1997  
Published online: March 15, 1997

### Abstract

**AIM:** To construct retroviral vector bringing *HSV-tk* gene under control of human AFP enhancer core sequence and human pgk promoter.

**METHODS:** Internal SV40 promoter was deleted by SalI from retroviral vector pMNSM to construct pMNM. *HSV-tk* gene driven by pgk promoter was released by *Bam*H I from an eukaryotic expression vector pBPGK-tk, and inserted into polylinker site of pMNM to construct pMNP-tk retroviral vector. Human  $\alpha$ -fetoprotein gene enhancer core sequence was released by *Eco*R I from pGEM. 7Z-AFPe plasmid was inserted into the immediate upstream of pgk promoter of pMNP-tk vector. Construction of hepatoma specific retroviral vector pMNAP-tk was completed.

**RESULTS:** The structure of pMNP-tk and pMNAP-tk vector was confirmed by restriction analysis.

**CONCLUSION:** The vector is of great significance for hepatoma specific prodrug transformation gene therapy.

**Key words:** Liver neoplasms; Herpes; Simplex virus; Retroviral; Alpha fetoproteins; Enhancer elements; Gene therapy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All

rights reserved.

Gao J, Cao GW, Qi ZT, Qiu XF, Wu ZD, Du P, Yang WG, Cui L. Construction of retroviral vector carrying *HSV-tk* gene under control of human AFP enhancer core sequence and human pgk promoter. *World J Gastroenterol* 1997; 3(1): 9-11 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/9.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.9>

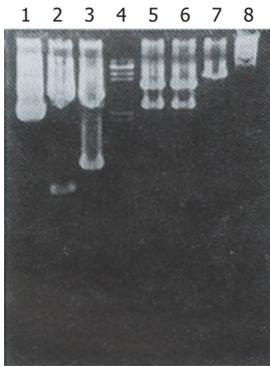
### COMMENTARY

Gene therapy for tumor is a modern biotherapy which is rapidly developing at present. The key point of gene therapy is to introduce the gene of interest into the tumor cells, and make the genes specifically express in tumor cells. Tumor targeted expression of genes was fully emphasized, especially to employ the cell type specific expression regulatory elements to direct the expression of the genes in certain target cells. American Recombinant DNA Advisory Committee (RAC) of NIH had passed a protocol which was suggested by K.W. Culver. That was to transfer HSV/tk gene into the murine packaging cells and introduce these cells into the human brain tumor bed, and followed by ganciclovir (GCV) treatment. GCV, by conversion into GCV triphosphate, can inhibit DNA polymerase, resulting in inability of the cells to proliferate. Now it has been brought into clinical experiments. Gao *et al* in this study improved this method, by constructing retroviral vector PMNAP-tk to the upstream of *HSV-tk* gene human pgk promoter using human AFP enhancer core sequence. This vector has the function of hepatoma specific prodrug transformation. This study on hepatoma gene therapy should be of great significance. Professor Zu-Yu LUO, Institute of Life Science, Fudan University, Shanghai, China.

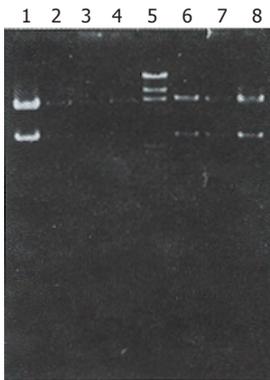
### INTRODUCTION

Herpes simplex virus thymidine kinase (*HSV-tk*) can catalyze deoxythymide to both deoxythymidylic acid and some nucleoside analogues (NAS) phosphorylation. These phosphorylated NAS is more toxic to mammalian cells, blocking cell DNA duplication<sup>[1]</sup>. Recently, numerous experiments *in vivo* or *in vitro* showed that *HSV-tk* gene transferred into tumor cells by some shuttle vectors produces efficiently anti-tumor effect using NAS as prodrug<sup>[2,3]</sup>. Furthermore, the clinical trials have been approved in some countries for the treatment of brain tumor with *HSV-tk* gene/prodrug system<sup>[4]</sup>.

Morbidity of hepatic cancer is very high in China. No effective treatment is available for the tumors in late stage. In order to establish an effective gene therapy against hepatic cancer and study the effect of household gene enhancer in the retroviral shuttle vector to regulate the gene of interest for hepatoma specific expression, we have constructed the general and the hepatoma



**Figure 1** Identification of insertion of *pgk* + *HSV-tk* segment into the pMNM retroviral vector and plasmid pGEM 7Z-AFP. Lane 1: plasmid pGEM. 7Z-AFP; Lane 2: pGEM. 7Z-AFP/*Hind* III 3.3 kb, 406 bp; Lane 3: pGEM. 7Z-AFP/*EcoR* I 3 kp, 727 bp; Lane 4: λDNA *Hind* III marker; Lane 5: pMNP-tk/*Bam*H I 6.1 kb, 2.8 kb; Lane 6: pMNP-tk/*EcoR* I and *Hind* III 6.1 kb, 2.8 kb; Lane 7: pMNP-tk/*EcoR* I 8.9 kb; Lane 8: plasmid pMNP-tk.



**Figure 2** The orientation of human AFP enhancer core sequence was defined with *Hind* III and *Bam*H I digestion of the colonies of pMNP-tk. Lane 1-4: right junction; Lane 5: λDNA *Hind* III marker; lane 6-8: reverse junction.

specific *HSV-tk* retroviral expression vector.

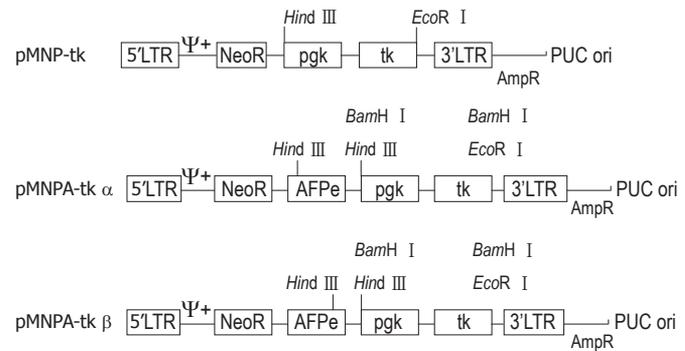
## MATERIALS AND METHODS

### Plasmids

Retroviral shuttle vector pMNSM was provided by Tokyo Medical and Dental University. Plasmid pBPGK-tk containing the human phosphoglycerokinase gene (*pgk*) promoter and *HSV-tk* gene was obtained from Nara Medical University. Plasmid pGEM 7Z-AFPe containing human  $\alpha$ -fetoprotein gene enhancer core sequence was provided by T. Tamaoki (University of Calgary, Calgary, Canada).

### Enzymes and bacterial cells

All restriction enzyme, T4 DNA ligase and Klenow fragment were purchased from Promega Corporation. *E. coli* host strain HB101 and *E. coli* JM109 were obtained from our department. The general and the hepatoma specific *HSV-tk* retroviral expression vectors were constructed<sup>[5]</sup>. After plasmid pMNSM and pBPGK-tk were introduced into *E. coli* HB101, respectively, ten ampicillin resistant colonies were selected and the plasmid DNAs were extracted. The correct plasmids identified by the restriction analysis were amplified through *E. coli* and purified by PEG 8000 (Sigma Corporation) method, respectively. After plasmid pMNSM was digested by endonuclease *Sal* I, the small SV40 promoter fragments were discarded and the big fragments were recovered from the low melting point agarose gel. The big fragments were self-circularized with T4 DNA ligase, and named pMNM. Plasmid pMNM DNAs amplified and purified were linearized by *Bam*H I digestion. A 2.8 kb BamHI fragment of plasmid pBPGK-tk containing the *pgk* gene promoter and *HSV-tk* gene was isolated and inserted at the *Bam*H I site of pMNM. The ligated molecules were used to transform *E. coli* HB101. Ampicillin resistant colonies were selected and the plasmid DNA was extracted. Through the combinative digestion of the *Hind* III site at the inserted fragment and the *EcoR* I site at the plasmid pMNM, the colon that appeared as a 28 kb and 61 kb fragment was correct, and named pMNP-tk (Figure 1).



**Figure 3** Structure of pMNP-tk, pMNP-tk $\alpha$ , pMNP-tk $\beta$  retroviral vectors. LTR: Retroviral long terminal repeat sequence; NeoR: Neomycin phosphotransferase gene (for G418 selection); *pgk*: Human phosphoglycerokinase promoter; *tk*: Herpes simplex virus thymidine kinase gene; AFPe: Human  $\alpha$  fetoprotein "home keeping" gene enhancer.

### Construction of hepatoma specific expression retroviral shuttle vector<sup>[5]</sup>

Plasmid pGEM 7Z-AFPe DNA was transformed into *E. coli* JM109. By  $\alpha$  complementation method, the white color colonies were selected and the correct plasmids were identified by the restriction analysis (Figure 1). The credible plasmid pGEM-7Z-AFPe was amplified. The AFP gene enhancer core sequence 727 bp *EcoR* I fragments, after being filled in by Klenow fragment and dNTP, were harvested from the low melting point agarose gel, and inserted into the *Sal* I linearized plasmid pMNP-tk. The recombinant DNA was introduced into the *E. coli* HB101 and combinative digestion of *Hind* III and *Bam*H I were used to identify the correct colonies. One colony exhibiting 406 bp, 2.8 kb and 6.4 kb fragments was in right junction, named pMNP-tk  $\alpha$ . Another colony exhibiting 321 bp, 2.8 kb and 6.5 kb fragments was reverse, named pMNP-tk  $\beta$  (Figure 2).

## RESULTS

### Construction of pMNP-tk

In order to make the chimeric *HSV-tk* gene express in eukaryotic cells, we isolated the DNA fragments containing both the *pgk* gene promoter and *HSV-tk* gene from the eukaryotic expression vector pBPGK-tk and removed the SV40 promoter from the retroviral vector pMNSM. The recombinant plasmid gene structure is shown in Figure 3.

### Construction of pMNP-tk $\alpha$ and pMNP-tk $\beta$

On the basis of structure of plasmid pMNP-tk, the AFP gene core sequence was inserted at the *Sal* I of pMNP-tk so that it can regulate the *pgk* gene promoter function and direct the *HSV-tk* gene hepatoma specific expression.

## DISCUSSION

The gene therapy for cancer is developing rapidly. Its mechanisms may be as follows: Increasing the anti-tumor immunity, and introducing tumor inhibitor gene, the antisense of oncogenes, the prodrug converting gene, MDR-1 gene for the protection of chemotherapy, and the anti-metastasis gene<sup>[6]</sup>. The prodrug genes, such as *HSV-tk*, *VZV-tk* and cytosine deaminase (*CD*), and encode proteins, can convert the nontoxic prodrug into intracellular toxins as non-mammalian metabolic enzymes<sup>[7]</sup>. Such enzymes can block the cell DNA duplication as a competitive inhibitor of DNA polymerase.

The gene expression in tumor cells is the first step for gene therapy. Eukaryotic expression plasmid vector and viral vector are commonly used for the expression of genes in tumor cells. In viral vectors, the recombined retroviral vector, which is constructed with Mo Mulv as the main skeleton, is more often used<sup>[8]</sup>. It is feasible that a gene will be expressed if it is regulated by two promoters. In our construction, we deleted the SV40 promoter of the retroviral vector, and made the *tk* gene under the control of the *pgk* gene promoter, which is a kind of eukaryotic promoters. These may decrease the presence of wild retrovirus<sup>[9]</sup> caused by recombined homologous product.

In *in vivo* gene therapy, it is important to make the gene specifically express in tumor tissues. Two approaches may be used.

One is to modify the shuttle vector by gene engineering, the other is to direct the gene expression in target cells by means of the tissue specific transcriptional regulatory sequence (TRS). Scharfmann *et al.*<sup>[9]</sup> considered that the transcriptional regulatory sequence of "household gene" will cause the chimeric gene to express at a high level in a specific tissue. Hubber *et al.*<sup>[7]</sup> reported that *VZV-tk* gene regulated by AFP TRS and mediated by retroviral vector, made the AFP-positive hepatoma cells, HepG2, H3B and HuH7 sensitive to the prodrug 6 methoxypurine arabinonucleoside (araM). The aim of our work was to regulate the *pgk + tk* gene by AFP enhancer core sequence 727 bp. The usage of the TRS for regulation of HSV *tk* gene can not only increase its transcription but also make its expression tissue specific. In our future experiments, we will assess their anti-tumor effects in nude mice.

## ACKNOWLEDGMENTS

We thank SUN Ru-Mei for his technical assistance.

## REFERENCES

- 1 Anderson WF. Human gene therapy. *Science* 1992; **256**: 808-813 [PMID: 1589762 DOI: 10.1126/science.1589762]
- 2 Culver KW, Ram Z, Wallbridge S, Ishii H, Oldfield EH, Blaese RM. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. *Science* 1992; **256**: 1550-1552 [PMID: 1317968 DOI: 10.1126/science.1317968]
- 3 Vile RG, Hart IR. Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA. *Cancer Res* 1993; **53**: 3860-3864 [PMID: 8395331]
- 4 Culver KW, Van Gilder J, Link CJ, Carlstrom T, Buroker T, Yuh W, Koch K, Schabold K, Doornbas S, Wetjen B. Gene therapy for the treatment of malignant brain tumors with in vivo tumor transduction with the herpes simplex thymidine kinase gene/ganciclovir system. *Hum Gene Ther* 1994; **5**: 343-379 [PMID: 8018748 DOI: 10.1089/hum.1994.5.3-343]
- 5 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd editor. New York: Cold Spring Harbor Laboratory Press, 1989: 1-75
- 6 Cao GW, Du P. Modern cancer biotherapeutics. Beijing: People's Military Press, 1995: 291-391
- 7 Huber BE, Richards CA, Krenitsky TA. Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. *Proc Natl Acad Sci USA* 1991; **88**: 8039-8043 [PMID: 1654555 DOI: 10.1073/pnas.88.18.8039]
- 8 Miller AD, Miller DG, Garcia JV, Lynch CM. Use of retroviral vectors for gene transfer and expression. *Methods Enzymol* 1993; **217**: 581-599 [PMID: 8386297]
- 9 Scharfmann R, Axelrod JH, Verma IM. Long-term in vivo expression of retrovirus-mediated gene transfer in mouse fibroblast implants. *Proc Natl Acad Sci USA* 1991; **88**: 4626-4630 [PMID: 1905011 DOI: 10.1073/pnas.88.11.4626]

S- Editor: Feng CY L- Editor: Ma JY E- Editor: Liu WX

## Sequencing of hepatitis C virus cDNA with polymerase chain reaction directed sequencing

Lai Wei, Yu Wang, Hong-Song Chen, Qi-Min Tao

Lai Wei, Yu Wang, Hong-Song Chen, Qi-Min Tao, Institute of Hepatology, People's Hospital of Beijing Medical University, Beijing 100044, China

Lai Wei, PhD, MD, male, born on November 14, 1961, from Nanjing in Jiangsu Province, graduated from the Department of Medicine of Xuzhou Medical College, Associate Professor, engaged in infectious diseases

Author contributions: All authors contributed equally to the work.

Supported by The China Medical Board of America (No.93-582)

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Qi-Min Tao, Professor Institute of Hepatology, People's Hospital of Beijing Medical University, Beijing 100044, China  
Telephone: +86-10-68314422-5722

Received: September 9, 1996

Revised: January 1, 1997

Accepted: March 1, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To explore a rapid and easy sequencing method for hepatitis C virus (HCV) genome and establish a new sequencing method in China.

**METHODS:** Polymerase chain reaction (PCR) was combined with a DNA sequencing technique. PCR products were purified by agarose gel electrophoresis, polyacrylamide gel electrophoresis (PAGE) and polyethylene glycol (PEG) respectively. Then, in the presence of a 5' labeling PCR primer, purified PCR products were directly sequenced. By this method, HCV NS5b cDNA from two HCV infected individuals (HC-42 and HC-49) were sequenced.

**RESULTS:** PCR directed sequencing worked best using PCR amplified DNA purified by electrophoresis as a sequencing template. When sequencing a large number of templates, the purification step can be bypassed by using a lower concentration of dNTPs (40  $\mu$ mol of each dNTP) and primers (10 pmol of each primer) in the first stage of PCR. The aliquot of the first stage of PCR mixture was then directly used for amplification of chain terminated products but the sequencing ladders generated were of low intensity. Polyethylene glycol (PEG) could not remove nonspecific products of PCR, which affected the sequencing result to a certain extent and generated a background in sequencing ladders. Compared with the reported HCVJ and HC-C2, a new three nucleotide deletion was found in HC-42.

**CONCLUSION:** PCR directed sequencing is a rapid, simple and effective method, especially for sequencing large samples. A three

nucleotide deletion was first reported.

**Key words:** Hepatitis C virus DNA; Viral DNA; Complementary polymerase chain reaction; Sequence analysis; DNA mutation

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wei L, Wang Y, Chen HS, Tao QM. Sequencing of hepatitis C virus cDNA with polymerase chain reaction directed sequencing. *World J Gastroenterol* 1997; 3(1): 12-15 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/12.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.12>

### INTRODUCTION

Hepatitis C virus (HCV) is a major causative agent of post transfusion non A and non B hepatitis and contains a positive stranded RNA molecule. Sequence analysis of the HCV genome plays an important role in the research on viral features, pathogenesis of chronic hepatitis C and discovery of a new genotype/subgenotype of HCV<sup>[1]</sup>. Traditional sequencing needs cloning which is time consuming and usually takes a lot of work. Recently, a novel DNA sequencing method, direct sequencing of polymerase chain reaction (PCR) amplified DNA, was described<sup>[2]</sup>. As a sequencing template, PCR amplified DNA was directly sequenced by a thermostable DNA polymerase from *Thermus flavus* in this method. In order to explore the possibility of sequencing HCV cDNA by this method and establish a new sequencing method in China, we purified and sequenced PCR amplified HCV NS5b cDNA. In the present study, this technique was optimized in ways so that it could be directly applicable to sequence a 376 bp fragment from patients with chronic hepatitis C. PCR direct sequencing was found to be easy, rapid and bypasses construction of the HCV cDNA clones. We believe that PCR direct sequencing is a practical method that would work well in research on the HCV genome and its variation.

### MATERIALS AND METHODS

#### Serum samples

Two sera were collected from 2 patients with chronic HCV infection. All of them were positive for anti HCV (2<sup>nd</sup> generation EIA, Institute of Hepatology, Beijing Medical University), containing HCV RNA detectable by the nested PCR method with two pairs of external and internal primers deduced from the 5' non coding region<sup>[3]</sup>.

#### Primers for PCR and sequencing

Primer no.51 (5'-GAGTTCCTGGTGAATACCTG-3'; nt 8189 to

8208, sense) and no.52 (5'-GATGTTATCAGCTCCAGGTC-3'; nt 8661 to 8680, antisense) were external primers. Primers no.53 (5'-CACCCGCTGTTTTGACTC-3'; nt 8246 to 8263, sense) and no.54 (5'-CCTAGTCATAGCCTCGGTC-3'; nt 8603 to 8621, antisense) were used as PCR primers as well as sequencing primers. All four primers were deduced from the putative nonstructural gene (NS) 5 b of the HCV-J isolate<sup>[4]</sup> and HC C2 isolate<sup>[5]</sup>.

#### Preparation of nucleic acid, cDNA synthesis and PCR

RNA from 50  $\mu$ L serum was extracted by the acid guanidium phenol/chloroform method. RNA obtained was denatured at 70 °C for 1 minute, chilled quickly on ice and used as a template for cDNA synthesis. Reverse transcription was carried out at 43 °C for 60 min in 10  $\mu$ L of Tris HCl buffer (50 mmol/L, pH8.4) containing 8 mmol/L MgCl<sub>2</sub>, 30 mmol/L KCl, 1 mmol/L dithiothreitol, 50 pmol or 10 pmol primer no 52, 10 mmol/L each of the four deoxyribonucleoside triphosphate (dNTPs) (Promega Corporation), 10 units of RNase inhibitor (Promega Corporation) and 2 units avian myeloblastosis virus reverse transcriptase (Promega Corporation). The cDNA was amplified in two stages in a DNA thermal cycler (Perkin Elmer Cetus, United States). Both stages were carried out in a final volume of 50  $\mu$ L containing 67 mmol/L Tris HCl, pH8.8, 1.5 mmol/L MgCl<sub>2</sub>, 1.5 units of Taq DNA polymerase (Promega Corporation), 2 or 10 mmol/L each of four dNTPs for the first stage, 10 mmol/L each of four dNTPs for the second stage, 10 or 50 pmol of each external primer for the first stage and 50 pmol of each internal primer for second stage). Both stages consisted of 35 cycles. In the first stage of PCR, each reaction cycle involved denaturation at 90 °C for 1 min, annealing of primers at 42 °C for 1.5 min and extension at 72 °C for 2 min. In the second stage of PCR, the program of the thermal cycle was similar to the first stage except that the annealing of primers was performed at 55 °C. Samples from the reaction mixture were subjected to electrophoresis on polyacrylamide gel (Promega Corporation) and DNA species on gel were stained with ethidium bromide for observation under ultraviolet light. The sizes of PCR products were estimated according to the migration pattern of pBR322 Hae III.

#### Purification of PCR products

**Purification by electrophoresis in agarose gel** PCR products were subjected to 2% agarose gel electrophoresis. The gel containing the positive band was cut off, wrapped up with PARA film (American National Can) and then stored at -70 °C for 30 min. After being eluted from the gel, the liquid was removed to a fresh tube, extracted by phenol/chloroform and precipitated by ethanol. At last, the precipitation was dissolved in ddH<sub>2</sub>O for sequencing.

#### Purification by PAG (polyacrylamide gel) electrophoresis

PCR products were subjected to 6% PAG electrophoresis. The gel containing the positive band was cut off and immersed in the elution solution containing 0.5 mmol/L NH<sub>4</sub>Ac and 1 mmol/L EDTA at 37 °C for 24 h. After this, the elution was treated as described above.

#### Purification by PEG (Polyethylene glycol) precipitation

PCR products were purified according to the reported method<sup>[6]</sup>.

#### Sequencing method<sup>[7]</sup>

**The primer labeling** Two internal primers were respectively used as sequencing primers in both directions. Ten pmol of sequencing primer, 50  $\mu$ Ci ( $r$ -<sup>32</sup>P) ATP (Ya Hui Company), 1  $\mu$ L of 10  $\times$  kinase buffer (70 mmol/L Tris HCl, pH7.5, 10 mmol/L MgCl<sub>2</sub>, 5 mmol/L dithiothreitol) and H<sub>2</sub>O to a final reaction volume of 10  $\mu$ L were mixed, then 5 units of T4 polynucleotide kinase was added. The mixture was incubated at 37 °C for 30 min.

**Sequencing** Two  $\mu$ L of each of the four dNTP/ddNTP (Boehringer Mannheim) extension termination mixtures were added to four tubes. In another fresh tube, purified PCR amplified DNA,

1.66 pmol of 5'-<sup>32</sup>P-labeled sequencing primer, 5  $\mu$ L of 5  $\times$  sequencing buffer and 5 units of Taq polymerase were mixed in a total reaction volume of 16  $\mu$ L. Then 4  $\mu$ L of the above mixture was transferred to each of the four tubes containing the dNTP/ddNTP mixture. At last, the tubes were placed in a thermal cycler that was preheated to 95 °C. PCR involving 30 cycles was performed. Each cycle included denaturation at 95 °C for 45 s, primer annealing at 55 °C for 45 s and primer extension at 70 °C for 1 min. After completion of thermal cycling, 3  $\mu$ L of stop solution (10 mmol/L NaOH, 95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol) were added to each reaction tube.

## RESULTS

#### Amplification of HCV cDNA by reverse transcription nested PCR

HCV cDNA was detected in two sera samples from patients with chronic HCV infection. The sizes of PCR products estimated in electrophoresis gel were about 376 bp as predicted.

#### Effect of different methods recovering a template on sequencing

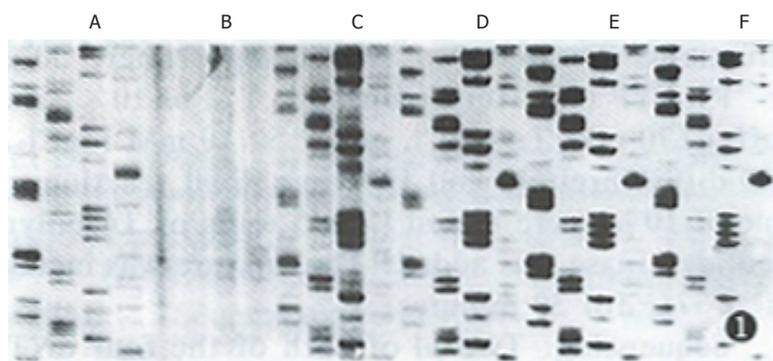
First stage PCR products with lower concentration dNTP (2 mmol/L each of four dNTPs) and primers (10 pmol of each external primer) and second stage PCR products were used as a sequencing template. PCR products purified by PEG precipitation, electrophoresis in agarose gel and PAG were sequenced respectively. The sequencing ladders are shown in Figure 1.

#### Sequence analysis of HCV NS5b cDNA

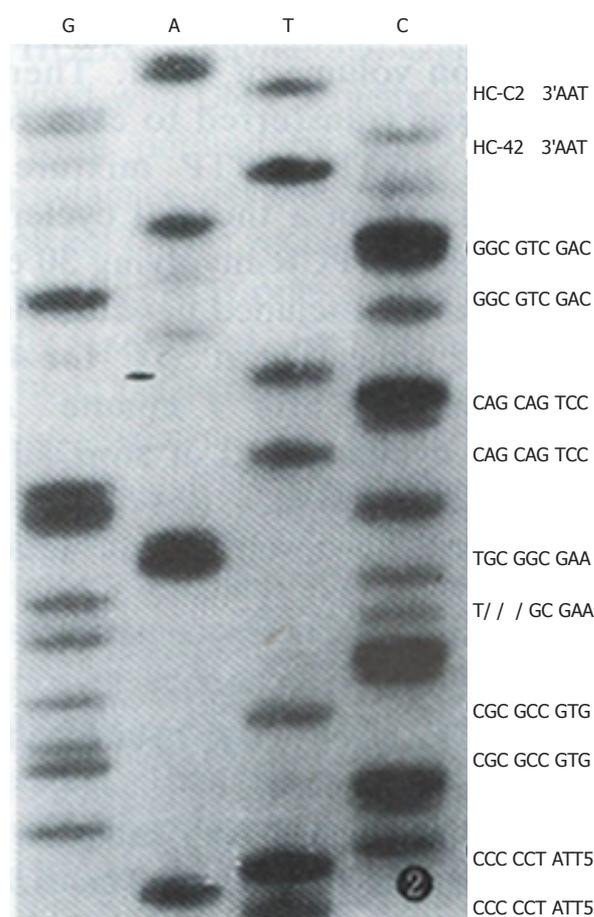
By the method described above, HCV NS5B cDNA from two patients with chronic HCV infection (HC-42 and HC-C11) were sequenced. Compared with HCV-J, 91.84% and 92.63% of nucleotide's homology, 91.84% and 95.79% with HC-C2, were found in HC-42 and HC-C11, respectively. A three nucleotide deletion occurred in HC-42 (Figure 2) and this deletion was reported for the first time.

## DISCUSSION

The first consideration about PCR directed sequencing is the presence of unused dNTPs and primers in the PCR products. Typically, about 200  $\mu$ mol of each dNTP are used in PCR reaction, whereas the sequencing reactions are routinely performed in the presence of around 10  $\mu$ mol of each dNTP and a 10.100-fold higher concentration of appropriate ddNTP depending on the polymerase used. In fact, only about 10% of the dNTPs are consumed in PCR. Therefore, about 90% of the dNTPs added to the PCR mixture would remain unused at the end of amplification. Unless these are removed, the routine sequencing reaction would fail to generate sequence ladders because of negligible ddNTP terminations. The presence of unused primers is another problem since approximately 90% of the primers from the PCR amplification would remain unused, which would consume the polymerase as well as the ddNTP and dNTPs in the sequencing reaction<sup>[2,8]</sup>. Because of these factors, sequencing with unpurified second stage PCR products is unable to obtain sequencing ladders (Figure 1B). Non specific PCR products are also an important factor affecting sequencing. Since PEG could precipitate large molecular weight nucleotides and low molecular weight primers and dNTPs would remain in the supernants, PEG was reported to be used to recover the PCR products<sup>[6]</sup>. However, PCR amplification produced expected DNA as well as some nonspecific products which were precipitated as expected DNA in PEG buffer. So PEG recovering PCR products could contain unremoved nonspecific products which would interfere with sequencing to some extent. With this as a sequencing template, sequencing ladders generated were not clear and had a dark background (Figure 1C). Purification by electrophoresis could remove unused primers and dNTPs. Moreover, this method could separate expected DNA fragments



**Figure 1** Effect of different methods recovering a template on sequencing. A: The first stage PCR products with lower concentration dNTPs and primers as a sequencing template; B: The second stage PCR products directly sequenced without purification; C: Sequencing of PCR amplified cDNA recovered by PEG; D: PCR products purified by agarose gel electrophoresis before sequencing; E: Sequencing of PCR products purified by PAG electrophoresis; F: Sequencing ladders of PCR products purified by agarose gel electrophoresis but without phenol/chloroform extraction.



**Figure 2** Deletion mutation in NS5B region of HC-42 and comparison with HC-C2. "—" denotes deletion.

from nonspecific products. Because of this, sequencing with PCR products purified by electrophoresis worked best (Figure 1D and E). Of course, extracting with phenol/chloroform and washing with 70% ethanol after electrophoresis were also needed (Figure 1F).

When sequencing a large number of templates, the purification step was heavy and complicated. Two strategies have been recommended to bypass this step<sup>[2]</sup>. In one strategy, lower concentrations of dNTPs (10-20  $\mu$ mol of each dNTP) and primers (10 pmol of each primer) are used to amplify target DNA. As a result, the carryover of unused dNTPs and primers into the sequencing reactions is minimized. Alternatively, the target DNA is first amplified for 25 cycles and then an aliquot of the first PCR mixture is used for amplification of chain terminated products in the presence of a 5' labeled sequencing primer positioned internally to the external

primer and an appropriate ddNTP; this strategy is similar to semi nested PCR. Considering nested PCR is needed to detect HCV RNA, two strategies described above were combined in the present study. We used 40  $\mu$ mol/L of each dNTPs and 10 pmol of each primer in the first stage PCR and the aliquot of the first PCR was then used as a sequencing template directly. By this method, the sequencing ladders could be read, although the overall quality of the sequence ladder was not as good as with purified second PCR products (Figure 1A).

Direct sequencing of PCR amplified DNA can lead to incorporation of errors in the DNA sequence due to Taq polymerase lacking proofreading 3'-5' exonuclease. Various estimates of misincorporation frequencies range from one error in 4000 nucleotides to one error in 400 nucleotides of the PCR amplified DNA<sup>[9]</sup>. Therefore, depending on the PCR conditions performed, it is possible that almost every molecule in a 1 kb size PCR amplified DNA could have one error<sup>[8]</sup>. The question is whether direct sequencing of the mutated PCR amplified DNA would result in the incorporation of errors in the DNA sequence generated. The answer to this question is that the copy number of starting DNA is in the order of  $10^3$ - $10^5$  molecules in most PCR amplifications. Consequently, any errors incorporated in each cycle reaction are randomized and therefore any specific mutant sequences constitute only a minute fraction of the final product. Since the DNA sequence generated is a consensus sequence of millions of template DNA molecules, these errors would not be accounted for in the final sequence. In addition, using a lower concentration of dNTPs and primers in the first stage PCR experiment could also reduce the possibility of misincorporation. HC-42 sequences were sequenced from three times individual PCR products with three sequences the same, as with HC-C11. The above results suggested that sequences generated from PCR direct sequencing are reliable.

A three nucleotide deletion was reported for the first time in this paper. It was found that a deletion mutation in a HCV sequence often occurs at the NS5 region<sup>[10]</sup>, as this deletion did in the present study. Since three nucleotides were deleted, the mutation did not result in significant codon changes. Further investigations and research are needed to elucidate the significance and epidemiology of this deletion.

The diversity of the HCV genome has been found to be related to the progression of liver disease<sup>[11]</sup>. Therefore, the variation of HCV awaits further studies to clarify the relationship so a rapid and easy sequencing technique is needed. PCR direct sequencing would be a very advantageous method to meet this need.

## REFERENCES

- 1 **Simmonds P.** Variability of hepatitis C virus. *Hepatology* 1995; **21**: 570-583 [PMID: 7531173 DOI: 10.1002/hep.1840210243]
- 2 **Du SC, Tao QM, Sun Y, Chen P, Xu XD, Liu JX.** Detection of hepatitis C virus RNA with nested PCR (in Chinese with English abstract). *Beijing Medical University* 1991; **23**: 429-431
- 3 **Rao VB, Saunders NB.** A rapid polymerase-chain-reaction-directed sequencing strategy using a thermostable DNA polymerase from *Thermus flavus*. *Gene* 1992; **113**: 17-23 [PMID: 1563631 DOI: 10.1016/0378-1119(92)90665-C]
- 4 **Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K.** Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci USA* 1990; **87**: 9524-9528 [PMID: 2175903 DOI: 10.1073/pnas.87.24.9524]
- 5 **Wang Y, Okamoto H, Tsuda F, Nagayama R, Tao QM, Mishiro S.** Prevalence, genotypes, and an isolate (HC-C2) of hepatitis C virus in Chinese patients with liver disease. *J Med Virol* 1993; **40**: 254-260 [PMID: 8394876 DOI: 10.1002/jmv.1890400316]
- 6 **Rosenthal A, Coutelle O, Craxton M.** Large-scale production of DNA sequencing templates by microtitre format PCR. *Nucleic Acids Res* 1993; **21**: 173-174 [PMID: 8441614 DOI: 10.1093/nar/21.1.173]
- 7 **Rao VB.** Strategies for direct sequencing of PCR-amplified DNA. *PCR Methods Appl* 1994; **4**: S15-S23 [PMID: 9018324 DOI: 10.1101/gr.4.1.S15]
- 8 **Rao VB.** Direct sequencing of polymerase chain reaction-amplified DNA. *Anal Biochem* 1994; **216**: 1-14 [PMID: 8135340 DOI: 10.1006/abio.1994.1001]
- 9 **Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA.** Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; **239**: 487-491 [PMID: 2448875 DOI: 10.1126/science.2448875]
- 10 **Martell M, Esteban JI, Quer J, Genescà J, Weiner A, Esteban R, Guardia J, Gómez**

J. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *J Virol* 1992; **66**: 3225-3229 [PMID: 1313927]

11 **Bukh J**, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. *Semin Liver Dis* 1995; **15**: 41-63 [PMID: 7597443 DOI: 10.1055/s-2007-100762]

**S- Editor:** Ma JY **L- Editor:** Ma JY **E- Editor:** Liu WX

## Inhibitory effect of sulindac against chemically-induced primary colonic tumors by N-methyl-N-nitrosourea in mice

Qiang Wang, Lie-Ying Fan, Jin He, Yuan-He Wang

Qiang Wang, Lie-Ying Fan, Jin He, Yuan-He Wang, Department of General Surgery, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

Dr. Qiang Wang, Associate Professor and Vice Director, specializing in the research of gastrointestinal surgery and gastrointestinal neoplasm, with 36 published papers

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Qiang Wang, Associate Professor, Vice Director, Department of General Surgery, Changzheng Hospital, Second Military Medical University, 415 Feng Yang Road, Shanghai 200003, China  
Telephone: +86-21-63275997-386  
Fax: +86-21-63275629

Received: August 9, 1996  
Revised: September 29, 1996  
Accepted: January 31, 1997  
Published online: March 15, 1997

### Abstract

**AIM:** To investigate the chemopreventive effect of sulindac, a nonsteroidal anti-inflammatory drug (NSAID), on the growth of N-methyl-N-nitrosourea (MNU)-induced mouse colonic tumors.

**METHODS:** The experimental colonic tumor model induced by intrarectal instillation of MNU in mice was used in the present study. In the first experiment, MNU intrarectal was instilled and sulindac administered concurrently to a group of mice for a period of 18 wk, while a control group of animals received MNU only for the same period. In the second experiment, two groups of mice that had already been treated with MNU for 12 wk received sulindac or not for another 18 wk.

**RESULTS:** The tumors induced in mice were all located in the distal part of the large intestine. There were no significant differences in the location and the gross appearance of the tumors in the MNU-induced group and control group in both experiments. In the first experiment, sulindac caused a significant reduction in both the number of mice with colonic tumors and the number of tumors per mouse. Sulindac had a significant inhibitory effect on the growth of the MNU-induced tumors. However, in the second experiment, the inhibitory effect of sulindac was less or disappeared.

**CONCLUSION:** Sulindac has a protective effect against the chemical induction of colonic tumors by MNU in mice. The chemopreventive effect is more significant in the initial stage of the tumor, while in the promotion stage this effect is less or disappeared. Sulindac can not cause the regression of established tumors.

**Key words:** Colonic neoplasms; Sulindac; Methyl-nitrosourea; Adenocarcinoma;

Disease models; Animal; Anti-inflammatory agents; Nonsteroidal

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang Q, Fan LY, He J, Wang YH. Inhibitory effect of sulindac against chemically-induced primary colonic tumors by N-methyl-N-nitrosourea in mice. *World J Gastroenterol* 1997; 3(1): 16-18 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/16.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.16>

### INTRODUCTION

Large bowel cancer is one of the leading causes of cancer deaths in humans. It was reported in recent years that sulindac, a nonsteroidal anti-inflammatory drug (NSAID), was capable of controlling tumor growth and reducing the numbers of adenoma in patients with familial polyposis (FAP), with the likelihood of reducing the risk of colon carcinogenesis<sup>[1,2]</sup>. In order to further understand these effects, the present study was designed to investigate the chemopreventive efficacy and modulating role of sulindac on colon carcinogenesis and on tumor growth in the colorectal cancer model induced by N-methyl-N-nitrosourea (MNU) in mice.

### MATERIALS AND METHODS

#### Colorectal cancer model induced by MNU

Randombred ICR female mice from the Shanghai SIPPR/BK Experimental Animal Ltd. Co. aged 5-6 wk and weighing 18-21 g were used in the experiments. They were fed with a standard pellet diet and allowed tap water ad libitum. The MNU (obtained from Sigma Chemical Co, St. Louis, MO., United States) was kept frozen in powdered form prior to use. The carcinogen needed to be reconstituted in distilled water and maintained at 4 °C until intrarectal instillation. MNU 0.1 mL of 0.4% (0.4 mg) was given using a 5.0 cm 18 gauge cannula needle which was inserted about halfway into the lumen of the colorectum through the anus, three times for the first six weeks and only once for the following six weeks. The method used for colorectal cancer induced in mice followed in detail the method described in our previous study<sup>[3]</sup>.

#### Treatment and grouping

Sulindac, cis-5-fluoro-2-methyl-1-[p-(methyl sulfinyl)benzylidene]indene-3-acetic acid, was dissolved in tap water in a concentration such that each animal received an average dose corresponding to 5 mg/kg a day. The study consisted of two experiments. In the first experiment, two groups of 48 animals were treated with MNU as described above, either with or without sulindac, for 24 wk. In the second experiment, 48 mice were treated with MNU for 12 wk at first. Then they were randomized, with 24 animals given sulindac solution for 18 wk while the other 24 animals only received water as

**Table 1** The experimental design and animal groups

Group	No.	MNU intraducal time (wk)	Sulindac time (wk)
Experiment one			
Sulindac group	24	12	18
Control group	24	12	No
Experiment two			
Sulindac group	24	12	18
Control group	24	12	No

**Table 2** Effect of sulindac on the morphological characteristics of colorectal tumors induced by N-methyl-N-nitrosourea

	Experiment one		Experiment two	
	Sulindac group	Control group	Control group	Sulindac group
Average number of adenoma	4.7	8.4	3.5	4.7
Average number of adenocarcinoma	9.3	3.9	8.7	9.4
Average ratio of adenoma to adenocarcinoma	0.51	2.15	0.40	0.50

**Table 3** Effect of sulindac on the number of colorectal tumors induced by N-methyl-N-nitrosourea

	Experiment one		Experiment two	
	Sulindac group	Control group	Control group	Sulindac group
No. of mice	24	24	24	24
No. of mice with tumors	19	9	21	20
No. of mice without tumors	5	15	3	4
Incidence of mice with colon tumors (%) <sup>1</sup>	79.2	37.5	87.5	83.3
No. of tumors <sup>2</sup>				
0	5	15	3	4
1-3	11	8	8	7
4-6	4	1	5	7
7-9	1	0	5	3
10-12	1	0	2	2
13-16	0	0	1	1

<sup>1</sup> $\chi^2$  test, Experiment one:  $\chi^2 = 7.563$ ,  $P < 0.01$ ; Experiment two:  $\chi^2 = 1.003$ ,  $P > 0.05$ . <sup>2</sup>Wilcoxon rank test, Experiment one:  $\mu = 5.078$ ,  $P < 0.01$ ; Experiment two:  $\mu = 1.611$ ,  $P > 0.05$ .

a control (Table 1).

Mice were killed by neck dislocation at the end of the experiments. The whole large intestine was removed and then fixed in 10% buffered formalin. For each colonic tumor the maximum longitudinal and transverse dimensions ( $d_1$  and  $d_2$ ) were obtained. Tumor average diameter ( $D$ ) and volume ( $V$ ) were estimated using the following formula:  $D = (d_1 + d_2)/2$ ,  $V = (\pi/96) (d_1 + d_2)^3$ . After 48 h fixation, the entire colon specimens were coiled up into "swiss rolls" and embedded in paraffin. The blocks were sectioned at 6  $\mu\text{m}$  and stained with hematoxylin and eosin (HE) for evaluation of histological changes.

### Statistical analysis

Statistical analysis was made using the  $\chi^2$  test and Wilcoxon rank test. The difference was regarded as significant if the  $P$  value was less than 0.05.

## RESULTS

The tumors induced in mice were all situated in the distal part of the large bowel, predominantly in the small polypoid adenocarcinoma. There were no significant differences in the location and the gross appearance of tumors in the MNU-induced group and control group in both experiments. In the first experiment, there were fewer adenocarcinomas and more adenomas in the sulindac-treated group

**Table 4** Effect of sulindac on the volume of the colorectal tumor induced by N-methyl-N-nitrosourea

	Experiment one		Experiment two	
	Sulindac group	Control group	Control group	Sulindac group
Median tumor diameter (mm) <sup>1</sup>				
< 1	5	5	8	6
1.1-1.5	4	1	4	5
1.6-2.0	4	2	5	4
2.1-2.5	3	1	2	3
2.6-2.9	2	0	1	1
> 3.0	1	0	1	1
Median tumor volume (mm <sup>3</sup> ) <sup>2</sup>				
1-3	5	4	7	7
4-6	4	3	3	3
7-9	3	1	4	4
10-13	2	1	2	3
14-16	1	0	2	1
17-20	2	0	1	0
21-24	1	0	1	1
> 25	1	0	1	1

<sup>1</sup>Wilcoxon rank test, Experiment one:  $\mu = 8.155$ ,  $P < 0.01$ ; Experiment two:  $\mu = 1.464$ ,  $P > 0.05$ .

<sup>2</sup>Wilcoxon rank test, Experiment one:  $\mu = 5.500$ ,  $P < 0.01$ ; Experiment two:  $\mu = 1.333$ ,  $P > 0.05$ .

than in the untreated group. In the second experiment, there were more adenocarcinomas and fewer adenomas in the sulindac-treated group than in the untreated group (Table 2).

In the first experiment, sulindac caused a significant reduction in both the number of mice with colonic tumors and the number of tumors per mouse. There were no significant differences in the number of mice with colonic tumors and the number of tumors per mouse between the sulindac-treated group and control group (Table 3). It was obvious that sulindac had a significant inhibitory effect on the growth of the MNU-induced tumors. However, in the second experiment the inhibitory effect of sulindac was less or disappeared after initiation of the colon carcinogenesis.

The median tumor diameter and median tumor volume were reduced in the sulindac-treated group in the first experiment. In the second experiment there were no differences in the median tumor diameter and volume between the sulindac-treated group and control group (Table 4). This finding reflects a rather more obvious reduction in tumor growth in the initiation stage, while in the second experiment the inhibitory effect on tumor growth is not significant in this promotion stage of colon carcinogenesis.

## DISCUSSION

The main purpose of this investigation is to study the potential chemopreventive properties of sulindac, a NSAID, in MNU-induced colon carcinogenesis. Several other NSAIDs have been studied for their chemopreventive efficacy in colon carcinogenesis. The studies by Spagnesi and Giardiello demonstrated that administration of sulindac causes regression of colon polyps in patients with FAP<sup>[1,2]</sup>. In the present study, we suggested that sulindac has an inhibitory effect on the development of MNU-induced colonic tumors in mice. Both the number of mice with tumors and the number of macroscopic tumors were reduced when MNU and sulindac were used together. It is more obvious that sulindac has a protective effect against the chemical induction of colonic tumors by MNU in mice. The chemopreventive effect was more significant in the initiation stage of the tumor, while in the promotion or progression stage this effect was less or disappeared. Sulindac could not cause the regression of established tumors induced by MNU.

The exact biochemical action of the sulindac in these experiments is not certain, but it is possible that it acts via inhibition of prostaglandin synthesis. Such an action may be used to explain the beneficial response obtained when indomethacin is administered to mice with transplantable NC carcinoma cell lines<sup>[4]</sup>. Tumor cells are thought to escape host immune surveillance through the production of prostaglandins in colonic tumors, which contain more

prostaglandins than the adjacent mucosa<sup>[5]</sup> and restore normal immunological mechanisms in the host. In addition, several studies also demonstrated that sulindac not only inhibits the prostanoid synthesis by acting on the cyclooxygenase (COX) activity but also modulates the activities of phospholipase C, lipoxygenase and arachidonic acid uptake, which are known to play a role in inflammation and cell proliferation<sup>[6,7]</sup>. Further experiments are now being conducted to investigate this phenomenon in terms of both the biochemical mechanisms involved and the changes in proliferative parameters in colonic tissues exposed to sulindac.

## REFERENCES

- 1 Spagnesi MT, Tonelli F, Dolara P, Caderni G, Valanzano R, Anastasi A, Bianchini F. Rectal proliferation and polyp occurrence in patients with familial adenomatous polyposis after sulindac treatment. *Gastroenterology* 1994; **106**: 362-366 [PMID: 8299902]
- 2 Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hyland LM, Celano P, Booker SV, Robinson CR, Offerhaus GJ. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med* 1993; **328**: 1313-1316 [PMID: 8385741 DOI: 10.1056/NEJM199305063281805]
- 3 Wang Q, Gao H, Wang YH, Chen YL, He J. Animal pattern of large intestinal cancer induced by MNU in mice. *Zhongguo Gangchangbing Zazhi* 1995; **15**: 6-8
- 4 Bennett A, Carroll MA, Melhuish PB, Stamford IF. Treatment of mouse carcinoma in vivo with a prostaglandin E2 analogue and indomethacin. *Br J Cancer* 1985; **52**: 245-249 [PMID: 4027166 DOI: 10.1038/bjc.1985.184]
- 5 Bennett A, Tacca MD, Stamford IF, Zebro T. Prostaglandins from tumours of human large bowel. *Br J Cancer* 1977; **35**: 881-884 [PMID: 871372 DOI: 10.1038/bjc.1977.132]
- 6 Marnett LJ. Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res* 1992; **52**: 5575-5589 [PMID: 1394181]
- 7 Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--differences and similarities. *N Engl J Med* 1991; **324**: 1716-1725 [PMID: 2034249]

S- Editor: Yang ZD L- Editor: Ma JY E- Editor: Liu WX

## Studies on the relationship between the point mutation of ras oncogenes and the prognosis of patients with gastric cancer

Dian-Chun Fang, Yuan-Hui Luo, Rong Lu, Wei-Wen Liu

Dian-Chun Fang, Yuan-Hui Luo, Rong Lu, Wei-Wen Liu, Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 630038, China

Dian-Chun Fang, Professor of internal medicine, with 102 published papers

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Dian-Chun Fang, Professor, Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 630038, China  
Telephone: +86-811-5318301

Received: August 21, 1996  
Revised: September 30, 1996  
Accepted: January 31, 1997  
Published online: March 15, 1997

### Abstract

**AIM:** To study the relationship between the point mutation of ras oncogenes and the prognosis of patients with gastric cancer.

**METHODS:** The point mutations at codon 12 and 61 of c-Ha-ras, at codon 12 and 13 of K-ras, and at codon 12 of N-ras were studied with PCR-RFLP in 88 formalin fixed and paraffin embedded specimens of gastric cancer.

**RESULTS:** It was found that the overall rate of point mutation of ras oncogenes was 18.2% and the positivity of the point mutation of ras oncogenes was related to the cancerous invasion of the serosa, the status of lymph node metastasis, the stage of cancer and the survival time after surgery.

**CONCLUSION:** The findings suggest that the determination of point mutations of ras oncogenes can be used to determine the prognosis of patients with gastric cancer.

**Key words:** Stomach neoplasms; Genes; Ras point mutation; Polymerase chain reaction; Polymorphism; Restriction fragment length; Prognosis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Fang DC, Luo YH, Lu R, Liu WW. Studies on the relationship between the point mutation of ras oncogenes and the prognosis of patients with gastric cancer. *World J Gastroenterol* 1997; 3(1): 19-21 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/19.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.19>

### INTRODUCTION

In recent years, the study of oncogenes has become one of the hot subjects in cancer research. It has been reported that the point mutation of ras oncogenes plays an important role in the development of certain cancers<sup>[1-3]</sup>. This study deals with the point mutation of c-Ha-ras, K-ras and N-ras in 88 formalin fixed and paraffin embedded specimens of gastric cancer. In addition, the relationship between the point mutation of the ras oncogenes and the prognosis of patients with gastric cancer was studied.

### MATERIALS AND METHODS

Eighty-eight specimens of gastric cancer were obtained during surgery in our hospital during the period from 1986 to 1991. All the specimens were fixed with 10% formalin and embedded in paraffin. Normal control tissue was collected from uninvolved gastric mucosa in all the patients. A 5 µm thick section was made, HE stained and examined under an optical microscope. Five similar sections were made from every specimen for DNA extraction.

For the extraction of DNA, 5 sections from each lesion were put into Eppendorf tubes and the paraffin was removed with xylene. After the tissue of the specimen was hydrolyzed and digested with proteinase K, the DNA content was extracted with the phenol chloroform method<sup>[4]</sup>. The samples of the purified DNA were diluted with TE buffer solution and their absorption value was determined on an ultraviolet ray spectrometer at the wavelength of 260 and 280 nm. The concentration and purity of nucleic acid were calculated.

The primers for the polymerase chain reactions (PCR) were synthesized by the Center of Science of Human Life of the Beijing University. The primer sets for ras mutation analysis were as follows:

P1A 5'-CAGGGCCCTCCTTGGCAGG-3'  
P1B 5'-GTCGTAGGCGTCCACAAAATGG-3'

P2A 5'-ACGTGCCTGTTGGACATCCT-3'  
P2B 5'-CACACAGGAAGCCCTCCCG-3'

P3A 5' ACTGAATATAAACTTGTGGTAGTTGGACCT-3'  
P3B 5'-TCAAAGAATGGTCTGGACC-3'

P4A 5'-AACTGGTGGTGGTTGGACCA-3'  
P4B 5'-CTCTATGGTGGGATCATATTC-3'

P1A and P1B is the primer to analyze codon 12 of c-Ha-ras. The amplified fragment had a length of 170 bp. After the cleavage with restriction endonuclease Hpa II or Msp I, 3 fragments with the length of 66, 56 and 48 bp were obtained in the wild type and 2 fragments with the length of 122 and 48 bp in the mutative type; P2A and P2B are the primers to analyze the point mutation at codon

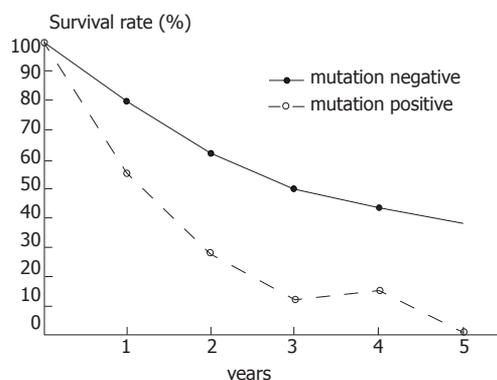


Figure 1 Five year survival rates of mutation negative and mutation positive after surgery for gastric cancer.

61 of c-Ha-ras. The amplified fragment had a length of 95 bp. After the cleavage with restriction endonuclease Bst N1, 3 fragments with the length of 15, 20 and 60 bp were obtained in the wild type and 2 fragments with the length of 20 and 75 bp were obtained in the mutative type. The primers for the analysis of the nonrestriction endonuclease point of the codon 12 and 13 of K-ras, P3A and P3B, were designed with the base mispairing method<sup>[5]</sup>. The amplified fragment was 157 bp in length. Endonuclease Bst N1 was used to analyze codon 12. The wild type was cleaved into 3 fragments of 114, 29 and 14 bp in length while the mutative type was cleaved into 2 fragments of 143 and 14 bp. Endonuclease Hph I was used to analyze codon 13. The fragment of the wild type was 157 bp in length while the mutative type was cleaved into 2 fragments of 114 and 43 bp; codon 12 of N-ras is also a nonrestriction endonuclease point and the primer was designed with the base mispairing method. The amplified fragment was 98 bp in length. The wild type was cleaved with Bst N1 into 2 fragments of 19 and 79 bp and the mutative type remained as a fragment of 98 bp.

PCR-RFLP was performed with the procedures as follows: DNA from 1 µg of tissue was dissolved in 50 µL PCR buffer solution containing 10 mmol/L Tris HCl, 50 mmol/L KCl, 1.5 mmol/L MgCl<sub>2</sub>, 0.2 mmol/L dATP, dGTP, dCTP and dTTP, 25 pmol/L primer and 1.3 units Taq DNA polymerase. The cycle of denaturation at 95 °C for 70 s, renaturation at 57 °C for 70 s and extension at 72 °C for 70 s was repeated for 35 times, with the last elongation step lengthened to 10 min. 10 µL of the PCR product was cleaved with the corresponding endonuclease (purchased from the BRL Company of United States), underwent 8% polyacrylamide gel electrophoresis and was stained with ethidium bromide. Eventually the product was observed under ultraviolet light and photographed.

All 88 cases were followed up and the longest follow up lasted 13 years in order that the outcome of each case was obtained.

## RESULTS

No point mutation (PM) of ras genes was found in specimens of normal gastric mucosa and 16 cases of the 88 with gastric cancer showed a PM rate of 18.2% (Figure 1). Among the 16 cases, PM at codon 12 of c-Ha-ras occurred in 12 cases (13.6%) and PM at codon 61 in 3 (3.4%); PM at codon 12 of K-ras in 5 (5.7%) and PM at codon 13 in none; and PM at codon 12 of N-ras in 1 (1.1%). PM at only 1 codon in the specimen was found in 11 cases and PM at 2 codons in the specimen was found in 5 cases. No case showed PM at 3 codons in the specimen simultaneously.

The 88 cases were divided into groups according to the pathological types of cancer, the size of cancer, the serosal invasion, the lymph node metastasis and the clinical staging of the case. It was found that the mutation rate was significantly higher in the group with serosal invasion than in that without ( $P < 0.01$ ), in the group with lymph node metastasis than in that without it ( $P < 0.05$ ) and in the group with clinical stage III and IV than in that with clinical stage I and II ( $P < 0.01$ ) (Table 1).

Survival analysis revealed a strong association between ras gene mutations of the tumor and patient survival time after surgery (Figure 1). The 5 year survival rate in the 72 cases without PM was 34.7% (25 cases) and compared with those with PM (0/16) the difference was

Table 1 Correlation between ras point mutation and clinical and pathological parameters of gastric cancer

	n	Point mutation negative	Point mutation positive (%)
Histological types			
Gland adenocarcinoma	20	18	2 (10.0)
Low differentiated carcinoma	29	22	7 (24.1)
Signet ring cell carcinoma	23	18	5 (21.7)
Mucinous carcinoma	16	14	2 (12.5)
Size			
< 5 cm	47	41	6 (12.8)
> 5 cm	41	31	10 (24.4)
Serosal invasion			
Without serosal invasion	40	38	2 (5.0)
With serosal invasion	48	34	14 (29.2) <sup>b</sup>
Lymph node metastases			
Without lymph node metastases	35	33	2 (5.7)
With lymph node metastases	53	39	14 (29.2) <sup>a</sup>
Staging			
Staging I and II	43	42	1 (2.3)
Staging III and IV	45	30	15 (33.3) <sup>c</sup>

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$

significant ( $P < 0.01$ ).

## DISCUSSION

This study shows that the activation of ras oncogenes is the molecular basis of the development of certain cancers. PM is one of the important patterns of ras gene activation. Therefore, the detection of PM becomes one of the hot topics in cancer research.

PCR-RFLP, a technique developed in recent years, greatly simplifies the procedures to detect PM<sup>[6]</sup>. It can be used to detect the PM in the DNA extracted from fresh specimens of gastric cancer as well as in the DNA from paraffin embedded specimens. The simplified technique determining PM makes it possible to determine the prognosis for patients with gastric cancer.

PM at the codons 12, 13 and 61 of ras oncogenes can enable the cells to possess the capacity of transformation<sup>[7]</sup>. However, points for the restriction endonuclease to cleave at the codons 12 and 13 for K-ras and codon 12 of N-ras and PCR-RFLP can not be performed. The authors, according to the 3' end region of the primers, introduced a mispaired base not affecting the amplification of PCR<sup>[5]</sup>. After the introduction of a mispaired base, a point to cleave is constituted for Bst N1. Thus, it is possible to study the PM at codon 12 and 13 of K-ras and codon 12 of N-ras.

It was reported that the mutation rate of ras genes was 90% in pancreatic cancer, 40% in colorectal cancer and below 10% in gastric cancer<sup>[8,9]</sup>. However, Deng *et al.*<sup>[6]</sup> found the mutation rate at codon 12 of Ha-ras was as high as 41% in gastric cancer. In our study, 88 paraffin embedded specimens of gastric cancer were studied with PCR-RFLP and the mutation rate of ras genes was found to be 18.2%. Japanese authors reported that PM of K-ras predominated in gastric cancer<sup>[10,11]</sup>. The difference of the PM rate between China and Japan might result from different geographical and environmental conditions, race and detection methods.

It was also found that the PM of ras genes was related to some biological factors of gastric cancer. Different types of cancers and their size play an insignificant role in the PM rate of ras oncogenes while those cases with serosal invasion and lymph node metastasis showed a far higher PM rate than those without them.

The cases of gastric cancer in clinical stage III and IV showed a significantly higher PM rate than those in the clinical stage I and II. This finding is consistent with the result reported by the authors who obtained it through the study on the molecular mechanism of cancer infiltration and metastasis<sup>[7,11]</sup>, which indicates the poor prognosis of the patients. Our results showed that the 5 year survival rate of patients without PM of ras genes was far higher than those with PM. Thus the determination of the PM of ras genes may be helpful for the prognosis of patients with gastric cancer and for the prescription of an efficient therapeutic plan for them.

The concept that cancer is a disease of genes has been widely accepted. Our study enables us to further understand the role

played by the PM of genes in the development of certain cancers. On the basis of the data of our study that most cases of gastric cancer did not show any evidence of PM of ras oncogenes, it seems obvious that PM of ras oncogenes is not the only factor to induce gastric cancer and there must be some other factors which need to be further investigated and explored.

## REFERENCES

- 1 **Koh EH**, Chung HC, Lee KB, Han EK, Oh SH, Min JS, Choi EM, Youn JK, Kim BS. Point mutation at codon 12 of the c-Ha-ras gene in human gastric cancers. *J Korean Med Sci* 1992; **7**: 110-115 [PMID: 1524724 DOI: 10.3346/jkms.1992.7.2.110]
- 2 **Stemmermann G**, Heffelfinger SC, Noffsinger A, Hui YZ, Miller MA, Fenoglio-Preiser CM. The molecular biology of esophageal and gastric cancer and their precursors: oncogenes, tumor suppressor genes, and growth factors. *Hum Pathol* 1994; **25**: 968-981 [PMID: 7927320 DOI: 10.1016/0046-8177(94)90056-6]
- 3 **Kato M**, Ito Y, Kobayashi S, Isono K. Detection of DCC and Ki-ras gene alterations in colorectal carcinoma tissue as prognostic markers for liver metastatic recurrence. *Cancer* 1996; **77**: 1729-1735 [PMID: 8608570 DOI: 10.1002/(SICI)1097-0142(19960415)77:8<1729::AID-CNCR47>3.0.CO;2-Z]
- 4 **Rogers BB**, Alpert LC, Hine EA, Buffone GJ. Analysis of DNA in fresh and fixed tissue by the polymerase chain reaction. *Am J Pathol* 1990; **136**: 541-548 [PMID: 2156429]
- 5 **Jiang W**, Kahn SM, Guillem JG, Lu SH, Weinstein IB. Rapid detection of ras oncogenes in human tumors: applications to colon, esophageal, and gastric cancer. *Oncogene* 1989; **4**: 923-928 [PMID: 2666911]
- 6 **Deng GR**, Liu XH, Wang JR. Correlation of mutations of oncogene C-Ha-ras at codon 12 with metastasis and survival of gastric cancer patients. *Oncogene Res* 1991; **6**: 33-38 [PMID: 1671796]
- 7 **Ranzani GN**, Pellegata NS, Previderè C, Saragoni A, Vio A, Maltoni M, Amadori D. Heterogeneous protooncogene amplification correlates with tumor progression and presence of metastases in gastric cancer patients. *Cancer Res* 1990; **50**: 7811-7814 [PMID: 2253224]
- 8 **Nanus DM**, Kelsen DP, Mentle IR, Altorki N, Albino AP. Infrequent point mutations of ras oncogenes in gastric cancers. *Gastroenterology* 1990; **98**: 955-960 [PMID: 2179035 DOI: 10.1016/0016-5085(90)90019-W]
- 9 **Wright PA**, Williams GT. Molecular biology and gastric carcinoma. *Gut* 1993; **34**: 145-147 [PMID: 8432461 DOI: 10.1136/gut.34.2.145]
- 10 **Kihana T**, Tsuda H, Hirota T, Shimosato Y, Sakamoto H, Terada M, Hirohashi S. Point mutation of c-Ki-ras oncogene in gastric adenoma and adenocarcinoma with tubular differentiation. *Jpn J Cancer Res* 1991; **82**: 308-314 [PMID: 1902452 DOI: 10.1111/j.1349-7006.1991.tb01847.x]
- 11 **Miki H**, Ohmori M, Perantoni AO, Enomoto T. K-ras activation in gastric epithelial tumors in Japanese. *Cancer Lett* 1991; **58**: 107-113 [PMID: 2049776]
- 12 **Neuman WL**, Wasylyshyn ML, Jacoby R, Erroi F, Angriman I, Montag A, Brasitus T, Michelassi F, Westbrook CA. Evidence for a common molecular pathogenesis in colorectal, gastric, and pancreatic cancer. *Genes Chromosomes Cancer* 1991; **3**: 468-473 [PMID: 1663781 DOI: 10.1002/gcc.2870030609]

S- Editor: Yang ZD L- Editor: Ma JY E- Editor: Liu WX

## Cellular immune function and liver damage in post-hepatic cirrhosis

Zhi-Jie Feng, Ran-Ming Niu, Xi-Ling Ren, Xi-Xian Yao

Zhi-Jie Feng, Ran-Ming Niu, Xi-Ling Ren, Xi-Xian Yao, Department of Internal Medicine, Second Affiliated Hospital, Hebei Medical University, Shijiazhuang 050000, Hebei Province, China

Dr. Zhi-Jie Feng, MD, Physician in Chief, with 21 papers and 3 books published.

Supported by The Foundation of Hebei Province Science Commission (No. 96216114), published in *Chin J Microbiol Immunol*, 1995; 15 (5):362 (in Chinese).

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Zhi-Jie Feng, Department of Internal Medicine, Second Affiliated Hospital, Hebei Medical University, Shijiazhuang 050000, Hebei Province, China  
Telephone: +86-311-7046975

Received: May 10, 1996

Revised: September 29, 1996

Accepted: January 31, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To study cellular immune function in patients with post-hepatic cirrhosis (PHC) and its relationship with different types of liver damage.

**METHODS:** Fifty-one patients with PHC, including 20 cases of Child-Pugh class A, 18 of class B, 13 of class C and 22 normal subjects as controls were studied. After peripheral blood mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation, lymphocyte transformation (LT) test, IL-2 activity and NK cell activity were measured by the <sup>3</sup>H-TdR incorporation technique.

**RESULTS:** Changes of LT stimulation index (SI), IL-2 activity (SI) and NK cell activity (%) in patients with PHC were significantly decreased compared with in the healthy controls ( $18.1 \pm 13.0$  vs  $34.9 \pm 21.7$ ,  $P < 0.01$ ;  $8.1 \pm 6.0$  vs  $13.6 \pm 5.8$ ,  $P < 0.01$ ;  $40.3 \pm 21.7$  vs  $61.3 \pm 20.5$ ,  $P < 0.01$ ; respectively). The defects of cellular immune function were closely related to Child-Pugh classification. The values in class C were much lower than those in B and A ( $P < 0.01$ ) and those in B were lower than those in A ( $P < 0.05$ ).

**CONCLUSION:** Defective cellular immune functions in patients with PHC are connected with the degree of liver damage.

**Key words:** Hepatitis; Liver cirrhosis; Immunology; Immunity, cellular; Killer cells, natural; Lymphocyte transformation; Interleukin

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Feng ZJ, Niu RM, Ren XL, Yao XX. Cellular immune function and liver damage in post-hepatic cirrhosis. *World J Gastroenterol* 1997; 3(1): 22-23 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/22.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.22>

### INTRODUCTION

Recent evidence has shown that deficiency of cell mediated immunity may play an important role in the pathogenesis of chronic HBV infection<sup>[1-3]</sup>. About 5%-10% cases of chronic hepatitis B develop into post-hepatic cirrhosis (PHC). In order to study the relationship between immune function and liver damage, a lymphocyte transformation (LT) test, IL-2 activity and NK cell activity were measured in peripheral blood mononuclear cells (PBMC) taken from 51 patients with PHC.

### MATERIALS AND METHODS

#### Subjects

Fifty-one PHC patients and 22 age and sex matched healthy controls were included. The diagnosis of PHC was based on clinical manifestations, serum biochemical findings and hepatitis B history or seropositive HBsAg for more than 12 mon. Diagnostic liver biopsy was performed in 12 patients. Patients with liver cirrhosis caused by other diseases were excluded. Of the 51 PHC patients, there were 36 males and 15 females, ranging in age from 22 to 68 years (mean 47.5 years). The course of disease varied from 1 mon to 30 years (mean 4.7 years). The complications of PHC included ascites (35 cases), upper gastrointestinal tract bleeding (28 cases), encephalopathy (5 cases) and hepatocellular carcinoma (7 cases). Twenty cases belonged to Child-Pugh class A, 18 cases to class B and 13 cases to class C. None of these patients had received corticosteroids or other immunosuppressive therapy within 6 mon.

#### Methods

**LT test** The PBMC were isolated by Ficoll-Hypaque gradient centrifugation, then washed and suspended at a concentration of  $1 \times 10^6$ /mL. The cells were stimulated with PHA (final concentration 25 µg/mL). The assay was made by the <sup>3</sup>H-TdR incorporation technique and individual sample results were expressed as a stimulation index (SI):

$$SI = \text{cpm (PHA)} / \text{cpm (control)}$$

**IL-2 activity** PBMC were stimulated with PHA (final concentration 150 µg/mL) to produce IL-2. After incubation, the supernatants were filtered and stored at 20 °C. The method of mouse thymus cell multiplication was used to assay IL-2 activity, using SI to express results.

$$SI = [\text{cpm (IL-2 supernatant)} - \text{cpm (background)}] / [\text{cpm (control)} - \text{cpm (background)}]$$

**NK cell activity** The activity was measured with the <sup>3</sup>H-TdR post

**Table 1 Immune function assay in post-hepatic cirrhosis patients ( $\bar{x} \pm s$ )**

Assays	PHC (n) <sup>b</sup>	Control (n)
LT test (SI)	18.1 ± 13.0 (48)	34.9 ± 21.7 (22)
IL-2 activity (SI)	8.1 ± 6.0 (41)	13.6 ± 5.8 (20)
NK activity (%)	40.3 ± 21.7 (49)	61.2 ± 20.5 (22)
IgG (g/L)	17.9 ± 7.7 (29)	10.0 ± 5.1 (22)
IgA (g/L)	3.2 ± 2.4 (29)	1.6 ± 0.8 (22)
IgM (g/L)	1.4 ± 0.8 (29)	0.8 ± 0.2 (22)
Complement C <sub>3</sub> (g/L)	0.5 ± 0.2 (29)	1 ± 0.6 (22)

<sup>b</sup>*P* < 0.01, *vs* control for all assays. PHC: Post-hepatic cirrhosis; LT: Lymphocyte transformation; SI: Stimulation index.

**Table 2 Cellular immune function in post-hepatic cirrhosis patients and Child-Pugh classification ( $\bar{x} \pm s$ )**

Assays	Child-Pugh class		
	A (n)	B (n)	C (n)
LT test (SI)	20.9 ± 16.7 (17)	19.9 ± 11.1 (18)	10.1 ± 5.4 (13) <sup>a</sup>
IL-2 activity (SI)	10.7 ± 7.9 (16)	7.4 ± 3.4 (13) <sup>a</sup>	5.2 ± 3.3 (12) <sup>b</sup>
NK activity (%)	46.5 ± 19.9 (18)	38.5 ± 15.4 (18) <sup>a</sup>	19.5 ± 11.3 (13) <sup>b</sup>

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, *vs* class A. LT: Lymphocyte transformation; SI: Stimulation index.

incorporative technique. The PBMC (effector cells) were suspended at a concentration of  $2 \times 10^6$ /mL. Target cells (Hep-2 cells), a cell line of carcinoma of the larynx, were at  $2 \times 10^4$ /mL. Effector/target ratio was 100:1. NK cell activity was calculated as follows:

$$\text{NK activity (\%)} = \{1 - [\text{cpm (effector+target)} - \text{cpm (effector)}] / [\text{cpm (target)} - \text{cpm (background)}]\} \times 100\%$$

The levels of serum IgG, IgA, IgM and complement C3 were measured by a simple agar immunodiffusion test.

### Statistical analysis

Results were expressed as  $\bar{x} \pm s$ . Comparisons between groups were made using the *t*-test. Linear regression analysis was performed to evaluate the correlation between LT test, IL-2 and NK cell activity.

## RESULTS

The results of the LT test, IL-2 activity and NK cell activity were significantly decreased in PHC patients compared with the healthy controls. The IgG, IgA and IgM were remarkably elevated and complement C3 was lowered in the PHC group (Table 1). The abnormalities of cellular immune function were closely related to the severity of liver damage determined by the Child-Pugh classification. The defects in patients with Child-Pugh class C were lower than that in patients with class A and B (Table 2). The linear regression analysis showed that there was strong positive correlation between LT test and NK cell activity ( $r = 0.4774$ ,  $P < 0.01$ ) and between IL-2 and NK cell activity ( $r = 0.3975$ ,  $P < 0.05$ ). There was no correlation between LT test and IL-2 activity ( $r = 0.3579$ ,  $P > 0.05$ ).

## DISCUSSION

Defective cellular immune function, for example, T cell blastogenesis,

CD4/CD8 ratio, lymphokine production, killer cell activity, *etc.*, was found in patients with chronic HBV infection<sup>[3-6]</sup>. The present study showed that there was a significant decrease in levels of the LT test, IL-2 activity and NK cell activity in PHC patients and that they were related to the Child-Pugh classification, suggesting that immunological abnormalities were closely related to the degree of liver damage. The more severely the liver was damaged, the more prominent the cellular immunological abnormalities. There was evidence to suggest that HBV-DNA may be duplicated and transcribed in PBMC, so that the immune function of T lymphocytes would be suppressed. It was shown that HBV-DNA was detected in PBMC of patients with HBV infection by the transfer hybridization technique<sup>[7]</sup>. The disturbance of T cell subsets, especially a decrease of CD4, may induce drawbacks of IL-2 production. IL-2 is a lymphokine which supports the immunoregulatory function of T cells and its deficiency may either suppress T cell proliferation or NK cell activity. Our present study showed that IL-2 activity was positively correlated with NK cell activity. Due to the destruction of cellular immunoregulation networks, hepatocyte damage was induced by impaired immune function of virus-infected hepatocytes and auto-immunoreaction. In addition, disturbances of immune function may also influence collagen metabolism and promote fibroplasia. Liver damage may lead to the defectiveness of hepatic immunoregulation and the decrease of number and function of Kupffer cells, which support the immune function of T, B and NK cells<sup>[8]</sup>. Therefore, cellular immunodeficiency was worsened more severely. Defectiveness of suppressor T cell function may lead to an increased B cell activation and its antibody production<sup>[9]</sup>.

## REFERENCES

- 1 Paronetto F. Cell-mediated immunity in liver disease. *Hum Pathol* 1986; **17**: 168-178 [PMID: 3512412 DOI: 10.1016/S0046-8177(86)80290-8]
- 2 Peters M, Vierling J, Gershwin ME, Milich D, Chisari FV, Hoofnagle JH. Immunology and the liver. *Hepatology* 1991; **13**: 977-994 [PMID: 2030002 DOI: 10.1002/hep.1840130529]
- 3 Löhr HF, Weber W, Schlaak J, Goergen B, Meyer zum Buschenfelde KH, Gerken G. Proliferative response of CD4+ T cells and hepatitis B virus clearance in chronic hepatitis with or without hepatitis B e-minus hepatitis B virus mutants. *Hepatology* 1995; **22**: 61-68 [PMID: 7601434 DOI: 10.1016/0270-9139(95)90353-4]
- 4 Saibara T, Maeda T, Miyazaki M, Onishi S, Yamamoto Y. Depressed immune function in patients with cirrhosis before emergence of hepatocellular carcinoma. *Hepatology* 1993; **18**: 315-319 [PMID: 7687981 DOI: 10.1002/hep.1840180215]
- 5 Lai KN, Leung JC, Tam JS, Leung NW. T lymphocyte activation in chronic hepatitis B infection: interleukin 2 release and its receptor expression. *Am J Gastroenterol* 1989; **84**: 1532-1537 [PMID: 2596455]
- 6 Chuang WL, Liu HW, Chang WY, Chen SC, Hsieh MY, Wang LY. Natural killer cell activity in patients with liver cirrhosis relative to severity of liver damage. *Dig Dis Sci* 1991; **36**: 299-302 [PMID: 1995265 DOI: 10.1007/BF01318200]
- 7 Pontisso P, Poon MC, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in mononuclear blood cells. *Br Med J (Clin Res Ed)* 1984; **288**: 1563-1566 [PMID: 6426645 DOI: 10.1136/bmj.288.6430.1563]
- 8 Okumura Y, Ishibashi H, Shirahama M, Kurokawa S, Kudo J, Okubo H, Niho Y. Kupffer cells modulate natural killer cell activity in vitro by producing prostaglandins. *Cell Immunol* 1987; **107**: 89-98 [PMID: 3581176 DOI: 10.1016/0008-8749(87)90268-1]
- 9 Kim SA, Lee SI, Choi IH, Shin JS, Uhm JR, Kim SJ, Choi HJ. Circulating immune complexes and cell-mediated immunity in patients with hepatitis B virus associated liver diseases. *Yonsei Med J* 1990; **31**: 347-358 [PMID: 2150250 DOI: 10.3349/ymj.1990.31.4.347]

S- Editor: Cao LB L- Editor: Ma JY E- Editor: Liu WX

## Surgical treatment of biliary ductal stricture complicating localized left hepatolithiasis

Wen-Bing Sun, Ben-Li Han, Jing-Xiu Cai, Zhen-Ping He

Wen-Bing Sun, Ben-Li Han, Jing-Xiu Cai, Zhen-Ping He, Hepatobiliary Surgery Center, Southwest Hospital, the Third Military Medical University, Chongqing 630038, China

Dr. Sun Wen Bing, male, born on July 12, 1964 and from Zhanhua County in Shandong Province, graduated from the Second Military Medical University, Associate Professor of Surgery, Vice Surgeon in Charge, specializing in the preventive study of cholangitis-induced liver damage, with 40 published papers

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: **Dr. Wen-Bing Sun**, the Third Military Medical University, Chongqing 630038, China  
Telephone: +86-811-5318301-73050.

Received: August 29, 1996  
Revised: September 29, 1996  
Accepted: January 31, 1997  
Published online: March 15, 1997

### Abstract

**AIM:** To summarize the experience in the clinical treatment of biliary duct strictures complicating localized left hepatolithiasis in the last two decades.

**METHODS:** A retrospective analysis of 67 cases of biliary duct strictures complicating localized left hepatolithiasis treated in our center in the last two decades was made with regards to each patient's age, gender, results of various preoperative examinations, operative findings, treatment and postoperative courses.

**RESULTS:** The incidence of left hepatic duct (LHD) stricture was 59.8% and that of a left external hepatic duct (LEHD) stricture was 84.0 % and 84.8% respectively, in which a severe degree dominated. Among the operative procedures used in the treatment of LHD strictures, plastic operation plus biliary enteric anastomosis ranks first in frequency (52.2%), with a re-stricture rate of 17.1%. Left lobectomy ranks third (19.4%) with no re-stricture. Simple plastic performance or dilation had a high occurrence rate of re-stricture and usually needed subsequent surgery. Most LEHD strictures were eradicated by lateral segmentectomy or lobectomy, whereas most LMHD strictures were just the opposite. The rate of preoperative diagnosis of LMHD by endoscopic retrograde cholangiography, percutaneous transhepatic cholangiography, computed tomography or intraoperative and postoperative trans-T-tube cholangiography was much lower than that of LEHD or extrahepatic duct.

**CONCLUSION:** Too much attention paid to LEHD disorders in the treatment of localized left hepatolithiasis potentially results in

negligence or omission in LMHD disorders. Malpractice treatments of LHD strictures are important factors affecting the long term results of localized left hepatolithiasis, for which left lobectomy is usually the therapy of choice.

**Key words:** Cholelithiasis/surgery; Bile duct diseases/surgery; Hepatic duct, common/surgery; Cholelithiasis/complication; Hepatectomy

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Sun WB, Han BL, Cai JX, He ZP. Surgical treatment of biliary ductal stricture complicating localized left hepatolithiasis. *World J Gastroenterol* 1997; 3(1): 24-26 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/24.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.24>

### INTRODUCTION

Solitary left hepatolithiasis with left intrahepatic ductal calculi is a common and peculiar type of hepatolithiasis which is prevalent in East Asia<sup>[1,2]</sup>. In order to treat left hepatolithiasis, it is necessary to deal with the intrahepatic biliary strictures<sup>[3]</sup>, the occurrence of which is a sign of complexity, difficulty, repetition and severity. Theoretically, lateral segmentectomy or left lobectomy could be used to solve the problem concerning the left hepatic duct (LHD) strictures<sup>[4]</sup>. However, recently some problems have been noticed, such as a high incidence of residual stones after surgery<sup>[5]</sup> and a high stone recurrence rate<sup>[6]</sup> in the treatment of patients with left hepatolithiasis, some of whom have had subsequent surgery. In this paper, 67 cases of left sided hepatic duct strictures complicating localized left hepatolithiasis surgically treated at our center in the last two decades were analyzed.

### MATERIALS AND METHODS

#### Subjects

One thousand and eighteen patients with primary intrahepatic lithiasis were treated at our center between June 1976 and June 1996, among whom 133 had localized left hepatolithiasis, 112 underwent operations with no deaths and 67 (29 males and 38 females) had complications with LHD strictures. A retrospective study of patients with LHD or second class hepatic ductal strictures was made with regards to each patient's age, gender, results of various preoperative examinations, operative findings, treatment and postoperative courses. The mean age was 40.7 years (range, 27 to 72 years).

#### Standard of inclusion

All diagnoses were confirmed by cholangiography or at surgery.

**Table 1** The occurrence rate of each hepatic duct stricture

Location	<i>n</i>	Mild stricture (%) <sup>a1</sup>	Severe stricture (%) <sup>a2</sup>	Total (%)
Left hepatic duct	112	26 (23.2)	41 (36.6)	67 (59.8)
Left medial hepatic duct	33	9 (27.3)	19 (42.4)	28 (84.8) <sup>b</sup>
Left external hepatic duct	75	29 (38.7)	34 (45.3)	63 (84.0) <sup>d</sup>

Mild or severe stricture refers to a stricture with a diameter > or ≤ respectively half of the greatest diameter of the dilated ducts proximal to the stricture. <sup>a1</sup> $\chi^2 = 5.26$ ,  $P > 0.05$ ; <sup>a2</sup> $\chi^2 = 4.90$ ,  $P > 0.05$ , among 3 groups. <sup>b</sup> $\chi^2 = 6.00$ ,  $P < 0.01$ ; <sup>d</sup> $\chi^2 = 11.82$ ,  $P < 0.01$ , vs left hepatic duct.

**Table 2** Surgical treatment of left hepatic duct stricture and the subsequent re-stricture rates ( $\bar{x} \pm s$ )

Operative procedures	Cases (%)	Mean follow up period (y)	Re-strictures (%)
Plastic operation plus			
Biliary enteric anastomosis	35 (52.2)	9.8 ± 2.7	6 (17.1) <sup>f</sup>
Severe degree	21 (51.2)	10.6 ± 2.3	4 (19.0)
Mild degree	14 (53.8)	8.6 ± 3.2	2 (14.3)
Left lobectomy	13 (19.4)	9.7 ± 3.0	0 (0.0) <sup>e</sup>
Severe degree	7 (17.1)	9.1 ± 3.1	0 (0.0)
Mild degree	6 (23.1)	10.3 ± 2.8	0 (0.0)
Stricture repair	6 (9.0)	8.5 ± 3.2	3 (50.0)
Severe degree	4 (9.8)	8.2 ± 3.5	2 (50.0)
Mild degree	2 (7.7)	7.8 ± 10.1	2 (100.0)
Stricture dilation	13 (19.4)	9.9 ± 2.2	12 (92.3)
Severe degree	9 (22.0)	10.7 ± 2.7	9 (100.0)
Mild degree	4 (15.4)	8.1 ± 2.4	3 (75.0)
Total	67 (100.0)	10.1 ± 2.9	21 (31.3)

<sup>f</sup> $\chi^2 = 19.76$ ,  $P < 0.01$ ; <sup>e</sup> $\chi^2 = 4.69$ ,  $P < 0.05$ , vs stricture dilation. No significant difference between severe degree and mild degree.

### Standard of stricture degree

The criteria for stricture degree used in this paper was proposed by the Chinese Surgical Association in 1983 (a mild or severe stricture refers to a stricture with a diameter > or ≤ respectively half of the greatest diameter of the dilated ducts proximal to the stricture).

### Statistical analysis

All data are expressed as  $\bar{x} \pm s$ . Statistical analysis was conducted with the Chi squared test.

## RESULTS

### Hepatic duct stricture

Left hepatolithiasis commonly becomes complicated due to biliary duct strictures and the occurrence rate of LHD stricture was 59.8%. In view of the difficulty and magnitude of the operation, the left external hepatic duct (LEHD) and the left medial hepatic duct (LMHD) were not routinely explored. Definitive diagnoses in some cases indicated that the occurrence rates of the second hepatic ducts are significantly higher than those of LHD ( $P < 0.01$ ) (Table 1).

### Treatment of LHD stricture

Table 2 shows the various procedures used for the treatment of LHD stricture in the current group, from which we can see that plastic operation plus biliary enteric anastomosis ranks first in frequency followed by stricture dilation, left lobectomy and simple repair. The incidence of re-stricture after left lobectomy was 0.0%, 17.1% following plastic operation plus biliary enteric anastomosis (not significantly higher than the former probably because of the limited cases). Simple plastic performance or dilation had a high occurrence rate of re-stricture and usually needed subsequent surgery, especially for the latter. The treatment profiles of strictures of severe and mild degrees were not significantly different.

### Treatment of LEHD and LMHD strictures

Practically, there is no specific procedure for strictures of LEHD and

**Table 3** Treatment of strictures of left external hepatic duct and left medial hepatic duct

	Cases	%
Lateral segmentectomy	66	58.9
+Bile duct exploration	39	34.8
+Biliary enteric anastomosis	27	24.1
Left lobectomy	14	12.5
+Bile duct exploration	10	8.9
+Biliary enteric anastomosis	4	3.6
Biliary enteric anastomosis	15	13.4
Bile duct exploration	17	15.2
Total	112	100

**Table 4** The rate of correct diagnosis of various bile ducts by different diagnostic procedures

	ERC (%)	PTC (%)	TTC (%)	CT (%)
Total	78	18	106	40
Left external hepatic duct	69 (88.5)	18 (100.0)	96 (90.6)	29 (72.5)
Left medial hepatic duct	16 (20.5)	6 (33.3)	21 (19.8)	12 (30.0)
Extrahepatic duct	78 (100.0)	15 (83.3)	106 (100.0)	36 (90.0)

ERC: Endoscopic retrograde cholangiography; PTC: Percutaneous transhepatic cholangiography; TTC: Trans-T-tube cholangiography; CT: Computed tomography.

LMHD, although in some ways, segmentectomy or lobectomy is the only practical method. Lateral segmentectomy can be used for LEHD stones, strictures, etc. while left lobectomy can be used for disorders of LEHD, LMHD or LHD. Based on this theory, we judged the effect of strictures of LEHD or LMHD on the treatment decision from the present data by analyzing the operative procedure profiles in combination with the incidence of strictures. Table 3 shows that the LEHD stricture is usually treated with segmentectomy or lobectomy. Eighty of 112 cases with left hepatolithiasis underwent lateral segmentectomy or lobectomy (71.4%), which was similar to the occurrence rate of LEHD stricture ( $P > 0.05$ ). Whereas, LMHD stricture is seldom treated with segmentectomy or lobectomy (12.5%), significantly lower than the incidence of LMHD stricture ( $P < 0.01$ ) and suggesting that the performance rate of left lobectomy was much less than clinically needed.

### Rate of preoperative diagnosis

The present data suggests a significantly lower chance for LMHD than LEHD disorders to undergo an eradication treatment. One of the reasons is the difference between the rates of preoperative diagnosis of LEHD and LMHD. The rate of preoperative diagnosis of LMHD by endoscopic retrograde cholangiography (ERC), percutaneous transhepatic cholangiography (PTC), computed tomography (CT) or intraoperative and postoperative trans-T-tube cholangiography (TTC) was much lower than that of LEHD or extrahepatic duct (Table 4).

## DISCUSSION

Localized left hepatolithiasis needs some special clinical treatment with the following characteristics: [WTBZ] (1)[WTB1] the intrahepatic calculi are localized in the left intrahepatic bile duct system; (2)the extrahepatic biliary duct may or may not be involved; and (3) theoretically, it can be cured by lateral segmentectomy or left lobectomy which brings about a thorough elimination of stones and the accompanying strictures. For the sake of acceptable results for clinical treatment in comparison with right hepatolithiasis<sup>[7]</sup>, no special consideration has been given.

Biliary stricture is defined by Matsumoto *et al.*<sup>[8]</sup> as a localized diminution in bile duct caliber proximal to the common hepatic duct. In this paper, the standard of classification of mild or severe degrees of stricture was suggested by the Chinese Surgical Association.

The stricture narrows the intraductal lumen and slows down the speed of bile flow<sup>[8,9]</sup>, leading to stasis of bile which leads to the greater generation of minute calculi and increases the size or number of stones. Besides, the increased pressure gradient of bile flow between the proximal duct and the stricture results in the

distention of the duct between the wave of contraction and the point of obstruction, resulting in the gradual increase of proximal ductal lumen. The increased size or number of stones and the discrepancy between ductal lumens may contribute to bile turbulence within the dilated ductal lumen. A vicious cycle thus develops. Moreover, bacteria grow further following chronic bile stasis<sup>[9]</sup>. So it is evident that bile duct stricture contributes to the complexity of pathological changes and difficulties in treating the recurrence of stones with unsatisfactory long term results.

In our limited experience, LHD stricture often occurs secondary to left hepatolithiasis, especially left lateral hepatolithiasis. Its occurrence implies a new stage of left hepatolithiasis in which LMHD becomes more susceptible to stricture, dilation or stones. Some factors contributing to the prognosis include calculi, stricture or dilation<sup>[7,10]</sup>, bile stasis and liver function damage<sup>[11]</sup>.

Although a bias in counting the incidence of LEHD and LMHD strictures resulting from the incomplete case records of the present group cannot be excluded, we can still deduce that LEHD and LMHD strictures are comparable in frequency although commonly different in severity, demonstrating that localized LMHD disorders are rare and that clinical treatment should be concentrated on the strictures of both LEHD and LMHD. So theoretically speaking, left lobectomy is sometimes the eradication treatment of choice for left biliary duct strictures, although the facts are almost quite the opposite. Left lobectomy is much less frequently performed than lateral segmentectomy in the clinical management of localized left hepatolithiasis.

Preoperative misdiagnosis is another important factor responsible for the current situation. The present data indicate that most of the omitted LMHD disorders result from no available radiological observations of LMHD preoperatively. So it is urgent to try to improve the preoperative diagnosis rate of LMHD. Since the mouth of LMHD is too low in a supine position to be filled up in cholangiography-inducing preoperative omission, attention should be paid to patients with intrahepatic lithiasis, especially left

hepatolithiasis, to achieve a definitive diagnosis of LMHD pre and intra-operatively with some improvements in cholangiography, including a prone ERC position, selective PTC and intraoperative B ultrasound. CT seems to be a great help in some patients by demonstrating the location of calculi directly and elucidating the bulk of hepatic lobe as an indirect sign of the disorders.

## REFERENCES

- 1 Nakayama F, Soloway RD, Nakama T, Miyazaki K, Ichimiya H, Sheen PC, Ker CG, Ong GB, Choi TK, Boey J. Hepatolithiasis in East Asia. Retrospective study. *Dig Dis Sci* 1986; **31**: 21-26 [PMID: 3940820 DOI: 10.1007/BF01347905]
- 2 Fan ST, Choi TK, Lo CM, Mok FP, Lai EC, Wong J. Treatment of hepatolithiasis: improvement of result by a systematic approach. *Surgery* 1991; **109**: 474-480 [PMID: 2008653]
- 3 Chijiwa K, Yamashita H, Yoshida J, Kuroki S, Tanaka M. Current management and long-term prognosis of hepatolithiasis. *Arch Surg* 1995; **130**: 194-197 [PMID: 7848091 DOI: 10.1001/archsurg.1995.01430020084016]
- 4 Jeng KS, Yang FS, Ohta I, Chiang HJ. Dilatation of intrahepatic biliary strictures in patients with hepatolithiasis. *World J Surg* 1990; **14**: 587-592; discussion 592-593 [PMID: 2238657 DOI: 10.1007/BF01658796]
- 5 Chang TM, Passaro E. Intrahepatic stones: the Taiwan experience. *Am J Surg* 1983; **146**: 241-244 [PMID: 6881448 DOI: 10.1016/0002-9610(83)90382-3]
- 6 Choi TK, Wong J, Ong GB. The surgical management of primary intrahepatic stones. *Br J Surg* 1982; **69**: 86-90 [PMID: 7059773 DOI: 10.1002/bjs.1800690210]
- 7 Jeng KS, Ohta I, Yang FS, Liu TP, Shih SC, Chang WS, Wan HY, Huang SH. Coexisting sharp ductal angulation with intrahepatic biliary strictures in right hepatolithiasis. *Arch Surg* 1994; **129**: 1097-1102 [PMID: 7944942 DOI: 10.1001/archsurg.1994.01420340111022]
- 8 Matsumoto Y, Fujii H, Yoshioka M, Sekikawa T, Wada T, Yamamoto M, Eguchi H, Sugahara K. Biliary strictures as a cause of primary intrahepatic bile duct stones. *World J Surg* 1986; **10**: 867-875 [PMID: 3776223 DOI: 10.1007/BF01655262]
- 9 Mercadier M, Fingerhut A. Strictures of the intrahepatic bile ducts. *World J Surg* 1984; **8**: 15-21 [PMID: 6367230 DOI: 10.1007/BF01658358]
- 10 Mueller PR, vanSonnenberg E, Ferrucci JT, Weyman PJ, Butch RJ, Malt RA, Burhenne HJ. Biliary stricture dilatation: multicenter review of clinical management in 73 patients. *Radiology* 1986; **160**: 17-22 [PMID: 3715030 DOI: 10.1148/radiology.160.1.3715030]
- 11 Sato T, Suzuki N, Takahashi W, Uematsu I. Surgical management of intrahepatic gallstones. *Ann Surg* 1980; **192**: 28-32 [PMID: 7406560 DOI: 10.1097/0000658-198007000-00005]

S- Editor: Yang ZD L- Editor: Ma JY E- Editor: Liu WX

## Study of the influence of hiatus hernia on gastroesophageal reflux

Hui-Ming Zhu

Hui-Ming Zhu, Department of Gastroenterology, Shenzhen People's Hospital, Shenzhen 51 8001, Guangdong Province, China

Dr. Hui-Ming Zhu, worked for postdoctoral research in Milan University, Italy during 1988-1992, with 54 published papers

Author contributions: The author solely contributed to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Hui-Ming Zhu, Department of Gastroenterology, Shenzhen People's Hospital, Shenzhen 518001, Guangdong Province, China

Received: September 9, 1996

Revised: January 1, 1997

Accepted: March 1, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To explore whether the presence of a sliding hiatus hernia influences gastroesophageal reflux.

**METHODS:** Endoscopy and 24 h pH monitoring were performed for 197 outpatients with gastroesophageal reflux symptoms.

**RESULTS:** Of the 197 patients with symptoms of gastroesophageal reflux, patients with hiatus hernia accounted for 36%. The incidence of esophagitis in patients with hiatus hernia was significantly higher than that in patients without hiatus hernia. The results of 24 h pH monitoring showed that 84 patients had physiological reflux, 37 had pathological reflux without esophagitis, 64 had reflux esophagitis and 12 had physiological reflux concomitant with esophagitis. All the patients with hiatus hernia had a longer percentage time with supine reflux and a higher frequency of episodes lasting over 5 min at night compared to those without hiatus hernia. The incidence of combined daytime and nocturnal reflux in patients with hiatus hernia was significantly higher than that in patients without hiatus hernia.

**CONCLUSION:** Pathological reflux and reflux esophagitis in some patients with symptoms of gastroesophageal reflux represent two different stages of gastroesophageal reflux disease. Pathological reflux is the first stage, in which the lower esophageal sphincter is incompetent but the esophageal mucosal resistance effectively prevents regurgitated acid from damaging the esophageal mucosa. Reflux esophagitis represents the second stage, in which the aggression of the regurgitated acid is so strong that the esophageal mucosa fails to resist it and the epithelium of the esophagus is damaged. Patients with hiatus hernia have a high incidence of combined daytime and nocturnal reflux, with the latter being responsible for esophagitis.

**Key words:** Hernia, hiatal; Gastroesophageal reflux; Endoscopy, gastrointestinal; Hydrogen ion concentration; Esophagitis, peptic

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhu HM. Study of the influence of hiatus hernia on gastroesophageal reflux. *World J Gastroenterol* 1997; 3(1): 27-30 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/27.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.27>

### INTRODUCTION

The relationship between hiatus hernia (HH) and gastroesophageal reflux (GER) disease remains controversial<sup>[1-4]</sup>. One opinion was that the demonstration of HH would frequently imply the presence of reflux esophagitis. Some investigators, for example, have claimed that HH can be found in almost all cases of esophagitis<sup>[5-6]</sup>. As a result, some authors believed that it was necessary to excise HH to cure GER disease. Another opinion was that the existence of HH did not affect esophageal sphincter competence<sup>[7-10]</sup>. The third opinion was that HH might play only a partial role in the development of esophagitis, that is, the absence of HH could exclude more severe forms of reflux esophagitis<sup>[11-14]</sup>. This study was, therefore, designed to evaluate the influence of the existence of HH on GER.

### MATERIALS AND METHODS

#### Patients

One hundred and ninety-seven consecutive outpatients (female, 75; male, 122) who had experienced the symptoms of heartburn, regurgitation and chest pain for at least 6 mon were included in this study. None had a past history of surgery or had taken H<sub>2</sub> receptor blockers or proton pump inhibitors during the 4 wk prior to endoscopy and 24 h esophageal pH monitoring.

#### Endoscopy and 24 h pH monitoring

Endoscopy was performed a week before 24 h esophageal pH monitoring. Esophagitis was graded from I to IV according to the Savary-Miller classification<sup>[15]</sup>. Only a few patients presented with grades II and III and therefore grades I and II, III and IV were grouped together, respectively. Twenty-four hour intraesophageal pH monitoring was carried out in accordance with a method described elsewhere<sup>[16,17]</sup>. Patients were advised to take a standard meal with approximately 2200 kilocalories during 24 h intraesophageal pH monitoring. A glass pH electrode with an incorporated potassium chloride reference electrode (Ingold Electrode, No.440) was passed by the nasoesophageal route and positioned with the tip 5 cm above the gastroesophageal junction identified by a pH meter. The results from the pH probe were recorded on a solid state recorder (Autronicord CM18), which was carried by the patients on a belt. A computer based analysis was used for the interpretation of the 24 h pH monitoring data. The parameters recorded included the number and percentage time of GER episodes and the number of

**Table 1 Demographic data and endoscopic findings in patients with and without hiatus hernia**

	Patients with HH (n = 71)	Patients without HH (n = 126)	P value
Age (yr)			
Patients with esophagitis	51.1 ± 1.5	40.7 ± 2.9	< 0.001
Patients without esophagitis	51.8 ± 4.2	41.0 ± 2.2	< 0.001
Sex (n, f/m)	25/46	50/76	> 0.05
Heartburn (%)	80.2	89.6	> 0.05
Regurgitation (%)	54.3	54.8	> 0.05
Chest pain (%)	60.6	61.1	> 0.05
Smoker (%)	21.1	25.4	> 0.05
Alcohol consumer (%)	36.6	27.0	> 0.05
Endoscopic findings (%)			
Normal	42.3	72.2	< 0.001
Grade I - II esophagitis	42.3	26.2	< 0.05
Grade III-IV esophagitis	15.4	1.6	< 0.001

HH = Hiatus hernia

**Table 2 Gastroesophageal reflux parameters in patients with and without hiatus hernia ( $\bar{x} \pm s$ )**

	RE (n = 64)	PR (n = 37)	PhR (n = 84)	PhRE (n = 12)
Patients with HH				
% time of reflux				
24-h	18.8 ± 15.4	14.9 ± 9.8	2.5 ± 0.2	3.9 ± 2.6
Upright	17.9 ± 5.0	14.4 ± 9.3	2.7 ± 2.2	4.4 ± 3.7
Supine	19.1 ± 2.2	15.1 ± 0.0	2.1 ± 3.5	3.6 ± 4.2
No. of episodes > 5 min				
24-h	9.1 ± 4.3	7.5 ± 3.2	0.9 ± 1.4	2.6 ± 4.1
Upright	5.8 ± 4.4	4.2 ± 3.2	0.5 ± 1.0	1.5 ± 1.3
Supine	3.3 ± 2.1	3.2 ± 2.3	0.3 ± 0.6	1.5 ± 1.8
Patients without HH				
% time of reflux				
24-h	17.1 ± 4.8	13.3 ± 6.7	2.7 ± 2.2	1.5 ± 1.7
Upright	18.9 ± 3.5	7.0 ± 8.0	3.4 ± 2.8	0.3 ± 0.5
Supine	13.5 ± 1.7	2.7 ± 2.2	1.5 ± 3.0	1.1 ± 1.6
No. of episodes > 5 min				
24-h	10.5 ± 8.0	6.1 ± 3.5	0.9 ± 1.4	2.0 ± 2.7
Upright	8.1 ± 6.9	4.5 ± 3.0	0.5 ± 1.0	1.6 ± 2.0
Supine	2.3 ± 2.1	1.6 ± 1.4	0.3 ± 0.6	0.3 ± 0.8

HH = Hiatus hernia; RE = Reflux esophagitis; PR = Pathological reflux; PhR = Physiological reflux; PhRE = Esophagitis with physiological reflux.

**Table 3 Statistical significance of gastroesophageal reflux parameters between patients with and without hiatus hernia in Table 2**

	RE	PR	PhR	PhRE
% time of reflux				
24-h	> 0.05	> 0.05	> 0.05	> 0.05
Upright	> 0.05	> 0.05	> 0.05	> 0.05
Supine	> 0.05	> 0.05	> 0.05	> 0.05
No. of episodes				
24-h	> 0.05	> 0.05	> 0.05	> 0.05
Upright	> 0.05	> 0.05	> 0.05	> 0.05
Supine	> 0.05	> 0.05	> 0.05	> 0.05
No. of episodes > 5 min				
24-h	> 0.05	> 0.05	> 0.05	> 0.05
Upright	> 0.05	> 0.05	> 0.05	> 0.05
Supine	> 0.05	> 0.05	> 0.05	> 0.05

HH = Hiatus hernia; RE = Reflux esophagitis; PR = Pathological reflux; PhR = Physiological reflux; PhRE = Esophagitis with physiological reflux.

GER episodes lasting over 5 min. A pathological reflux (PR) was diagnosed if 1) the pH value in the regurgitated contents was less than 4.0 and 2) the complete reflux duration was more than 7% in 24 h<sup>[16,17]</sup>; physiological reflux (PhR) was defined if a reflux event did not fulfill the above criteria; reflux esophagitis (RE) means a pathological reflux event associated with esophageal inflammatory lesions; and physiological reflux esophagitis concomitant with esophagitis (PhRE) means an esophageal inflammatory lesion without pathological GER.

### Statistical analysis

The Wilcoxon test was used for the analysis of GER parameters in the patients with and without HH. Student's *t* test was applied to analyze the age and the duration of GER symptoms. The remaining data were analyzed using the Chi square test.

### RESULTS

Of 197 patients with symptoms of GER, patients with HH accounted for 36%. Demographic data and endoscopic findings in patients with and without HH are listed in Table 1. The incidence of esophagitis in patients with HH was significantly higher than that in patients without HH (grade III esophagitis, *P* < 0.05; and grade III-IV esophagitis, *P* < 0.001). There was no significant difference between patients with HH and without HH concerning sex, GER symptoms, smoking and alcohol consumption (*P* > 0.05).

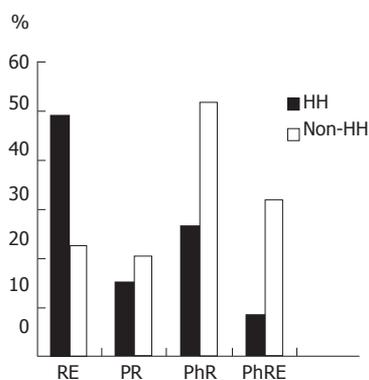
On the basis of the results of 24 h pH monitoring and endoscopy, the patients with HH and without HH were divided into 8 subgroups: HH patients with RE, non HH patients with RE, HH patients with PR, non HH patients with PR, HH patients with PhR, non HH patients with PhR, HH patients with PhRE and non HH patients with PhRE. GER parameters in patients with and without HH are shown in Table 2. The statistical analysis for the GER parameters in patients with and without HH is given in Table 3. A mean percentage time with GER in HH patients with PR was longer than that in non HH patients with PR and a frequency of nocturnal reflux lasting over 5 min was higher in HH patients with PR than that in non HH patients with PR.

The incidences of reflux esophagitis, pathological reflux, physiological reflux and physiological reflux with esophagitis in patients with and without HH are shown in Figure 1. The incidence of reflux esophagitis in patients with HH was significantly higher than that in patients without HH (*P* < 0.01); on the other hand, the incidence of physiological reflux in patients without HH was significantly higher than that in patients with HH (*P* < 0.01). The incidence of pathological reflux and physiological reflux with esophagitis in the two groups showed no statistically significant difference (*P* > 0.05). Figure 2 shows the percentages of upright, supine and combined upright and supine reflux in patients with and without HH. There was a significant difference between patients with and without HH regarding upright and combined GER (*P* < 0.01). There was no statistically significant difference in supine GER in patients with and without HH (*P* > 0.05).

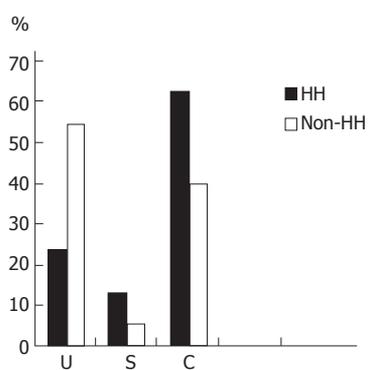
### DISCUSSION

Because the relationship between HH and GER disease is still controversial, it is necessary to calculate the incidence of sliding HH and to compare GER parameters in HH patients with those without HH. Clagett *et al*<sup>[18]</sup> reported that the incidence of HH in the general population is far more than the number of patients who present clinically with symptoms of GER. In 95 asymptomatic subjects examined by Dyer *et al*<sup>[19]</sup>, the incidence of HH was 33% and only 16% of the subjects complained of symptoms of GER. Of 102 patients with symptoms of GER studied by DeMeester, 52% had endoscopic evidence of HH<sup>[20]</sup>. Kaul *et al*<sup>[11]</sup> reported that HH was found in 50 of 101 patients with symptoms of GER. In our 197 patients with symptoms of GER, 36% had HH. The incidence of reflux esophagitis in patients with HH was significantly higher than in patients without HH, while the incidence of physiological GER was significantly higher in patients without HH than that in patients with HH.

The number of GER episodes, percentage time with GER and the number of episodes lasting over 5 min in patients with HH were compared with those in patients without HH. The results showed that the percentage time with nocturnal reflux in HH patients with PR was longer than that in non HH patients with PR and the frequency of episodes lasting over 5 min at night in HH patients with PR was higher than that in non HH patients with PR, whereas there was no significant difference in the frequency of GER, percentage time with GER and number of episodes lasting over 5 min between HH patients and non HH patients with RE, PhR and PhRE. In a previous study, Sloan *et al*<sup>[21]</sup> found that impaired emptying in



**Figure 1** The incidences of Reflux esophagitis, Pathological reflux, Physiological reflux and Esophagitis with physiological reflux in patients with and without hiatus hernia. RE: reflux esophagitis, PR: Pathological reflux; PhR: Physiological reflux; PhRE: esophagitis with physiological reflux; HH: Hiatus hernia, Non HH: Patients without HH.



**Figure 2** Percentages of upright, supine and combined reflux in patients with and without hiatus hernia. U: Upright reflux, S: Supine reflux; C: Combined daytime and nocturnal reflux, HH: Hiatus hernia, Non HH: Patients without HH.

patients with HH was attributable to an early retrograde flow, occurring immediately after lower esophageal sphincter relaxation. The results reported by Mittal *et al*<sup>[22]</sup> showed that acid clearance at 5 cm above the lower esophageal sphincter was faster in non HH patients than in HH patients with GER. Our data revealed that the presence of HH in patients with PR impaired the acid clearance function of the esophagus, while in patients with RE, no noteworthy influence of HH on the parameters of GER was observed. One question arising from our results is why the presence of HH had a different influence on the parameters of GER in patients with PR and RE. It seems that PR and RE in some patients with symptoms of GER represented two different stages of GER disease, with PR being the first stage, in which the lower esophageal sphincter was incompetent but the esophageal mucosal resistance effectively prevented regurgitated acid from damaging the esophageal mucosa. RE in some patients with symptoms of GER represented the second stage, in which the aggression of the regurgitated acid was so strong that the esophageal mucosa failed to resist it and the epithelium of the esophagus was damaged. There is evidence that in the first stage HH had considerable influence on GER and esophageal clearance of refluxed acid. In the second stage, the interaction between lower esophageal sphincter incompetence and esophagitis might be involved in the pathogenesis, *i.e.* GER might develop into esophagitis, which, in turn, impairs lower esophageal sphincter competence and aggravates the GER. In this stage, a vicious cycle of the conditions might have a crucial influence on GER<sup>[23]</sup>, whereas the effect of etiological factors such as HH on GER in this stage appeared to be less important.

It is currently accepted that there are a number of factors involved in the pathogenesis of GER disease but the most important one is the contact time between regurgitated acid and the esophageal mucosa<sup>[24-27]</sup>. In the present study, the exposure time of esophageal mucosa to regurgitated acid and patterns of GER in patients with HH were compared with those without HH. However, we were unable to demonstrate any significant difference in the percentage time with GER between HH patients with RE and those without HH. Our results revealed that the incidence of combined

daytime and nocturnal reflux was significantly higher in patients with HH than that in patients without HH and that the incidence of upright reflux, on the other hand, was significantly higher in patients without HH than that in patients with HH. Attention has long been paid to the relationship between the development of esophagitis and patterns of GER since the advent of 24 h pH monitoring. Some investigators, for example, have revealed that the development of esophagitis is related to increased supine reflux<sup>[5,24,28-30]</sup>. Others have found evidence to support the opinion that daytime gastroesophageal reflux plays a more important role in the development of esophagitis<sup>[31-34]</sup>. Although there are different opinions as to the relationship between patterns of GER and esophagitis, it is generally recognised that a difference in patterns of GER is related to the development of esophagitis. Our results, however, suggested that combined daytime and nocturnal reflux may be responsible for the increased incidence of esophagitis in HH patients.

## REFERENCES

- 1 **Pope CE.** Pathophysiology and diagnosis of reflux esophagitis. *Gastroenterology* 1976; **70**: 445-454 [PMID: 765192]
- 2 **Dodds WJ, Hogan WJ, Miller WN.** Reflux esophagitis. *Am J Dig Dis* 1976; **21**: 49-67 [PMID: 3966 DOI: 10.1007/BF01074140]
- 3 **Ott DJ, Dodds WJ, Wu WC, Gelfand DW, Hogan WJ, Stewart ET.** Current status of radiology in evaluating for gastroesophageal reflux disease. *J Clin Gastroenterol* 1982; **4**: 365-375 [PMID: 7119414]
- 4 **Dodds WJ.** 1976 Walter B. Cannon Lecture: current concepts of esophageal motor function: clinical implications for radiology. *AJR Am J Roentgenol* 1977; **128**: 549-561 [PMID: 403780 DOI: 10.2214/ajr.128.4.549]
- 5 **Lichter I.** Measurement of gastro-oesophageal acid reflux: its significance in hiatus hernia. *Br J Surg* 1974; **61**: 253-258 [PMID: 4598976 DOI: 10.1002/bjs.1800610402]
- 6 **Brennan TG, Trindade LM, Rozycki ZJ, Giles GR.** The influence of the lower oesophageal sphincter pressure on the outcome of hiatus hernia repair. *Br J Surg* 1974; **61**: 201-205 [PMID: 4820996 DOI: 10.1002/bjs.1800610308]
- 7 **Cohen S, Harris LD.** Does hiatus hernia affect competence of the gastroesophageal sphincter? *N Engl J Med* 1971; **284**: 1053-1056 [PMID: 5553194 DOI: 10.1056/NEJM197105132841902]
- 8 **HIEBERT CA, BELSEY R.** Incompetency of the gastric cardia without radiologic evidence of hiatal hernia. The diagnosis and management of 71 cases. *J Thorac Cardiovasc Surg* 1961; **42**: 352-362 [PMID: 13714147]
- 9 **Kramer P.** Does a sliding hiatus hernia constitute a distinct clinical entity? *Gastroenterology* 1969; **57**: 442-448 [PMID: 4951149]
- 10 **Field P, Stalker MJ.** Incompetence of the cardiac sphincter without radiologic demonstration of hiatus hernia. *Can J Surg* 1968; **11**: 412-419 [PMID: 5683598]
- 11 **Kaul B, Petersen H, Myrvold HE, Grette K, Røysland P, Halvorsen T.** Hiatus hernia in gastroesophageal reflux disease. *Scand J Gastroenterol* 1986; **21**: 31-34 [PMID: 3952449 DOI: 10.3109/00365528609034617]
- 12 **Berstad A, Weberg R, Frøyshov Larsen I, Hoel B, Hauer-Jensen M.** Relationship of hiatus hernia to reflux oesophagitis. A prospective study of coincidence, using endoscopy. *Scand J Gastroenterol* 1986; **21**: 55-58 [PMID: 3952452 DOI: 10.3109/00365528609034622]
- 13 **Ott DJ, Wu WC, Gelfand DW.** Reflux esophagitis revisited: prospective analysis of radiologic accuracy. *Gastrointest Radiol* 1981; **6**: 1-7 [PMID: 7262493 DOI: 10.1007/BF01890213]
- 14 **Wright RA, Hurwitz AL.** Relationship of hiatal hernia to endoscopically proved reflux esophagitis. *Dig Dis Sci* 1979; **24**: 311-313 [PMID: 456217 DOI: 10.1007/BF01296546]
- 15 **Savary M, Miller G.** The Esophagus. Handbook and Atlas of Endoscopy. Switzerland: Gassmann 1978: 125-132
- 16 **Bianchi Porro G, Pace F.** Comparison of three methods of intraesophageal pH recordings in the diagnosis of gastroesophageal reflux. *Scand J Gastroenterol* 1988; **23**: 743-750 [PMID: 3175534 DOI: 10.3109/00365528809093943]
- 17 **Pace F, Sangaletti O, Bianchi Porro G.** Daytime reduction of gastro-oesophageal reflux after healing of oesophagitis and its value as an indicator of favourable response to maintenance treatment. *Gut* 1990; **31**: 1025-1029 [PMID: 2210448 DOI: 10.1136/gut.31.9.1025]
- 18 **Clagett OT.** Present concepts regarding the surgical treatment of oesophageal hiatal hernia. *Ann R Coll Surg Engl* 1966; **38**: 195-209 [PMID: 5931109]
- 19 **Dyer NH, Pridie RB.** Incidence of hiatus hernia in asymptomatic subjects. *Gut* 1968; **9**: 696-699 [PMID: 5717971 DOI: 10.1136/gut.9.6.696]
- 20 **DeMeester TR, Lafontaine E, Joelsson BE, Skinner DB, Ryan JW, O'Sullivan GC, Brunnsden BS, Johnson LF.** Relationship of a hiatal hernia to the function of the body of the esophagus and the gastroesophageal junction. *J Thorac Cardiovasc Surg* 1981; **82**: 547-558 [PMID: 7278346]
- 21 **Sloan S, Kahrilas PJ.** Impairment of esophageal emptying with hiatal hernia. *Gastroenterology* 1991; **100**: 596-605 [PMID: 1993483]
- 22 **Mittal RK, Lange RC, McCallum RW.** Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. *Gastroenterology* 1987; **92**: 130-135 [PMID: 3781181]
- 23 **Mueller-Lissner SA.** When is oesophagitis healed? In Tytgat GN (ed). The medical management of oesophageal reflux disease. Royal Society of Medicine? aRound Table series N.22-oxford: Alden Press, 1990: 106-115

- 24 **Kruse-Andersen S**, Wallin L, Madsen T. Reflux patterns and related oesophageal motor activity in gastro-oesophageal reflux disease. *Gut* 1990; **31**: 633-638 [PMID: 2379866 DOI: 10.1136/gut.31.6.633]
- 25 **Demeester TR**, Johnson LF, Joseph GJ, Toscano MS, Hall AW, Skinner DB. Patterns of gastroesophageal reflux in health and disease. *Ann Surg* 1976; **184**: 459-470 [PMID: 13747 DOI: 10.1097/0000658-197610000-00009]
- 26 **Dodds WJ**, Hogan WJ, Helm JF, Dent J. Pathogenesis of reflux esophagitis. *Gastroenterology* 1981; **81**: 376-394 [PMID: 7016659]
- 27 **Richter JE**, Castell DO. Gastroesophageal reflux. Pathogenesis, diagnosis, and therapy. *Ann Intern Med* 1982; **97**: 93-103 [PMID: 6124198 DOI: 10.7326/0003-4819-97-1-93]
- 28 **Robertson D**, Aldersley M, Shepherd H, Smith CL. Patterns of acid reflux in complicated oesophagitis. *Gut* 1987; **28**: 1484-1488 [PMID: 3428675 DOI: 10.1136/gut.28.11.1484]
- 29 **Pujol A**, Grande L, Ros E, Pera C. Utility of inpatient 24-hour intraesophageal pH monitoring in diagnosis of gastroesophageal reflux. *Dig Dis Sci* 1988; **33**: 1134-1140 [PMID: 3409799 DOI: 10.1007/BF01535790]
- 30 **Orr WC**, Robinson MG, Johnson LF. Acid clearance during sleep in the pathogenesis of reflux esophagitis. *Dig Dis Sci* 1981; **26**: 423-427 [PMID: 7249882 DOI: 10.1007/BF01313584]
- 31 **de Caestecker JS**, Blackwell JN, Pryde A, Heading RC. Daytime gastro-oesophageal reflux is important in oesophagitis. *Gut* 1987; **28**: 519-526 [PMID: 3596333 DOI: 10.1136/gut.28.5.519]
- 32 **Branicki FJ**, Evans DF, Jones JA, Ogilvie AL, Atkinson M, Hardcastle JD. A frequency-duration index (FDI) for the evaluation of ambulatory recordings of gastro-oesophageal reflux. *Br J Surg* 1984; **71**: 425-430 [PMID: 6722476 DOI: 10.1002/bjs.1800710607]
- 33 **Blackwell JN**, Heading RC. When does gastro-oesophageal reflux occur in patients with peptic oesophagitis. (Abstract). *Gut* 1980; **21**: 922
- 34 **Rokkas T**, Anggiansah A, Uzoechina E, Owen WJ, Sladen GE. The role of shorter than 24-h pH monitoring periods in the diagnosis of gastro-oesophageal reflux. *Scand J Gastroenterol* 1986; **21**: 614-620 [PMID: 3749799 DOI: 10.3109/00365528609003108]

S- Editor: Ma JY L- Editor: Ma JY E- Editor: Liu WX

## Reduced secretion of epidermal growth factor in duodenal ulcer patients with *Helicobacter pylori* infection

Xue-Qing Chen, Wan-Dai Zhang, Bo Jiang, Yu-Gang Song, Ri-Zi Reng, Dian-Yuan Zhou

Xue-Qing Chen, Wan-Dai Zhang, Bo Jiang, Yu-Gang Song, Ri-Zi Reng, Dian-Yuan Zhou, PLA Institute for Digestive Diseases and Department of Gastroenterology, Nanfang Hospital, First Military Medical University, Gangzhou 510515, Guangdong Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Xue-Qing Chen, MD, PLA Institute for Digestive Diseases and Department of Gastroenterology, Nanfang Hospital, First Military Medical University, Gangzhou 510515, Guangdong Province, China  
Telephone: +86-20-87705577-3017  
Fax: +86-20-87705671

Received: July 13, 1996  
Revised: October 1, 1996  
Accepted: January 1, 1997  
Published online: March 15, 1997

### Abstract

**AIM:** To investigate the concentration changes of epidermal growth factor (EGF) in duodenal ulcer patients with *Helicobacter pylori* (*H. pylori*) infection.

**METHODS:** Immunoreactive concentration of somatostatin, gastrin and epidermal growth factor of gastric and saliva juice in healthy volunteers, and chronic gastritis and duodenal ulcer patients with *H. pylori* infection were measured by radioimmunoassay.

**RESULTS:** Gastrin concentration of gastric juice in *H. pylori*-positive chronic gastritis ( $P > 0.05$ ) and duodenal ulcer patients ( $P < 0.01$ ) was higher than that of healthy volunteers ( $P < 0.05$ ), whereas somatostatin concentration of gastric juice in chronic gastritis ( $P < 0.05$ ) and duodenal ulcer patients ( $P < 0.01$ ) was lower than that in healthy volunteers. Furthermore, EGF levels of gastric and saliva juice in duodenal ulcer patients with *H. pylori* infection ( $n = 10$ ,  $272.0 \text{ ng/L} \pm 96.3 \text{ ng/L}$  and  $8.3 \text{ ng/L} \pm 2.4 \text{ ng/L}$ , respectively) were significantly lower than that in healthy volunteers ( $n = 12$ ,  $405.6 \text{ ng/L} \pm 35.6 \text{ ng/mL}$  and  $22.0 \text{ ng/L} \pm 17.0 \text{ ng/L}$ , respectively) and in *H. pylori*-positive chronic gastritis patients ( $n = 25$ ,  $423.0 \text{ ng/L} \pm 104.0 \text{ ng/L}$  and  $22.0 \text{ ng/L} \pm 11.1 \text{ ng/L}$ , respectively) ( $P < 0.05$ ).

**CONCLUSION:** A lower secretion of EGF may be a causative factor in the pathogenesis of *H. pylori*-positive duodenal ulcer.

**Key words:** Duodenal ulcer; *Helicobacter pylori*; Gastritis epidermal growth factor; Gastrins; Somatostatin

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen XQ, Zhang WD, Jiang B, Song YG, Reng RZ, Zhou DY. Reduced secretion of epidermal growth factor in duodenal ulcer patients with *Helicobacter pylori* infection. *World J Gastroenterol* 1997; 3(1): 31-34 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/31.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.31>

### INTRODUCTION

Increasing evidence suggests that *Helicobacter pylori* (*H. pylori*) may play a role in the pathogenesis of duodenal ulcer although the mechanism remains unsolved<sup>[1-3]</sup>. More than 90% of patients with duodenal ulcer are concomitant with *H. pylori* infection in gastric antrum which leads to chronic active gastritis. *H. pylori*-positive patients with duodenal ulcer are known to have a high gastric acid output, an increased parietal cell mass and a raised basal as well as a bombesin/gastrin releasing peptide and meal stimulated gastrin secretion<sup>[4-6]</sup>. The patients also have a decreased somatostatin level in gastric juice and antral mucosal tissue<sup>[7]</sup>. Eradication of *H. pylori* may decrease hypergastrinemia, enhance antral somatostatin secretion, and then reduce gastric acid secretion<sup>[8-10]</sup>. However, hypergastrinemia and decreased antral somatostatin secretion cannot explain the relation between *H. pylori* and duodenal ulcer, because there was no consistent relationship between chronic *H. pylori* infection and acid secretion (either basal or stimulated) observed<sup>[11,12]</sup>.

Epidermal growth factor (EGF) is a polypeptide of 6000 daltons containing 53 amino acid residues<sup>[13]</sup>. The peptide has been proved to have an action to inhibit the gastric acid secretion in several species including humans, prevent the gastric mucosa from damage, and promote the healing of experimental

gastric ulcers in rats<sup>[14-16]</sup>. Sialoadenectomy in rats damages the integrity of gastric mucosa<sup>[16,17]</sup>. Mucosal ulceration can induce a novel EGF secreting cell lineage in human gastrointestinal stem cells<sup>[18]</sup>. These evidences suggested that EGF may play an important role in the pathogenesis of duodenal ulcer.

The present study was to determine the changes of basal acid output, the secretion of gastrin, somatostatin and EGF in normal subjects, and in patients with chronic gastritis and duodenal ulcer due to *H. pylori* infection, so as to elucidate the possible role of EGF in the pathogenesis of duodenal ulcer.

### MATERIALS AND METHODS

#### Subjects

Thirty-nine *H. pylori*-positive patients and 14 healthy volunteers were studied. None of them had taken steroids, nonsteroidal anti-inflammatory drugs, antibiotics, anticoagulants, or any investigational medication during the previous 4 wk. Exclusion criteria included the history of chronic gastrointestinal and other

active illnesses. Eligible patients gave informed written consent before enrolment. They were assigned to two groups based on the endoscopic results: Chronic gastritis group, consisting of 10 men and 17 women (mean age 38.5 years) and duodenal ulcer group, including 5 men and 7 women (mean age 32.8 years). Healthy group included 4 men and 10 women (mean age 37.4 years).

#### Endoscopy and basal acid output measurement

Gastroduodenoscopy was performed using an Olympus endoscope with standard biopsy forceps. Four fragments from the lesser curvature of the antrum at 1-2 cm from the pylorus and 2 fragments from the greater curvature of the corpus were obtained. The biopsy forceps were disinfected with 70% ethanol after each use. Before the biopsy, sampling of gastric juice as much as possible was obtained. The basal acid output was measured by titration of gastric juice in one hour with 10 mmol/L NaOH.

#### *H. pylori* diagnosis

The presence of *H. pylori* in the antrum and corpus was evaluated by microbiologic methods including culture<sup>[19]</sup>, rapid urease test (commercial kits from Sanqing Biological Reagents Co, Fuzhou, China) and Warthin starry or Giemsa stains<sup>[20]</sup>. At least two of these tests should be positive in patients with *H. pylori* infection. *H. pylori* identified by Warthin Starry or Giemsa stains was graded as<sup>[21]</sup>: 0, null; 1+, a small number of bacteria (up to 20/gastric pit) presenting in a few of gastric pits; 2+, a large number of bacteria (more than 20/gastric pit) presenting in several gastric pits or a small number of bacteria in many gastric pits; 3+, a large number of bacteria presenting in nearly all gastric pits.

#### Histology

Biopsy fragments taken from the antral and oxyntic mucosa were fixed in 10% buffered formalin (pH7.4), dehydrated and embedded in paraffin, and stained 5 µm thick with hematoxylin and eosin for histological evaluation. All sections were examined by one of the investigators (R.Z.R.), who was unaware of the previous histologic results, endoscopic findings, rapid urease tests and culture results.

#### Extract and assay of somatostatin, gastrin and EGF from gastric and saliva juice

Samples from gastric juice and saliva juice were boiled in water for 10 min, centrifuged, and pH adjusted to 7.0-7.5 by 10 mmol/L NaOH titration, then transferred to plastic tubes, and frozen with 500 KU/mL trasyolol at 30 °C. Gastrin, somatostatin and EGF were measured using radioimmunoassay kit from National Institute of Atomic Energy, Beijing, China, and Beijing Hai-Ke-Ri Biotech Centre, Beijing, China, respectively. Somatostatin, gastrin and EGF concentrations of gastric or saliva juice were expressed as ng/L.

#### Statistical analysis

Data of somatostatin, gastrin and EGF were expressed as mean ± SD and analyzed using one-way ANOVA (SNK test). The differences were considered significant when  $P < 0.05$ .

## RESULTS

*H. pylori* status, gastric histology, and basal acid output were investigated in 27 gastritis patients, 12 duodenal ulcer patients, and 14 volunteers. *H. pylori* were found in 2 of 14 healthy volunteers, 25 of 27 patients with chronic active gastritis, and 10 of 12 duodenal ulcer patients, and in none of those with normal gastric mucosa (Table 1). Presence of *H. pylori* in chronic active gastritis patients was more common than in duodenal ulcer patients. In addition, active inflammatory infiltration tended to attack corpus mucosa in chronic gastritis patients (14/27), and those who had low basal acid output. Four of 12 duodenal ulcer patients had corpus inflammation and high basal acid output. Table 2 summarizes the concentration of somatostatin, gastrin and EGF of gastric and saliva juice in *H. pylori*-positive patients and *H. pylori*-negative healthy volunteers. Gastrin concentration of gastric juice in duodenal ulcer was significantly higher than that in control group ( $P < 0.01$ ). There

were no significant differences in gastrin concentration between the chronic gastritis group and the control group. On the other hand, the somatostatin concentration of gastric juice in chronic gastritis and duodenal ulcer group was lower than that in the control group ( $P < 0.05$  or 0.01). In *H. pylori*-positive chronic gastritis group, the levels of EGF in saliva juice and gastric juice were 423.0 ng/L ± 104.0 ng/L, and 22.0 ng/L ± 11.1 ng/L, with no differences as compared with those in the control group (405.6 ng/L ± 35.6 ng/L and 22.0 ng/L ± 17.0 ng/L, respectively), ( $P > 0.05$ ). However, the levels of EGF in saliva and gastric juice in chronic gastritis group and control group were both significantly higher than those in the duodenal ulcer group (272.0 ng/L ± 96.3 ng/L and 8.3 ng/L ± 2.4 ng/L, respectively), ( $P < 0.05$ ).

## DISCUSSION

This study showed for the first time that the levels of EGF in duodenal ulcer patients with *H. pylori* infection were much lower than those of the healthy volunteers and chronic gastritis patients with *H. pylori* infection, and also confirmed the previous findings that *H. pylori* infection can enhance gastrin secretion and lower somatostatin level, which can cause abnormal secretion of gastric acid<sup>[4,7-10]</sup>.

A strong association between *H. pylori* and diseases of upper gastrointestinal tract has been reported<sup>[1,2]</sup>. The causal relationship between *H. pylori* and chronic superficial gastritis is well established, but that between *H. pylori* and peptic ulcer is rather difficult to establish on the basis of the available data<sup>[1]</sup>. The suggested mechanisms in antral organism cause a duodenal lesion including bacterial colonization of gastric metaplasia in the duodenum<sup>[22]</sup>, secondary changes in gastric acid or duodenal bicarbonate secretion<sup>[23,24]</sup>, or the changes caused by the infected organism and/or the inflammatory response to the host<sup>[25,26]</sup>. Recently, the changes in gastric acid caused by *H. pylori* infection have drawn more attention, for inhibition of gastric acid secretion promoted duodenal ulcer healing even in the presence of *H. pylori* and inflammation of gastric antrum<sup>[27]</sup>.

The possible hypotheses in explaining the relationship between *H. pylori* infection and duodenal ulcer have been described as "gastrin link"<sup>[2,28,29]</sup> or "somatostatin link"<sup>[10,30]</sup>, as duodenal ulcer patients with *H. pylori* infection often have hypergastrinemia, which may increase parietal cell mass and reduce somatostatin secretion known to promote the gastric secretion<sup>[4-6,27-30]</sup>. On the contrary, there was no consistent relationship between chronic *H. pylori* infection and acid secretion observed<sup>[11,12,30,31]</sup>. Kang *et al*<sup>[11]</sup> showed that patients with duodenal ulcer or combined gastric and duodenal ulcer had similar gastric acid outputs irrespective of the presence or absence of *H. pylori*. However, gastric ulcer patients with *H. pylori* had higher basal and maximal acid output when compared to patients without *H. pylori*. McColl *et al*<sup>[31]</sup> have observed that after eradication of *H. pylori* in duodenal ulcer, daytime intragastric pH and nocturnal acid secretion were unchanged, even after 7 mo. Our results showed that hypochlorohydrin in chronic gastritis patients and high acidity in duodenal ulcer patients with *H. pylori* infection, both had enhanced gastrin secretion and reduced somatostatin secretion. Low gastric acidity in chronic gastritis may be elicited by the action of "protein inhibitor of gastric acid"<sup>[2,32]</sup>, but it would not be excluded that parietal cells may be damaged or inhibited by active inflammation of oxyntic mucosa because of host's response to *H. pylori*. The above results suggested that "gastrin link" or "somatostatin link" could not elucidate the mechanism of *H. pylori* in the pathogenesis of duodenal ulcer<sup>[30]</sup>, and other factor (s) should be taken into account.

The pathogenesis of peptic ulcer can be considered in terms of aggressive factors overwhelming mucosal defense. EGF should be one of such factors. In the previous studies it was shown that the EGF is localized in the submandibular and Brunner's glands of the rats and humans, and exerts protection of gastric mucosa and inhibition of gastric acid secretion<sup>[14,16]</sup>. Olsen *et al*<sup>[15]</sup> showed that the oral administration of human EGF/URO may benefit the healing of chronic duodenal ulcers in rats. Gastric mucosal integrity in rats of removed submandibular gland to reduce EGF levels in gastric juice was prone to be damaged<sup>[16]</sup>. Chen *et al*<sup>[33]</sup> compared patients with

Table 1 Profile of *Helicobacter Pylori* infection and basal acid output

Group	n	Symptom duration (> 4 mo)	<i>H. Pylori</i> infection			Mucosal active inflammation			Basal acid output (mmol/h)		
			0	+	++	+++	corpus	antrum	0-9	2-5	> 5
Con	14	0	12	2	0	0	2	0	0	14	0
CG	27	3	2	9	5	10	27	14	13	9	5
DU	12	3	2	8	1	1	12	4	0	1	11

Con: Healthy volunteers; CG: Chronic gastritis; DU: Duodenal ulcer.

Table 2 Concentration of somatostatin, gastrin and epidermal growth factor in *Helicobacter Pylori*-positive patients and *Helicobacter Pylori*-negative healthy volunteers (ng/L)

Group	n	Gastrin	Somatostatin	Epidermal growth factor	
		Gastric juice	Gastric juice	Saliva juice	Gastric juice
Con	12	71.2 ± 18.3	105.2 ± 33.5	405.6 ± 35.6	22.0 ± 17.0
CG	25	84.1 ± 24.0	88.6 ± 24.8 <sup>a</sup>	423.0 ± 104.0	22.0 ± 11.1
DU	10	109.2 ± 24.5 <sup>b</sup>	52.4 ± 13.8 <sup>b</sup>	272.0 ± 96.3 <sup>b</sup>	8.3 ± 2.4 <sup>a</sup>

<sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, compared with control group. Con: Healthy volunteers, CG: Chronic gastritis, DU: Duodenal ulcer.

gastric ulcer and duodenal ulcer to healthy subjects and observed that EGF levels of plasma and saliva juice in the former were lower than that in the latter. Their results are similar to ours, but different from those of Hirasawa *et al.*<sup>[34]</sup> who observed that salivary EGF output in patients with gastric, duodenal and gastroduodenal ulcers was higher than that in normal subjects, however, salivary EGF output in refractory peptic ulcer patients was much lower. Therefore, it is reasonable that any factor (s) which reduce or inhibit EGF secretion may be able to promote gastric mucosal damage. Based on the above evidences, we think that reduced EGF secretion may play an important role in the development of duodenal ulcer with *H. pylori* infection.

The secretion of less EGF is postulated to be predominated by genetic factors or effects of eradication of *H. pylori* on the changes of EGF levels in saliva and gastric juice (unpublished data), which showed that EGF levels in 3 of 4 patients became normal and one remained unchanged in one month. We presume that if less EGF secretion is caused by *H. pylori* infection, alternative explanations for the phenomenon are that cytokines or antibodies resulting from the host's defensive response to *H. pylori* infection should be the inhibitor of secretion of EGF.

In conclusion, our study shows that gastric acidity is higher in *H. pylori*-positive duodenal ulcer patients than that in *H. pylori*-positive chronic gastritis and healthy subjects. Contents of gastrin of gastric juice in *H. pylori* positive chronic gastritis and duodenal ulcer patients were higher than in healthy subjects, and the somatostatin concentration was lower in healthy subjects. Levels of EGF of gastric and salivary juice were also lower than those in the chronic gastritis patients and duodenal ulcer patients with *H. pylori* infection. Based on these results, we assume that EGF may play a causal role in the pathogenesis of *H. pylori*-positive duodenal ulcer.

## REFERENCES

- 1 NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. *JAMA* 1994; **272**: 65-69 [PMID: 8007082 DOI: 10.1001/jama.272.1.65]
- 2 Dunn BE. Pathogenic mechanisms of *Helicobacter pylori*. *Gastroenterol Clin North Am* 1993; **22**: 43-57 [PMID: 8449569]
- 3 Tytgat GN, Noach LA, Rauws EA. *Helicobacter pylori* infection and duodenal ulcer disease. *Gastroenterol Clin North Am* 1993; **22**: 127-139 [PMID: 8449562]
- 4 Beardshall K, Moss S, Gill J, Levi S, Ghosh P, Playford RJ, Calam J. Suppression of *Helicobacter pylori* reduces gastrin releasing peptide stimulated gastrin release in duodenal ulcer patients. *Gut* 1992; **33**: 601-603 [PMID: 1612474 DOI: 10.1136/gut.33.5.601]
- 5 McColl KE, Fullarton GM, el Nujumi AM, Macdonald AM, Brown IL, Hilditch TE. Lowered gastrin and gastric acidity after eradication of *Campylobacter pylori* in duodenal ulcer. *Lancet* 1989; **2**: 499-500 [PMID: 2570202 DOI: 10.1016/S0140-6736(89)92105-3]
- 6 Graham DY, Opekun A, Lew GM, Evans DJ, Klein PD, Evans DG. Ablation of exaggerated meal-stimulated gastrin release in duodenal ulcer patients after clearance of *Helicobacter (Campylobacter) pylori* infection. *Am J Gastroenterol* 1990; **85**: 394-398 [PMID: 2327380]
- 7 Kaneko H, Nakada K, Mitsuma T, Uchida K, Furusawa A, Maeda Y, Morise K. *Helicobacter pylori* infection induces a decrease in immunoreactive-somatostatin concentrations of human stomach. *Dig Dis Sci* 1992; **37**: 409-416 [PMID: 1346517 DOI: 10.1007/BF01307736]
- 8 Graham DY, Lew GM, Lechago J. Antral G-cell and D-cell numbers in *Helicobacter pylori* infection: effect of *H. pylori* eradication. *Gastroenterology* 1993; **104**: 1655-1660 [PMID: 8500723]
- 9 Queiroz DM, Mendes EN, Rocha GA, Moura SB, Resende LM, Barbosa AJ, Coelho LG, Passos MC, Castro LP, Oliveira CA. Effect of *Helicobacter pylori* eradication on antral gastrin- and somatostatin-immunoreactive cell density and gastrin and somatostatin concentrations. *Scand J Gastroenterol* 1993; **28**: 858-864 [PMID: 7903471 DOI: 10.3109/00365529309103125]
- 10 Moss SF, Legon S, Bishop AE, Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. *Lancet* 1992; **340**: 930-932 [PMID: 1357347 DOI: 10.1016/0140-6736(92)92816-X]
- 11 Kang JY, Wee A. *Helicobacter pylori* and gastric acid output in peptic ulcer disease. *Dig Dis Sci* 1991; **36**: 5-9 [PMID: 1985005 DOI: 10.1007/BF01300078]
- 12 Chittajallu RS, Howie CA, McColl KE. Effect of *Helicobacter pylori* on parietal cell sensitivity to pentagastrin in duodenal ulcer subjects. *Scand J Gastroenterol* 1992; **27**: 857-862 [PMID: 1439539 DOI: 10.3109/00365529209000154]
- 13 Cohen S, Carpenter G. Human epidermal growth factor: isolation and chemical and biological properties. *Proc Natl Acad Sci USA* 1975; **72**: 1317-1321 [PMID: 1055407 DOI: 10.1073/pnas.72.4.1317]
- 14 Dembiński A, Drozdowicz D, Gregory H, Konturek SJ, Warzecha Z. Inhibition of acid formation by epidermal growth factor in the isolated rabbit gastric glands. *J Physiol* 1986; **378**: 347-357 [PMID: 3025433 DOI: 10.1113/jphysiol.1986.sp016223]
- 15 Olsen PS, Poulsen SS, Therkelsen K, Nexø E. Effect of sialoadenectomy and synthetic human urogastone on healing of chronic gastric ulcers in rats. *Gut* 1986; **27**: 1443-1449 [PMID: 3492412 DOI: 10.1136/gut.27.12.1443]
- 16 Skinner KA, Tepperman BL. Influence of desalivation on acid secretory output and gastric mucosal integrity in the rat. *Gastroenterology* 1981; **81**: 335-339 [PMID: 7239140]
- 17 Amagase H, Murakami T, Misaki M, Higashi Y, Hashimoto K, Fuwa T, Yata N. Possible mechanism of gastric mucosal protection by epidermal growth factor in rats. *Life Sci* 1990; **47**: 1203-1211 [PMID: 2243536 DOI: 10.1016/0024-3205(90)90212-A]
- 18 Wright NA, Pike C, Elia G. Induction of a novel epidermal growth factor-secreting cell lineage by mucosal ulceration in human gastrointestinal stem cells. *Nature* 1990; **343**: 82-85 [PMID: 2296294 DOI: 10.1038/343082a0]
- 19 Queiroz DM, Mendes EN, Rocha GA. Indicator medium for isolation of *Campylobacter pylori*. *J Clin Microbiol* 1987; **25**: 2378-2379 [PMID: 3429628]
- 20 Potters HV, Loffeld RJ, Stobberingh E, van Spreuwel JP, Arends JW. Rapid staining of *Campylobacter pyloridis*. *Histopathology* 1987; **11**: 1223 [PMID: 2447004 DOI: 10.1111/j.1365-2559.1987.tb01863.x]
- 21 Satoh K, Kimura K, Yoshida Y, Kasano T, Kihira K, Taniguchi Y. A topographical relationship between *Helicobacter pylori* and gastritis: quantitative assessment of *Helicobacter pylori* in the gastric mucosa. *Am J Gastroenterol* 1991; **86**: 285-291 [PMID: 1998309]
- 22 Wyatt JI, Dixon MF. Chronic gastritis--a pathogenetic approach. *J Pathol* 1988; **154**: 113-124 [PMID: 3280764 DOI: 10.1002/path.1711540203]
- 23 Tarnasky PR, Kovacs TO, Sytnik B, Walsh JH. Asymptomatic *H. pylori* infection impairs pH inhibition of gastrin and acid secretion during second hour of peptone meal stimulation. *Dig Dis Sci* 1993; **38**: 1681-1687 [PMID: 8359081 DOI: 10.1007/BF01303178]
- 24 Kelly SM, Crampton JR, Hunter JO. *Helicobacter pylori* increases gastric antral juxtamucosal pH. *Dig Dis Sci* 1993; **38**: 129-131 [PMID: 8420744 DOI: 10.1007/BF01296784]
- 25 Graham DY, Go MF, Lew GM, Genta RM, Rehfeld JF. *Helicobacter pylori* infection and exaggerated gastrin release. Effects of inflammation and progastrin processing. *Scand J Gastroenterol* 1993; **28**: 690-694 [PMID: 8210984 DOI: 10.3109/00365529309098274]
- 26 Murakami M, Saita H, Teramura S, Dekigai H, Asagoe K, Kusaka S, Kita T. Gastric ammonia has a potent ulcerogenic action on the rat stomach. *Gastroenterology* 1993;

- 105: 1710-1715 [PMID: 8253347]
- 27 **Hu FL**. [Comparison of acid and Helicobacter pylori in ulcerogenesis of duodenal ulcer disease]. *Zhonghua Yixue Zazhi* 1993; **73**: 217-29, 253 [PMID: 8395315]
- 28 **Levi S**, Beardshall K, Swift I, Foulkes W, Playford R, Ghosh P, Calam J. Antral Helicobacter pylori, hypergastrinaemia, and duodenal ulcers: effect of eradicating the organism. *BMJ* 1989; **299**: 1504-1505 [PMID: 2514864 DOI: 10.1136/bmj.299.6714.1504]
- 29 **Levi S**, Beardshall K, Haddad G, Playford R, Ghosh P, Calam J. Campylobacter pylori and duodenal ulcers: the gastrin link. *Lancet* 1989; **1**: 1167-1168 [PMID: 2566737 DOI: 10.1016/S0140-6736(89)92752-9]
- 30 **McHenry L**, Vuyyuru L, Schubert ML. Helicobacter pylori and duodenal ulcer disease: the somatostatin link? *Gastroenterology* 1993; **104**: 1573-1575 [PMID: 8097735]
- 31 **McCull KE**, Fullarton GM, Chittajalu R, el Nujumi AM, MacDonald AM, Dahill SW, Hilditch TE. Plasma gastrin, daytime intragastric pH, and nocturnal acid output before and at 1 and 7 mon after eradication of Helicobacter pylori in duodenal ulcer subjects. *Scand J Gastroenterol* 1991; **26**: 339-346 [PMID: 1853158 DOI: 10.3109/00365529109025052]
- 32 **Vargas M**, Lee A, Fox JG, Cave DR. Inhibition of acid secretion from parietal cells by non-human-infecting Helicobacter species: a factor in colonization of gastric mucosa? *Infect Immun* 1991; **59**: 3694-3699 [PMID: 1894369]
- 33 **Chen SP**, Lu GJ, Wen SH. Study on epidermal growth factor levels of saliva, gastric juice and serum in patients with peptic ulcer disease. *Zhonghua Xiaohua Zazhi* 1994; **14**: 15-17
- 34 **Hirasawa Y**, Asaki S, Hongo M, Ohara S, Shibuya D, Yamaguchi N, Matsuda K, Toyota T. [Salivary epidermal growth factor in patients with peptic ulcer]. *Nihon Shokakibyō Gakkai Zasshi* 1991; **88**: 1043-1050 [PMID: 1856997]

S- Editor: Yang RC L- Editor: Ma JY E- Editor: Liu WX

## Adherent properties of *Helicobacter pylori* to human epithelial cells

Zheng-Xiang Wang, Hou-Feng Shen, Hong-Ju Chen

Zheng-Xiang Wang, Hou-Feng Shen, Hong-Ju Chen, Department of Microbiology and Immunology, Yangzhou University Medical College, Yangzhou 225001, Jiangsu Province, China

Author contributions: All authors contributed equally to the work.

Supported by Jiangsu Natural Science Foundation (No. BK93155315).

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Zheng-Xiang Wang, Yangzhou University Medical College, Yangzhou 225001, Jiangsu Province, China  
Telephone: +86-514-7312921

Received: July 26, 1996

Revised: October 1, 1996

Accepted: January 1, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To study the properties and factors of *Helicobacter pylori* (*H. pylori*) adherence to human epithelial cells.

**METHODS:** The adherent properties of human epithelial cells were studied using a group of isolated *H. pylori* strains, anti-*H. pylori* monoclonal antibodies and varied pH environment in *in vitro* adherence model with HEp2 cells.

**RESULTS:** *H. pylori* YC 11A was able to adhere to HEp2 cells specifically and its adherence efficiency reached the highest (81%) within 3 h after incubation with HEp2 cells. There was no significant difference between adherence in air and in 5% oxygen. The monoclonal antibodies specific to *H. pylori* predominant antigens did not inhibit activities on adherence of *H. pylori* to HEp2 cells. The pH value significantly affected the adherence process and the optimal pH was 3.0-4.6.

**CONCLUSION:** *H. pylori* specifically adheres to HEp2 cells, and pH value significantly affects this process. A high level of anti-*H. pylori* predominant antibodies in serum may have no protective activities against *H. pylori* infection.

**Key words:** *Helicobacter pylori*; Epithelial cells; Antibodies; Monoclonal antibodies; Hydrogen ion concentration

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang ZX, Shen HF, Chen HJ. Adherent properties of *Helicobacter pylori* to human epithelial cells. *World J Gastroenterol* 1997; 3(1): 35-37 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/35.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.35>

### INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a pathogen of nearly all duodenal ulcers and most gastric ulcers and is associated with an increased risk of gastric adenocarcinoma<sup>[1,2]</sup>. *H. pylori* has been found in intercellular junctions as well as on the surface of natural cells *in vivo*, but never inside the cells for its poor invasive properties, yet its adherent properties are rarely identical and could generate the characteristic histopathological lesions. This study aims to develop an *in vitro* model of adherence of *H. pylori* and analyze the properties and the factors of adherence of *H. pylori* to human epithelial cells.

### MATERIALS AND METHODS

#### Strains and cells

The *H. pylori* strains used were isolated initially from patients with chronic active gastritis or digestive ulcers and stored at 70 °C<sup>[3,4]</sup>. HEp2, an epithelial cell line, was obtained from the Chinese Academy of Preventive Medicine and has passed 23 generations in culture.

#### Adherence tests

HEp2 cells were grown in 24 well microplates (Nunc, Roskilde, Denmark) with cover slips in 1.5 mL of Delbacco's modified Eagle's medium with 10% fetal calf serum without antibiotics to obtain a subconfluent monolayer. The bacteria were cultured for 48-72 h on Skirrow's blood medium at 35 °C under 5% O<sub>2</sub>, 10% CO<sub>2</sub> and 85% N<sub>2</sub> and were gently harvested in brucella broth to give a cell density of 10.7/mL. The HEp2 cell slips were washed three times with Hank's solution, one time with 0.2 mol/L (pH3.6) citrate buffer, followed by addition of 0.9 mL of 0.2 mol/L (pH3.6) citrate buffer and 0.1 mL of the bacteria suspension. The microplates were then reincubated under microaerobic condition for 8 h and subsequently washed 5 times with strong agitation with 0.9% saline solution to remove nonadherent bacteria and fixed with 2.5% glutaraldehyde solution for 15 min at room temperature. The slides were stained and examined under light microscope.

To estimate the factors affecting the adherence, the adherence tests were carried out in air, in varied pH or in the system containing 0.1 mL of 1:10 monoclonal antibodies specific to *H. pylori* predominant antigens<sup>[5]</sup>.

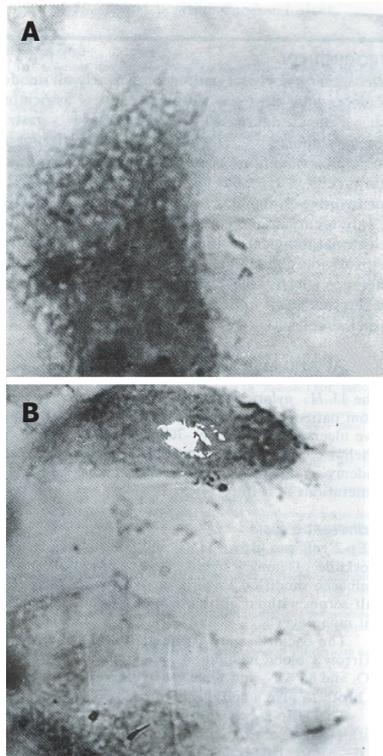
### RESULTS

The results obtained for *H. pylori* YC 11A adherence to HEp2 are shown in Table 1. The adherence of *H. pylori* to HEp2 began 5 min after coincubation and peaked at the 3<sup>rd</sup> hour. There was no significant difference between adherence in air or in microaerobic atmosphere ( $P > 0.01$ ).

*H. pylori* YC-11A started to adhere to HEp2 with its terminal portion, and after a long time of incubation, it could adhere to every part of the surface of HEp2, yet adherence to apicals of HEp2 cells was more frequent (Figure 1).

**Table 1** Levels of sIL-2R

Time	Adherence efficiency (%)	
	In microaerobic conditions	In air
5 min	3 ± 2	4 ± 2
40 min	16.5 ± 4.0	14.0 ± 3.5
1.5 h	38 ± 5	34 ± 6
3 h	81 ± 3	78 ± 4
4 h	84 ± 5	76 ± 3
5 h	82 ± 4	81 ± 4



**Figure 1** Adherence of *Helicobacter pylori* YC 11A to HEp2 cells (1000 ×). A: Incubation for 40 min; B: Incubation for 5 h.

The adherence efficiency obtained with 11 strains of *H. pylori* isolates is listed in Table 2. The pH of adherence environment remarkably affected the adherence of *H. pylori* YC-11A to HEp2 cell (Figure 2). The optimal adherent pH was 2.6-4.6 and the maximum adherence efficiency was obtained with pH at 3.0. The results of inhibition of monoclonal antibodies specific to *H. pylori* on adherence are listed in Table 3 and there was no inhibited activity at pH3.6 in microaerobic atmosphere.

## DISCUSSION

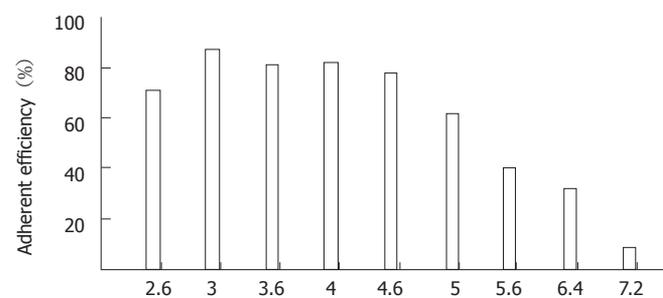
To colonize luminal mucus, *H. pylori* adheres to the apical plasma membrane of the epithelial cell surface in the antrum *in vivo* by the specific compounds on its surface. These specific structures include flagella and adhesins. All the eleven strains of *H. pylori* isolates showed different adherent efficiency, indicating that the expression level of adhesin and mobility by various isolates differed.

Current evidence suggested that there are a number of adhesins on the surface of *H. pylori*. These include fibrillar hemagglutinin<sup>[6]</sup> and M (microbial) selectins<sup>[7]</sup>. Fibrillar hemagglutinin specifically binds sialylactose<sup>[6]</sup>. M selectin is similar to exoenzyme S from *Pseudomonas aeruginosa* in structure, and immunogenity and monoclonal antibodies against this adhesin prevent the attachment of *H. pylori in vitro* to its lipid receptors—gangliotetraosylceramide, gangliotriaosylceramide and phosphatidylethanolamine<sup>[8]</sup>. Yet, the gastric acidic environment has not been considered. Adherence of *H. pylori* to HEp2 cell was pH restricted and the low pH benefited the adherence, suggesting that the binding properties of adhesins of *H. pylori* to its receptor and the natural properties of the adhesins and their receptors possibly possess specificities, which differ greatly from those of other enteropathogens, such as enterotoxigenic *Escherichia coli*, whose virulence can be easily neutralized by

**Table 2** Adherent efficiency of different *Helicobacter Pylori* strains to HEp2 cells

Strains	Adherence efficiency (%)
<i>H. pylori</i> YC-1	74
<i>H. pylori</i> YC-2	76
<i>H. pylori</i> YC-3	52
<i>H. pylori</i> YC-4	64
<i>H. pylori</i> YC-5	61
<i>H. pylori</i> YC-6	58
<i>H. pylori</i> YC-7	71
<i>H. pylori</i> YC-8	85
<i>H. pylori</i> YC-9	80
<i>H. pylori</i> YC-11A	81
<i>H. pylori</i> YC-11B	79

*H. pylori*: *Helicobacter pylori*



**Figure 2** Effect of pH on adherence of *Helicobacter pylori* to HEp2 cells.

**Table 3** Monoclonal antibodies inhibited adherence of *Helicobacter pylori* YC-11A to HEp2 cells

Monoclonal antibody	Adherence efficiency (%)
21A5-3	81
22C6-3	84
23C2-2	78
31A10-1	83
31A11-3	74
31B1-1	76
31B1-2	80
31D12-2	78
Control	81

antibodies specific to its adhesin<sup>[9]</sup>. *H. pylori* infection stimulates immune response, leading to a much higher level of antibodies in sera<sup>[4]</sup>. The monoclonal antibodies used were a cluster of antibodies specific to the predominant antigens of *H. pylori*<sup>[5]</sup>, but they all had no inhibitory actions on adherence of *H. pylori* and even more promoted adherence of *H. pylori*. These results further indicated that a high level of antibodies in human serum against *H. pylori* predominant antigens might not benefit the clearance of *H. pylori* in gastric mucus and may be a factor for persistence of *H. pylori* infection.

## REFERENCES

- 1 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315 [PMID: 6145023 DOI: 10.1016/S0140-6736(84)91816-6]
- 2 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelmann JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131 [PMID: 1891020 DOI: 10.1056/NEJM199110173251603]
- 3 Wang ZX, Wang XL and Wu Y. A singular procedure for culture isolation of Campylobacter pylori. *Yangzhou Yixueyuan Xuebao* 1990; **2**: 148-149
- 4 Wang ZX. Long-term storage of Helicobacter pylori. *Microbiol* 1991; **18**: 118-119
- 5 Wang ZX, Shen HF, Chen HJ and Tong K. Establishment and preliminary characterization of the hybridoma cell lines secreting anti-Helicobacter pylori monoclonal antibodies. *J Monoclonal Antibody* 1994; **19**: 56-57
- 6 Evans DG, Karjalainen TK, Evans DJ, Graham DY, Lee CH. Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of Helicobacter pylori. *J Bacteriol* 1993; **175**: 674-683 [PMID: 7678592]
- 7 Lingwood CA, Wasfy G, Han H, Huesca M. Receptor affinity purification of a lipid-binding adhesin from Helicobacter pylori. *Infect Immun* 1993; **61**: 2474-2478 [PMID: 8500882]
- 8 Gold BD, Huesca M, Sherman PM, Lingwood CA. Helicobacter mustelae and

Helicobacter pylori bind to common lipid receptors in vitro. *Infect Immun* 1993; **61**: 2632-2638 [PMID: 8500901]

9 Tacket CO, Losonsky G, Link H, Hoang Y, Guesry P, Hilpert H, Levine MM.

Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic Escherichia coli. *N Engl J Med* 1988; **318**: 1240-1243 [PMID: 3283555 DOI: 10.1056/NEJM198805123181904]

S- Editor: Yang RC L- Editor: Ma JY E- Editor: Liu WX

## Evaluation of a fecal occult blood test with reverse passive hemagglutination for colorectal neoplasm screening

Lun Zhou, Hai Yu, Shu Zheng

Lun Zhou, Hai Yu, Shu Zheng, Cancer Institute, Zhejiang Medical University, Hangzhou 310009, Zhejiang Province, China

Dr. Lun Zhou, Associate Professor, engaged in prevention and treatment research on cancer, with 25 published papers

Author contributions: All authors contributed equally to the work.

Supported by The National "Eighth Five Year Plan" Key Research Project (No. 85-914-01-09)

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Lun Zhou, Associate Professor, Zhejiang Medical University, Hangzhou 310009, Zhejiang Province, China  
Telephone: +86-571-7027427

Received: July 28, 1996

Revised: September 1, 1996

Accepted: January 1, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To evaluate the one and three sampling reverse passive hemagglutination fecal occult blood test (RPHA FOBT) for colorectal neoplasm screening.

**METHODS:** A group of 3034 individuals with histories of colorectal polyps and/or ulcers were screened for colorectal cancer. Three day fecal samples were collected and 60 cm fiberoptic colonoscopy was conducted for each subject. The fecal samples were tested for occult blood with the RPHA method and the endoscopic and histopathological diagnoses were used as standard reference for evaluation. The sensitivity, specificity and positive and negative predictive values of different samplings were compared.

**RESULTS:** About 521 cases of colorectal neoplasms were detected, including 12 cases of colorectal cancer and 509 cases of polyps. Results showed that the mean sensitivity of one sampling RPHA FOBT for colorectal neoplasm was only 13.2%, the specificity was 90.3% and the positive and negative predictive values were 21.3% and 83.4%, respectively; while for the three sampling, taking one positivity as positive, the sensitivity increased to 22.0%, the specificity decreased to 81.6% and the positive and negative predictive values were 19.7% and 83.6%, respectively.

**CONCLUSION:** A single RPHA FOBT seems to be less sensitive for screening for colorectal neoplasms. Since it is convenient and economical, RPHA FOBT remains the most practical procedure for detection of early colorectal cancer and polyps if it is combined with other screening methods.

**Key words:** Colonic neoplasms; Rectal neoplasms; Colonic polyps; Hemagglutination tests; Occult blood

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhou L, Yu H, Zheng S. Evaluation of a fecal occult blood test with reverse passive hemagglutination for colorectal neoplasm screening. *World J Gastroenterol* 1997; 3(1): 38-40 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/38.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.38>

### INTRODUCTION

Fecal occult blood test (FOBT) has been the most important procedure for population screening for colorectal cancer since Greegor first reported it in 1967<sup>[1,2]</sup>. During screening, both early colorectal cancer and precancerous lesions such as adenomatous polyps can be detected, so screening for colorectal neoplasms with FOBT is a procedure of secondary prevention as well as a measure for primary prevention of colorectal cancer. The conventional chemical FOBT (e.g. hemocult) requires dietary control to reduce the false positive rate and the three day sampling to reduce the false negative rate. Although the reverse passive hemagglutination (RPHA) method which was developed in the 1980s raised the sensitivity and specificity of screening, Saito *et al*<sup>[3]</sup> suggested that a single sampling RPHA may be adequate for screening. This has not been verified in large population screening. In the present study, we screened a high risk population for colorectal cancer with RPHA FOBT and 60 cm fiberoptic colonoscopy and evaluated various protocols of RPHA FOBT (one, two and three sampling) for screening for colorectal neoplasms.

### MATERIALS AND METHODS

#### Subjects

A total of 3034 individuals with a history of rectal polyps and ulcers detected in mass screening 10 years ago in Haining and Jiashan counties in Zhejiang province included 1716 males and 1318 females, aged 32-72, with a mean age of 49.2 years.

#### Methods

All subjects had a 60 cm fiberoptic colonoscopy and those with positive endoscopic findings had a biopsy for histopathological examination. The bowel preparation and endoscopic procedures were reported in another paper<sup>[4]</sup>. No dietary control was required and three day fecal samples were collected and submitted for laboratory testing before endoscopy. The RPHA FOBT was performed for each sample, according to Zhu *et al*<sup>[5]</sup>. During analysis, subjects were divided into two groups: Neoplasm (cancer, adenoma

**Table 1 Relationship between the 1<sup>st</sup> reverse passive hemagglutination fecal occult blood test and colorectal neoplasm**

		Colorectal neoplasm		Total
		+	-	
RPHA FOBT	+	60	250	310
	-	380	1863	2243
Total		440	2113	2553

Sensitivity: 13.6% (60/440), specificity: 88.2% (1863/2113), positive predictive value: 16.1% (60/310), negative predictive value: 83.1% (1863/2243).

**Table 2 Relationship between the 2<sup>nd</sup> reverse passive hemagglutination fecal occult blood test and colorectal neoplasm**

		Colorectal neoplasm		Total
		+	-	
RPHA FOBT	+	50	168	236
	-	372	1823	2195
Total		422	2009	2431

Sensitivity: 11.8% (50/422), specificity: 90.7% (1823/2009), positive predictive value: 21.2% (50/236), negative predictive value: 83.1% (1823/2195).

**Table 3 Relationship between the 3<sup>rd</sup> reverse passive hemagglutination fecal occult blood test and colorectal neoplasm**

		Colorectal neoplasm		Total
		+	-	
RPHA FOBT	+	55	151	206
	-	333	1733	2066
Total		388	1884	2272

Sensitivity: 14.2% (55/388), specificity: 92.0% (1733/1884), positive predictive value: 26.7% (55/206), negative predictive value: 83.9% (1773/2066).

and polyps) and non neoplasm, according to endoscopic and histopathological diagnoses. For those who completed three RPHA FOBT, three test thresholds were used: (1) all tests positive as positive [3/3 (+)]; (2) two positive as positive [2/3 (+)]; and (3) one positive as positive [1/3 (+)]. The sensitivity, specificity and positive and negative predictive values were used as efficacy indicators for evaluation. For comparison,  $\chi^2$  test was performed with Epi info software and the Mantel Haenszel or Yate's correction was used for significance analysis.

## RESULTS

Out of 3034 subjects, 521 cases of colorectal neoplasms were diagnosed by endoscopic and histopathological examination. There were 12 cases of cancer and 509 polyps, in which adenoma accounted for 45.0% (229/509). Among 2553 subjects who completed at least one FOB test, there were 440 neoplasias (12 cancers and 428 polyps); in 2431 subjects who had at least 2 FOB tests, there were 422 neoplasias (12 cancers and 410 polyps); in 2272 subjects who completed three FOB tests, 388 cases of neoplasia were detected, including 11 cancers and 377 polyps. The correlation between neoplasia and three FOBT results is presented in Tables 1-3. The mean sensitivity, specificity and positive and negative predictive values for three tests were 13.2%, 90.3%, 21.3% and 83.4%, respectively. In 2272 subjects who had three FOB tests, the sensitivity and specificity of the FOB test to neoplasia using different positive thresholds are shown in Table 4, Table 5 and Table 6. It was demonstrated that with the elevation of a positive threshold [1/3 (+) to 3/3 (+)], the sensitivity decreased from 22.0% to only 5.4%, while the specificity increased from 81.6% to 96.5%.

## DISCUSSION

Occult bleeding of the lower digestive tract is the most common symptom of early cancer or polyps of the large bowel. Meanwhile, in physiological conditions, there can be a small amount of bleeding

**Table 4 Relationship between three sampling 1/3 (+) and colorectal neoplasm**

		Colorectal neoplasm		Total
		+	-	
RPHA FOBT	+	85	346	431
	-	302	1539	1841
Total		387	1885	2272

Sensitivity: 22.0% (85/387), specificity: 81.6% (1539/1885), positive predictive value: 19.7% (85/431), negative predictive value: 83.6% (1539/1841).

**Table 5 Relationship between three sampling 2/3 (+) and colorectal neoplasm**

		Colorectal neoplasm		Total
		+	-	
RPHA FOBT	+	46	138	184
	-	341	1747	2088
Total		387	1885	2272

Sensitivity: 11.9% (46/341), specificity: 92.7% (1747/1885), positive predictive value: 25.0% (46/184), negative predictive value: 83.7% (1747/2088).

**Table 6 Relationship between three sampling 3/3 (+) and colorectal neoplasm**

		Colorectal neoplasm		Total
		+	-	
RPHA FOBT	+	21	66	87
	-	366	1819	2185
Total		387	1885	2272

Sensitivity: 5.4% (21/387), specificity: 96.5% (1819/1885), positive predictive value: 24.1% (21/87), negative predictive value: 83.2% (1819/2185).

in an apparently normal digestive tract; it is, however, seldom more than 2 mL over 24 h. The conventional chemical FOB test has a low sensitivity and can only detect more than 10 mL/24 h of bleeding in the lower digestive tract<sup>[6]</sup>. Therefore, a three day sampling has been recommended clinically to elevate the sensitivity of the test. However, it is difficult to implement in large population screening because of the greatly increased work load of sample collection, lab tests, the data process and the cost for reagents. In 1984, Saito first reported the application of RPHA FOBT in screening colorectal cancer, suggesting that one sampling RPHA might replace the three day hemoccult test<sup>[3]</sup> because the former had a higher sensitivity. In China, Zhou *et al.*<sup>[7]</sup> also successfully developed RPHA FOB test kits that can detect intestinal bleeding as small as 0.48 mL/24 h.

In order to objectively evaluate the efficacy of RPHA in screening for colorectal neoplasias, the present study compared the results of one with three sampling RPHA FOBT, using 60 cm fiberoptic colonoscopy as a standard reference. Our study revealed that the mean sensitivity of one sampling RPHA FOBT was only 13.2%, which means that as many as 86.8% of colorectal neoplasias might be missed if FOBT is used as the only measure for screening. However, when the three sampling method is used with taking 1/3 (+) as positive criteria, 22.0% of colorectal neoplasias can be detected. The authors previously reported that the sensitivity of three sampling RPHA FOBT for colorectal cancer was 63.6% and was 40% for villous or tubulovillous adenoma, which has an increasing tendency to malignant transformation<sup>[8]</sup>.

The low sensitivity of one sampling RPHA FOBT may result from the variation of bleeding status of early colorectal neoplasia, particularly polyps. Ahlquist *et al.*<sup>[9]</sup> measured FOB with a hemoccult test consecutively for 2 wk in a group of patients with colorectal cancer. They found that only one quarter of the patients presented with consistent positivity and that the FOB fluctuated day by day in the remainder. Therefore, three day sampling is more likely to find occult intestinal bleeding; on the other hand, it will definitely increase the cost and work load of screening, particularly in a large population. To cope with this dilemma, we designed a new screening protocol comprising one sampling RPHA FOBT plus a computerized

risk assessment as a primary screening procedure. With the combination of two methods, the sensitivity of primary screening will be raised. In a high incidence area, we screened 62, 667 individuals aged 30 and above using this protocol. Among them, 4299 subjects required endoscopic examination; in 3162 people who underwent endoscopy, 397 cases of colorectal neoplasia were detected, including 41 cancers and 356 polyps. In all cases of neoplasia, 172 (43.3%) had a positive FOBT and the remaining 56.7% of cases were screened endoscopically only according to risk assessment<sup>[10]</sup>. In conclusion, RPHA FOBT is a convenient, economical and noninvasive method for screening colorectal neoplasia, although it is less sensitive. If used in combination with other screening measures, RPHA FOBT can still be an effective method for detecting early colorectal cancer and polyps. Whether to choose one or three sampling methods depends on the size of the screened population and the availability of resources.

## REFERENCES

- 1 Greegor DH. Occult blood testing for detection of asymptomatic colon cancer. *Cancer* 1971; **28**: 131-134 [PMID: 5110619 DOI: 10.1002/1097-0142(197107)28:1<131::AID-CNCR2820280125>3.0.CO;2-I]
- 2 Ahlquist DA. Occult blood screening. Obstacles to effectiveness. *Cancer* 1992; **70**: 1259-1265 [PMID: 1511373 DOI: 10.1002/1097-0142(19920901)70:3]
- 3 Saito H, Kawaquchi H, Uro Y, Aisqwa T, Munakata A, Yoshida Y. Mass screening for colorectal cancer by an immunological occult blood test employing reversed passive hemagglutination reaction. Proceedings of First Shanghai International Symposium on Gastrointestinal Cancer, 1988: P172-174
- 4 Yu H. [Evaluation of RPHA fecal occult blood test in screening for colorectal cancer]. *Zhonghua Zhongliu Zazhi* 1990; **12**: 108-110 [PMID: 2401172]
- 5 Zhu WX, Tang HX, Jiang X. The preparation and application of reverse passive hemagglutination fecal occult blood test kits. *J Practical Oncology* 1988; **3**: 146-148
- 6 Stroehlein JR, Fairbanks VF, McGill DB, Go VL. Hemocult detection of fecal occult blood quantitated by radioassay. *Am J Dig Dis* 1976; **21**: 841-844 [PMID: 1015491 DOI: 10.1007/BF01072074]
- 7 Zhou PH, Zhang WZ. [Experimental study on reverse passive hemagglutination for the detection of human fecal occult blood]. *Zhonghua Yixue Zazhi* 1987; **67**: 671-672 [PMID: 3130160]
- 8 Yu H, Zhou L, Zheng PY, Qiu PL, Zheng S, Sun QR. Evaluation of reverse passive hemagglutination (RPHA) fecal occult blood test in screening of colorectal neoplasia. *Chin J Cancer Res* 1994; **6**: 274-278 [DOI: 10.1007/BF03025581]
- 9 Ahlquist DA, McGill DB, Fleming JL, Schwartz S, Wieand HS, Rubin J, Moertel CG. Patterns of occult bleeding in asymptomatic colorectal cancer. *Cancer* 1989; **63**: 1826-1830 [PMID: 2702590 DOI: 10.1002/1097-0142(19900501)63:9<1826::AID-CNCR2820630928>3.0.CO;2-P]
- 10 Zheng S. [The screening model for early diagnosis of colorectal cancer in general population]. *Zhonghua Yixue Zazhi* 1991; **71**: 381-384, 28 [PMID: 1659480]

S- Editor: Cao LB L- Editor: Ma JY E- Editor: Liu WX

## Analysis of lactate dehydrogenase activities and isoenzyme patterns in colorectal cancer tissues

Chun-Hua Zhao, Chun-Ying Jiang, Yu-Yi Zhang, Xian-Xi Liu, Dao-Chun Luo, Xiao-Ting Zhang, Yu-Qin Lin

Chun-Hua Zhao, Yu-Yi Zhang, Xian-Xi Liu, Dao-Chun Luo, Yu-Qin Lin, The Department of Biochemistry, Shandong Medical University, Jinan 250012, Shandong Province, China

Chun-Ying Jiang, Xiao-Ting Zhang, Shandong Traditional Hospital

Chun-Hua Zhao, with 8 published papers, Lecturer of Biochemistry

Presented at Acta Academiae Medicinae Shandong, 1996; 34 (1):12-14 (in Chinese)

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Chun-Hua Zhao, The Department of Biochemistry, Shandong Medical University, Jinan 250012, Shandong Province, China  
Telephone: +86-531-2952424-693

Received: July 1, 1996  
Revised: September 1, 1996  
Accepted: January 1, 1997  
Published online: March 15, 1997

### Abstract

**AIM:** To investigate the relationship between lactate dehydrogenase (LDH) activity or LDH isoenzyme patterns and the pathogenesis of colorectal cancer.

**METHODS:** Activities of tissue LDH and LDH isoenzyme patterns in 16 patients with colorectal cancer were assayed using spectrophotometric procedures and agarose gel electrophoresis, respectively.

**RESULTS:** The total and specific activities of LDH were significantly higher in colorectal cancer tissues than those in adjacent noncancerous tissues ( $P < 0.001$ ). The LDH isoenzyme pattern was also different from that in the control. The percentage of LDH<sub>5</sub> doubled and the ratio of LDH<sub>4</sub> + LDH<sub>5</sub>/LDH<sub>1</sub> + LDH<sub>2</sub> was  $3.6 \pm 1.4$  in cancer tissue, significantly greater than in the control.

**CONCLUSIONS:** The increased LDH activity in colorectal cancer tissues resulted mainly from the increased LDH<sub>5</sub>, suggesting that the alteration of LDH activity and isoenzyme patterns were related to the pathogenesis of colorectal cancer.

**Key words:** Colonic neoplasms; Rectal neoplasms; Lactate dehydrogenase; Lactate dehydrogenase isoenzymes

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhao CH, Jiang CY, Zhang YY, Liu XX, Luo DC, Zhang XT, Lin YQ. Analysis of lactate dehydrogenase activities and isoenzyme patterns in colorectal cancer tissues. *World J Gastroenterol* 1997; 3(1): 41-42 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/41.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.41>

### INTRODUCTION

Studies on lactate dehydrogenase (LDH) isoenzyme patterns in colorectal cancer tissues have rarely been reported although its total and specific activities have been measured by many authors<sup>[1,2]</sup>. To study the pathogenesis of colorectal cancer and provide a certain theoretical basis for diagnosis, in the present study we determined the total and specific activities and isoenzyme patterns of LDH in colorectal cancer tissues and in adjacent noncancerous tissues.

### MATERIALS AND METHODS

#### Materials

All samples were obtained surgically and histological examinations were made routinely. The samples were washed with ice cold normal saline to remove contaminated blood and stored at 30 °C.

In our experiment specimens were obtained from rectal cancer (13 cases), colonic cancer (3 cases) and noncancerous tissues taken at 5-8 cm proximal or distal to the edges of the tumor of the same patient. Nine men and seven women were included in the group. All reagents used were "Anala R" grade.

#### Methods

**Preparation of tissue homogenate supernatants** 0.3 g tissues were homogenised in 3 mL of 0.01 mol/L Tris HCl buffer (containing 0.001 mol/L DTT, 0.001 mol/L EDTA, pH7.5) and centrifuged at  $20000 \times g$  for 20 min at 4 °C (DUPONT RC5C). The supernatants were collected for assay.

**Determination of LDH activity** Enzymatic activities in tissue extracts were measured by spectrophotometric procedures with 2,4-dinitrophenylhydrazine<sup>[3]</sup>. 1 μmol pyruvate produced at 37 °C for 15 min represented one unit.

**Isoenzyme patterns** Isoenzyme patterns were assayed by agarose electrophoresis modified according to Lou *et al*<sup>[4]</sup>. Gels were scanned at 500 nm using a Dual Wavelength Chromato Scanner (Shimadzu CS-930).

**Determination of protein content** Protein content was measured by the method of Bradford<sup>[5]</sup>, with bovine serum albumin as standard.

#### Statistical analysis

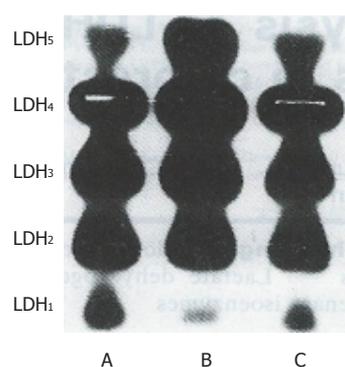
All values were expressed as  $\bar{x} \pm s$  and Student's *t* test was used

**Table 1** Lactate dehydrogenase activities in colorectal cancer tissues and adjacent noncancerous tissues ( $\bar{x} \pm s$ )

Tissues	n	Total activities (u/g tissue)	Specific activities (u/mg protein)
Cancer tissue	16	62.70 ± 13.50	63.41 ± 12.41
Adjacent Control			
Proximal tissue	16	43.15 ± 22.95 <sup>d</sup>	38.22 ± 19.77 <sup>b</sup>
Distal tissue	16	44.81 ± 17.24 <sup>b</sup>	39.92 ± 15.15 <sup>b</sup>

<sup>b</sup>*P* < 0.01, <sup>d</sup>*P* < 0.01 vs cancer tissue**Table 2** Lactate dehydrogenase isoenzyme patterns in colorectal cancer tissues and adjacent noncancerous tissues ( $\bar{x} \pm s$ )

Tissues	n	LDH isoenzyme (%)					LDH <sub>4</sub> + LDH <sub>5</sub> /LDH <sub>1</sub> + LDH <sub>2</sub>
		1	2	3	4	5	
Cancer tissue	16	1.65 ± 1.42	15.54 ± 3.80	26.59 ± 6.25	36.63 ± 6.80	19.13 ± 8.05	3.6 ± 1.4
Adjacent control							
Proximal tissue	16	4.93 ± 6.19	19.18 ± 5.29 <sup>a</sup>	30.05 ± 4.04	37.03 ± 8.24	8.76 ± 6.04 <sup>e</sup>	2.3 ± 1.2 <sup>a</sup>
Distal tissue	16	4.28 ± 2.55 <sup>b</sup>	22.17 ± 4.57 <sup>b</sup>	34.38 ± 5.75 <sup>c</sup>	30.76 ± 5.83 <sup>a</sup>	8.11 ± 6.32 <sup>c</sup>	1.7 ± 0.9 <sup>e</sup>

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, <sup>c</sup>*P* < 0.001 vs cancer tissue. LDH: lactate dehydrogenase.**Figure 1** Lactate dehydrogenase isoenzyme patterns. A: proximal tissue; B: cancer tissue; C: distal tissue. LDH: lactate dehydrogenase.

for intergroup comparison.

## RESULTS

### LDH activities

Table 1 shows that both the total and specific LDH activities in tumors were significantly higher than those in the adjacent noncancerous tissues (*P* < 0.001).

### LDH isoenzyme patterns

The electrophoretograms of LDH isoenzymes in the diseased foci showed a shift towards the M type (Figure 1). The percentages of LDH<sub>1</sub> and LDH<sub>2</sub> in tumors decreased significantly in comparison with proximal and distal noncancerous tissues; the percentage of LDH<sub>3</sub> decreased while that of LDH<sub>4</sub> increased in comparison with distal tissues; the percentage of LDH<sub>5</sub> was 2.2 and 2.4-fold higher than that in proximal and distal tissues, respectively. The ratio of LDH<sub>4</sub> + LDH<sub>5</sub>/LDH<sub>1</sub> + LDH<sub>2</sub> was 3.6 ± 1.4, above the control (Table 2).

## DISCUSSION

It is well known that glycolysis in cancer tissue increases significantly as a consequence of an important enzyme of the glycolytic pathway LDH that may manifest with a higher activity in a cancer patient's serum and tissues. Our data showed a significant increase of total and specific LDH activities in cancer tissues, about 140% of the control. These results were consistent with the reports by Carda-Abella *et al.*<sup>[6]</sup> and Hong *et al.*<sup>[7]</sup>.

Because of the tissue distribution specificity, LDH isoenzymes may be expressed in different levels. It was necessary to assay LDH isoenzyme patterns while total and specific activities were deter-

mined. Our results indicated that the increased LDH<sub>5</sub> contributes to the increase of total LDH activity in tumors; the ratio of LDH<sub>4</sub> + LDH<sub>5</sub>/LDH<sub>1</sub> + LDH<sub>2</sub> also increased greatly, *i.e.* 3.6 ± 1.4, suggesting that LDH isoenzyme pattern shifts towards the M type. It is the M type LDH that promotes the conversion of pyruvate to lactate, while the H type LDH mainly catabolizes the utilization of lactate. Therefore M type LDH can be found predominantly in colorectal cancer tissues in which anaerobic glycolysis is increased abnormally. Market *et al.* thought that the patterns of isoenzymes were biochemical phenotypes of genes. H and M subunits were controlled by A and B genes, respectively. The findings that LDH isoenzyme patterns shift towards the M type may be related to its abnormal expression of genes, suggesting that studying the expression of LDH genes in colorectal tumors will help to elucidate its pathogenesis. In the comparison of malignant tissues with the control at the distance of 1, 2, 4, 6 and 8 cm from the edge of cancer, Onos<sup>[8]</sup> found that LDH activity in cancer tissues was very high and it gradually decreased in control tissues surrounding the tumor with a distance from cancer. By studying LDH isoenzyme patterns in precancerous polyps, Onos also found that it shifts towards the M type, indicating that the deviation of LDH isoenzyme patterns in normal tissue could be regarded as early signs of malignancy before the morphological changes.

Our results suggest that the alteration of LDH activity and its isoenzyme patterns are related to the pathogenesis of colorectal cancer and more details will be studied in our laboratory.

## REFERENCES

- Munjal DD. Concurrent measurements of carcinoembryonic antigen, glucosephosphate isomerase, gamma-glutamyltransferase, and lactate dehydrogenase in malignant, normal adult, and fetal colon tissues. *Clin Chem* 1980; **26**: 1809-1812 [PMID: 6108167]
- Han B, Yu JP, Shen ZX, Luo HS, Yang YM, Wang ZW. Enzymatic analysis of colorectal biopsy specimens in polyps and carcinomas. *Shijie Huaren Xiaohua Zazhi* 1989; **9**: 342-345
- Li QY, Xu MZ, Kong XY. Practices of Medical Laboratory Sciences. Wuhan: Hubei People's Publishing House, 1980: 341-343
- Luo L, Yang ZH. A high sensitive method for determination of LDH isoenzymes. *Zhonghua Jianshan Yixue Zazhi* 1992; **15**: 6-7
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254 [PMID: 942051 DOI: 10.1016/0003-2697(76)90527-3]
- Carda-Abella P, Perez-Cuadrado S, Lara-Baruque S, Gil-Grande L, Nuñez-Puertas A. LDH isoenzyme patterns in tumors, polyps, and uninvolvement mucosa of human cancerous colon. *Cancer* 1982; **49**: 80-83 [PMID: 7053822 DOI: 10.1002/1097-0142(19820101)49:1<80::AID-CNCR2820490118>3.0.CO;2-C]
- Hong GY, Li JW, Xiao NQ. Systematic studies of human LDH isoenzymes. *Acta Sci Nat Univ Pekin* 1988; **24**: 195-201
- Ono S. [Studies on carcinoembryonic antigen (CEA), lactate dehydrogenase (LDH), and LDH isozymes in the tissue of colorectal carcinoma]. *Nihon Geka Gakkai Zasshi* 1983; **84**: 336-348 [PMID: 6325865]

S- Editor: Yang ZD L- Editor: Ma JY E- Editor: Liu WX

## Clinical observation of $^{125}\text{I}$ -labeled anti-alpha fetoprotein antibody radioimmunotherapy in hepatocellular carcinoma

Ying-De Wu, Ke-Zeng Yang, De-Nan Zhou, You-Quan Gang, Xiang-Qun Song, Xiao-Hua Hu, Bing-Yan Huang

Ying-De Wu, Ke-Zeng Yang, You-Quan Gang, Xiang-Qun Song, Xiao-Hua Hu, Bing-Yan Huang, Department of Chemotherapy, Affiliated Cancer Hospital, Guangxi Medical University, Nanning 530021, Guangxi Province, China

De-Nan Zhou, Guangxi Cancer Institute, Nanning, Guangxi Province, China

Dr. Ying-De Wu, male, born on February 10, 1936, Fu Sui County, Zhuang nationality, graduated from the Faculty of Medicine, Guangxi Medical University, professor and chief physician of the Department of Medical Oncology; former head of the Department of Cancer Prevention and Director of the Department of Chemotherapy. Since 1980 he has been engaged mainly in the study of targeted therapy for hepatocellular carcinoma, radiation therapy and tumor chemotherapy, hepatocellular carcinoma research at state and provincial level, and has 31 papers published. At present, he is the Director of the Department of Immunity, Cancer Institute, Secretary General of Guangxi Anticancer Association and Executive Editor of the Journal of Chinese Medical Abstract — **Oncology**.

Author contributions: All authors contributed equally to the work.

Supported by Grants from Guangxi Commission of Science and Technology Research Items, No.900226.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Ying-De Wu, Department of Chemotherapy, Affiliated Cancer Hospital, Guangxi Medical University, Nanning 530021, Guangxi Province, China  
Telephone: +86-771-5313022-3137 (Home), 3051 (Office)

Received: August 29, 1996

Revised: December 1, 1996

Accepted: December 30, 1996

Published online: March 15, 1997

### Abstract

**AIM:** To observe the therapeutic effects and toxic side effects of  $^{125}\text{I}$  labeled horse anti-human alpha fetoprotein (AFP) polyclonal antibodies in immune targeted therapy against hepatocellular carcinoma (HCC).

**METHODS:** A modified chloramine-T method to produce nuclide  $^{125}\text{I}$  labeled horse anti-human AFP polyclonal antibodies was used to treat 22 cases of HCC. Drugs were administered by intravenous drip. The median dose of  $^{125}\text{I}$  in the whole group was 289.3 (100.3-708.9) MBq. In this series of 22 cases, 19 were evaluated. HCC cases of the same period treated by  $^{131}\text{I}$  anti AFP (A group), anti-cancer drugs and anti AFP conjugates (B group) and chemotherapy alone (C group) were used as controls.

**RESULTS:** The effective rate (CR + PR) was 31.6%, tumor shrinkage rate was 63.2% (12/19), AFP descending rate 64.7% (11/17) and 6 cases became AFP negative. The post treatment 1 year survival rate was 47.1% (8/17). Seven cases are still alive. Five cases survived 14.33 mo, showing good therapeutic tolerance and minimal toxic side effects.

**CONCLUSION:** The therapeutic effect in the treatment group was significantly better than that of the control groups. This may be due to the effect of the continuous radiation of the long half life  $^{125}\text{I}$  within the tumor cells.

**Key words:** Liver neoplasms/therapy; Iodine radioisotopes; Radioimmunotherapy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wu YD, Yang KZ, Zhou DN, Gang YQ, Song XQ, Hu XH, Huang BY. Clinical observation of  $^{125}\text{I}$ -labeled anti-alpha fetoprotein antibody radioimmunotherapy in hepatocellular carcinoma. *World J Gastroenterol* 1997; 3(1): 43-46 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/43.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.43>

### INTRODUCTION

There have been many experimental and clinical reports about targeted treatment of hepatocellular carcinoma (HCC)<sup>[1-5]</sup>. In 1980 the authors in 1980 started to use  $^{131}\text{I}$  anti alpha fetoprotein (AFP) for the treatment of HCC, reported preliminary results in 1983<sup>[5]</sup> and published the results of 72 cases in 1994, which showed rather good curative effects<sup>[6]</sup>. However,  $^{131}\text{I}$  has several short comings, such as requiring highly protective measures, a short half-life, apparent time limits, etc. Hence, the authors selected nuclide  $^{125}\text{I}$  as the warhead. Although its energy is low, it has a long half-life, is easy to operate and only requires comparatively simple protective measures. In this paper, the authors reported the use of  $^{125}\text{I}$  anti AFP antibodies to treat 22 cases of unresectable HCC (treatment group) from March 1991 to November 1993. HCC cases of the same period treated by intravenous  $^{131}\text{I}$  anti AFP targeted therapy (A group), intravenous anticancer drugs, anti AFP conjugates (B group) and chemotherapy alone (C group) were used for control studies.

### MATERIALS AND METHODS

#### Preparation of $^{125}\text{I}$ anti AFP antibodies

Horse anti-human AFP polyclonal antibody serum was extracted by

the ammonium sulfate precipitating method and purified by affinity chromatography. The purified antibodies were labeled with <sup>125</sup>I (supplied by the China Atomic Energy Research Institute) by the chloramine-T method. The product <sup>125</sup>I anti AFP was isolated by a Sephadex G column and filtered to remove bacteria. After cultivation and limulus testing to ascertain the lack of bacteria and pyrogens, the product was prepared for clinical use. Altogether 22 batches were prepared, with a <sup>125</sup>I recovery rate of 60%-80%, labeled rate of 65%-83%, and comparative radioactivity of 56.74 GBq/g IgG.

### Cases

All cases were diagnosed as primary HCC on the basis of CT or hepatic arteriography, B ultrasonic examination or AFP test. Except for 2 cases which were AFP negative (confirmed by pathological examination), all cases were AFP positive (> 400 µg/L). The heart, liver and kidney functions of these 22 patients on admission were basically normal. Their pretreatment clinical characteristics are illustrated in Table 1.

### Methods of treatment

Treatment group: 3-6 d before and 7 d after treatment the patients were given compound iodine solution to block the thyroid gland. Skin tests were performed prior to <sup>125</sup>I anti AFP intravenous drip and prednisone 30 mg was taken fractionally to prevent allergic reactions. The labeled drug was given once per month. The <sup>125</sup>I median dose of the whole group was 289.2 (100.3-708.9) MBq. The IgG amount of anti AFP antibodies was 5.2 (1.8-12.8) mg. Ten of the treatment group were treated only by <sup>125</sup>I anti AFP, while the remaining 12, after receiving 1.2 targeted treatments, were given intravenous chemotherapy, transarterial infusion (TAI) or transarterial chemoembolization (TAE).

Control groups: (1) the A group was treated by <sup>131</sup>I anti AFP. The method of preparation followed that of Wu *et al.*<sup>[6]</sup>. The administration of drug was the same as the treatment group (Table 2); (2) the B group was treated by conjugates of 5 Fu, DDP or MMC with anti AFP IgG [method of preparation follows that of Tumor, 1992; 12 (1):1]. The dose was 10.20 mL per day, diluted with normal saline, by intravenous drip once a day or on alternate days, consecutively for 20-50 d; and (3) the C group was treated with DDP 100 mg, within 3 or 5 d by intravenous drip, MMC 6.10 mg/wk, intravenous drip for 2 wk and 5-Fu 0.5-0.75 g/d for 5 d. Some of the cases adopted TAI or TAE. All were repeated once every 4 wk and the treatment ran for 2-4 cycles (Table 2).

### Therapeutic observations and criteria of curative and toxic side effects

The blood pictures were examined every week. Each patient had heart, liver and kidney function tests and AFP tested pretreatment. B ultrasound or CT examinations were carried out monthly or bimonthly. Determination of the blood thyroid function T<sub>3</sub> and T<sub>4</sub> before treatment and during the monitoring of the blood, <sup>125</sup>I turned to zero. Detection of the biological bodily distribution of <sup>125</sup>I by FT-3120 isotope activity was performed in some cases to determine the blood <sup>125</sup>I concentration (% dose/mL) at different times after treatment and the urinary excretion rate (the percentage of 24 h urine <sup>125</sup>I administered dose amount). The organ surface cpm·min<sup>-1</sup> of the liver and some other organs were also determined. The evaluation of the short term therapeutic effects was carried out according to CR, PR, MR, SD and PD standards. The toxic side effects and reactions were evaluated according to the WHO toxic reaction graduated standard.

## RESULTS

All patients in the treatment group were followed up for 10.33 mo. In 2 cases, the duration of treatment was less than one year. Nineteen cases in the treatment group were evaluated and 3 cases in which the tumor mass was either too big, numerous or complicated with tumor thrombus of the portal vein were not evaluated. Of these 3 cases, 2 were discharged from hospital on the 14<sup>th</sup> and 19<sup>th</sup> day after one targeted treatment because of the

appearance of jaundice and worsening illness. One patient who left the hospital due to liver rupture was not reexamined after treatment. In the control groups, A, B and C, all the patients died except one in control group A. The effective rate and one year survival rate in the treatment group were higher than those in the control groups (Table 3). In the treatment group, the tumor shrinkage rate was 63.2% (12/19), median shrinkage rate was 45.0% (18%-100%) and 6 cases had a shrinkage rate of more than 50%. Of the 7 cases of the treatment group who survived, 5 cases had already survived for 14, 14, 22, 30 and 33 mo. One patient, a 29 year old male, received 3 targeted therapies with the following results: The tumor shrank in size from 8.9 cm × 8.1 cm to 4.9 cm × 3.8 cm; AFP of more than 1600 µg/L became negative; the tumor gouged out surgically was 4.5 cm × 4.5 cm × 4 cm in size and found to have nodular liver cirrhotic changes; cross section of the tumor showed a necrotic substance; and pathological examination showed that the tumor was a highly differentiated hepatocellular carcinoma. Electron microscopy (Figures 1-4) revealed the vacuolation of cellular organelles, nuclear dissolution or pyknosis of the nuclei, cellular degeneration, necrosis and the disappearance of normal structures.

### AFP changes

The AFP descending rate in the treatment group was 64.7% (11/17, in 6 cases the AFP became negative) and was higher than those in the control groups, A, B and C. The AFP descending rates of the control groups were 44.0% (12/25), 38.1% (8/12) and 46.6% (7/15), respectively.

### Toxic side effects

There were no allergic reactions in the treatment group. Two cases (receiving the same batch of labeled material) had fever (38.8 °C and 39.5 °C) one hour after the intravenous drip. The fever was related to the pyrogens. There were 5 cases whose SGPT was increased slightly. Leukopenia grade I, II and III occurred in 4, 3, 2 cases, respectively, which was significantly lower than that in the chemotherapy only group and in chemotherapy group it occurred in 19, 4 and 3 cases, respectively. In the treatment group, T<sub>3</sub> and T<sub>4</sub> were determined in 27 cases and there was a decrease in 8 and 6 cases, respectively. One case was medicated with thyroid tablets because he had clinical manifestations of mild "hypothyroidism". The patient had returned to normal when he was reexamined 2 mo after treatment.

## DISCUSSION

People use lipiodol as a carrier for antitumor drugs, nuclide <sup>131</sup>I or <sup>125</sup>I as warheads in targeted therapy for HCC. This therapeutic method was indicated for the treatment of unresectable HCC. Bretagne<sup>[7]</sup>, Baoul *et al.*<sup>[8]</sup>, Lu *et al.*<sup>[4]</sup> and Wang *et al.*<sup>[2]</sup> used <sup>131</sup>I (or <sup>125</sup>I) labeled lipiodol infusion via the hepatic artery or combined with antitumor drugs for the treatment of HCC. The AFP descending rate was 37%-86.7%, the objective effective rate was 40%-75%, and the post treatment 1 year survival rate was 31%. However, it was necessary to deliver the drugs via the hepatic artery. Regarding using antibodies as a carrier, many scientists have reported the usage of anti-ferritin antibody. Order<sup>[9]</sup> used <sup>131</sup>I anti-ferritin antibodies in treating 105 cases of HCC, applying targeted therapy after radiotherapy combined with chemotherapy. The effective rate was 48% (CR 7%, PR 41%). Tang<sup>[1]</sup>, using <sup>131</sup>I anti-human HCC isoferritin antibodies as the main tool, integrated this tool with radiotherapy, chemotherapy and immunotherapy in the treatment of 47 cases of HCC. The results were that in 13 cases the tumors shrank and were resected and 7 cases survived for more than 3 years. We used <sup>131</sup>I anti AFP ≥ 2 times for 47 cases of HCC, achieving a one year survival rate of 45.7%. However, in that group of patients, 14 cases received drugs via the hepatic artery. The effective rate was higher in treatment via the hepatic artery than via the intravenous route (64.3% vs 15.2%). Also, the 1 year survival rate was higher in the former than the latter (64.3% vs 37.5%). In our group of 22 cases, most were in the advanced stage (Table 1) and the drugs were all administered by the intravenous route. In cases treated ≥ 2 times, the 1 year survival rate (57.1%) was

**Table 1** Pretreatment clinical characteristics of the treatment and control (A, B, C) groups

Characteristics	Treatment group	Control groups		
		A	B	C
Cases	22	25	21	25
Sex (male/female)	20/2	25/0	20/1	24/1
Average age (yr)	46.4	42.6	43.7	45.3
Age range	27.65	28.71	30.6	31.64
Type, stage (II/III)				
Simple type (case)	14/1	15/1	15/1	17/1
Cirrhotic type (case)	7/0	8/1	5/0	6/1
Tumor mass ≤ 7	2	7	6	8
Size (cm) 7.1 - 10	10	13	8	13
> 10	10	5	7	4
Node single	9	12	6	16
multiple	13	13	15	9
Portal vein tumor thrombus	9	11	6	9
AFP > 400 (μg/L)	20	25	21	18
< 25	2	0	0	7
HBsAg (+)	19	21	18	22

**Table 2** Dose, cycle (times) number of <sup>125</sup>I group and control (A, B, C) groups

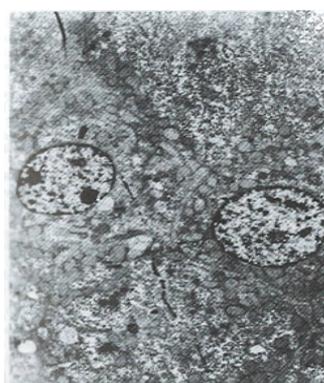
Treatment program	<sup>125</sup> I		A		B		C	
	I	≥ II	I	≥ II	I	≥ II	I	≥ II
Targeted times, treatment (case)	6	16	8	17				
Average/case (MBq)	163.5	382	388.5	984.9	treatment for 20-50 d			
Median/case (MBq)	207.2p	340.4	392.2	936.1				
Chemotherapy (case)	7	5	11	6	2		0	25 <sup>1</sup>
Including TAI, E (case)	7	3	4	1	2		13	3

<sup>1</sup>25 cases treated by 2 cycles, among them 13 cases had once of TAI.

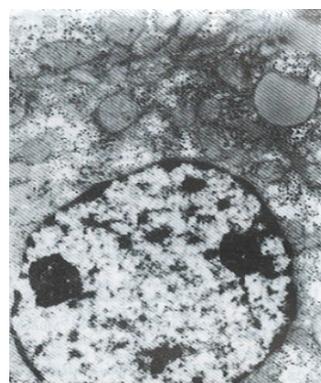
**Table 3** Short term therapeutic effects, post treatment survival rate of the treatment group and control (A, B, C) groups

Therapeutic effect	Treatment group <sup>1</sup>		Control groups			
	22	16 <sup>2</sup>	25	A	B	C
CR + PR	31.6 (6/19)	37.5 (6/16)	4.0 (1/25)	5.9 (1/17)	4.8 (1/21)	8.0 (2/25)
CR + PR + MR	42.1 (8/19)	43.8 (7/16)	16.0 (4/25)	23.5 (4/17)	28.6 (6/21)	20.0 (5/25)
Survival ≥ 6 mon	68.4 (13/19)	75.0 (12/16)	28.0 (7/25)	41.2 (7/17)	42.9 (9/21)	52.0 (13/25)
Survival ≥ 12 mon	47.1 (8/17)	57.1 (8/14)	16.0 (4/25)	23.5 (4/17)	9.5 (2/21)	8.0 (2/25)
Still alive	7	7	1	1	0	0

<sup>1</sup>Only in <sup>125</sup>I group 1 case reached CR; <sup>2</sup>by 2 or more than 2 times of treatment.



**Figure 1** Electron microscope observation of resected specimen <sup>125</sup>I anti alpha fetoprotein treatment. (Li, male, 27 years old, HCC, admittance No.10206 tumor cross section, necrotic material, capsule intact electron microscope: Degeneration, necrosis of tumor tissue). Normal liver cell, from juxta tumor. × 2200



**Figure 2** Electron microscope observation of resected specimen <sup>125</sup>I anti alpha fetoprotein treatment. (Li, male, 27 years old, HCC, admittance No.10206 tumor cross section, necrotic material, capsule intact electron microscope: Degeneration, necrosis of tumor tissue). Dissolution of nucleus. × 7700

significantly higher than those of the control groups ( $P < 0.05$ ). Besides, 7 were still alive. Electron microscopy of the surgical specimen obtained from one post treated patient showed cellular degeneration and necrosis, indicating that such morphological changes were related to the selective action of the targeted drugs. Koji *et al.*<sup>[10]</sup> reported that on the 7th day after the infusion of <sup>125</sup>I labeled anti AFP and F (ab') into the rat liver cancer model, within the tumor, 30%-60% of the radioactive materials localized on the cell membrane and nuclear membrane of the cancer cells. Such localization within the tumor was 4 times higher than that in the

control group IgG. He further observed that the AFP of advanced stage HCC patients dropped to normal level and was maintained at that level for more than 4 mo after receiving <sup>125</sup>I anti AFP treatment. He believed that this might be due to the attack and inhibition of the cancer cells by the antibodies. Judging from the observation of the blood <sup>125</sup>I levels and the urinary excretion rates determined at different times in some of our treated patients, and as time passes, such blood <sup>125</sup>I levels and urinary excretion rates declined but rather slowly, showing that the radiation action of <sup>125</sup>I within the cancer cells might persist for quite a long time, thereby producing



**Figure 3** Electron microscope observation of resected specimen <sup>125</sup>I anti alpha fetoprotein treatment. (Li, male, 27 years old, HCC, admittance No.10206 tumor cross section, necrotic material, capsule intact EM: Degeneration, necrosis of tumor tissue). Nuclear dissolution, organelle vacuolation. × 4000



**Figure 4** Electron microscope observation of resected specimen <sup>125</sup>I anti alpha fetoprotein treatment. (Li, male, 27 years old, HCC, admittance No.10206 tumor cross section, necrotic material, capsule intact EM: Degeneration, necrosis of tumor tissue). Pyknotic nucleus, organelle vacuolation. × 7700

comparatively good therapeutic results. This paper and the Koji experimental investigation suggest that <sup>125</sup>I anti AFP possesses a certain degree of specificity in the treatment of HCC. Of course, this awaits further clinical verification. Although <sup>131</sup>I possesses certain merits, such as a strong tumoricidal effect, it has short comings such as a short half-life, time limits, needing rather expensive preventive measures and patients having to be isolated. On the other hand, <sup>125</sup>I has several advantages, *e.g.* long half-life, low energy, short X-ray penetration distance (about the diameter of a cell), little adverse effect on normal cells, easy to operate, simple protective measures and having significant practical value. It can be prepared as HCC targeted treatment medical kits to be used extensively in the clinical field.

## REFERENCES

- 1 **Tang ZY**, Liu KD, Fan Z, Lu JZ, Zhang YJ, Hou Z. Targeting therapy of hepatocellular carcinoma aexperimental and clinical studies. *Tumor* 1990; **10**: 241-244
- 2 **Wang XH**, Du JH, Qian JM, Wen YJ, Shao YN. A study of targeted therapy for primary liver cancer. *Jiangsu Yiyao* 1991; **17**: 534-535
- 3 **Du JH**, Wang XH, Qian JM, Chen D, Shao YN, Zhou SH. A study of <sup>125</sup>I-lipoidal injection through hepatic artery. *Jiangsu Yiyao* 1989; **15**: 63-65
- 4 **Lu JZ**, Tang ZY, Liu KD, Zhao G, Yuan AN, Zhou YG. Hepatic artery injection of <sup>131</sup>I (or <sup>125</sup>I) labeled lipid contrast medium: preliminary observation of therapeutic use in patients with primary liver cancer. *Zhonghua Heyixue Zazhi* 1991; **11**: 93-95
- 5 **Liu YK**, Yang KZ, Wu YD, Gang YQ, Zhu DN. Treatment of advanced primary hepatocellular carcinoma by <sup>131</sup>I-anti-AFP. *Lancet* 1983; **1**: 531-532 [PMID: 6186874 DOI: 10.1016/S0140-6736(83)92220-1]
- 6 **Wu YD**, Yang KZ, Zhou DN, Liu YK, Gang YQ, Song XQ. Clinical study of <sup>131</sup>I-labeled anti-AFP antibody for targeting therapy. *Tumor* 1994; **14**: 200-203
- 7 **Bretagne JF**, Raoul JL, Bourguet P, Duvauferrier R, Deugnier Y, Faroux R, Ramée A, Herry JY, Gastard J. Hepatic artery injection of I-<sup>131</sup>-labeled lipiodol. Part II. Preliminary results of therapeutic use in patients with hepatocellular carcinoma and liver metastases. *Radiology* 1988; **168**: 547-550 [PMID: 2839867 DOI: 10.1148/radiology.168.2.2839867]
- 8 **Raoul JI**, Bretagne JF, Caucanas JP, Pariente EA, Boyer J, Paris JC, Michel H, Bourguet P, Victor G, Therain F. Internal radiation therapy for hepatocellular carcinoma. Results of a French multicenter phase II trial of transarterial injection of iodine <sup>131</sup>-labeled Lipiodol. *Cancer* 1992; **69**: 346-352 [PMID: 1309429 DOI: 10.1002/1097-0142(19920115)69:2<346::AID-CNCR2820690212>3.0.CO;2-E]
- 9 **Order SE**, Stillwagon GB, Klein JL, Leichner PK, Siegelman SS, Fishman EK, Ettinger DS, Haulk T, Kopher K, Finney K. Iodine <sup>131</sup> antiferritin, a new treatment modality in hepatoma: a Radiation Therapy Oncology Group study. *J Clin Oncol* 1985; **3**: 1573-1582 [PMID: 2415692]
- 10 **Koji T**, Ishii N, Munehisa T, Kusumoto Y, Nakamura S, Tamenishi A, Hara A, Kobayashi K, Tsukada Y, Nishi S, Hirai H. Localization of radioiodinated antibody to alpha-fetoprotein in hepatoma transplanted in rats and a case report of alpha-fetoprotein antibody treatment of a hepatoma patient. *Cancer Res* 1980; **40**: 3013-3015 [PMID: 6156759]

S- Editor: Yang RC L- Editor: Ma JY E- Editor: Liu WX

## Drinking water and liver cancer

Cui-Cai Ruan, Yan-Hua Chen, Zhen-Quan Zhang

Cui-Cai Ruan, Yan-Hua Chen, Zhen-Quan Zhang, Department of Chemistry and Mycology, Guangxi Cancer Institute, Nanning 530027, Guangxi Province, China

Cui-Cai Ruan, MD, Associate Professor, Vice Editor in Chief and Director of Chinese Medical Abstracts Oncology, with 68 published papers

Yan-Hua Chen, Assistant Prof and Prof Zhang Zhen Quan, Guangxi Cancer Institute

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China (No.49163012)

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Ruan-Cui Cai, Department of Chemistry and Mycology, Guangxi Cancer Institute, Nanning 530027, Guangxi Province, China Telephone: +86-771-5313022-3031

Received: August 8, 1996

Revised: September 9, 1996

Accepted: January 31, 1997

Published online: March 15, 1997

### Abstract

**AIM:** To study the relationship between the mutagenicity of drinking water and incidence of liver cancer in high liver cancer incidence areas in Guangxi.

**METHODS:** A relationship between the mutagenicity of drinking water and incidence of liver cancer was studied in Fusui County, a high liver cancer incidence area in China. Thirty-two samples of different kinds of drinking water (13 samples of pond water, 3 samples of well water near the ponds, 5 samples of well water, 6 samples of river water and 5 samples of tap water) were tested with a micronuclear technique in the root tips of *Vicia faba*.

**RESULTS:** Among the 32 samples of different kinds of drinking water, 12 samples of pond water and 2 samples of well water near the ponds induced micronucleus frequencies on the root tips of *Vicia faba* to increase ( $P < 0.01$ ), with the average micronucleus rate being 15.8% and 11.7%, respectively, while there was no difference between the micronucleus frequencies on the root tips of *Vicia faba* induced by well water (4.3%), river water (3.9%) or tap water (4.2%) and that on the control group ( $P > 0.05$ ). Micronuclear effects on the root tips of *Vicia faba* in different kinds of drinking water were positively related to the incidence of liver cancer ( $r = 0.86$ ,  $P < 0.05$ ).

**CONCLUSION:** There were substances that caused chromosomal aberrations in the drinking pond water in high liver cancer incidence areas of Guangxi. Different kinds of drinking water were closely related to the incidence of liver cancer. Chemical mutagens in the water may be an important factor in the high incidence of human liver cancer.

**Key words:** Drinking water; Liver neoplasms; Legumes; Micronucleus tests

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Ruan CC, Chen YH, Zhang ZQ. Drinking water and liver cancer. *World J Gastroenterol* 1997; 3(1): 47-49 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/47.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.47>

### INTRODUCTION

AFB<sub>1</sub>, HBV infection and polluted drinking water are three important risk factors for the high incidence of liver cancer which have received extensive attention in the research of the pathogen for primary hepatic carcinoma (PHC). The epidemiological investigation has shown that there is a close relationship between polluted drinking water and the incidence of liver cancer. This relationship is especially common in the high liver cancer incidence area in Guangxi<sup>[1]</sup> where we found that people who drank the pond water daily had a higher incidence of liver cancer than those who drank the well and river water<sup>[2]</sup>.

The purpose of our research was to observe the relationship between the drinking water and liver cancer using a micronuclear technique in the root tips of *Vicia faba* in order to provide a scientific basis for an epidemiological investigation.

### MATERIALS AND METHODS

#### Spots and water sample collection

There are about 80 thousand people in Fusui County. We set up 32 spots in 23 villages according to the types of drinking water in the area and water samples were collected from each spot. The drinking water included pond water, well water near the ponds, well water, river water and tap water where Cl<sub>2</sub> (chlorine) had not been added. The water samples were put in plastic buckets separately and then kept at 10 °C for the experiment after being numbered. The *Vicia faba* is a green bean and the purely bred seed was provided by the Biology Department of Huazhong Teachers' University in China. The micronuclear background was 3%-5%. **The incidence of liver cancer**

**Table 1** Micronuclear effects on the root tips of *Vicia faba* induced by different kinds of water

Water samples	The number of samples	MCN‰ ( $\bar{x} \pm s$ )	Induced effects
Pond water	13	15.8 ± 4.05 <sup>b</sup>	+
Well water by the ponds	3	11.7 ± 6.15	+
Well water	5	4.3 ± 0.74	-
River water	6	3.9 ± 0.87	-
Tap water	5	4.2 ± 0.85	-
Distilled water	12	4.5 ± 0.58	-
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	12	25.1 ± 5.10 <sup>b</sup>	+

Each sample was compared with the distilled water (*t* test). <sup>b</sup>*P* < 0.01.

**Table 2** Micronuclear effects on the root tips of *Vicia faba* induced by pond water

Spots	MCN‰ ( $\bar{x} \pm s$ )	Induced effects
Mumin	13.3 ± 1.24 <sup>b</sup>	+
Lingfan	16.3 ± 4.73 <sup>a</sup>	+
Bayang	15.3 ± 2.52 <sup>b</sup>	+
Liansui	18.0 ± 2.00 <sup>a</sup>	+
Ruilong	9.7 ± 0.58 <sup>a</sup>	+
Zhongyuan	15.3 ± 2.50 <sup>b</sup>	+
Busha	16.0 ± 1.60 <sup>a</sup>	+
Sairen	20.0 ± 1.63 <sup>a</sup>	+
Lailu	5.0 ± 1.00	-
Sanhe	8.7 ± 1.15 <sup>b</sup>	+
Dubang	21.7 ± 4.16 <sup>b</sup>	+
Jutun	19.3 ± 1.53 <sup>b</sup>	+
Tanlong	18.0 ± 2.16 <sup>b</sup>	+
Distilled water	4.4 ± 0.58	-
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	25.0 ± 3.24 <sup>b</sup>	+

MCN of water from each spot was compared with distilled water (*t* test). <sup>b</sup>*P* < 0.01, <sup>a</sup>*P* < 0.05

**Table 3** Micronuclear effects on the root tips of *Vicia faba* induced by well water by the ponds

Spots	MCN‰ ( $\bar{x} \pm s$ )	Induced effects
Tangan	17.7 ± 8.14 <sup>a</sup>	+
Mumin	3.0 ± 1.00	-
Jucheng	14.3 ± 3.05 <sup>a</sup>	+
distilled water	4.1 ± 0.25	-
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	25.6 ± 5.20 <sup>b</sup>	+

MCN of water from each spot was compared with distilled water (*t* test), <sup>b</sup>*P* < 0.01, <sup>a</sup>*P* < 0.05.

was the annual rate per 100000 derived from the reports from the Fusui Cancer Institute from 1973 to 1987. All the cases in the reports had been investigated and checked by researchers in this specific field.

**Micronuclear experiments on the root tips of *Vicia faba***

The tests were carried out according to the method, procedure and requirements of Degress and other reports<sup>[3,4]</sup>. Routine germinating, handling, pruning, fixing and hydrolysis were conducted. After that, we took 3 root tips from each sample, observed 1000 cells under the super microscope, calculated the number of the micronucleus cells (MCN) and the micronucleus rate (MCN %). **Statistical analysis** of MCN% was carried out on the tested and control group. Distilled water was used as a negative control and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (3.4 × 10<sup>-6</sup> mol/L) as a positive control by each assay.

**RESULTS**

Table 1 shows the micronuclear effects on the root tips of *Vicia faba* induced by different kinds of water samples. Tests of 32 samples of different water samples showed that micronucleus frequencies of *Vicia faba* root tips induced by pond water were the highest (*P* < 0.01), next was well water near the ponds (*P* < 0.05), while there was no difference between the micronucleus frequencies induced by well water, river water or tap water and the negative controls (*P* > 0.05).

**Table 4** Micronuclear effects on the root tips of *Vicia faba* induced by well water

Spots	MCN‰ ( $\bar{x} \pm s$ )	Induced effects
Ruilong	5.00 ± 1.00 <sup>a</sup>	-
Sairen	3.30 ± 0.47 <sup>a</sup>	-
Dubang	4.67 ± 1.15 <sup>a</sup>	-
Tangan	3.85 ± 0.57 <sup>a</sup>	-
Zhongyuan	4.51 ± 0.51 <sup>a</sup>	-
Distilled water	4.33 ± 0.52	-
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	24.9 ± 3.10 <sup>b</sup>	+

MCN of water from each spot was compared with distilled water (*t* test), <sup>b</sup>*P* < 0.01, <sup>a</sup>*P* < 0.05.

**Table 5** Micronuclear effects on the root tips of *Vicia faba* induced by river water

Spots	MCN‰ ( $\bar{x} \pm s$ )	Induced effects
Longtun	5.33 ± 0.57	-
Nale	4.00 ± 1.00	-
Wangzhuang	4.10 ± 0.85	-
Liuqiao	3.66 ± 0.75	-
Funong	5.67 ± 0.50	-
Longtou	4.66 ± 1.10	-
Distilled water	4.23 ± 0.52	-
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	25.70 ± 2.30 <sup>b</sup>	+

MCN of water from each spot was compared with distilled water (*t* test), <sup>b</sup>*P* < 0.01.

**Table 6** Micronuclear effects on the root tips of *Vicia faba* induced by tap water

Spots	MCN‰ ( $\bar{x} \pm s$ )	Induced effects
Changping	3.33 ± 0.57	-
Zhongdong	4.33 ± 0.55	-
Nongyang	5.00 ± 1.00	-
Tangan	4.12 ± 0.87	-
Yiqiao	3.80 ± 0.51	-
Distilled water	4.30 ± 0.50	-
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	25.71 ± 2.91 <sup>b</sup>	+

<sup>b</sup>*P* < 0.01.

**Table 7** Relationship between different kinds of drinking water and the incidence of liver cancer

Type of drinking water	Incidence of liver cancer (/100 thousand)	RR
Pond water	82.56 <sup>b</sup>	2.34
Well water by the ponds	75.85 <sup>b</sup>	2.17
Well water	63.12 <sup>a</sup>	1.80
River water	45.69 <sup>a</sup>	1.31
Tap water	35.01	1.00

Comparison between the incidence of liver cancer induced by different kinds of water and that induced by tap water, <sup>b</sup>*P* < 0.01, <sup>a</sup>*P* < 0.05

Table 2 shows the micronuclear effects on the root tips of *Vicia faba* induced by pond water. Tests of 13 pond water samples showed that 12 samples of water induced increased micronucleus frequencies on the root tips of *Vicia faba*, with a positive rate of 92.3% and the average micronucleus rate between 8.7%-21.7%.

Table 3 shows the micronuclear effects on the root tips of *Vicia faba* induced by well water by the ponds. Tests of 3 water samples showed that 2 samples induced increased micronucleus frequencies on the root tips of *Vicia faba*.

Table 4, Table 5 and Table 6 show the micronuclear effects on the root tips of *Vicia faba* induced by well water, river water and tap water separately. There was no difference between the micronucleus frequencies induced by these water samples and the controls (*P* > 0.05).

Table 7 shows the relationship between the different kinds of drinking water and the incidence of liver cancer. 40000 people who drank pond water had a higher incidence of liver cancer than the 40000 who drank well water by the ponds, well water and river

**Table 8 Relationship between micronuclear effects induced by different kinds of drinking water and the incidence of liver cancer**

Type of drinking water	MCN‰ ( $\bar{x} \pm s$ )	Incidence of liver cancer (/100 thousand)
Pond water	15.8 ± 4.05	82.56
Well water by the ponds	11.7 ± 6.15	75.85
Well water	4.3 ± 0.74	63.12
River water	3.9 ± 0.87	45.69
Tap water	4.2 ± 0.85	35.01

MCN induced by different kinds of drinking water was compared with the incidence of liver cancer, ( $r = 0.86, P < 0.05$ ).

water, while those who drank tap water had the lowest incidence of liver cancer. There is a marked difference between the incidence of liver cancer induced by different kinds of drinking water other than tap water and that induced by tap water.

Table 8 shows the relationship between micronuclear effects on the root tips of *Vicia faba* induced by different kinds of drinking water and the incidence of liver cancer. The micronuclear effects induced by pond water samples was strong, so the people who drank pond water had a higher incidence of liver cancer, while the incidence of liver cancer among those who drank well water, river water and tap water was low. Micronuclear effects on the root tips of *Vicia faba* induced by the substances in different kinds of drinking water coincided with the incidence of liver cancer ( $r = 0.86, P < 0.05$ ).

Comparing the micronuclear effects induced by different pond water samples with the incidence of liver cancer among the local people, we found that the micronuclear effects induced by pond water agreed with the changing incidence of liver cancer on the whole. However, the micronuclear effects induced by different pond water samples changed greatly because of the varying degrees of pollution of the pond water and its interrelation with the incidence of liver cancer was not significant ( $r = 0.53, P > 0.05$ ).

## DISCUSSION

The relationship between polluted drinking water and liver cancer is difficult to understand and has still not been elucidated due to the limited experimental means. Many scholars have noticed the common phenomenon that polluted drinking water will cause a high incidence of liver cancer. In our study we found that 12 samples of pond water and 2 samples of well water by the ponds in 32 samples of different kinds of drinking water collected in Fusui County induced micronucleus frequencies on the root tips of *Vicia faba* to increase, indicating that chemical mutagens polluted the drinking pond water in Fusui County. This kind of substance can distort chromosomes in plant cells and has mutagenicity. It suggested that ingesting these mutagens and/or carcinogens may increase the latent danger for

people to develop liver cancer and provided experimental evidence for the etiology of liver cancer.

Research shows that drinking highly polluted surface water, water with added  $Cl_2$  and water with a high concentration of  $CHCl_3$  will increase the risk of liver cancer. The risk may come from many mutagens and/or carcinogens which have interrelated and cooperative roles in the polluted drinking water<sup>[5]</sup>. In our research, we found mutagenic substances in the drinking pond water of Fusui County. The people who drink polluted pond water have the highest incidence of liver cancer, which is 2.34 times as much as the incidence of liver cancer in those who drink tap water. The degree of the micronuclear effects induced by different kinds of water coincided with the incidence of liver cancer ( $r = 0.86, P < 0.05$ ), that is to say, people who drink highly polluted water and water with a strong micronuclear effect have a high incidence of liver cancer. Although a single chemical mutagen and/or carcinogenic substance may have a very low concentration in the polluted water at a PPb or PPM level, this will harm the DNA and reach a carcinogenic threshold for animals and humans drinking it long term. Frequent exposure to the carcinogenic environment with AFB<sub>1</sub> and mycotoxins and so on<sup>[6]</sup> may be another important risk factor for the high incidence of liver cancer.

There has been no definitive final conclusion about the cause of liver cancer to date but many scholars think that liver cancer is caused by the complementary effects of multiple carcinogenic factors. Therefore, preventing hepatitis, reducing intake of AFB<sub>1</sub> and drinking hygienic water<sup>[7]</sup> are basic strategies to prevent liver cancer. Our research shows that there may be some specific carcinogens in the drinking water of high liver cancer incidence areas. So, the incidence of liver cancer will be lowered by controlling and preventing pollution of water with all kinds of mutagens and/or carcinogens. It is predicted that liver cancer will be the first cancer that man can prevent. Certainly we can.

## ACKNOWLEDGMENTS

We gratefully acknowledge Dr Huang Zhong-Shi for assisting us with spot investigation and the water sample collection.

## REFERENCES

- 1 Su DL. Drinking water and liver cancer. *Zhonghua Yufang Yixue Zazhi* 1980; **14**:65-70
- 2 Ye FS. The research progress of preventing liver cancer in Guangxi since the liberation of China. *Guangxi Yixue Zazhi* 1984; **5**: 226-231
- 3 Degreessi F. Micronucleus test in *Vicia faba* root tips to detect mutagen damage in fresh water pollution. *Mut Res* 1982; **997**: 9-14
- 4 Ruan CC, Liang Y, Liu JL, Tu WS, Liu ZH. Study of micronucleus test in *Vicia faba* root tips in the detection of mutagenic environmental pollutants. *Environ Sci* 1992; **4**: 56-59
- 5 Ruan CC. Mutagens of foods and liver cancer. *Nature J* 1991; **14**: 774-781
- 6 Ruan CC, Liang Y, Liu JL, Tu WS, Liu ZH. The comutagenic effect of metabolic extracts of fungi grown on the main grain in high liver cancer incidence areas a Fusui County. *Zhonghua Yufang Yixue Zazhi* 1991; **5**: 288-291
- 7 Ruan CC, Chen YH. Asynthetical policy of preventing liver cancer. *Zhonghua Yufang Yixue Zazhi* 1992; **5**: 300-304

S- Editor: Ma JY L- Editor: Ma JY E- Editor: Liu WX

## Co-regulative effects of the cAMP/PKA and DAG/PKC signal pathways on human gastric cancer cells during differentiation induced by traditional Chinese medicines

Shan-Qing Gu, Yun-Yan Liang, Li-Ren Fan, Bao-Yuan Li, Dai-Shu Wang

Shan-Qing Gu, Li-Ren Fan, Lab of Oncology, Qingdao No.6 People's Hospital, Qingdao 266033, Shandong Province, China

Yun-Yan Liang, Bao-Yuan Li, Dai-Shu Wang, Lab of Cell Biology, Beijing Institute for Cancer Research, Beijing 100034, China

Shan-Qing Gu, male, born in June 1970 in Weifang, Shandong Province, admitted to a postgraduate program of oncology in Beijing Medical University, has 4 papers published

Author contributions: All authors contributed equally to the work.

Supported by National Natural Science Foundation of China, No. 938800202.

Presented at The International Conference of Gastroenterology in Shanghai in November, 1996; at The 7<sup>th</sup> Chinese Symposium on Oncology in Integrated Chinese and Western Medicine in Hangzhou in September 1996; and at the 8<sup>th</sup> Chinese Symposium on Digestive Diseases in Integrated Chinese and Western Medicine in Xi'an in October 1996

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: **Dr. Dai-Shu Wang, Professor**, Lab of Oncology, Qingdao No.6 People's Hospital, Qingdao 266033, Shandong Province, China  
Telephone: +86-532-3833866

Received: September 1, 1996  
Revised: November 5, 1996  
Accepted: December 14, 1996  
Published online: March 15, 1997

### Abstract

**AIM:** To evaluate the role of cAMP/PKA and DAG/PKC pathways of MGc80-3 cells treated with a traditional Chinese medicine compound, bailong preparation (bailong).

**METHODS:** cAMP level, DAG content and activities of PKA and PKC were measured in different groups: control; 1.8 g/L bailong; 1.8 g/L bailong + 20 mg/L PKA inhibitor; and 5 µmol/L PKC inhibitor.

**RESULTS:** When MGc80-3 cells were treated with bailong for 3 h, cAMP level and PKA activity were 113% and 19.7% higher than those of the control, while DAG content and PKC activity were 47.0% and 64.2% lower than those of the control. When the PKC pathway was blocked by PKC inhibitor GF-109203 X, cAMP level and PKA activity were increased by 78.8% and 33.5% compared to inhibitor GF-109203 X, and cAMP

level and PKA activity were increased by 78.8% and 33.5% compared to the control, while the DAG content and PKC activity were decreased by 40.3% and 56.3%. When MGc80-3 cells were treated with bailong and PKA inhibitor blocked PKA pathways at the same time, cAMP level and PKA activity were decreased by 46.0% and 28.9%. On the other hand, DAG content and PKC activity were increased by 50.7% and 51.6% compared to the bailong group.

**CONCLUSION:** There is a relationship of cause and effect between differentiation of MGc80-3 cells and the signal pathways. The results of this study are similar to that of hexamethylenebisacetamide (HMBA), suggesting that the two signal systems are the foundation of proliferative regulation of MGc80-3 cells treated with Chinese medicine bailong or HMBA.

**Key words:** Chinese medicine; cAMP/PKA; DAG/PKC; Gastric cancer

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Gu SQ, Liang YY, Fan LR, Li BY, Wang DS. Co-regulative effects of the cAMP/PKA and DAG/PKC signal pathways on human gastric cancer cells during differentiation induced by traditional Chinese medicines. *World J Gastroenterol* 1997; 3(1): 50-53 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/50.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.50>

### INTRODUCTION

Most extracellular agonists exert their effects on cells by activating or inhibiting transmembrane signaling systems that control the production of second messengers. Recently, the biochemistry of cellular signaling systems have come to the fore as potential targets for the discovery and development of new anticancer drugs<sup>[1,2]</sup>. In general, cAMP plays a negative role in cell proliferation and a positive role in differentiation of cancer cells. On the other hand, the role of two second messengers, DAG and IP<sub>3</sub>, act against cAMP. As we know, malignant phenotypes of some cancer cells can be changed by cAMP and its derivatives through the regulation of the PKA system. Actually, it is the result of the combined regulation of several signal systems. Furthermore, an increasing body of literature suggests that PKC may play a role in the signal transduction pathways mediating hexamethylenebisacetamide (HMBA) induced changes in cellular growth and differentiation<sup>[3]</sup>. Nishizuka pointed out that there were crosstalk between the cAMP/PKA and DAG/PKC pathways

and provided two models for their interaction<sup>[4]</sup>. Tumor therapy by TCM has been reported<sup>[5,6]</sup>, but its mechanism of function is not clear and there are no papers on the co-regulative effects on different signal systems. Previous research showed that bailong responded well in tumor therapy and that its function was related to a signaling system. Further investigations on signal pathways of MGc80-3 cells treated with bailong were carried out in this study in order to evaluate its positive and negative regulation compared to that of HMBA.

## MATERIALS AND METHODS

### Preparation of bailong medium

Bailong, a traditional Chinese medical formula, is composed of 8 Chinese medicinal herbs, including *Solanum lyratum* thumb and *Solanum nigrum* L. The end product is produced by Tianjin Pharmaceutical Factory of Chinese Medicine. 300 g/L powder of bailong (its efficacy is 6 times higher than the crude drugs) was dissolved in 100 mL distilled water, then sterilized at 0.07MP for 20 min and centrifuged at 3000 r/min for 15 min after cooling down. The supernatant was collected as stock buffer and its concentration was 18 g/L, while the working buffer was 1.8 g/L. Normal medium was the same as the bailong medium.

### Other reagents

[<sup>3</sup>H] glycerol was purchased from Amersham, [<sup>3</sup>H] cAMP and [r-<sup>32</sup>P] ATP were obtained from the Institute of Nuclear Energy of China, PKA inhibitor (type III) was purchased from Sigma and PKC inhibitor (GF-109203 X) was obtained from Boehringer Mannheim.

### Cell culture

MGc80-3 cells were maintained at 37 °C in the presence of 5% CO<sub>2</sub> in RPMI-1640 (Gibco) medium containing 15% bovine serum.

### Determination of intracellular cAMP level 1

To take advantage of the competitive combination between cAMP and <sup>3</sup>H-cAMP into specific protein kinase, intracellular cAMP level was measured using a radioimmunoassay kit, as described by the manufacturer, which was supplied by the Chinese Academy of Science. Samples were extracted according to Jakobsen<sup>[7]</sup>. cAMP level was expressed as pmol/10.6 cells.

### DAG measurement

To label MGc80-3 cells with <sup>3</sup>H-glycerol for 15 h according to Wolfman *et al.*<sup>[7]</sup>, cells were collected and extracted, then extended on Silica gel G, followed by scintillation counting in Pico-fluor (Packard).

### Protein extraction

Cells were washed and scraped into cold phosphate buffered saline (PBS). After being centrifuged at 1000 r/min for 5 min, sediment was dissolved in cell lysis buffer (20 mmol/L Tris HCl, 4 mmol/L EDTA, 1 mmol/L EGTA, pH7.5) at 0 °C, lysed by sonication without breaking the nuclear membrane, and then added to the same volume sucrose Tris buffer (40 mmol/L Tris HCl, pH7.5, 0.66 mol/L sucrose, 0.1 mol/L β-mercaptoethanol, 2 mmol/L PMSF). Centrifuged at 4 °C for 5 min in order to precipitate the nucleus, partial supernatant was used to measure the activity of PKA. The remaining sediment was re-suspended in the surplus, sonicated nuclear membrane at 0 °C, 10% Triton X 100 was added to final concentration of 1%, shaken on ice for 1 h and centrifuged again at 34000 r/min for 1 h. Supernatant was purified by DEAE-52 (Whatman) and the column was balanced with buffer (5 mmol/L Tris HCl, pH7.5, 1 mmol/L EGTA, 50 mmol/L β-mercaptoethanol) previously. The column was washed with 10 mL balance buffer after samples were added, then washed out with balance buffer containing 0.15 mol/L NaCl so that the washed buffer could be used for a PKC activity assay.

### Assay of activities of PKA and PKC

The activities of PKA and PKC were determined according to Plet *et*

*al.*<sup>[9]</sup> and Dabon *et al.*<sup>[10]</sup> and the protein content in these extracts was determined according to Lowry's assay<sup>[11]</sup>.

## RESULTS

### Effects on cAMP-PKA pathway in MGc80-3 cells treated with bailong

From Figure 1 and Figure 2, both the bailong and PKC inhibitor could enforce the cAMP-PKA pathway in MGc80-3 cells when treated for 3 h. When treated with bailong, cAMP level and PKA activity increased by 112.7% and 19.7%, while when treated with PKC inhibitor, it increased by 78.9% and 33.5% compared to the control. When treated with the bailong and PKA inhibitor, cAMP level and PKA activity decreased by 46.4% and 28.9% compared to the bailong group.

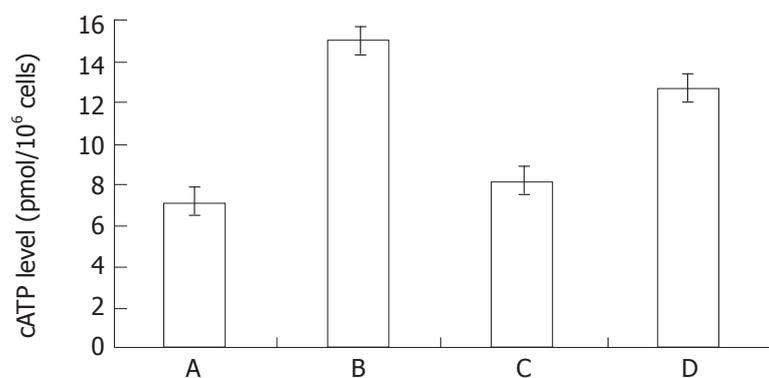
### Effects on DAG-PKC pathways in MGc80-3 cells treated with bailong

From Figure 3 and Figure 4, both the bailong and PKC inhibitor could block the DAG-PKC pathway in MGc80-3 cells treated with bailong for 3 h. When treated with bailong, DAG and PKA activity decreased by 47.0% and 64.2%, while when treated with PKC inhibitor it decreased by 40.3% and 56.3% compared to the control. When treated with bailong and PKA inhibitor, DAG and PKA activity increased by 50.7% and 51.6%.

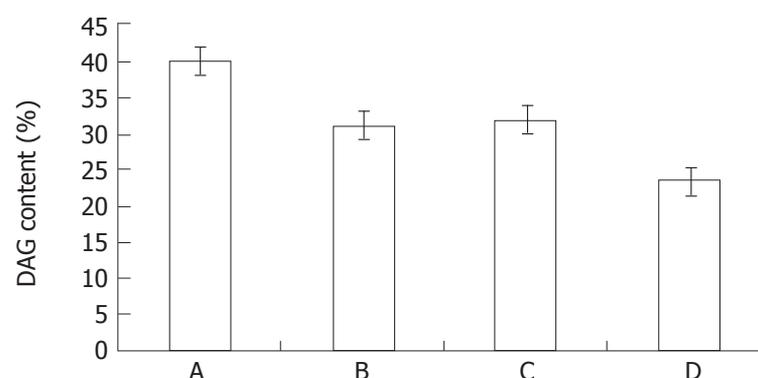
## DISCUSSION

According to the theory of traditional Chinese medicine (TCM), tumorigenesis and development always follow a process of blood stasis due to stagnation of Qi, causing accumulation of phlegm-dampness, stagnation of heat, disorder of the viscera and their interrelations, and deficiency of both blood and Qi. Upon this, bailong, a prescription of TCM, with 8 herbs including *Solanum lyratum* thumb and *Solanum nigrum* L. as ingredients, functions on fostering the original essence, strengthening the body defense, promoting blood circulation so as to remove the blood stasis and clear away pathogenic heat and toxic materials. After several modifications of its composition and much research on the production line, bailong was not made into a medicinal preparation until 1991. Clinical and experimental studies suggested that bailong could inhibit tumor growth *in vivo* and *in vitro*. Based on previous works, further investigations on the co-regulation and their interaction between cAMP/PKA and DAG/PKC signal systems of MGc80-3 cells treated with bailong was carried out in order to ascertain the malignancy reduction of cancer cells by regulating the expression of signal systems, which helps to integrate the theory of TCM and Western medicine to regulate tumor growth and differentiation at the molecular level.

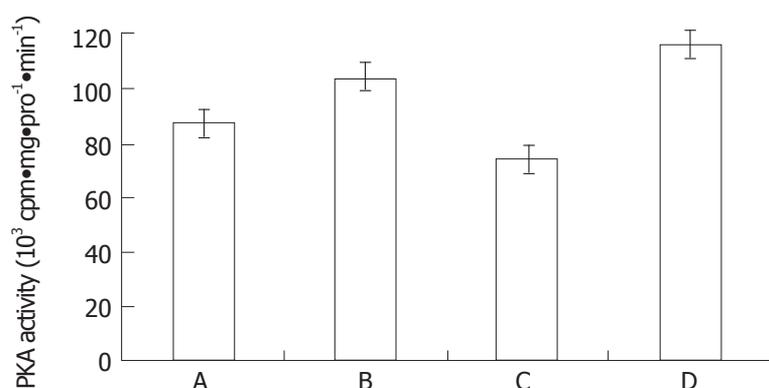
In measuring the efficacy of bailong, remarkable growth inhibition of tumor cells occurred at the concentration of 1.0 g/L. At the concentration of 1.8 g/L, growth inhibition of MGc80-3 cells was 52.9% on the 3rd day and 75.8% on the 4<sup>th</sup> day. MGc80-3 cells were still treated with bailong at the concentration of 1.8 g/L. Three hours later, we found that cAMP level and PKA activity increased while the DAG content and PKC activity decreased. This suggests that: 1. the two signal systems were closely related to growth inhibition of MGc80-3 cells resulting from bailong. Picus thought that activation of PKC could be the common pathway in process of tumor promotion<sup>[12]</sup> and the role of differentiation of PKA II has been universally acknowledged. High expression of PKA related to differentiation and low expression of PKC related to proliferation are the reasons for growth inhibition of MGc80-3 cells. It is worth pointing out that bailong could translocate PKA II from cytoplasm to the nucleus in EAC cells, which is very similar to that of HMBA. This is the first time the role of TCM in regulating cell proliferation and differentiation of cancer cells through the cell signaling systems has been reported; 2. When MGc80-3 cells were cultured with bailong for 3 h, remarkable changes of the two signal systems were observed, but the growth inhibition was not obvious until the 3<sup>rd</sup> or 4<sup>th</sup> day, indicating that the changes of messenger molecules appeared earlier than that of cell proliferation by several cell cycles<sup>[13]</sup>. Regulation of



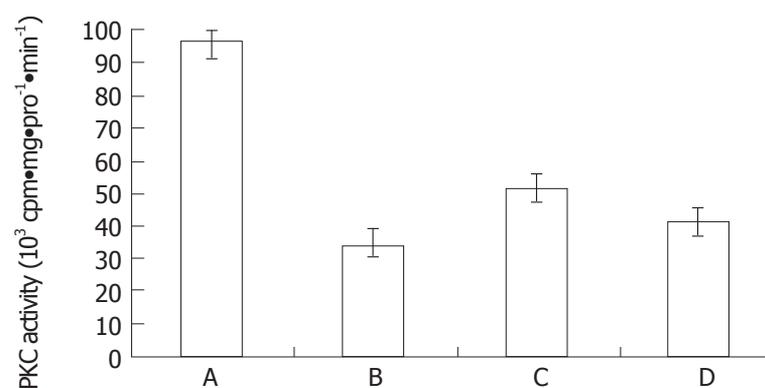
**Figure 1** Changes of cAMP level in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ( $n = 3$ )



**Figure 3** Changes of DAG activity in MGc80-3 cells treated in different conditions. A: control; B: 1.8 mg/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L ( $n = 3$ )



**Figure 2** Changes of PKA activity in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ( $n = 3$ )



**Figure 4** Changes of PKC activity in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ( $n = 3$ )

bailong was similar to that of HMBA in signal transduction of MGc80-3 cells<sup>[14]</sup>, which suggested both of them may have the same mechanism. To ascertain if the relationship of cause and effect between regulation of bailong and two signal systems existed in MGc80-3 cells, different blockers of signal pathways were used to inhibit signal transduction. When the cAMP/PKA pathway of MGc80-3 cells was blocked by PKA inhibitor (type III), the efficacy of bailong was obvious, that is to say, cAMP level and PKA activity did not rise compared to the bailong group and the DAG/PKC pathway returned to the level of the control. If the PKC inhibitor GF-109203 X was used as positive standard instead of treatment with bailong, it was found that the role of blocking the DAG/PKC pathway was similar to that of bailong. Thus it proved that cAMP/PKA and DAG/PKC signal pathways were related to the anticancer action of bailong. In view of recent research<sup>[15,16]</sup>, cAMP and its derivatives (8-CI-cAMP, db-cAMP, etc.) and PKC inhibitor (such as staurosporine analogues: CGP41 251 and UCN-01) conspicuously reduce malignancy of cancer cells. Cellular signaling systems have been regarded as one of the potential targets for the discovery and development of new anticancer drugs, which suggested that the analysis of bailong should conform to international studies on cellular signaling systems, especially to the results of HMBA. So the promotion of differentiation of MGc80-3 cells induced by bailong might be the positive and negative result of the two signal pathways. Tony Hunter pointed out that the interaction of protein kinases and phosphatases conformed to Nishizuka's<sup>[4]</sup> model, in which the interaction of protein kinases and phosphatases were suited to the TCM theory of Yin Yang<sup>[17]</sup>. The positive and negative regulation of cAMP/PKA and DAG/PKC in MGc80-3 cells could also be explained by the Yin Yang theory. All of these suggest that the same targets are shared by Chinese and Western medicine and further investigations are needed.

## REFERENCES

- Powis G. Signalling targets for anticancer drug development. *Trends Pharmacol Sci* 1991; **12**: 188-194 [PMID: 1862534 DOI: 10.1016/0165-6147(91)90545-4]
- Tritton TR, Hickman JA. How to kill cancer cells: membranes and cell signaling as targets in cancer chemotherapy. *Cancer Cells* 1990; **2**: 95-105 [PMID: 2167715]
- Melloni E, Pontremoli S, Michetti M, Sacco O, Cakiroglu AG, Jackson JF, Rifkind RA, Marks PA. Protein kinase C activity and hexamethylenebisacetamide-induced erythroleukemia cell differentiation. *Proc Natl Acad Sci USA* 1987; **84**: 5282-5286 [PMID: 3474654 DOI: 10.1073/pnas.84.15.5282]
- Nishizuka Y. Studies and perspectives of protein kinase C. *Science* 1986; **233**: 305-312 [PMID: 3014651 DOI: 10.1126/science.3014651]
- Yu QS, Zhang FZ, Tang XR. [Clinical study on early use of Chinese medicinal herbs and chemotherapy after operation of gastric cancer]. *Zhongguo Zhongxiyi Jiehe Zazhi* 1995; **15**: 459-461 [PMID: 8580690]
- Wang GM, Chen CH, Sun GZ. [Clinical and experimental study in treating gastric cancer with replenishing qi and invigorating spleen oral liquid combined with chemotherapy]. *Zhongguo Zhongxiyi Jiehe Zazhi* 1994; **14**: 661-663 [PMID: 7703634]
- Jakobsen A. Cellular cyclic AMP content independent of ascites tumour growth rate in vivo. *Eur J Cancer* 1975; **11**: 203-204 [PMID: 165941 DOI: 10.1016/0014-2964(75)90118-8]
- Wolfman A, Macara IG. Elevated levels of diacylglycerol and decreased phorbol ester sensitivity in ras-transformed fibroblasts. *Nature* 1987; **325**: 359-361 [PMID: 3027568 DOI: 10.1038/325359a0]
- Plet A, Evain-Brion D, Gerbaud P, Anderson WB. Retinoic acid-induced rapid loss of nuclear cyclic AMP-dependent protein kinase in teratocarcinoma cells. *Cancer Res* 1987; **47**: 5831-5834 [PMID: 3664484]
- Darbon JM, Issandou M, Delassus F, Bayard F. Phorbol esters induce both intracellular translocation and down-regulation of protein kinase C in MCF-7 cells. *Biochem Biophys Res Commun* 1986; **137**: 1159-1166 [PMID: 3729953 DOI: 10.1016/0006-291X(86)90347-5]
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275 [PMID: 14907713]
- Pincus SM, Beckman BS, George WJ. Inhibition of dimethylsulfoxide-induced differentiation in Friend erythroleukemic cells by diacylglycerols and phospholipase C. *Biochem Biophys Res Commun* 1984; **125**: 491-499 [PMID: 6596101 DOI: 10.1016/0006-291X(84)90567-9]
- Shen DW, Real FX, DeLeo AB, Old LJ, Marks PA, Rifkind RA. Protein p53 and inducer-mediated erythroleukemia cell commitment to terminal cell division. *Proc Natl Acad Sci USA* 1983; **80**: 5919-5922 [PMID: 6351070 DOI: 10.1073/pnas.80.19.5919]
- Peng J, Liang YY, Fang JC, Shi YJ, Wang DS. [Study on the relationship between two second-messenger pathways on the regulation of proliferation and differentiation in MGc 80-3 cells]. *Shiyan Shengwu Xuebao* 1993; **26**: 187-195 [PMID: 8191797]
- Meyer T, Regenass U, Fabbro D, Alteri E, Rösler J, Müller M, Caravatti G, Matter A. A derivative of staurosporine (CGP 41 251) shows selectivity for protein kinase C inhibition and in vitro anti-proliferative as well as in vivo anti-tumor activity. *Int J Cancer* 1989; **43**: 851-856 [PMID: 2714889 DOI: 10.1002/ijc.2910430519]

- 16 **Akinaga S**, Gomi K, Morimoto M, Tamaoki T, Okabe M. Antitumor activity of UCN-01, a selective inhibitor of protein kinase C, in murine and human tumor models. *Cancer Res* 1991; **51**: 4888-4892 [PMID: 1893379]
- 17 **Hunter T**. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* 1995; **80**: 225-236 [PMID: 7834742 DOI: 10.1016/0092-8674(95)90405-0]

S- Editor: Yang RC L- Editor: Ma JY E- Editor: Hu S

## Study of the regulatory effect of acupuncture on rotation-induced gastric dysrhythmia in rabbits

An-Li Zhang, Ri-Xin Chen, Ming-Fei Kang, Hong-Li Fan, Wan-Ling Wang

An-Li Zhang, Ri-Xin Chen, Ming-Fei Kang, Department of Acupuncture Moxibustion and Osteo-traumatology of Jiangxi College of TCM, Nanchang 330006, Jiangxi Province, China

Hong-Li Fan, Wan-Ling Wang, Postgraduate student of Jiangxi College of TCM, Nanchang 330006, Jiangxi Province, China

An-Li Zhang, female, born in Nanchang, Jiangxi Province, graduated from the TCM, Department of Jiangxi College of TCM in 1967, Professor, Director of the Teaching, Department of Acupuncture Moxibustion and Osteo-traumatology and Acupuncture, Department, Affiliated Hospital of Jiangxi College of TCM; Adviser to master degree postgraduate students, engaged in studying the diagnosis and treatment of gastroenteropathy, with more than 20 papers and one book (ZI WU LIU ZHU KAI XUE ZHI NAN) (Editor in Chief) published, 20 Yang Ming Rd. Nanchang 330006, Jiangxi Province, China

Author contributions: All authors contributed equally to the paper.

Supported by Jiangxi Provincial Natural Science Fund, No. 9281.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr An-Li Zhang, Professor, Director of the Teaching, Department of Acupuncture Moxibustion and Osteo-traumatology and Acupuncture, 20 Yang Ming Road. Nanchang 330006, Jiangxi Province, China  
Telephone: +86-791-6816996

Received: March 8, 1996

Revised: September 1, 1996

Accepted: January 1, 1997

Published online: March 15, 1997

### Abstract

**AIM:** A model of experimental gastric dysrhythmia in rabbits was set up to evaluate the effect of different acupoints on regulating gastric dysrhythmia in rabbits so as to promote acupuncture treatment for this kind of disease.

**METHODS:** A model of gastric dysrhythmia in rabbits was established by the rotation method using the basic electrical rhythm (BER) as an objective index. After puncturing at the points of Zusanli (ST36), Neiguan (PC6), Tiaokou (ST38) and Tianquan (PC2) in the four groups of experimental gastric dysrhythmia rabbits, the difference in regulatory effects on the disturbance and frequency of the gastric electric slow wave was observed.

**RESULTS:** Before needling at the specific acupoints Zusanli and Neiguan, the percentage of disturbance electric slow wave for the Zusanli and Neiguan groups was  $57.0785 \pm 10.644$  and  $55.5173 \pm 6.0500$ , respectively; after such needling, the percentage was  $43.7823 \pm 10.1518$  and  $43.5147 \pm 6.8983$  for the Zusanli and Neiguan groups, respectively, while the frequency of electric slow

wave for the Zusanli and Neiguan groups was  $2.2870 \pm 0.3800$  and  $2.4020 \pm 0.3536$ , respectively, before needling and after needling, the frequency was  $2.7090 \pm 0.5865$  and  $2.9220 \pm 0.4923$  for the Zusanli and Neiguan groups, respectively. Comparing the percentage and frequency for the Zusanli and Neiguan groups before and after needling, the result shows that both groups have a significant difference statistically ( $P < 0.05$ ) but between the Zusanli and Neiguan groups, there was no significant difference. Before and after needling the nonspecific acupoints of Tiaokou and Tianquan, there was no difference between the Tiaokou and Tianquan groups. Between the Zusanli and Tiaokou groups and the Neiguan and Tianquan groups, there are significant differences in regulating gastric dysrhythmia.

**CONCLUSION:** This model is suitable for the observation of gastric dysrhythmia. The specific acupoints of Zusanli and Neiguan have good effects on the treatment of gastric dysrhythmia.

**Key words:** Stomach; Acupuncture; Electrophysiology; Zusanli Neiguan disease models, Animal

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Zhang AL, Chen RX, Kang MF, Fan HL, Wang WL. Study of the regulatory effect of acupuncture on rotation-induced gastric dysrhythmia in rabbits. *World J Gastroenterol* 1997; 3(1): 54-55 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/54.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.54>

### INTRODUCTION

Our previous studies demonstrated that acupuncture can treat gastric dysrhythmia very well. In order to observe the differences of different acupoints in treating gastric dysrhythmia, we established a model of gastric dysrhythmia by the rotation method and treated the dysrhythmia by puncturing at different acupoints. By comparing the differences of regulating gastric dysrhythmia between using specific and nonspecific acupoints, an experimental basis is provided for treating motion sickness and gastric dysrhythmia syndrome by acupuncture treatment.

### MATERIALS AND METHODS

All 60 rabbits were implanted with two silver electrodes beneath the serous coat 2 cm from the pylorus of the curvatura ventriculi major with the distance of the two electrodes at 1 cm. The conducting wire goes out from the neck. The experiment was carried out after the rabbits recovered.

The gastric dysrhythmia model was established by the rotation method; the speed of rotating apparatus was 0.5C/S, rotating in the same direction for 20 min.

The gastric dysrhythmia rabbits were divided randomly into four groups of 15 rabbits; the specific acupoint Zusanli (ST36), Neiguan (PC6), nonspecific acupoint Tiaokou (ST38) and Tianquan (PC2) groups. Each acupoint was stimulated for 10 min with manipulation given evenly. An electrogastrogram (EGG) was recorded for 5 min before and after acupuncture. In accordance with our previous study, we regarded 3.00-3.95 C.P.M as a normal slow wave frequency, with out of this range regarded as an abnormal slow wave frequency. The experimental data were calculated using the *t* test.

## RESULTS

Rabbits were studied after fasting for 12 h. Head and body shaking vibrated the rabbits' eyes after the rabbits were rotated for 20 min. The EGG showed that before rotation, the percentage of disturbance slow wave was  $36.051 \pm 8.0388$  and frequency was  $3.3426 \pm 0.2523$  C.P.M. After rotation, the percentage of disturbance wave was  $51.6914 \pm 5.9842$  and frequency was  $2.670 \pm 0.4541$  C.P.M. Comparing before and after rotation, the percentage of disturbance wave and frequency was significantly improved ( $P < 0.001$ ), with the disturbance of EGG lasting more than two hours.

### **Regulatory effect of acupuncture on gastric dysrhythmia by using different acupoints**

Before acupuncture, there was no significant difference in the percentage of disturbance slow wave and frequency of the four groups ( $P > 0.05$ ). After acupuncture, the gastric dysrhythmia in the Zusanli and Neiguan groups was greatly improved and almost recovered to normal. Zusanli and Neiguan groups had a similar regulatory effect. In comparing before and after acupuncture in the Tiaokou and Tianquan groups, there was no significant

improvement of gastric dysrhythmia ( $P > 0.05$ ). There appeared to be some differences between the Zusanli and Tiaokou groups. Zusanli had a better regulatory effect than Tiaokou and Neiguan had a better regulatory effect than Tianquan.

### **The significance of the rotation induced model**

In previous studies, researchers often used injections or surgery to establish a gastric dysrhythmia model. These methods are useful but have limitations to a certain degree as they may disturb or even destroy the subjects' neurohormonal regulatory system and are usually not consistent with the disease in clinical practice.

By viewing the situation as a whole, our study imitated the pathogenesis of motion sickness and disturbed the vestibular system by rotating so as to establish a gastric dysrhythmia model in rabbits. The model is steady and easy to repeat and suitable to investigate the acupuncture effects on gastric dysrhythmia.

### **Specific acupoints**

Based on TCM theory, acupoints have functions in common and specific acupoints have their specific character. The effects of acupuncture are closely related to different acupoints for different diseases. Zusanli, with a sophisticated function of regulating gastric dysrhythmia, is indicated for treating gastric disorders. The Tiaokou group served as a control group. The results showed that the acupuncture effect of Zusanli is better than its same channel nonspecific acupoint Tiaokou and thus Zusanli has its specific effect on gastric disorders.

For a long time, many researchers have paid more attention to using Neiguan to treat cardiac disease, but there are few reports on treating gastric diseases. Our study showed that Neiguan has fine effects on treating gastric diseases and a better effect than the nonspecific acupoint Tianquan.

S- Editor: Yang RC L- Editor: Ma JY E- Editor: Hu S

## Current status of basic and clinical research in the field of gastroenterology in China

Xie-Ning Wu

Xie-Ning Wu, Department of Gastroenterology, Shanghai First People's Hospital, Shanghai 200080, China

Author contributions: The author solely contributed to the work.

Dr. Xie-Ning Wu, Professor of Medicine, Shanghai Medical University, Chief of the Department of Gastroenterology, Shanghai First People's Hospital; Member of the Academic Committee of Shanghai Gastroenterological Association, and Executive Committee Member of the Shanghai Hepatology Association; Member of the APASL and International Gastro-surgical Club.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Xie-Ning Wu, Professor, Department of Gastroenterology, Shanghai First People's Hospital, Shanghai 200080, China  
Telephone: +86-21-63240090

Received: August 12, 1996  
Revised: September 30, 1996  
Accepted: January 1, 1997  
Published online: March 15, 1997

**Key words:** Gastroenterology; Clinical studies; Review literature; Experimental research

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wu XN. Current status of basic and clinical research in the field of gastroenterology in China. *World J Gastroenterol* 1997; 3(1): 56-60 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i1/56.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i1.56>

This article discusses the major scientific advances in the field of gastroenterology in China, including some investigations into the mechanisms of diseases, new clues and new approaches to treatment.

### ESOPHAGUS

An atlas of esophageal motility was published at the end of 1994 in which more than 160 typical manometric graphs were selected from a total of 2500 graphs of various esophageal motility disorders and the perioperative period, which is useful and practical for clinicians<sup>[1]</sup>. 24 h esophageal pH monitoring has been carried out in many hospitals among healthy Chinese adults and peptic ulcer patients. In the former, the obtained values were different from healthy Western adults reported in the Western medical literature. Among those with pH < 4, the total fraction time was 3.4%, upright fraction time 4.3%, supine fraction time 4.3%, the number of reflux episodes > 5.0 min less than two, the longest reflux episode 16 min and the number of reflux episodes 60, giving a composite score of 12.7. These might be due to racial differences, LES function, living habits

and dietary composition<sup>[2]</sup>. Recently, an esophageal manometric study was conducted in asymptomatic esophageal diabetic patients that showed the following abnormalities: Diminution of resting LES pressure and amplitude of contraction of the lower segment of the esophageal body, increase of tertiary and segmental contractions and frequent double peak and multi peak contractions<sup>[3]</sup>.

### STOMACH

The gastric and gallbladder emptying time was reduced after long term acid inhibition by omeprazole in the treatment of reflux esophagitis combined with concomitant reduction of postprandial release of pancreatic polypeptides and normal serum VIP and CCK. It was suggested that the diminished vagal tone was responsible for the long term use of omeprazole in reflux esophagitis patients<sup>[4]</sup>. *Helicobacter pylori* (*Hp*) is now also a subject of emphasis in China and has become a rapidly expanding specialty. In 43 strains of *Hp*, an analysis of the expression of *CagA* and *VacA* virulence factors was carried out. According to their gastric and phenotypic properties, type I bacteria had the gene coding for *CagA* and expression of protein together with vacuolating cytotoxins, whereas type II bacteria did not. There was an intermediate phenotype expressing *CagA* independently of *VagA* or vice versa and the authors concluded that *CagA* is not necessary for the expression of vacuolating cytotoxins<sup>[5]</sup>. On differentiation of *Hp* isolated from recrudescence and reinfection after dual or triple therapy, some used PCR and single strand conformation polymorphism analysis was also found to be useful in the epidemiological study of *Hp* infection<sup>[6]</sup>. On a study of local humoral immunity of gastric *Hp* infection, research in the Shanghai Institute of Digestive Diseases studied the immunoglobulin antibodies to *Hp* immunoblot analysis in 167 patients with various gastroduodenal diseases. Protein bands 138, 71 kDa and 100.31 kDa were most frequently found (92%-100%) and 64.67 kDa protein was found in 72.5% of duodenal ulcers, 64.2% of gastric ulcers and 92.9% of gastric cancer. The author concluded that 64.67 kDa protein band might be a marker for predicting and assessing the severity of gastric disease<sup>[7]</sup>. Among IgG, IgA and IgM bands in children, the IgM bands were more numerous than those in adults, indicating an early stage of *Hp* infection<sup>[8]</sup>. Another basic study was conducted on hydrophobicity changes of the gastric mucosal surface to search for the influence of *Hp* in patients with erosive gastritis and peptic ulcer and the contact angle was found to be decreased in *Hp* positive patients, which reflected hydrophobicity changes and phospholipase levels in the mucosa. It suggests weakening of the mucosal barrier and disturbed mucosal phospholipid metabolism<sup>[9]</sup>.

In *Hp* infection, the levels of ascorbic acid and copper zine superoxide dismutase in gastric juice were significantly lower in *Hp* positive than in *Hp* negative patients and the lipoperoxide level was the highest. In patients with gastric cancer, the CuZnSOD levels in gastric juice and plasma were much lower than in those with chronic gastric or peptic ulcers. The degree of decrease in ascorbic acid and CuZnSOD level in gastric juice was in decreasing order in patients

with CSG, CAG and gastric cancer<sup>[10]</sup>. Electromicroscopic features of *Hp* infection of gastric mucosa revealed *Hp* aggregation on the epithelial cell membrane in the form of hairy sticks or pseudopodia-like protrusions. Transmission electromicroscopy also showed that mucus granules aggregated at the inner side of the cell membrane. Scanning electromicroscopy disclosed a nibbling phenomenon of *Hp*, adhering beside the ulcerative area of the cell membrane and some island-like granules floating on the surface<sup>[11]</sup>. Investigating the pathogenesis of *Hp* infection showed that the tissue TNF- $\alpha$  and gastrin production were increased but both were diminished after *Hp* eradication, which might be an important mechanism of gastrin linked hypothesis in patients with *Hp* infection. With regards to the relationship between *Hp* and gastric cancer, several articles focused on the molecular aspects. It was found that the mutation rate of H-ras oncogene was higher in *Hp* infected groups than in those without infection, showing that the *Hp* infection was associated with an increased expression of ras p21 protein which increased the risk of ras oncogene activation, DNA damage and S-phase cells, indicating the rapid turnover of cells from injury<sup>[12]</sup>. Another experimental study showed DNA damage in gastric mucosal cells in *Hp* infection of an animal model, manifested as a decrease in the percentage of double strand DNA, chromaticity, fluorescent intensity of DNA EB complex, with EB as a fluorescence probe, and its resistance to hydrolysis by DNase I. This indicates *Hp* infection may play a causative role in gastric cancer<sup>[13]</sup>. Clinically, transmission of *Hp* is probably from human to human, from patients to medical staff. In a survey of one hospital, the overall prevalence of *Hp* among medical staff was 70% compared to 41.7% of the general population ( $P < 0.01$ ). Among endoscopists, its prevalence was 82.4%, higher than medical doctors in general (66.4%) and nurses 65% ( $P < 0.05$ )<sup>[14]</sup>. To assess the therapeutic efficiency of single, dual and triple therapy for eradication of *Hp* infection, <sup>14</sup>C and <sup>13</sup>C breath tests are presently used in large medical centers. A capsule-based modified microdose <sup>14</sup>C urea breath test proved to be more simple, accurate and economical than the conventional ones. Its sensitivity and specificity were 93.3% and 92.3%, respectively, and the positive and negative prediction values were similar<sup>[15]</sup>. In a newly discovered test, <sup>15</sup>N-urea excretion, which is devised here and can be used as a tracer for detection of clinical *Hp* infection, the <sup>15</sup>N-excretion rate in urine ammonium was much higher in *Hp* positive than in *Hp* negative subjects. A single dose of <sup>15</sup>N was taken orally and urine samples were collected every 30 min for two hours. The normal value was  $< 5\%$ , its sensitivity was 96% and specificity 97%, indicating it was simple, accurate and non invasive<sup>[16]</sup>.

Basic research of gastric cancer inclines towards the molecular level. Point mutation of c-Ha-ras at codon 12 and 61, N-ras at codon 12 and K-ras at codon 12 and 13 were observed in formalin fixed paraffin embedded specimens of 43 cases of gastric cancer by using PCR-RFLP. Mutation of c-Ha-ras at codon 12 in 33.3% and K-ras at codon 12 in 4.8% was found. Only one case with mutation of ras gene survived five years, about 50% without this point mutation survived five years or more. With ras gene point mutation, local lymph nodes metastases were present in 100% and lymph node involvement was only 69% in those with no point mutation. Furthermore, there were much fewer stage I and II cases of ras gene mutation, indicating that point mutation of ras oncogene in cancer tissue signifies a poor prognosis<sup>[17]</sup>. Another advance was the study of the plasma and intracellular concentrations of vitamin A, C, E,  $\beta$  carotene, folic acid and B12 in gastric precancerous lesions and gastric cancer patients with a status of DNA methylation. It was found that folic acid was most effective for atrophy and intestinal dysplasia, natural  $\beta$  carotene could ameliorate the histological changes and the total genomic DNA methylation was enhanced. As we know, human EGF plays an important role in the growth of gastric cancer. EGF is found to be increased in serum and saliva of patients with gastric cancer but not in urine<sup>[18]</sup> and it is higher in stage III and IV than in stage I and II, according to the TNM classification. A new treatment advance has been made, successful local adjuvant therapy in experimental gastric cancer, *i.e.* the parvovirus<sup>[19]</sup>. The parvoviral NS gene expression was studied in human gastric cancer cells transfected with a plasmid carrying MVMPNS gene which showed the following effectiveness: Nucleus/cytoplasm ratio decreased, cancer cell replication time was prolonged, rate of cloning diminished, intracellular adhesion ability increased and tumor formation in nude mice suppressed as some of the cancer cells died. In another study, expression of ras and myc oncogenes of gastric cancer cells were suppressed by the above-mentioned NS gene with augmentation

of expression of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and TGF $\beta$ . It is anticipated that the parvoviral NS gene can influence the expression of many important intracellular genes and interfere with differentiation and proliferation of cancer cells, in which the direct cytotoxic effect or induction of apoptosis might be its mechanism. In the diagnosis of gastric and colorectal cancer, a 40 kDa glycoside oligosaccharide structure glycoprotein was found to be a tumor associated antigen, its rapid ELISA kit showed positive rates in gastric, colonic and rectal cancer of 64%, 67.7% and 60%, respectively, and 41.4% in benign gastric diseases<sup>[20]</sup>. For detection of micrometastasis of bone marrow in patients with gastric cancer, an anti-epithelial cell membrane antibody examined in the marrow blood was performed by immunohistochemical staining and its positive rates were found to be correlated to the degree of cell differentiation, location of gastric cancer, the TNM staging and age of patients. The positive cells of the epithelial cell membrane antibody in the marrow blood were significantly higher than CEA positive cells ( $P < 0.01$ ). The five year survival rate was much lower and the cause of death was mainly due to dissemination<sup>[21]</sup>. An experiment of estradiol in human gastric cancer cell lines showed the number of cells in various stages and proliferative indices increased and its stimulative effect could be inhibited by tamoxifen, which should be combined with chemotherapy in gastric cancer patients<sup>[22]</sup>. Furthermore, LAK cells when given together with anti-gastric cancer monoclonal antibody MGB2 increased the killing effect and the combined use of the two is more promising<sup>[23]</sup>.

## SMALL AND LARGE INTESTINE

Enteroclysis, angiography, radionuclide scanning and enteroscopy were used to diagnose gastrointestinal bleeding of obscure origin, confirmed by surgery and pathology. Among these, 70% were diagnosed solely by enteroclysis and leiomyoma was the most common finding, the nonspecific inflammation of the ileum and Meckel's diverticulum was the next, followed by angiodysplasia. Jejunal mucosal biopsy could be complementary in certain difficult to diagnose small intestinal lesions. Enteroclysis is also helpful to diagnose chronic idiopathic pseudo intestinal obstruction<sup>[24]</sup>. An experimental transplantation of human colonic tubular adenocarcinoma into BALB/C nu/nu mice was conducted, with five generations of transplanted mice showing xenograft cancer in each generation of nude mice manifesting the same ploidy status, DNA content (mostly aneuploid), distribution of estrogen and progesterone receptors and cell kinetics as human colorectal cancer<sup>[25]</sup>. Another study on the somatostatin level of carcinoma and precancerous tissue showed that the mean somatostatin level in well differentiated cancerous tissues was higher than that in poorly differentiated and that in distant mucosa (5.10 cm from the cancerous tissue) it was lower than that in the adjacent mucosal area ( $< 2$  cm). This indicates that the somatostatin level is correlated to the differentiation of cancer and the presence of some sort of host defense reaction to delay its growth<sup>[26]</sup>.

## LIVER

The three main targets of research in hepatology are hepatitis C, hepatic fibrosis and hepatic cancer, mainly the aspects of pathogenesis and approaches in treatment. By immunohistochemical staining with monoclonal antibody of HCV-Ag (NS4) in patients with hepatitis C (acute, chronic and severe types), it was shown that HCV-Ag granules were distributed sporadically or in clusters in the cytoplasm of liver cells in acute and chronic hepatitis patients but was different in severe hepatitis. Cases with both seropositive HCV RNA and anti-HCV had HCV-Ag detected in contrast to cases with seropositive anti-HCV only. This shows that the expression of hepatic HCV-Ag is closely related to the presence of serum HCV RNA<sup>[27]</sup>. Cloning and sequencing of c-DNA of C33c protein gene in the NS 3 region of HCV from Shanghai and Jiangsu Province showed that the two isolates were homologous and this homology was higher than 99% at the nucleotide level. If compared with other isolates, the homology (94%-97.7%) at the amino acid level was much higher than the homology (81.4%-94.5%) at the nucleotide level. It suggests that C33c protein is suitable for diagnostic use<sup>[28]</sup>. In another study, the NS4 antigen of HCV in liver tissue from 9 patients treated with IFN- $\alpha$  was studied immunohistochemically using the LAB method of HCV NS4 McAb. The pattern of HCV-Ab staining was the same in pre and

post therapy liver specimens and less HCV-Ag positive cells were seen in those with a beneficial response to INF- $\alpha$ . This shows that the pattern of HCV-Ag staining and the histological activity index are more useful to predict and assess the therapeutic response to IFN- $\alpha$  than serum ALT and HCV RNA<sup>[29]</sup>. In a nationwide epidemiological survey of 67, 153 subjects, the infection rate of HCV was 3.2%, with about 40 million people infected. Analysis of HCV in Chinese patients showed most strains were Okamoto type II and III (Simmond type 1b and 2a) and only some strains were type I (1a). Most of the strains in northern, western and southern China were type II but in northwestern and northeastern areas, predominantly HCV strains were type III. The difference of HCV genotypes was not correlated with clinical severity and course of illness. The histopathological characteristics of chronic hepatitis C and B were somewhat different with most HCV specimens showing mild to moderate hepatitis, steatosis 61% vs 29% ( $P < 0.001$ ), bile duct damage 75% vs 29% ( $P < 0.01$ ), lymphocyte aggregation/follicle 43% vs 21% ( $P < 0.01$ ), increase of mononuclear cells in sinusoids 49% vs 27% ( $P < 0.05$ ) and less frequent ground glass hepatocytes, 14% vs 53% ( $P < 0.01$ ). The major ultrastructural changes were shown in the endoplasmic reticulum and mitochondria<sup>[30]</sup>. In another report, the pathological picture of hepatitis C in Chinese adult acute hepatitis patients was the same as in Westerners and steatosis was prominent despite mild necrosis and inflammation. Dense lymphoid aggregates and fibrosis in the portal tract were frequent, most cases exhibited cytoplasmic positivity in the form of diffuse or inclusion body. Electromicroscopy showed some intercellular and perisinusoidal fibrosis and the latter in the hepatic lobules and portal tracts was an indication of a trend toward chronicity. Significant pathological discrepancies were present between hepatitis C and B<sup>[31]</sup>. Regarding a treatment regimen, a randomized control study was carried out comparing 3Mu IFN- $\alpha$  2 $\alpha$  t.i.w. regimen for 6 mo and 6Mu IFN t.i.w. for 3 mo followed by 3Mu t.i.w. for another 3 mo. The complete response rate at the end of 6 mo was 67.6% and 62% respectively, and the clearance rate of serum HCV RNA was 71.4% and 72%, respectively. The normalization of serum ALT was also similar in the two groups (67.6% and 62.1%). The recommended dosage in most Chinese patients is 3Mu t.i.w. for 6 mo and for those with a poor response, an escalating dosage and prolonged schedule is necessary<sup>[30]</sup>. Until December 1992, there were 602 cases of hepatitis D coexisting with hepatitis B, with a seropositive HDAG marker in 6773 cases of hepatitis B, and the positive rate varied in different regions of the country, 1.73% and 37.5%, with an average of 8.89%. In 2797 hepatic specimens of hepatitis B, 223 were positive for HDAG, the average positive rate was 33%. A positive HDAG marker was most frequently seen in chronic hepatitis B with moderate activity<sup>[32]</sup>.

Chronic hepatitis B treated with domestic recombinant human interferon alpha-1 was conducted in a randomized double blind sequential clinical trial of 225 cases in matched pairs. This domestic product is a medical engineering product. The serum HBeAg, HBV DNA and both HBeAg and HBV DNA were 40.5%, 57.1% and 39.3% in 37 pairs of the treated group and 57.6%, 64% and 45.3% in the extended treated group, compared with controlled group ( $P < 0.01$ ). In the 6 mo follow up, the seronegative conversion rate of HBeAg and HBV DNA remained at 54% and 50.9%, respectively, and for 12 mo, they were 59.8% and 56.9%, respectively. The seropositive conversion rates of anti HBe were 29.7%, 2.7% and 33.3% in the 3 allocated groups. These demonstrated that the domestic product of recombinant human IFN $\alpha$ -1 at a dosage of 40  $\mu$ g/d for three months has a similar effect as the Western product<sup>[33]</sup>. Another series of the therapeutic vaccine of viral hepatitis B was conducted in infected one day old ducklings as an animal model. The DHBs was complexed to anti DHBsAg and attached to staphylococcus aureus Conan 1 strain, then serum viral DNA was converted to negative in 60%-80% of treated ducks, DHBsAg was converted to negative in 40% and in some, anti DHBs could be detected. This is the result of complexing DHBsAg with anti DHBs which is immunogenic<sup>[34]</sup>.

Hepatic fibrosis is another topic focused on with the consensus that the cirrhotic stage is irreversible and therapy should be aimed at the early stage of hepatic fibrosis as chronic hepatitis B and C are prevalent in this part of the world and alcoholic liver disease is rising.

Separation and cultivation of rat Ito cells was successfully established ten years ago in the Shanghai Institute of Digestive

Disease. Collagen I, III, IV, V and various components of extracellular matrix (ECM), such as fibronectin (FN), laminin (LN), undulin, integrin, hyaluronic acid (HA) etc., were all evolved from Ito cells, playing an important role in hepatic fibrosis. Collagen III is more abundant in early cirrhosis, whereas collagen I is predominant in advanced cirrhosis. Expression of the HBV genome and its effect on the modulation of ECM was studied in Changzhen Hospital of the 2<sup>nd</sup> Military Medical University with fruitful results. Hormones such as insulin and glucagon and growth factors such as EGF and FGF all stimulate the growth of fibroblasts<sup>[35]</sup>. Many Chinese medicinal herbs with remarkable effectiveness, homologous to colchicine, have been developed to revolutionize treatment. Collagenolytic enzymes are being investigated at present and many medicinal herbs and herbal mixtures have been found to be effective in promoting the activity of matrix metalloproteinase. The intrahepatic deposition of collagen was found to have a corresponding increase of collagenase activity at the early stage of hepatic fibrosis and the increase of degradation paralleled the abnormal collagen synthesis by a feedback mechanism which led to the formation of the continuation of sinusoidal basement membrane, i.e. the capillarization of sinusoids, forming the pathological basis of progression to cirrhosis. The activities of lysosomal and microsomal enzymes such as  $\beta$  N-acetyl glucosamidase ( $\beta$ -NAG) and glycyproline dipetidyl aminopeptidase (GPDA) were also found to be increased significantly at the early stage of fibrosis. All this indicated that degradation of collagen metabolism was very active in the active stage of chronic hepatitis. P III P, PC III, HA, FN, LN, Collagen IV and VI are the principal markers used in certain medical centers in Shanghai. The 7S segment of collagen IV was found to be increased and paralleled with the increment of collagen IV mRNA, which is considered to be a better marker than P-III-P in hepatic fibrosis<sup>[36]</sup>. On culture of lipocytes, IFN- $\gamma$  and tocopherol were found to have an inhibitory action on <sup>3</sup>H hydroxyproline incorporation, whereas IL-2, IL-6 promoted proliferation of lipocytes and synthesis of collagen. Furthermore, IL-6 had a bidirectional effect, it increased expression of  $\alpha$ 2 macroglobulin to inhibit the collagenase activity and thereby the degradation of collagen. Tocopherol is a co-repressor enzyme and inhibits replication of lipocyte DNA and selectively inhibits the synthesis of collagen<sup>[37]</sup>. pHGF derived from fetal liver promoted the hepatocyte proliferation and has an anti-hepatic fibrosis effect, as seen by the reduction of hyaluronic acid. It also increases the immunological function of macrophages, T and NK cells activities and diminishes the peripheral blood mononuclear cells to produce TNF<sup>[38]</sup>. Tetrandrine had an inhibitory effect on <sup>3</sup>H-proline incorporation at a concentration of 10  $\mu$ g/mL-50  $\mu$ g/mL in an experimental study of DNA and collagen synthesis of 3T3 cells and was considered antifibrotic<sup>[39]</sup>. Another drug, cinnarizine was found to have the effect of blocking the G<sub>1</sub> phase cells of 3T3 fibroblasts from progressing to the S-phase and diminished the DNA content and mitosis, as demonstrated by flow cytometry<sup>[40]</sup>. Retinoic acid when co-cultured with 3T3 fibroblasts and Ito cells was shown to inhibit the procollagen III mRNA expression at the same time, which was the mechanism of action of its antifibrogenic effect<sup>[41]</sup>. In the nutritional therapy for post-hepatic cirrhosis patients, a high calorie vegetarian diet can provide a daily intake of 2263 Kcal and 95 g protein with 2/3 of vegetarian origin. By a <sup>15</sup>N-glycine tracer kinetic study, 24 h urinary creatinine output increased, that of urea nitrogen decreased, serum albumin and transferrin increased and the body nitrogen balance became positive<sup>[42]</sup>. In portal hypertension, transjugular intrahepatic portosystemic stent shunts were performed in Beijing and Nanjing PLA General Hospital in over two hundred cases, with the rates of success of 92.2% and 94.5%, respectively, and the mortality rates of 0% and 5.5%, respectively. The velocity of portal blood flow increased, esophageal varices disappeared and only a few developed stenosis and occlusion of the shunt but angioplasty and stent institution resulted in secondary patency. Hepatic failure and rebleeding were rare<sup>[43,44]</sup>. Recently, an experimental study was conducted on the role of nitric oxide in arterial vasodilatation of cirrhotic rats. It was believed that endogenous nitric oxide could increase mean arterial pressure, reduce peripheral vascular resistance and cardiac index, leading to hyperdynamic circulatory status, but correct treatment awaits further investigation<sup>[45]</sup>.

With the advances of molecular biological technology and genetic effects, the study of molecular events in hepatocarcinogenesis is

rapidly progressing in China. In addition to the seven oncogenes, namely, N-ras, C-myc, c-ets2, IGF II, IGF II R, IGF I R and CSF- I R, activated in human hepatic cancer, Gu found that transthyretin was deleted in the gene structure and suppressed in mRNA expression in primary hepatic cancer. When it was transfected into human hepatoma cells, cell growth was retarded, indicating that this might be a novel candidate as a cancer suppressor gene for primary hepatoma<sup>[46]</sup>. Studies at the molecular level also included methylation of c-myc oncogene, expression of BCL-2 oncoprotein, the interacting site of hepatitis B virus X gene and tumor suppressor gene *p53*, alterations of *p16* gene, expression of *IL-2* gene, expression of chimeric anti-HBx antibody, retroviral vector mediated gene, transfer of TNF gene, *p53* mutational point dimorphism, ras and *p53* gene mutation, etc.<sup>[47]</sup>. The precise pathogenesis is still not completely elucidated. More recently, the HBxAg gene has been suspected to link HBV infection and HCC, which might inhibit the function of tumor suppressor gene *p53*. Detection of the expression of HBxAg was shown by using anti recombinant HBV X protein antibody and an immunohistochemical method, LSAB. The HBxAg was localized primarily in the cytoplasm with some in the nucleus in cancerous and precancerous tissues in all 38 cases. Detection rate of HBsAg in cancer was even higher than HBsAg in both serum and cancerous tissue. This indicates that HBxAg is closely related to hepatic cancer and might be used as a carrier in targeted therapy in the future<sup>[48]</sup>. Also by immunohistochemical staining, the positive signals of HBsAg and HBcAg were observed in 10.9% (9/43) and 14.54% (8/55) of hepatic cancer cells, the latter appeared as fine brown granules in the cytoplasm and only one was in the pattern of the inclusion body. The relationship of HBcAg and HBsAg in the induction of hepatocellular carcinoma remains unknown<sup>[49]</sup>. HCV RNA by a non-radioactive in situ hybridization and immunohistochemical method could also be demonstrated in the cytoplasm, nucleus or both. HCAg molecules were found and expressed in both cytoplasmic and inclusion types. HCV infected cells, including hepatic cancer cells and hepatocytes, were in diffuse, clustered or discrete forms in both cancer and precancerous tissues<sup>[50]</sup>. In another study, in 102 liver cancer specimens by immunohistochemistry, HCV antigen C33c and HBxAg were found to be positive in 81.4% and 74.5%, respectively, both were positive in 61.8% and when added together, 94.1%. In the precancerous tissue of 50 cases, the aforementioned antigens were 63% and 92%, respectively. HCV C33c antigen was localized intracytoplasmically in cancer cells and distributed in focal or diffuse form, some were accessible to the nucleus and C33c antigen positive cells were scattered or focal in cancer cells and diffuse in precancerous tissue<sup>[51]</sup>. Expression of EGF and EGFR was found to be positive in noncancerous tissue and less were found in cancer tissue, indicating that during the cancerous process, with EGFR a loss of normal membranous structures<sup>[52]</sup>. EGFab, EGFR-McAb and somatostatin all exerted an inhibitory effect on the growth of hepatic cells. Somatostatin was shown to antagonize the growth stimulating effect of EGF and cause down regulation of EGFR<sup>[53]</sup>.

Experimental gene therapy is best exemplified by introducing *IL-2* gene into the mouse hepatoma cell line by means of a retroviral vector. The transfected cells showed diminution of tumorigenicity when *IL-2* gene transfected cells were inactivated with mitomycin and inoculated several times, antitumor activity was apparent and growth of tumor nodules were retarded and more vulnerable to liquefaction and necrosis, which might open a new avenue to the treatment of hepatic carcinoma<sup>[54]</sup>. Another study of human *IL-2* gene transduced into a hepatoma cell line of mice by lipofectamine DNA complex showed *IL-2* secreted by these cancer cells and necrosis at the tumor center and inflammatory cells infiltrating along the tumor border, which indicates that a direct transfer of *IL-2* gene into cancer cells produced an antitumor effect<sup>[55]</sup>. Expression of *p53* and PCNA in hepatocellular carcinoma was found to have close relationship with portal vein tumor thrombogenesis. The PCNA labeled index was higher in positive than negative *p53* expression. This indicates that *p53* mutation may result in highly proliferative and invasive potentials which might be one of the mechanisms of genesis of portal vein tumor thrombus<sup>[56]</sup>. Regarding sensitivity and specificity, ten tumor markers were compared in the diagnosis of hepatoma. It was found that AFP-variant LCA reactive AFP, des-gamma carboxyprothrombin and GGT- II were superior to  $\alpha$  L-fucosidase,  $\alpha$ -1-AT, ALP- II, aldolase

isoenzyme and acidic ferritin. The sensitivity of the aforementioned markers was 84.4%, 72.3% and 79.7% and their specificity was 89.4%, 97% and 96.4%. The sensitivity of serum pyruvate kinase M2, hepatic cancer specific protein and HA<sub>18.1</sub> was 95.3%, 91.6% and 86.7%, respectively. The best screening procedures in the detection of primary hepatoma are AFP and ultrasound plus one of the three and this combination will cover AFP negative or low level of AFP patients to obtain an early diagnosis<sup>[57]</sup>. Serum soluble TNFR I level in liver cancer patients was found to correlate well with staging of the disease and response to chemotherapy. The frequency of increase of sTNFR I was 89.16%, greatly exceeding that of serum AFP (54.22%), and its determination could serve as a diagnostic aid in the detection of cancer and in the assessment of prognosis<sup>[58]</sup>. A high level of PC III was also seen in patients with hepatic carcinoma, PC III could be demonstrated within carcinoma cells by an immunofluorescence technique and carcinoma cells could produce PC III directly, hence its high level might also be taken as a marker of hepatic carcinoma.

## BILIARY SYSTEM

In a study of the phagocytic function of hepatic and pulmonary macrophages, 12 h after the onset of acute cholangitis caused by <sup>14</sup>C labeled living B. Coli, the phagocytic function of Kupffer cells decreased progressively and in contrast, the function of pulmonary alveolar macrophages increased continuously. The TNF secretion was increased in both cases. Another study on the effect of somatostatin on the sphincter of Oddi through endoscopic manometry found that somatostatin had significant inhibitory effect on the activity of the sphincter of Oddi and is beneficial to biliary and pancreatic flow. Epithelial tumor markers for extrahepatic bile ducts have been studied, 54.0% (22/42) were found to be positive for epithelial membrane antigen and 76.2% (32/42) positive for cytokeratin. The well differentiated adenocarcinoma had higher positive rate than the poorly differentiated. The two antigens were slightly more frequently present in precancerous than cancerous tissues but was lower in those with metastasis than those without. The absence of these markers in bile duct carcinoma signifies a poor prognosis. In another study using polyclonal antibody against C-erbB-2 protein by an immunohistochemical method, 26/41 cases of adenocarcinoma of the extrahepatic bile duct exhibited overexpression of C-erbB-2 on the cell membrane, indicating amplification of this gene in these cancers, and overexpression of this gene was also correlated with metastasis. Microvessels were found more abundantly by an immunohistochemical method of factor VIII related antigen in poorly differentiated gallbladder carcinoma than in well differentiated ones and in those with metastasis than those without. This indicates angiogenesis in gallbladder carcinoma is related to the histological pattern, the degree of differentiation and presence of metastasis. Therapeutic endoscopy is now widely used all over the country and sphincterotomy, dilatation and stent placement for stenosis of bile duct have all been performed.

## PANCREAS

In experimental acute hemorrhagic necrotizing pancreatitis, there was alteration of platelets and fibrinolytic function, the plasma granular membrane protein (GMP-140) and plasminogen activated inhibitor activity were found to be increased, platelet electrophoretic time much prolonged and tissue plasminogen activator activity much lower compared to sham operated dogs after induction of the disease. With treatment with Chinese herbal medicine tetramethylpyrazine, all the aforementioned alterations were absent, indicating that platelet activation and decreased fibrinolytic function play an important role in pancreatic microcirculatory disturbance and possible pancreatic microthrombosis. Tetramethylpyrazine has beneficial effects in correcting these disturbances, providing a therapeutic basis for its clinical use. Aside from the above, another herb, rhubarb (Da Huang) has been used in China for years in acute pancreatitis, including both the edematous and necrotizing forms, with great success before the advent of octreotide. In acute edematous pancreatitis, the cure rate is 100% and the time of the disappearance of abdominal pain, subsidence of fever and recovery of urinary amylase to normal were much shorter compared to the conventional Western therapy. In acute hemorrhagic necrotizing pancreatitis, rhubarb plus conventional

Western therapy without octreotide, atropine and gastrointestinal decompression resulted in the operative and mortality rates being much lower, 22.2% vs 66.6% and 3.3% vs 22.8%, respectively ( $P < 0.01$ ). The mechanisms of the actions of rhubarb are: Inhibiting trypsin, pancreatic lipase, elastase, kininogens, etc.; a broad spectrum antibiotic action for both aerobes and anaerobes; increasing the level of SOD; inhibiting absorption of endotoxins; lowering the blood viscosity, elevating the osmotic pressure and decreasing TXB<sub>2</sub>, improving the ratio of TXB<sub>2</sub>/PGF-1 $\alpha$  and pancreatic microcirculation; a hemostatic action; and in animal models, the intercellular tight junction and nuclear structure of the cells are restored to normal. Nowadays, we treat acute hemorrhagic necrotizing pancreatitis with either octreotide or a rhubarb mixture or both. This Chinese herbal medicine can also abolish intestinal paralysis and restore gastrointestinal tract function.

## REFERENCES

- Lo JY, Ed. Atlas of esophageal motility. Shanxi Science and Technology Publisher. September 1994
- Guo P, Xu GM, Zhou DW. 24 h esophageal pH monitoring in 50 healthy Chinese volunteers. *Zhonghua Xiaohua Zazhi* 1996; **16**: 32-34
- Liu M, Wu XN, Wang GL, Yeh RS. Esophageal manometric studies in patients with diabetes mellitus. *Zhonghua Xiaohua Zazhi* 1995; **15**: 200-202
- Xing JH, Ke MY, Chen YF, Wang ZF, Sun K, Zhang SJ. Effect of potent acid inhibition on the gastric and gallbladder emptying in man: its possible mechanism. *Changdao Bingxue* 1996; **1**: 7-10
- Xiang Z, Censini S, Bayeli PF, Telford JL, Figura N, Rappuoli R, Covacci A. Analysis of expression of CagA and VacA virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. *Infect Immun* 1995; **63**: 94-98 [PMID: 7806390]
- Wang WH, Hu FL, Jia BG, Ju FJ, Zhu LH. Differentiation of different *Helicobacter pylori* isolate using polymerase chain reaction and single-strand conformation polymorphism. *Zhonghua Xiaohua Zazhi* 1995; **15**: 9-12
- Pan ZJ, Xiao SD. Immunoblot analysis of immunoglobulin G antibodies to *Helicobacter pylori* and its clinical application. *Changdao Bingxue* 1996; **1**: 23-25
- Pan ZJ, Xiao SD, Jiang SJ, Kang HZ. Immune response to *Helicobacter pylori* in children with dysplasia. *Zhonghua Xiaohua Zazhi* 1995; **15**: 36-38
- Ma SH, Li S, Zhang XK, Zhang XR, Li XH, Ho J. Hydrophobicity changes of gastric mucosal surface and influence of *Helicobacter pylori* in patients with erosive gastritis and peptic ulcer. *Zhonghua Xiaohua Zazhi* 1995; **15**: 13-15
- Chen JX, Chen Q, Xu YR, Jiang ZM, Lu HM. Changes of ascorbic acid, Cu Zn SOD and LPO in gastric juice and blood in patients with *Helicobacter pylori* infection. *Zhonghua Xiaohua Zazhi* 1995; **15**: 389-341
- Liang H, Ding XT, Shi XL, Jia XL, Zhu KM. Electromicroscopic analysis of *Helicobacter pylori* infection in gastric mucosa. *Zhonghua Xiaohua Zazhi* 1995; **15**: 42-43
- Yu J, Zhang JK. Study on the relationship between *Helicobacter pylori* infection and the pathogenesis of gastric cancer by using molecular biologic techniques. *Zhonghua Xiaohua Zazhi* 1995; **15**: 28-30
- Chen MH, Hu PJ, Lee A. Experimental study of immunization against *Helicobacter pylori* in animal model. *Zhonghua Xiaohua Zazhi* 1995; **15**: 19-20
- Liu WZ, Xiao SD, Jiang SJ, Li RR, Pang ZJ. Seroprevalence of *Helicobacter pylori* infection in medical staff in Shanghai. *Scand J Gastroenterol* 1996; **31**: 749-752 [PMID: 8858741]
- Cheng JP, Xu CP, Chen SJ, Yu QW. Microdose capsule-based <sup>14</sup>C-urea breath test for the diagnosis of Hp infection. *Zhonghua Xiaohua Zazhi* 1995; **15**: 44-46
- Liu GL, Wu JC, Zhang ZH, Mou YL, Chen Q, Yang QQ. Detection of *Helicobacter pylori* in human using <sup>15</sup>N-area method. *Zhonghua Xiaohua Zazhi* 1995; **15**: 18-19
- Wang JR, Liu WW, Deng GR, Leu YY, Li JY. A study of relationship of point mutation of ras oncogene at codon 12 with prognosis of gastric cancer patients. *Zhonghua Xiaohua Zazhi* 1995; **15**: 133-135
- Liu GM, Jiang R, Zhang YG. Changes in blood, urine, saliva hEGF in patients with gastric carcinoma. *Zhonghua Xiaohua Zazhi* 1996; **16**: 79-81
- Shu XZ, Yang QH, Cong XQ, Jiang SJ, Xiao SD. Effect of parvoviral NS gene expression on gastric cancer cells. *Changdao Bingxue* 1996; **1**: 26-28
- Zhang G, Zhong HM, Xing PJ, Wang SX. Measurement of serum tumor-associated oligosaccharide antigen-G by rapid ELISA in patients with gastric and colonic cancer. *Zhongguo Xin Xiaohuabingxue Zazhi* 1996; **4**: 69-70
- Zhao ZS, Liu FK, Liu JH, Tang HD, Wang SW, Zhong J. Detection of bone marrow micrometastasis in patients with gastric cancer by antihuman epithelial membrane antibody and its clinical value. *Zhonghua Xiaohua Zazhi* 1995; **15**: 342-344
- Tan DJ, Wang HR, Wang HE, Chen CG. The effect of estrogen and its receptor antagonist on multiplication of gastric cancer cells. *Zhongguo Xin Xiaohuabingxue Zazhi* 1996; **4**: 64-65
- Be F, Zhang XY, Mu ZX, Wu ZP, Fan DM, Hu JL. The cytotoxic effect of gastric cancer manifested by LAK cells together with antigastric cancer monoclonal antibody. *Changdao Bingxue* 1995; **1**: 20-22
- Ran ZH, Shan MJ, Xiao SD. Gastrointestinal bleeding of obscure origin analysis of 50 cases. *Zhonghua Xiaohua Zazhi* 1996; **16**: 266-268
- Fu H, MoSJ, Cao SL, Zhu WJ, Liu SL, Tang JX. Investigation of DNA ploidy status, cytokinetic and female hormonal receptors on nude mice with human colonic cancer. *Zhonghua Xiaohua Zazhi* 1996; **16**: 148-151
- Zhao RH, Wang YH, Chen YL, Ye TL, Chen T. Changes of the somatostatin levels in tumors and surrounding mucosa in colorectal cancer patients and its clinical significance. *Zhonghua Xiaohua Zazhi* 1995; **15**: 149-152
- Yuan HJ, Hu DC, Zhai WR, Xu YH, Liu I. Expression of hepatitis C virus NS4 antigen in liver tissue of patients with hepatitis. *Liver* 1996; **1**: 21-24
- Qiu JH, Lu ZM, Zhang DH, Chen Z. Cloning and sequencing of cDNA of C33c protein gene in NS3 region of HCV from Shanghai and Jiangsu Province. *Zhonghua Chuanranbingxue Zazhi* 1995; **13**: 133-136
- Yuan HJ, Hu DC, Qu WR, Zhang QP, Yu YH, Zhong K. Changes in hepatitis C virus NS4 antigen in liver induced by IFN therapy. *Changdao Bingxue* 1996; **1**: 14-16
- Yao GB. Clinical aspect of viral hepatitis in China. In: Tang ZY, Ye SL, Qiu SJ, editors. Recent Progress in Liver Cancer and Hepatitis. Beijing: International Academic Publisher, 1996: 43-45
- Zhou XJ, Zhang TH. Pathologic finding in acute hepatitis C. *Liver* 1996; **1**: 25-27
- Zhao QR, Huang SS, Zhao DT. Epidemiological study of hepatitis D in China. *Linchuang Gandanbing Zazhi* 1995; **11**: 179-180
- Yiang SS, Yao GB, Xu DZ, Zhang DF, Loh ZM, Fu SS. A clinical study on treatment of chronic viral hepatitis B using domestic recombinant human interferon alpha-1. *Zhonghua Xiaohua Zazhi* 1995; **15**: 194-197
- Wen YM, Ma ZM, Wang Y, Kong YY. Studies on therapeutic vaccine of viral hepatitis B. In: Recent progress in liver cancer and hepatitis, Tang ZY, Ye SL, Qiu SJ, editors. Beijing: International Academic Publisher, 1996: 38-39
- Zeng MD, Zhang JG, Qiu DK, Li JQ, Wu ZH, Xiao SD. Experimental studies of the effects of hormones and growth factors on the growth of fibroblasts in serum-free cultured medium. *Zhonghua Xiaohua Zazhi* 1995; **15**: 153-155
- Zhang FQ, Wang SZ. Diagnostic value of serum collagen I in hepatic fibrosis. *Linchuang Gandanbing Zazhi* 1996; **12**: 17-18
- Yang YP, Sun SS, Chen DY, Chen JM, Zhang B. Lipocyte proliferation and modulation of collagen synthesis of IFN-Y, IL-2, IL-6 and tocopherol. *Linchuang Gandanbing Zazhi* 1995; **11**: 72-74
- Zhang YJ. Advances in research and clinical use of hepatocyte growth factor. *Linchuang Gandanbing Zazhi* 1995; **11**: 80-82
- Fan LY, Kung ST, Gao F, Gao ZF, Hou J. Influence of tetrandrine on DNA and collagen synthesis of fibrosis and human fetal hepatocytes. *Linchuang Gandanbing Zazhi* 1996; **12**: 25-26
- Li DG, Liu YL, Lu HM, Pan XL, Xu JF. The modulatory effects of cinnarizine on fibroblasts analyzed by flow cytometry. *Zhonghua Xiaohua Zazhi* 1995; **15**: 156-158
- Gao CF, Wang H, Kong XT. The effect of vitamin A on 3T3 cells. *Zhongguo Xin Xiaohuabingxue Zazhi* 1996; **4**: 9-11
- Tang ZD, Jin XH, Xie JL, Shen HL, Bai JH, Xiao ZQ. Protein calorie malnutrition in patients with posthepatic cirrhosis of the liver and its treatment. The Third International Conference of Gastroenterology, Hong Kong and Shanghai, 1995: 61
- Wang MQ, Zhang JS, Yu M, Xing ZZ, Yang L, Huang YZ. Transjugular intrahepatic protosystemic shunt stent: Results in 102 patients. *Zhonghua Xiaohua Zazhi* 1996; **16**: 128-131
- Wu SJ, Li JS, Cao JM, Wu XH, Chen JK, Han JM. Clinical study of transjugular intrahepatic protosystemic stent in portal hyperkinetic. *Zhonghua Xiaohua Zazhi* 1996; **16**: 132-133
- Zhang PL, Liang KH, Zhang WY, Liang JS, Yang ZL. The role of nitric oxide in arterial vasodilatation of cirrhotic rats. *Zhonghua Xiaohua Zazhi* 1995; **15**: 333-335
- Gu JR. Gene related to human hepatic cancer. Recent progress in liver cancer and hepatitis. Shanghai International Symposium, Tang ZY, Ye SL, Qiu SJ, editors. Beijing: International Academic Publisher, March 1996: 4
- Ibid 1996: 64-84
- Yang XB, Wang MW, You WD, Yu G. Detection of HBxAg in hepatic cancer tissue with immunohistochemical (LSAB) method and its significance. *Linchuang Gandanbing Zazhi* 1996; **12**: 68-70
- Liang YR, Wu MY, Liu Y, Huang ZZ, Tan DM. Expression of HBcAg on hepatocellular carcinoma and paracancerous tissue. *Linchuang Gandanbing Zazhi* 1995; **11**: 20-22
- Shou ZP, Dai YM, Ni CR, Wang NJ, Zhang SP. Expression of hepatitis C virus RNA and HcAg in human hepatocellular carcinoma and its surrounding liver tissue. *Zhonghua Xiaohua Zazhi* 1995; **15**: 191
- Wang CT, Wang NL, Wang BY. Localization and significance of hepatitis C viral antigen C33c and HBxAg in human primary hepatic carcinoma. *Zhonghua Xiaohua Zazhi* 1996; **16**: 72-74
- Zhen JL, Liu BL, Nie ZS, Huang XL, Du XD. Expression of EGF in hepatic cirrhosis with hepatocellular carcinoma and their biological significance. *Linchuang Gandanbing Zazhi* 1996; **12**: 15-16
- Kong KY, Zhang J, Lu GJ, Xu SP, Chen YF. The effect of EGF on the growth of human hepatoma cells and the regulatory action of somatostatin on EGF receptors. *Zhonghua Xiaohua Zazhi* 1995; **15**: 129-132
- Wu ZH, Zhan MD, Chen SH, Li JQ, Qiu DK. Growth characteristics of mouse hepatoma cells transfected with IL-2 gene, an in vitro and in vivo study. *Liver* 1996; **1**: 6-10
- Fu QC, Xu DH, Zhang ZC, Ge K, Liu SH, Chen ZW. Preliminary studies on the effects of direct gene transfer with liposome interleukin-2 gene complex on hepatocellular carcinoma in mice. *Liver* 1996; **1**: 11-15
- Zheng YX, Yu YQ, Liu KD, Shi DR, Zhou HQ, Lou HF. Correlation between portal vein tumor thrombogenesis and expression of p53 and PCNA in hepatocellular carcinoma. *Zhonghua Xiaohua Zazhi* 1996; **16**: 75-78
- Shen DM. Present status of primary liver cancer related tumor markers in China. Recent progress in liver cancer and hepatitis. Shanghai International Symposium, Tang ZY, Ye SL, Qiu SJ editors. Beijing: International Academic Publisher, March 1996: 14-15
- Wang YF, Wu XN, Wu Q, Zhang XQ, Chen XF, Zhou XH. The biological significance of serum soluble tumor necrosis factor receptor I in hepatoma patients. Recent progress in liver cancer and hepatitis. Shanghai International Symposium, Tang ZY, Ye SL, Qiu SJ, editors. Beijing: International Academic Publisher, March 1996: 76



Published by **Baishideng Publishing Group Inc**  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

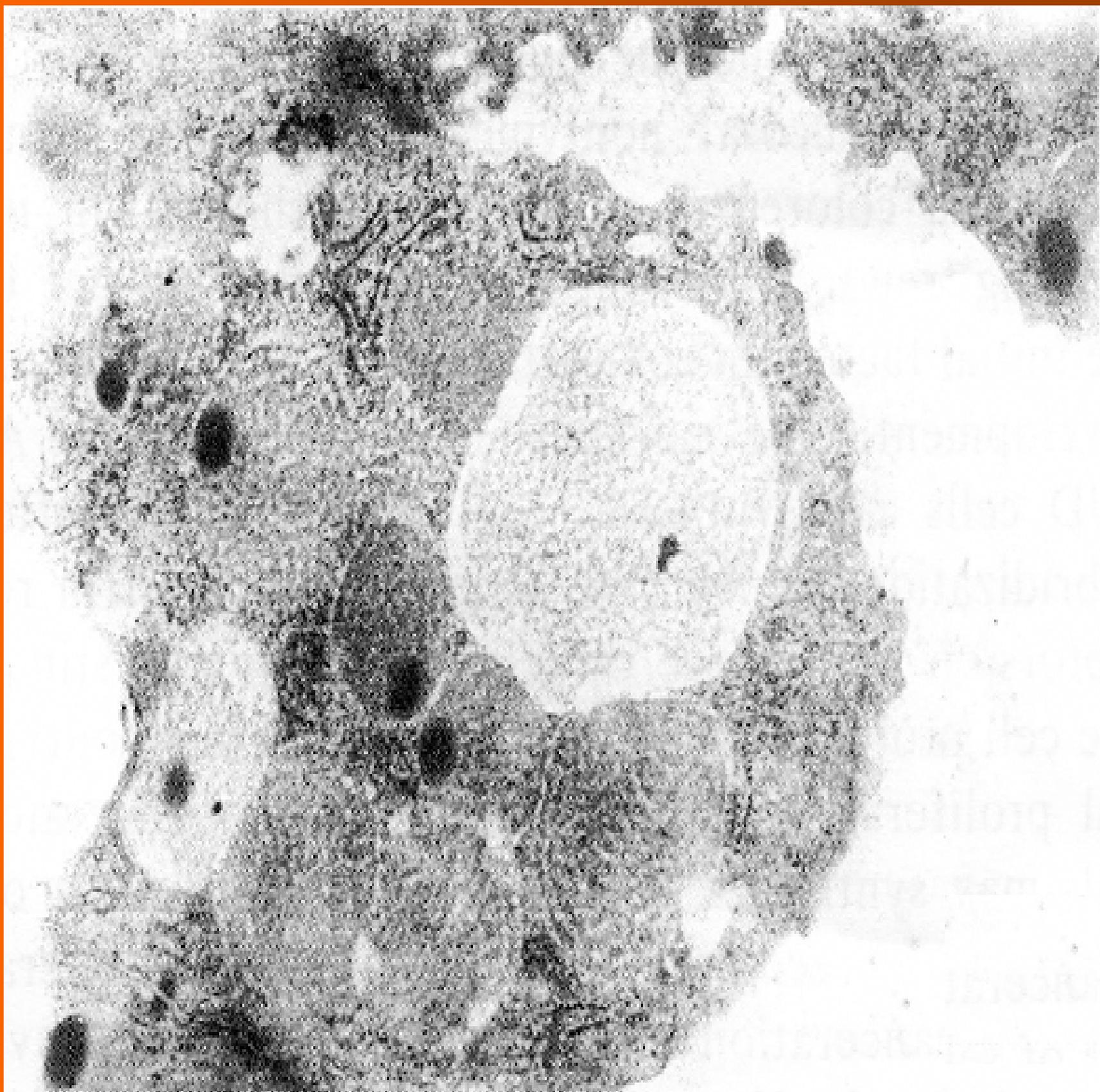


ISSN 1007-9327



# World Journal of *Gastroenterology*

*World J Gastroenterol* 1997 June 15; 3(2): 61-130



**COMMENTARY**

- 61 Iron, HCV and the liver  
*Maier KP*

**ORIGINAL RESEARCH**

- 64 Histopathology and immunohistochemistry of large hepatocellular carcinoma with undetectable or low serum levels of alpha-fetoprotein  
*Wu QM, Hu MH, Tan YS*
- 67 Recovery of allografted small intestine function  
*Jiang ZW, Li JS, Li N, Li YS, Liu FL, Sheng XQ, Cheng YM.*
- 69 Effect of octreotide on cell-cycle kinetics and serum carcinoembryonic antigen level in hepatic metastases of colonic adenocarcinoma  
*Liu R, Wang YH, Tang Y, Cao GS*
- 72 Fos expression in catecholaminergic medullary neurons induced by chemical stimulation of stomach projecting to the paraventricular nucleus of the hypothalamus in rats  
*Dong YX, Xiong KH, Rao ZR, Shi JW*
- 75 Characteristics of DNA repair induced by DNA polymerase  $\beta$  in hepatoma cells after  $\gamma$ -ray irradiation  
*Cai JM, Zheng XL, Luo CJ, Gao JG, Cheng TM*
- 78 Protective effect of vitamin E on age-related alterations of Kupffer cell energy metabolism  
*Sun WB, Ma RL, Peng ZM, Li K, Duan HC, Han BL*
- 81 Protective effect of rhubarb on the intestinal mucosal barrier  
*Chen DC, Yang XY, Zhang XY, Chen XY*
- 84 Immunohistochemical study of gastrin in colorectal carcinoma tissues and adjacent mucosa  
*Jiang CP, Chen YQ, Zhu JW, Shen HX, Yu X*
- 87 Changes in *p53* and *Waf1p21* expression and cell proliferation in esophageal carcinogenesis  
*Wang LD, Yang WC, Zhou Q, Xing Y, Jia YY, Zhao X*
- 90 Morphological analysis of lymph vessels and capillaries in gastric carcinoma  
*Liao XB, Tang WP, Zhang QM, Fu ZG, Zhao YH*
- 93 Pathogenetic effects of salted pork in an area of China with high-risk for stomach cancer  
*Yuan Y, Lin HZ, Zhang YC, Wang XJ, Wu YQ, Gao H, Wang L, Liu YH, Lu F, Lou SQ*
- 95 Evaluation of preoperative staging in advanced gastric cancer with MRI  
*Tang GY, Guo QL, Xin PP*
- 98 Clinical significance of PCR in *Helicobacter pylori* DNA detection in human gastric disorders  
*Xu GM, Ji XH, Li ZS, Man XH, Zhang HF*

- 101 Immunohistochemical detection of proliferating cell nuclear antigen in hepatocellular carcinoma  
*Wang D, Shi JQ, Liu FX*
- 104 Hepatic arterial infusion chemotherapy and embolization in the treatment of primary hepatic carcinoma  
*Xu LM, Liu C, Liu P*
- 108 Hepatitis C virus RNA detection in serum and peripheral blood mononuclear cells of patients with hepatitis C  
*Zhou P, Cai Q, Chen YC, Zhang MS, Guan J, Li XJ*
- 111 A prospective study of vertical transmission of hepatitis C virus  
*Sun DG, Liu CY, Meng ZD, Sun YD, Wang SC, Yang YQ, Liang ZL, Zhuang H*
- 114 Preparation and application of monoclonal antibodies against hepatitis C virus nonstructural proteins  
*Gao JE, Tao QM, Guo JP, Ji HP, Lang ZW, Ji Y, Feng BF*
- 117 Expression of insulin-like growth factor II and its receptor in liver cells from patients with chronic liver diseases  
*Yang DH, Xiu C, Yang B, Gu JR, Qian LF, Qu SM*
- 119 Inflammatory bowel disease in the Hubei Province of China  
*Xia B, Shivananda S, Zhang GS, Yi JY*
- 121 Relationship between loss of heterozygosity of *deleted in colorectal carcinoma* gene microsatellites and prognosis of colorectal adenocarcinoma  
*Zhao P, Hu YC, Wang DW, Wang ZP, Xu XZ, Yi PY, Gao YB, Yang GH*
- 123 Radiotherapy of 180 cases of operable esophageal carcinoma  
*Chen DF, Yang ZY, Yin WB*

**TRADITIONAL MEDICINE**

- 127 Effect of muscarinic blocker on enhancing the action of fructus aurantii immaturus on intestinal myoelectric activity in dogs  
*Huang ZH, Yang DZ, Wei YQ, Luo YH*
- 129 Effect of remedies for enhancing resistance and relieving blood stasis on metastasis in postoperative gastric cancer and ornithine decarboxylase levels  
*Bu P*

**BRIEF REPORT**

- 68 Effects of tetrandrine and verapamil on fibroblastic growth and proliferation  
*Liu XS, Li DG, Lu HM, Xu QF*
- 71 Effect of faeces troglodyterorum extract B<sub>1</sub> on the experimental gastric ulcer and gastric secretion in rats  
*Wang XW, Cheng ZA, Li QM, Liu WZ*
- 77 Short- and long-term effect of interferon therapy for chronic hepatitis C  
*Tang ZY, Qi JY, Shen HX, Yang DL, Hao LJ*
- 80 Alterations in p53 expression and cell proliferation in esophageal epithelia among patients from geographical areas with high or low incidence of esophageal cancer  
*Wang LD, Zhou Q, Zhang YC, Li XF, Wang WP, He L, Gao SS, Li YX*

- 83 Exploration of the immunoreactivity of the Traditional Chinese medicine Shenrouyangzhentang to vasoactive intestinal polypeptide  
*Gu YC, Chen DZ*
- 86 Ultrastructural cytochemical study of enzymes expressed by signet ring cells in gastric cancer  
*Yang GL, Dong YM, Du WD, Su YH, Zhang H, Wu JF, Wang DB, Xu AL*
- 103 Effect of amygdalin on proliferation of rat hepatic fat-storing cells and collagen production *in vitro*  
*Xu LM, Liu C, Liu P*
- 110 Expression and analysis of McAbs antigen against human hepatocellular carcinoma  
*Mi L, Chen ZN*
- 113 Study on clinical pathology and immunohistochemistry of chronic erosive gastritis  
*Ke CS, Li DF, Wang W, Wang YM*
- 120 Preliminary study on the pathological model of Piyinxu in rats  
*Chen DZ, Wei MX*
- 116 Clinical and experimental study on therapeutic effect of Weixibaonizhuanwan on gastric precancerous lesions  
*Zhang XC, Gao RF, Li BQ, Ma LS, Mei LX, Wu YZ, Liu FQ, Liao ZL*
- 122 Expression of the c-erbB-2 proto-oncogene product in gastric carcinoma and precancerous lesions  
*Mi JQ, Yang SQ, Shen MC*

**ABOUT COVER**

Jiang CP, Chen YQ, Zhu JW, Shen HX, Yu X. Immunohistochemical study of gastrin in colorectal carcinoma tissues and adjacent mucosa. *World J Gastroenterol* 1997; 3(2): 84-86

**AIMS AND SCOPE**

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup> each month. The *WJG* Editorial Board consists of 1376 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

**INDEXING/ABSTRACTING**

*World Journal of Gastroenterology* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. According to the 2014 Journal Citation Reports® released by Thomson Reuters (ISI), the 2014 impact factor for *WJG* is 2.369, ranking 41 among 76 journals in gastroenterology and hepatology, quartile in category Q2.

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Wen-Xi Liu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ze-Mao Gong*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Gastroenterology*

**ISSN**  
ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

**LAUNCH DATE**  
October 1, 1995

**FREQUENCY**  
Quarterly

**EDITORS-IN-CHIEF**  
**Bo-Yong Pan**, President of China Speciality Council of Gastrology and China Association of Huatuo Medicine, Room 12, Building 621, the Fourth Military Medical University, Xi'an 710033, Shaanxi Province, China

**Lian-Sheng**, Member of the Speciality Committee of Digestive Diseases, Chinese Association of Combined Traditional Chinese and Western Medicine, Taiyuan Research and Treatment Centre for Digestive Diseases, Taiyuan 030001, Shanxi Province, China

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Gastroenterology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: bpgoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esp/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
June 15, 1997

**COPYRIGHT**  
© 1997 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_19970116143427.htm](http://www.wjgnet.com/bpg/g_info_19970116143427.htm)

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esp/>

## Iron, HCV and the liver

KP Maier

KP Maier, MD, Department of Medicine, Division of Gastroenterology, City Hospital Esslingen, Acad. Department of University of Tuebingen, Germany

Author contributions: The author solely contributed to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: KP Maier, MD, Professor, Department of Medicine, Division of Gastroenterology, City Hospital Esslingen, Acad. Department of University of Tuebingen, Germany

Received: October 25, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

**Key words:** Iron; Hepatitis C virus; Liver; Hepatitis C© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.Maier KP. Iron, HCV and the liver. *World J Gastroenterol* 1997; 3(2): 61-63  
Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/61.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.61>

### IRON

Iron is an essential element necessary for the survival of cells. Excess or deficiency of iron leads to diseases. The liver is a major target organ of injury in diseases causing iron overload, which is consistent with its central role in iron storage and metabolism<sup>[1]</sup>. Most of the iron is absorbed in the non-heme form, largely in the upper part of the small intestine. There is no physiological mechanism for the excretion of excessive iron. Therefore, normal iron balance is maintained by the regulation of iron absorption, although the underlying mechanism still remains unclear<sup>[2]</sup>. In blood, iron is bound to transferrin, a glycoprotein synthesized in the liver. Transferrin is responsible for the "total iron-binding capacity" of serum. Physiologically, this is saturated to one-thirds with iron. The hepatic uptake of iron from the blood is receptor mediated<sup>[3]</sup>. The receptor-iron complex is internalized and iron is released. However, alternative pathways of iron uptake across the plasma membrane of the liver cell seem to play important roles in iron-overload states<sup>[3,4]</sup>. In the liver, iron depots in the form of ferritin or hemosiderin are available for metabolization and hemoglobin formation, should the demand arise. In diseases of iron overload, the binding capacity of the iron-storage proteins (ferritin and hemosiderin) are saturated, leading to the accumulation of ionized ("free") iron in the cytosol.

Why is free iron ("prooxidant iron") toxic to the liver?

Despite convincing clinical evidence for the occurrence of liver

injury as a result of excessive iron accumulation, the specific pathophysiological mechanisms involved in this process are poorly understood. Some aspects, however, have aroused interests: (1) Iron-induced oxidative injury to phospholipid of subcellular membranes of liver cells seems to play a central role in initiating liver damage. Experimentally, iron overload leads to peroxidation, especially in mitochondria and microsomal membranes<sup>[5,6]</sup>, thereby resulting in decreased energy production by interference with the electron transport chain<sup>[7]</sup>. Therefore, one of the key characteristics of the pathogenesis of iron-induced liver damage is increased oxidative stress, which ultimately leads to cell death and secondary fibrogenesis; (2) There appears to be a direct iron-induced oxidative damage of nucleic acid. DNA strand breaks in rat hepatic nuclei subjected to iron-induced lipid peroxidation have been demonstrated *in vitro*<sup>[8]</sup>. Hepatocellular carcinoma (HCC) is a common complication of long-standing cirrhosis in genetic hemochromatosis, and iron-induced damage to DNA may play an important role in its development; and (3) A fibrous tissue reaction is observed wherever the iron is deposited. This is particularly true for patients with genetic hemochromatosis<sup>[9]</sup>. Therefore, it has been suggested that the triggering factor of fibrogenesis appears to be the accumulation of iron in the form of aggregates of activated non-parenchymal cells<sup>[10]</sup>.

It should be noted that in genetic hemochromatosis, hepatic fibrosis appears only subsequent to iron overload of Kupffer cells after the occurrence of sideronecrosis of periportal hepatocytes<sup>[11]</sup>. Thus, activation of Kupffer cells by a necrotic event might be required for initiation of the fibrogenetic process in iron overload states<sup>[10]</sup>.

Thus far, increased lipid peroxidation, and hence fibrosis, has been clearly demonstrated in several experimental *in vivo* models of iron overload<sup>[10,11]</sup>. Recent data in the gerbil model provide deeper insight into the molecular events underlying the profibrogenic action of iron and have shown that parenteral iron overload leads to hepatic cirrhosis<sup>[12]</sup>. In the animal model of iron overload, a dramatic increase of signals for collagen mRNA around iron foci in fat-storing liver cells has been documented<sup>[10]</sup>. The pattern of iron distribution in the liver of this animal model resembles that of secondary iron overload states, with the metal being mainly present in non-parenchymal cell foci<sup>[10]</sup>. The concept of the need for Kupffer cell activation by a necrogenic event for the initiation of the fibrogenic process is consistent with the findings obtained in genetic hemochromatosis, in which iron itself causes sideronecrosis of hepatocytes and activation of Kupffer cells. Interestingly, at least in the animal model, antioxidative treatment with dietary vitamin E supplementation arrested fibrogenesis and completely reversed hepatic cirrhosis, possibly by a blocking activity exerted on hepatic non-parenchymal cell proliferation triggered by iron<sup>[10]</sup>.

### IRON AND HEPATITIS C VIRUS

It has been reported that iron is also an important factor involved in

**Table 1 Laboratory and histological findings in transfusion-dependent patients with thalassemia major and chronic hepatitis C before interferon therapy<sup>[25]</sup>**

Lab and histologic findings	Responders (n = 24)	Non-responders (n = 30)	Control subjects (n = 14)
Age (yr)	153 ± 34	145 ± 29	115 ± 29
Units of blood received	369 ± 145	318 ± 117	243 ± 86
ALT (U/L)	270 ± 180	255 ± 46	244 ± 50
Serum ferritin (µg/L)	1812 ± 962	2463 ± 850	1621 ± 745
Hepatitis C virus-RNA	Positive	Positive	Positive
Hepatic histologic findings	CAH	CAH	CAH

the progression of chronic liver disease and that the effect of iron is concomitant with that of other hepatotoxins (viruses, alcohol) which are able to initiate a damaging event in the liver<sup>[10]</sup>.

Markers of viral infection are present in about one-fourth of all Italian patients with genetic hemochromatosis<sup>[13]</sup>. The prevalence of HBsAg in Italian patients with genetic hemochromatosis has been reported to be slightly more than double (5%) that in a healthy population<sup>[14]</sup>. The prevalence of anti-HCV in this group, however, reached 20.5%. Interestingly, although most of the patients with associated viral hepatitis had cirrhosis and their serum ferritin levels and amount of mobilizable iron were significantly lower than those in patients with fibrosis/cirrhosis ( $P < 0.01$ ) without concomitant viral infection; this suggests that hepatitis viruses may act synergistically with iron in accelerating the liver damage<sup>[14]</sup>. It has recently been shown that some viruses may promote hepatocyte damage by activating lipid peroxidation *via* an iron-mediated mechanism<sup>[15]</sup>. This evidence is consistent with the finding that Japanese encephalitis virus causes the accumulation of iron in the liver and spleen, apparently by stimulating the release of a macrophage-derived iron-regulating factor that could be related to IL-8<sup>[16]</sup>. Another consideration is that chronic viral hepatitis might alter hepatocyte function in such a manner that there is increased hepatocellular iron concentration, perhaps by upregulating transferrin receptor expression<sup>[17]</sup>.

Elevated iron and ferritin levels have been reported in the serum of some patients with hepatitis B virus (HBV) and hepatitis C virus (HCV) infections<sup>[18]</sup>. Moreover, parenchymal iron overload has been shown in the nontumorous liver of most patients presenting with HCC developed in a non-cirrhotic liver<sup>[19]</sup>. It must be stressed, however, that in a large series of 80 patients with chronic viral hepatitis, only very few had elevated hepatic iron concentrations, despite the fact that elevated iron and ferritin levels in the serum were present in about one-third of the patients<sup>[18]</sup>. Thus, it has been concluded that abnormal results of serum iron status tests are clinically insignificant. Other authors, however, found significantly elevated serum ferritin concentration and elevated tissue iron specifically in some patients with HCV infection<sup>[20,21]</sup>. In fact, 59% of patients with HCV infection had high ( $> 26.85 \mu\text{mol/L}$ ) serum iron levels, whereas only 14% of the HBV-infected patients and 21% of the patients with a non-viral etiology had abnormal serum iron levels<sup>[21]</sup>. Further, serum transferrin saturation was significantly higher in HCV patients than in others<sup>[21]</sup>. Interestingly, there was no significant correlation with the degree of inflammation or transaminases, thus confirming that increased storage of iron is not a function of the inflammatory process *per se*<sup>[20]</sup>. Surprisingly, HBV infection was not associated with increased markers of iron metabolism. Therefore, it has been speculated that in contrast to HBV infection, HCV infection triggers free radical production, which may lead to interference with mitochondrial function<sup>[19]</sup>.

## HCV, IRON AND INTERFERON (IFN) TREATMENT

There is some evidence that patients chronically infected with HCV have a decreased response to (IFN) therapy in the presence of iron overload<sup>[22,23]</sup>.

This feature seems to be specific for HCV infection. It has been discussed that both the increased serum iron concentration and transferrin saturation blunt the action of IFN, since they have opposite effects on the immune system<sup>[22]</sup>. Bacon *et al*<sup>[24]</sup> studied

8 non-responders to IFN therapy, who were subjected to repetitive phlebotomies. Seven of them had a significant decrease in serum alanine transaminase (ALT) activity. After a second course of IFN (3 million units three times per week for 4 mo), the serum ALT level restored to the normal range in 3 of 8 patients. This led the authors to conclude that iron depletion seems to improve the response of chronic HCV infection to IFN therapy. In patients with thalassemia major and chronic hepatitis C, non-responders and responders to IFN did not differ with regard to mean ALT activity (Table 1). However, serum ferritin concentration was markedly elevated in non-responders, and response to therapy was inversely related ( $P < 0.002$ ) to the liver iron burden<sup>[25]</sup>.

Olynik *et al*<sup>[26]</sup> found that hepatic iron concentration (HIC) was a good predictor of response to IFN therapy in 58 patients with chronic hepatitis C. Overall, 41% of their patients responded to therapy. However, in a subgroup of patients with an HIC of  $> 1100 \mu\text{g/g}$  liver, 88% failed to respond favorably to IFN therapy. The reason for the higher HIC in patients not responding to IFN therapy remained unclear. The authors thought that iron could impair host lymphocyte-dependent clearance of HCV. They proposed to determine HIC before starting IFN treatment in cases of chronic HCV infection. In another study<sup>[27]</sup>, the mean liver iron concentration of non-responders was also significantly higher than that of the responders (1252  $\mu\text{g/g}$  dry weight vs 828  $\mu\text{g/g}$  dry weight). It should be mentioned, however, that both of these mean values were within the normal range of HIC. The authors thought that an "iron threshold" of 600  $\mu\text{g/g}$  dry weight would characterize patients not responding to IFN therapy. Similar results were obtained in a study of 44 patients with chronic HCV infection<sup>[28]</sup>. Again, liver iron concentration was in the normal range, but nearly twice as high in non-responders as compared to responders. Thus far, there is no agreement regarding which of the iron indices most accurately predicts response to therapy. In a recent study<sup>[29]</sup>, total hepatic iron scores, mean serum ferritin level as well as mean quantitative HIC were higher in patients with incomplete IFN response, however without reaching statistical significance. Interestingly, the morphologic distribution of iron in liver biopsies and not total liver iron differed significantly in IFN responders and non responders. Scores for stainable iron in sinusoidal cells and portal tracts were significantly lower in responders ( $P = 0.02$  and  $P = 0.05$ , respectively) to IFN therapy as compared to non-responders.

At present, it is unclear whether iron removal in patients with chronic HCV infection enhances the effect of IFN. In a pilot study conducted on 26 patients, repeated phlebotomies were performed to remove excessive iron (with the endpoint set at serum ferritin concentration below 10  $\mu\text{g/L}$ ) before initiation of IFN treatment. Again, iron removal alone effectively reduced the serum ALT levels [from 125 U/L to 49 U/L (mean)], but did not enhance the effect of treating viremia<sup>[30]</sup>. Whatever the practical consequences of this studies are, it should be mentioned that significant reductions in serum ALT levels were achieved by phlebotomy alone<sup>[24]</sup> and that compared to IFN therapy alone, phlebotomy plus IFN therapy resulted in greater reduction of serum ALT levels and improvement in histological picture<sup>[31]</sup>.

## CONCLUSIONS

Recent studies have suggested that there is a close link between iron metabolism and viral hepatitis, especially hepatitis C. Some studies seem to indicate that the total quantity of iron present in the liver as well as the lobular and cellular distribution of iron are important determinants of the long-term outcome. Interestingly, Kupffer cell function may play a critical role, since the degree of stainable iron in these cells or cells in the portal tracts is significantly lower in complete responders as compared to those in non-responders or incomplete responders<sup>[29]</sup>.

These findings lead to the conclusion that patients with lesser amounts of hepatic iron respond better to antiviral therapy than those with larger amounts of hepatic iron. It is assumed that differences in the pretreatment levels of total hepatic iron and serum ferritin are less striking than those in the cellular and zonal

distribution of iron. However, whether iron removal prior to IFN therapy enhances the percentage of IFN responders in chronic HCV infection is open to discussion. An ongoing randomized controlled study (phlebotomy ribavirin interferon; PRINT) in IFN non-responders with chronic hepatitis C is expected to further clarify whether iron removal will prove useful in the long-term management of this disease.

## ACKNOWLEDGMENTS

This work has been generously supported by the Breuninger Stiftung, Stuttgart, Germany.

## REFERENCES

- 1 **Deugnier YM**, Loréal O, Turlin B, Guyader D, Jouanolle H, Moirand R, Jacquelinet C, Brissot P. Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their biochemical correlations. *Gastroenterology* 1992; **102**: 2050-2059 [PMID: 1587423]
- 2 **Halliday JW**. The regulation of iron absorption: one more piece in the puzzle? *Gastroenterology* 1992; **102**: 1071-1073 [PMID: 1537499]
- 3 **Bonkovsky HL**. Iron and the liver. *Am J Med Sci* 1991; **301**: 32-43 [PMID: 1847276 DOI: 10.1097/0000441-199101000-00006]
- 4 **Adams PC**, Powell LW, Halliday JW. Isolation of a human hepatic ferritin receptor. *Hepatology* 1988; **8**: 719-721 [PMID: 2839401 DOI: 10.1002/hep.1840080402]
- 5 **Britton RS**, Ferrali M, Magiera CJ, Recknagel RO, Bacon BR. Increased prooxidant action of hepatic cytosolic low-molecular-weight iron in experimental iron overload. *Hepatology* 1990; **11**: 1038-1043 [PMID: 2365281 DOI: 10.1002/hep.1840110620]
- 6 **Hanstein WG**, Sacks PV, Muller-Eberhard U. Properties of liver mitochondria from iron-loaded rats. *Biochem Biophys Res Commun* 1975; **67**: 1175-1184 [PMID: 1201066 DOI: 10.1016/0006-291X(75)90797-4]
- 7 **Bacon BR**, Healey JF, Brittenham GM, Park CH, Nunnari J, Tavill AS, Bonkovsky HL. Hepatic microsomal function in rats with chronic dietary iron overload. *Gastroenterology* 1986; **90**: 1844-1853 [PMID: 3009259]
- 8 **Shires TK**. Iron-induced DNA damage and synthesis in isolated rat liver nuclei. *Biochem J* 1982; **205**: 321-329 [PMID: 7138506 DOI: 10.1042/bj2050321]
- 9 **Gordeuk VR**, Bacon BR, Brittenham GM. Iron overload: causes and consequences. *Annu Rev Nutr* 1987; **7**: 485-508 [PMID: 3300744 DOI: 10.1146/annurev.nu.07.070187.002413]
- 10 **Pietrangelo A**, Gualdi R, Casalgrandi G, Montosi G, Ventura E. Molecular and cellular aspects of iron-induced hepatic cirrhosis in rodents. *J Clin Invest* 1995; **95**: 1824-1831 [PMID: 7706489]
- 11 **Bacon BR**, Britton RS. The pathology of hepatic iron overload: a free radical-mediated process? *Hepatology* 1990; **11**: 127-137 [PMID: 2153094 DOI: 10.1002/hep.1840110122]
- 12 **Iancu TC**, Rabinowitz H, Brissot P, Guillouzo A, Deugnier Y, Bourel M. Iron overload of the liver in the baboon. An ultrastructural study. *J Hepatol* 1985; **1**: 261-275 [PMID: 4067258 DOI: 10.1016/S0168-8278(85)80054-4]
- 13 **Lustbader ED**, Hann HW, Blumberg BS. Serum ferritin as a predictor of host response to hepatitis B virus infection. *Science* 1983; **220**: 423-425 [PMID: 6301008 DOI: 10.1126/science.6301008]
- 14 **Piperno A**, Fargion S, D'Alba R, Roffi L, Fracanzani AL, Vecchi L, Failla M, Fiorelli G. Liver damage in Italian patients with hereditary hemochromatosis is highly influenced by hepatitis B and C virus infection. *J Hepatol* 1992; **16**: 364-368 [PMID: 1487615 DOI: 10.1016/S0168-8278(05)80671-3]
- 15 **Schwarz KB**, Larroya S, Vogler C, Sippel CJ, Homan S, Cockrell R, Schulze I. Role of influenza B virus in hepatic steatosis and mitochondrial abnormalities in a mouse model of Reye syndrome. *Hepatology* 1991; **13**: 96-103 [PMID: 1846348 DOI: 10.1002/hep.1840130114]
- 16 **Bharadwaj M**, Khanna N, Mathur A, Chaturvedi UC. Effect of macrophage-derived factor on hypoferraemia induced by Japanese encephalitis virus in mice. *Clin Exp Immunol* 1991; **83**: 215-218 [PMID: 1847096 DOI: 10.1111/j.1365-2249.1991.tb05617.x]
- 17 **Bacon BR**, Fried MW, DiBisceglie AM. A 39-year-old man with chronic hepatitis, elevated serum ferritin values, and a family history of hemochromatosis. *Semin Liver Dis* 1993; **13**: 101-105 [PMID: 8446904 DOI: 10.1055/s-2007-1007342]
- 18 **Di Bisceglie AM**, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. *Gastroenterology* 1992; **102**: 2108-2113 [PMID: 1587431]
- 19 **Turlin B**, Juguet F, Moirand R, Le Quilleuc D, Loréal O, Champion JP, Launois B, Ramée MP, Brissot P, Deugnier Y. Increased liver iron stores in patients with hepatocellular carcinoma developed on a noncirrhotic liver. *Hepatology* 1995; **22**: 446-450 [PMID: 7635411 DOI: 10.1016/0270-9139(95)90564-2]
- 20 **Farinati F**, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. *J Hepatol* 1995; **22**: 449-456 [PMID: 7545199 DOI: 10.1016/0168-8278(95)80108-1]
- 21 **Arber N**, Konikoff FM, Moshkowitz M, Baratz M, Hallak A, Santo M, Halpern Z, Weiss H, Gilat T. Increased serum iron and iron saturation without liver iron accumulation distinguish chronic hepatitis C from other chronic liver diseases. *Dig Dis Sci* 1994; **39**: 2656-2659 [PMID: 7995192 DOI: 10.1007/BF02087705]
- 22 **Arber N**, Moshkowitz M, Konikoff F, Halpern Z, Hallak A, Santo M, Tiomny E, Baratz M, Gilat T. Elevated serum iron predicts poor response to interferon treatment in patients with chronic HCV infection. *Dig Dis Sci* 1995; **40**: 2431-2433 [PMID: 7587826 DOI: 10.1007/BF02063249]
- 23 **Piperano A**, D'Alba R, Roffi L, Fargion S, Mancina G, Fiorelli G. Relation between alpha interferon response and liver iron stores in chronic hepatitis C. *Hepatology* 1993; **18**: 250A [DOI: 10.1016/0270-9139(93)92524-4]
- 24 **Bacon BR**, Rebholz AE, Fried M, Di Bisceglie AM. Beneficial effect of iron reduction therapy in patients with chronic hepatitis C who failed to respond to interferon. *Hepatology* 1990; **12**: 105A
- 25 **Clemente MG**, Congia M, Lai ME, Lilliu F, Lampis R, Frau F, Frau MR, Faa G, Diana G, Dessi C. Effect of iron overload on the response to recombinant interferon-alpha treatment in transfusion-dependent patients with thalassemia major and chronic hepatitis C. *J Pediatr* 1994; **125**: 123-128 [PMID: 8021761 DOI: 10.1016/S0022-3476(94)70138-5]
- 26 **Olynyk JK**, Reddy KR, Di Bisceglie AM, Jeffers LJ, Parker TI, Radick JL, Schiff ER, Bacon BR. Hepatic iron concentration as a predictor of response to interferon alpha therapy in chronic hepatitis C. *Gastroenterology* 1995; **108**: 1104-1109 [PMID: 7698578 DOI: 10.1016/0016-5085(95)90209-0]
- 27 **Van Thiel DH**, Friedlander L, Fagiuoli S, Wright HI, Irish W, Gavalier JS. Response to interferon alpha therapy is influenced by the iron content of the liver. *J Hepatol* 1994; **20**: 410-415 [PMID: 8014455 DOI: 10.1016/S0168-8278(94)80017-0]
- 28 **Olynyk J**, Reddy R, Di Bisceglie AM. Hepatic iron concentration as a predictor of response to alpha interferon therapy in chronic hepatitis C. *Hepatology* 1993; **18**: A90
- 29 **Barton AL**, Banner BF, Cable EE, Bonkovsky HL. Distribution of iron in the liver predicts the response of chronic hepatitis C infection to interferon therapy. *Am J Clin Pathol* 1995; **103**: 419-424 [PMID: 7537017]
- 30 **Hayashi H**, Takikawa T, Nishimura N, Yano M. Iron removal as premedication for treatment of chronic active hepatitis C (abstract). *Hepatology* 1994; **20**: 72J [DOI: 10.1016/0270-9139(94)90455-3]
- 31 **Van Thiel DH**, Friedlander L, Molloy PJ, Kania RJ, Fagiuoli S, Wright HI, Gasbarrini A, Caraceni P. Retreatment of hepatitis C interferon non-responders with larger doses of interferon with and without phlebotomy. *Hepatogastroenterology* 1996; **43**: 1557-1561 [PMID: 8975965]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Histopathology and immunohistochemistry of large hepatocellular carcinoma with undetectable or low serum levels of alpha-fetoprotein

Qi-Ming Wu, Ming-Hua Hu, Yun-Shan Tan

Qi-Ming Wu, Ming-Hua Hu, Yun-Shan Tan, Department of Pathology, Jiangxi Medical College, Nanchang 330006, Jiangxi Province, China

Qi-Ming Wu, female, born on May 5, 1963, in Nanchang, Jiangxi Province, graduated from the Department of Medicine, Jiangxi Medical College. Technician in charge, having 5 papers published

Author contributions: All authors contributed equally to the work.

Supported by The National Science Foundation of China, No. 39160034.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Qi-Ming Wu, Department of Pathology, Jiangxi Medical College, Nanchang 330006, Jiangxi Province, China.  
Telephone: +86-791-8627217-2317

Received: September 22, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To explore the pathomorphological characteristics of large hepatocellular carcinoma (LHCC) with low serum alpha-fetoprotein (AFP) level.

**METHODS:** Specimens obtained from surgically resected LHCC with undetectable or low levels of serum AFP were fixed in formalin, embedded in paraffin, and prepared as serial sections. Routine hematoxylin and eosin as well as immunohistochemical stains (LSAB method) were used to test for expression of AFP, alpha-1-antitrypsin, epithelial membrane antigen, and vimentin. Some characteristics of the histopathological changes and immunohistochemical reactions of the cancerous tissues were observed under the light microscope.

**RESULTS:** The majority of the cases (19/30) of LHCC with undetectable or low levels of serum AFP were of the clear-cell-type HCC, with 2 being positive for AFP expression at the periphery of the cytoplasm.

**CONCLUSION:** The clear cell is the morphological manifestation of disturbance in glycogen and/or lipid metabolism of hepatoma cells. Such changes might be one of the factors hindering the synthesis of AFP and resulting in negative or low level serum AFP of the patient.

**Key words:** Liver neoplasms/pathology; Alpha fetoproteins/analysis; Immunohistochemistry

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All

rights reserved.

Wu QM, Hu MH, Tan YS. Histopathology and immunohistochemistry of large hepatocellular carcinoma with undetectable or low serum levels of alpha-fetoprotein. *World J Gastroenterol* 1997; 3(2): 64-66 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/64.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.64>

### INTRODUCTION

Serum alpha-fetoprotein (AFP) is generally detected and often elevated in patients with hepatocellular carcinoma (HCC); however, some patients with small HCC (SHCC, diameter  $\leq 3$  cm) in early stage or large HCC (LHCC; Diameter,  $\geq 3$  cm) in the mid and late stages show absence or low levels of serum AFP. In the present study, 30 cases of LHCC with undetectable or low levels serum AFP were assessed histopathologically and immunohistochemically, and the relationship between some pathomorphological characteristics and the level of serum AFP was evaluated.

### MATERIALS AND METHODS

Specimens were obtained from the surgical resection of 30 LHCC with diameter of  $> 3$  cm and undetectable or low levels of serum AFP (counter immunoelectrophoresis, negative; Rocket electrophoresis,  $\leq 399$   $\mu\text{g/L}$ ). Eight specimens from cases positive for serum AFP with high serum AFP levels (rocket electrophoresis  $\geq 400$   $\mu\text{g/L}$ ) were used as controls.

All specimens were fixed in 10% formalin normal saline, embedded in paraffin, prepared as serial sections, stained by routine hematoxylin-eosin and immunohistochemical technique with labeled streptavidin biotin (LSAB), and observed under the light microscope.

Immunohistochemistry was performed to detect the following: AFP, alpha-1-antitrypsin (AAT), epithelial membrane antigen (EMA), and vimentin. All antibodies and agents were products from DAKO Company.

### RESULTS

#### *Degree of cell differentiation of HCC tissue*

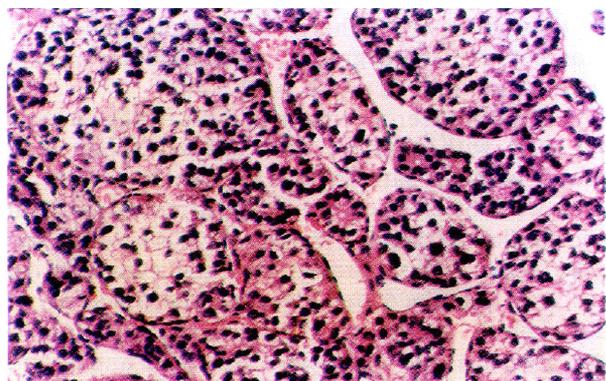
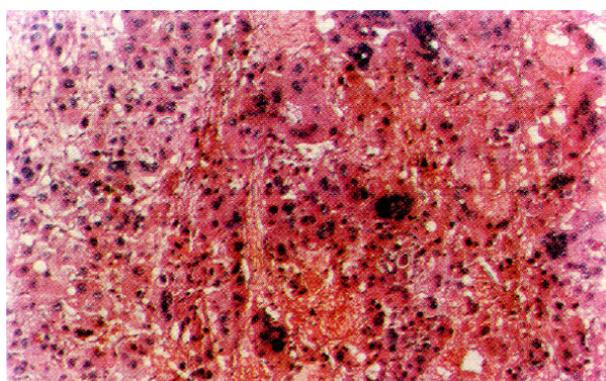
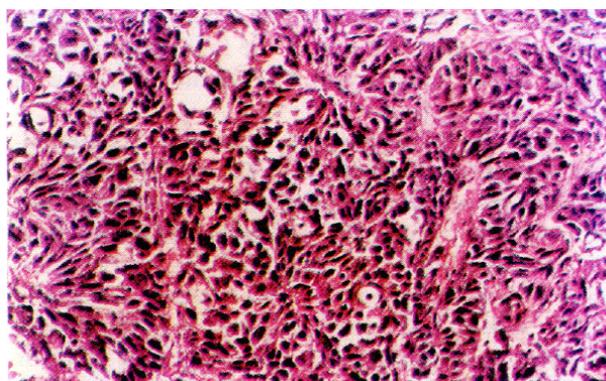
Classification of the 30 cases of LHCC with undetectable or low levels of serum AFP according to the Edmondson 4-grade method<sup>[1]</sup> revealed that cell differentiation was of grade I in 1 case, grade II in 1 case, grade III in 18 cases, and grade IV in 10 cases (Table 1).

#### *Pathomorphological types of cells in hepatocarcinomatous tissue*

According to the WHO classification system<sup>[1]</sup>, cells of carcinomatous tissues in the 30 cases of LHCC with undetectable or low levels of

**Table 1** Degree of cell differentiation of hepatocarcinomatous tissues

Serum alpha-fetoprotein level ( $\mu\text{g/L}$ )	Cases	Degree of differentiation			
		I	II	III	IV
$\leq 20$	18	1	9	7	1
21-299	12	0	9	3	0
Total	30	1	18	10	1

**Figure 1** Clear cell hepatocellular carcinoma. HE  $\times 400$ .**Figure 2** Giant cell hepatocellular carcinoma. HE  $\times 400$ .**Figure 3** Spindle cell hepatocellular carcinoma. HE  $\times 400$ .

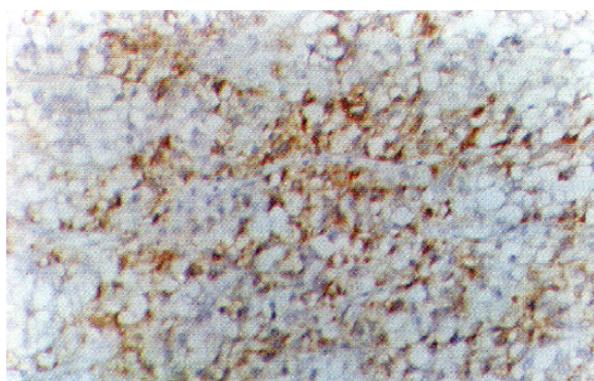
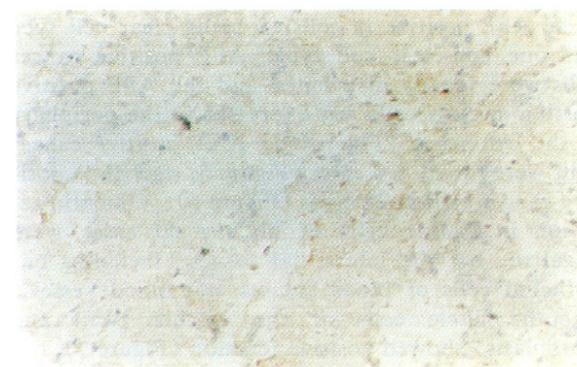
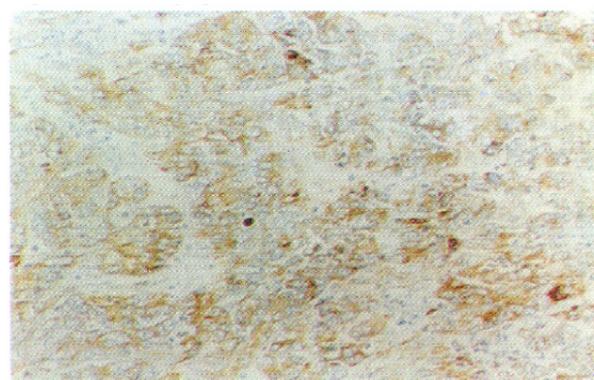
serum AFP showed peculiar morphological types. Among them, 19 were of the clear-cell type (clear cells occupied 30%-90% of total cancer cells), (Figure 1), 1 was of the giant-cell type (Figure 2), and 1 was of the spindle-cell type (Figure 3, Table 2).

#### Immunohistochemical reactions of cells in hepatocarcinomatous tissues

In histological sections of the 30 cases of LHCC with undetectable or low levels of serum AFP, only 3 were found to be positive for serum AFP in cells, 2 were of the clear-cell type, and 1 was of the non-clear-cell type. AFP-positive cancer cells were focally distributed and the AFP-positive portion in the clear cells was expressed in the periphery of the cytoplasm (Figure 4). AAT-positive cancer cells were found in 8 cases (Figure 5) and EMA-positive cancer cells in 28 cases (Figure 6). Tests for vimentin expression in cancer cells of all the 30 cases were negative, while those in the interstitial tissues (including

**Table 2** Cytomorphological types of hepatocarcinomatous tissue

Serum alpha-fetoprotein level ( $\mu\text{g/L}$ )	Number of cases	Morphological types				
		Trabecular compact	Clear cell	Giant cell	Spindle cell	
$\leq 20$	18	6	10	1	1	
21-299	12	3	9	0	0	
Total	30	9	19	1	1	

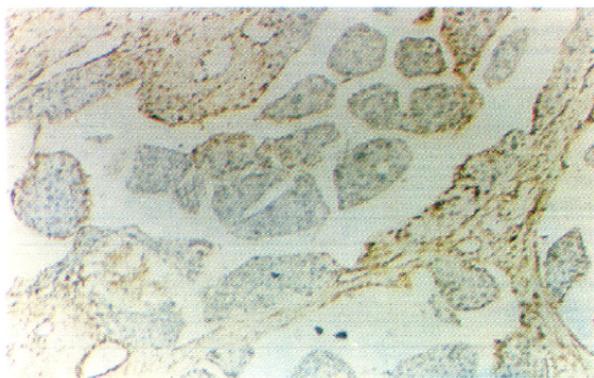
**Figure 4** Clear cell hepatocellular carcinoma. The AFP-positive portion in clear cells was expressed in the periphery of the cytoplasm. Immunohistochemical stains  $\times 400$ . AFP: Alpha-fetoprotein.**Figure 5** Clear cell hepatocellular carcinoma. The AAT-positive portion was expressed in the cytoplasm. Immunohistochemical stains  $\times 100$ . AAT: Alpha-1-antitrypsin.**Figure 6** Clear cell hepatocellular carcinoma. The EMA-positive portion was expressed in the cancer cell membrane. Immunohistochemical stains  $\times 100$ . EMA: Epithelial membrane antigen.

endothelial cells of blood sinuses and blood vessels, smooth muscle cells of the vascular wall, and fibroblasts) were positive (Figure 7).

In paraffin sections of 8 cases of LHCC with positive and high expression levels of serum AFP observed immunohistochemically, various concentrations of AFP-positive, AAT-positive, and EMA-positive cancer cells were found in all cases; tests for vimentin expression in the 8 cases yielded the same results as those obtained in the cases with undetectable serum AFP levels, *i.e.* negative results for cancer cells and positive results for interstitial tissues (Table 3).

## DISCUSSION

Different degrees of cell differentiation were observed in the 30 cases investigated in this study, with Grade III ranking the first and



**Figure 7 Clear cell hepatocellular carcinoma.** Tests for vimentin expression in cancer cells were negative, but those in the interstitial tissues appear to be positive. Immunohistochemical stains  $\times 100$

**Table 3 Immunohistochemical reactions of hepatocarcinoma**

Serum alpha-fetoprotein level ( $\mu\text{g/L}$ )	Number of cases	Morphological types				
		Trabecular	compact	Clear cell	Giant cell	Spindle cell
$\leq 20$	18	6	10	1	1	1
21-299	12	3	9	0	0	0
Total	30	9	19	1	1	1

Grade IV the next. Grade III and IV constituted the great majority of the cases, but this grade distribution was not only peculiar for those cases with undetectable or low levels of serum AFP. Two massive case reports from our country<sup>[2,3]</sup> indicated that Grades III and IV occupied the great majority of HCC, including both cases positive and negative for serum AFP expression. The distribution of Grades III and IV was also predominant in this study of 30 cases of LHCC that had undetectable or low levels of serum AFP, without any significant difference from that observed in HCC that were positive or negative for serum AFP expression. There was no significant correlation between the degree of differentiation of cancer cells and serum AFP level.

Cells of cancerous tissues in most of the 30 cases of LHCC with undetectable or low levels of serum AFP showed peculiar pathological morphology, most of which were of the clear-cell type, with a few belonging to the giant- and spindle-cell types. Wu *et al.*<sup>[4]</sup> used periodic acid-Schiff and Sudan black stains and proved that the cytoplasm of clear cells were rich in glycogen and lipid, partly as

ordinary cytoplasmic constituents (the glycogen and lipid dissolved and disappeared during the preparation of histological slides, which were left clear). On electron microscopy, cell organelles decreased, the rough surfaced endoplasmic reticulum (RER), free ribosomes and polyribosomes seemed to have been pushed aside by glycogen and lipid. Zhang *et al.*<sup>[5]</sup> observed by immune-electronmicroscopy that AFP was mainly located in the membrane of RER and ribosomes. Our immunohistochemical study on 19 cases of clear-cell carcinoma showed positive results for AFP expression in only 2 cases, wherein AFP was focally distributed with positive staining of the peripheral cytoplasm. The latter findings coincided with the above mentioned phenomenon, *i.e.*, the pushing of RER and ribosomes aside. It is suggested that clear cells are morphological manifestations of glycometabolic and lipometabolic disturbances in hepatocarcinomatous cells. It is a type of degeneration of the carcinoma cells that possibly hinders the synthesis of AFP. This might be one of the factors responsible for the undetectable or low levels of serum AFP in patients with HCC.

Thus, samples testing negative and positive for serum AFP expression did not show any significant differences in the results of the immunohistochemical studies such as those for EMA and vimentin. The positive results for EMA expression and negative results for vimentin expression in the cancer cells are indicative of their epithelial origin. Further, AAT-positive cases among those testing negative for serum AFP expression were apparently fewer than those testing positive for serum AFP for control. The reasons for these findings remain obscure and further studies are required to elucidate them.

## REFERENCES

- 1 **Kojiro M.** Pathology of hepatocellular carcinoma. In: Okuda K, Ishak KG (editors), Neoplasms of the liver. Tokyo: Springer Verlag 1987 [DOI: 10.1007/978-4-431-68349-0\_7]
- 2 **National Cooperation Group of Pathology of Hepatoma.** Pathology of primary hepatocellular carcinoma: an analysis of 500 autopsies. *Jiangsu Yiyao Zazhi (in Chinese)* 1985; **1**: 2-7
- 3 **Wu MC,** Gong WM, Zhang XH, Chen H, Zhang XZ. Clinico-pathological study of 1000 cases of hepatocellular carcinoma. *Aizheng Fangzhi Yu Zhiliao* 1993; **20**: 137-139
- 4 **Wu PC,** Lai CL, Lam KC, Lok AS, Lin HJ. Clear cell carcinoma of liver. An ultrastructural study. *Cancer* 1983; **52**: 504-507 [PMID: 6305477 DOI: 10.1002/1097-0142(19830801)52:3<504::AID-CNCR2820520321>3.0.CO;2-N]
- 5 **Zhang JS,** Xu YD, Zhang XR, Yin YY. Pathological basis of serum AFP elevation during carcinomatous change in hepatoma of rats. *Zhonghua Binglixue Zazhi* 1987; **16**: 278-280

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Recovery of allografted small intestine function

Zhi-Wei Jiang, Jie-Shou Li, Nin Li, You-Sheng Li, Fang-Lan Liu, Xue-Qin Sheng, Yong-Min Cheng

Zhi-Wei Jiang, Jie-Shou Li, Nin Li, You-Sheng Li, Fang-Lan Liu, Xue-Qin Sheng, Yong-Min Cheng, Department of Abdominal Surgery, General Hospital of Chinese PLA Nanjing Command Area, Nanjing 210002, Jiangsu Province, China

Zhi-Wei Jiang, male, born on Jan 10, 1969 in Nanjing County, Jiangsu Province, graduated from the Department of Medicine, Secondary Military Medical University, Surgeon in Chief, engaged in the study on abdominal organ transplantation, having 10 papers published.

Author contributions: All authors contributed equally to the work

Supported by The National Science Foundation of China, No. 39070828.

Original title: *China National Journal of New Gastroenterology* (1995-1997) re-named *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Zhi-Wei Jiang, Department of Abdominal Surgery, General Hospital of Chinese PLA Nanjing Command Area, Nanjing 210002, Jiangsu Province, China  
Telephone: +86-25-4403110

Received: October 6, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate recovery of the allografted small intestine function after clinical small bowel transplantation (SBT).

**METHODS:** The structure of the graft was evaluated by endoscopic biopsy and histopathologic examination. Graft functions were assessed by D-xylose absorption, barium studies, nitrogen balance calculation, and blood and stool cultures. Nutritional status of the recipients was judged by measurement of body weight and serum protein concentrations.

**RESULTS:** The recipient discontinued total parenteral nutrition (TPN) and resumed oral nutrition 100 d after SBT. On oral diet, the patient maintained a normal nutritional status, gained weight by 3 kg, and had a normal serum albumin concentration (40.2 g/L  $\pm$  0.2 g/L). Satisfactory D-xylose absorption was achieved 8 wk after the operation. Nitrogen balance of the gut was maintained well and increased gradually. Serial mucosal biopsy showed normal structures 2 wk after grafting, without evidence of rejection and graft versus host diseases (GVHD). Barium studies conducted on the 10<sup>th</sup> day and 38<sup>th</sup> day by barium studies revealed that the grafted small bowel motility showed normal patterns of peristalsis and transit. No bacterial translocations were noted.

**CONCLUSION:** Function of the grafted small intestine recovered satisfactorily 100 d after transplantation, indicating good clinical outcome of SBT.

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Nutritional support; Small intestine/transplantation; Small intestine/physiopathology

Jiang ZW, Li JS, Li N, Li YS, Liu FL, Sheng XQ, Cheng YM. Recovery of allografted small intestine function. *World J Gastroenterol* 1997; 3(2): 67-68 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/67.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.67>

### INTRODUCTION

Successful small bowel transplantation (SBT) can improve the life quality for patients dependent on long-term total parenteral nutrition (TPN). The ultimate goal of SBT is to restore normal gastrointestinal function. The purpose of this study was to evaluate the function of allografted small intestine after a clinical SBT.

### MATERIALS AND METHODS

A clinical SBT was performed on March 12, 1994. The recipient was a 31-year-old woman who had severe short bowel syndrome and was dependent on long-term TPN. The donor was a 28-year-old man who had died of brain injury. Both the donor and the recipient were of blood type O. The graft was transplanted in an orthotopic location. The donor superior mesenteric artery (SMA) with a Carrel patch was anastomosed to the recipient aorta, while the donor portal vein was anastomosed to the recipient superior mesenteric vein (SMV). The grafted small intestine was anastomosed to the native gastrointestinal tract, exteriorizing the distal segment of the graft about 10 cm in length as a stoma<sup>[1]</sup>. Immunosuppression was achieved with cyclosporine A (CsA), tripterygium wilfordii (GTW), methylprednisolone, and prostaglandin E<sub>1</sub> (PGE<sub>1</sub>). Histopathologic studies of endoscopy-guided biopsies were performed twice a week. Enteral feeding was initiated 4 d after surgery and then gradually increased in volume and concentration as the parenteral feeding was reduced and eventually stopped. Further, 2% glutamine (Glu) was added to the enteral feeds. The patient's nutritional status was assessed principally by measurement of body weight and serum protein levels. D-xylose absorption<sup>[2]</sup> and nitrogen balance were measured to assess the graft absorption function. Motility of the small bowel was assessed by barium studies on the 10<sup>th</sup> and 38<sup>th</sup> postoperative days. Bacterial translocation was judged by frequent blood and stool cultures.

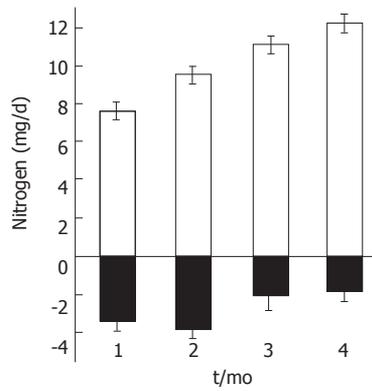


Figure 1 Calculation of nitrogen balance of the gut.

## RESULTS

The patient was free from dependency on TPN 100 d after the operation. Steady weight gain (3 kg) and normal albumin concentration ( $40.2 \text{ g/L} \pm 0.2 \text{ g/L}$ ) had been achieved on enteral feeding alone. Satisfactory absorption of D-xylose was documented 8 wk after surgery. Positive nitrogen balance of the gut was achieved in the post-operative course (Figure 1). Barium studies showed that the transit and peristalsis patterns of the graft were normal. Serial intestinal biopsies showed that the mucosa was mildly edematous, with villous blunting and infiltration of some mononuclear cells into the lamina propria and submucosa in the first 2 wk. However, the mucosa was restored to its normal appearance without any evidence of rejection or graft versus host disease (GVHD) at 2 wk after the operation. No bacterial translocation occurred postoperatively.

## DISCUSSION

We treated the short bowel syndrome successfully by SBT for the first time in our country. One hundred days after the transplantation, the functions of the allografted small intestine were restored satisfactorily, and the patient could maintain a normal nutritional status by oral diet

only, without the need for further parenteral supplement. The graft had a normal structure with good performance in its functions of absorption, motility, and barrier protection.

Factors impairing the functions of intestinal graft include surgery, rejection, and infection. The measures taken to improve the graft functions in our patient included shortening the ischemic period of the graft, improving the surgical technique, preventing rejection and infection, and protecting the intestinal barrier function. Skilled execution of the vascular anastomosing technique could avoid the stenosis of the anastomosis. After revascularization, the use of low-dose dopamine would promote the graft blood flow. To shorten the graft ischemic time, especially warm ischemic time, the quality of the graft could be improved by ice cooling of the abdominal cavity and immediate *in situ* perfusion through an aortic cannula with cold Eurocollins solution. The graft was transported to the operating room as soon as possible and the retrieval was done carefully. A matched donor was selected, and a reasonable immunosuppressive protocol was adopted to prevent rejection. Additionally, to improve gut barrier function after SBT, early enteral feeding was provided to stimulate mucosal growth and preserve the tight junctions of intestinal epithelial cells. Glu was supplemented to supply the gut with essential nutrients<sup>[3]</sup>, and oral antibiotics were used to reduce the microbial flora in the graft.

Thus, this case demonstrated that SBT is a feasible option for patients with short bowel syndrome.

## REFERENCES

- 1 Li N, Li JS, Li YS, Jiang ZW, Yin LU, Zhang LH. Perioperative management for the first case of small bowel transplantation. *Zhonghua Qiguan Yizhi Zazhi* 1994; **15**: 112-114
- 2 Billiar TR, Garberoglio C, Schraut WH. Maltose absorption as an indicator of small-intestinal allograft rejection. *J Surg Res* 1984; **37**: 75-82 [PMID: 6376953 DOI: 10.1016/0022-4804(84)90164-1]
- 3 Frankel WL, Zhang W, Afonso J, Klurfeld DM, Don SH, Laitin E, Deaton D, Furth EE, Pietra GG, Naji A. Glutamine enhancement of structure and function in transplanted small intestine in the rat. *JPEN J Parenter Enteral Nutr* 1993; **17**: 47-55 [PMID: 8437324 DOI: 10.1177/014860719301700147]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Effects of tetrandrine and verapamil on fibroblastic growth and proliferation

Xue-Song Liu, Ding-Guo Li, Han-Ming Lu, Qin-Fang Wu

Xue-Song Liu, Ding-Guo Li, Han-Ming Lu, Qin-Fang Wu, Research Laboratory of Digestive Diseases, Xinhua Hospital, Shanghai Second Medical University, Shanghai 200092, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: September 22, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the effect of tetrandrine (Tet) and verapamil (Ver) on liver fibrosis by evaluating their effects on the growth and proliferation of fibroblasts.

**METHODS:** Flow cytometry and imaging analysis were used to study the effects of combined and individual treatment of Tet and Ver on fibroblastic proliferation *in vitro*. Untreated control cells were used

for comparison.

**RESULTS:** Compared to untreated fibroblasts, those treated with both Tet and Ver showed higher percentages of cells in the G<sub>1</sub> and G<sub>2</sub>+M phase in the cell cycle of 3T6 fibroblasts ( $P < 0.01$ ) and markedly elevated protein content, while those treated with Tet only showed significantly lower intracellular RNA content (1.5-2.0  $\mu\text{g}$ ,  $P < 0.01$ ) and those treated with Ver only showed lower intracellular DNA content (15-20  $\mu\text{g}$ ,  $P < 0.01$ ).

**CONCLUSION:** The inhibitory action of Tet and Ver on fibroblastic growth and proliferation might be responsible for the antifibrotic effects of these medications in liver fibrosis.

**Key words:** Tetrandrine/pharmacology; Verapamil/pharmacology; Fibroblasts/drug effects; Liver cirrhosis/drug therapy

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Liu XS, Li DG, Lu HM, Xu QF. Effects of tetrandrine and verapamil on fibroblastic growth and proliferation. *World J Gastroenterol* 1997; 3(2): 68 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/68.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.68>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Effect of octreotide on cell-cycle kinetics and serum carcinoembryonic antigen level in hepatic metastases of colonic adenocarcinoma

Rui Liu, Yuan-He Wang, Yan Tang, Gui-Song Cao

Rui Liu, Yuan-He Wang, Yan Tang, Gui-Song Cao, Department of Surgery, Changhai Hospital, Shanghai 200433, China

Yuan-He Wang, General Surgery, Changzheng Hospital, Shanghai 200003, China

Dr. Rui Liu, male, born on June 11, 1962 in You County, Hunan Province, graduated from the Department of Medicine in the Second Military Medical University, Attending doctor, engaged in clinical and experimental study on gastroenterologic, pancreatic and splenic diseases, having 16 papers published.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Rui Liu, Department of Surgery, Changhai Hospital, 170 Changhailu, Shanghai 200433, China

Received: September 30, 1996  
Revised: February 15, 1997  
Accepted: April 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the inhibitory effect of somatostatin analogue (octreotide) on tumor growth.

**METHODS:** The influence of cell-cycle kinetics on hepatic metastases of BALB/c mice colonic adenocarcinoma (CT26) with octreotide treatment *in vivo* was investigated by flow cytometry. The serum carcinoembryonic antigen (CEA) levels were also determined.

**RESULTS:** The results showed that the proliferative index (PI) and the S-phase fraction in hepatic tumors of mice treated with octreotide decreased markedly and that the G<sub>0</sub>/G<sub>1</sub> serum CEA phase fraction increased significantly in comparison with the control ( $P < 0.01$ ). After administration of octreotide, the serum CEA levels were also lower than those in the control group. The incidence of liver metastases in the treated group was lower than that in the control. The body weight loss in the mice was slower and survival was longer in the treated group than in the control group. Furthermore, the changes in PI and the fraction distribution of S-phase or G<sub>0</sub>/G<sub>1</sub>-phase in cell cycle were closely related to the serum CEA levels.

**CONCLUSION:** Octreotide may be useful for inhibiting the hepatic metastases of colonic carcinoma.

**Key words:** Colonic neoplasms; Liver neoplasms/secondary; adenocarcinoma; Cytometry; Octreotide; Carcinoembryonic antigen/analysis

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Liu R, Wang YH, Tang Y, Cao GS. Effect of octreotide on cell-cycle kinetics and serum CEA level in hepatic metastases of colonic adenocarcinoma. *World J Gastroenterol* 1997; 3(2): 69-71 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/69.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.69>

### INTRODUCTION

Somatostatin analogues (e.g. octreotide, RC-160 and others) are widely used to treat neuroendocrine tumors (such as carcinoids, vasoactive intestinal peptide (VIP) omas, insulinomas and glucagonomas), acute pancreatitis and fistulae of gastrointestinal tract<sup>[1]</sup>. Recently, these drugs have also been considered to have some therapeutic effect for non-endocrine gastrointestinal tumors<sup>[2,3]</sup>. In order to study the mechanism of octreotide and its anticancer effect, the proliferative index (PI) and the fraction distribution of cell cycle were investigated by flow cytometry. The changes in the serum carcinoembryonic antigen (CEA) level, incidence of hepatic metastases, body weight, and survival time were also determined. Furthermore, the relationship between the parameters of cell-cycle kinetics and the changes of serum CEA level were analyzed.

### MATERIALS AND METHODS

#### **Peptides, tumor cell line, and instrumentation**

Octreotide was provided as a gift from Sandoz Company (Basel, Switzerland). The tumor cell line CT26, from the colonic adenocarcinoma of mice, was obtained from the Immunological Department of Second Military Medical University (Shanghai, China). The type of flow cytometry (FCM) apparatus used in this study was FACStar Plus, manufactured by Becton Dickinson Company (United States). The CEA ELISA kit was produced by Sanye Company of Shanghai Second Medical University.

#### **Animals and treatment**

Thirty-two male BALB/c mice, aged 5-7 wk, were used in this study. The mice were randomized into the following four groups: Group A ( $n = 10$ ): Liver metastases treated with octreotide for tumor measurement and FCM analysis; group B ( $n = 10$ ): Liver metastases used as control for tumor measurement and FCM analysis; group C ( $n = 6$ ): Liver metastases treated with octreotide for survival time; group D ( $n = 6$ ): Liver metastases used as control for survival time.

The mice were placed under ether anesthesia, and a small incision was made in the left subcostal area. The spleen was injected with  $1 \times 10^6$  cultured CT26 in 100  $\mu$ L of culture medium. The mice

**Table 1 Comparison of body weight and incidence of hepatic metastases**

	Group A	Group B
Body weight ( $\bar{x} \pm s$ , g)		
0	16.74 $\pm$ 0.94	16.96 $\pm$ 0.90
1 <sup>st</sup> week	16.55 $\pm$ 0.82	16.64 $\pm$ 0.80
2 <sup>nd</sup> week	16.11 $\pm$ 0.79	15.68 $\pm$ 0.68 <sup>b</sup>
3 <sup>rd</sup> week	15.35 $\pm$ 0.81 <sup>b</sup>	15.05 $\pm$ 0.87 <sup>b</sup>
Weight decreasing rate ( $\bar{x} \pm s$ , %)		
1 <sup>st</sup> week	1.23 $\pm$ 0.82	1.74 $\pm$ 1.40
2 <sup>nd</sup> week	3.70 $\pm$ 1.12 <sup>d</sup>	7.48 $\pm$ 2.14
3 <sup>rd</sup> week	8.25 $\pm$ 2.65 <sup>c</sup>	11.27 $\pm$ 2.79
Incidence of hepatic tumor		
Grade I	0	0
Grade II	70% (7/10) <sup>e</sup>	20% (2/10)
Grade III	30% (3/10) <sup>e</sup>	80% (8/10)

<sup>b</sup> $P < 0.01$  vs before experiment; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , group A vs group B.

**Table 2 Changes in proliferative index and phase fractions in tumor cell cycle ( $\bar{x} \pm s$ )**

	Group A	Group B
PI	0.24 $\pm$ 0.06 <sup>b</sup>	0.31 $\pm$ 0.02
S (%)	17.89 $\pm$ 5.11 <sup>b</sup>	24.54 $\pm$ 2.58
G <sub>0</sub> /G <sub>1</sub> (%)	76.41 $\pm$ 5.51 <sup>b</sup>	69.38 $\pm$ 2.30
G <sub>2</sub> /M (%)	6.11 $\pm$ 1.71	5.67 $\pm$ 1.76

PI: Proliferative index. <sup>b</sup> $P < 0.01$ , group A vs group B.

in groups A and C were administered daily subcutaneous injection of octreotide at a dose of 50  $\mu$ g/kg daily, while those in groups B and D were injected with saline solution.

#### Measurement and analysis of FCM

The mice were weighed once a week. The animals in groups A and B were killed after 3 wk. The incidence of hepatic metastases and the liver weight were determined. The hepatic metastases were classified into grades I -III according to the number of visible liver tumors: Grade I : 0; grade II : 1-50 liver tumors mass; grade III : > 50 liver tumors with mass or diffuse growth. Blood samples were collected by ophthalmectomy and the serum level of CEA was measured. Tumor samples of volume approximately 0.5 cm<sup>3</sup> were dissected from the hepatic metastases was subjected to FCM analysis.

The mice in groups C and D were treated continuously until they died naturally.

#### Statistical analysis

Statistical analysis included both univariate ( $\chi^2$  and Student's *t*-test) and multivariate (logistic regression) tests.

## RESULTS

#### Change in body weight

The changes in the body weight of groups A and B are shown in Table 1. Compared to status before initiation of treatment, the weight in group A did not change significantly even 1 and 2 wk after treatment; however, the weight in group B decreased markedly after the second week. The difference in the rate of weight loss between groups A and B was statistically significant.

#### Change of hepatic metastases

Administration of octreotide for 3 wk significantly lowered the incidence of hepatic metastases and inhibited the growth of CT26 (Table 1). The incidence of grade II liver metastases in the control group decreased markedly, while that of grade III increased significantly in comparison with the treated group ( $P < 0.05$ ).

#### PI and phase fraction in tumor cell cycle

The PI and each phase fraction of tumor cell cycle, as determined by FCM, are shown in Table 2. After administration of octreotide, the PI and the S-phase fraction in hepatic tumors of mice decreased markedly, while the G<sub>0</sub>/G<sub>1</sub>-phase fraction increased significantly in com-

parison with the control group ( $P < 0.01$ ).

#### Change of serum CEA and the relationship between CEA and cell cycle kinetics

After the third treatment session, the serum level of CEA was 89.60  $\mu$ g/L  $\pm$  31.72  $\mu$ g/L in group A and 140.50  $\mu$ g/L  $\pm$  49.97  $\mu$ g/L in group B ( $P < 0.05$ ). Statistical regression analysis showed that the changes in the PI and the fraction distribution of S-phase and G<sub>0</sub>/G<sub>1</sub>-phase in cell cycle were closely related to the levels of serum CEA in the treated group ( $r = 0.6677$ ,  $P < 0.05$ ;  $r = 0.7071$ ,  $P < 0.01$ ;  $r = -0.6703$ ,  $P < 0.05$ , respectively).

#### Evaluation of survival time

After 3 wk of octreotide treatment, the median survival time was 49.3  $\pm$  13.0 d in group A and 34.2  $\pm$  7.3 d in group B ( $P < 0.05$ ).

## DISCUSSION

Somatostatin analogue, designated as octreotide with eight amino acids, is long acting and much more potent than somatostatin in inhibiting insulin, glucagon, and growth hormone. Clinical studies have demonstrated that octreotide is an effective pharmaceutical agent in treating various endocrine tumors (e.g. carcinoids, insulinomas, VIPomas, etc.). In recent years, several investigators have described that somatostatin analogues can exert inhibitory effect in gastrointestinal "non-endocrine" tumors by means of suppressing the growth of tumors and prolonging the survival time<sup>[4,5]</sup>.

In view of cell-cycle kinetics, PI and S-phase fraction always reflect the proliferative state of tumor cells. This study demonstrated that octreotide could markedly decrease the PI and the S-phase fraction of hepatic tumors and significantly increase the G<sub>0</sub>/G<sub>1</sub>-phase fraction in comparison with the control. Administration of octreotide significantly lowered the incidence and inhibited hepatic metastases of CT26 colonic adenocarcinoma cell line. The animal's body weight decreased slowly and the survival time of mice prolonged markedly. Therefore, octreotide can inhibit the proliferation of CT26 tumor cell line and block the G<sub>0</sub>/G<sub>1</sub>-phase cells of CT26 adenocarcinoma from entering the S-phase. These experimental results from the view of cellular kinetics suggest that octreotide has the antitumor effect.

The possible mechanism of the antitumor action of somatostatin is still unknown. From the available data, the following are proposed as likely mechanisms: (1) The antitumor action of somatostatin could be mediated directly by specific receptors located on the tumor cell membrane. (2) Somatostatin analogues can suppress the release of gastrointestinal hormones (e.g. insulin, gastrin, cholecystokinin and others), interfere with the synthesis of autocrine or paracrine growth factors by tumor cells (such as epidermal growth factor, platelet derived growth factor, transforming growth factor and others). (3) Somatostatin also exerts an inhibitory effect on DNA synthesis in tumor cells. And (4) Furthermore, somatostatin analogues can also inhibit the angiogenesis of neoplasm<sup>[6-8]</sup>.

Our data suggested that octreotide can also influence serum CEA. The serum CEA levels in the group treated with octreotide decreased markedly and were positively related with PI and S-phase fraction of cell cycle and negatively related with G<sub>0</sub>/G<sub>1</sub>-phase fraction. CEA, an oncofetal glycoprotein, was first described in 1965. The present study shows that the CEA is not only a tumor associated cancer antigen but also a member of immunoglobulin supergene family<sup>[9]</sup>. The nucleotide sequence of the CEA gene was homologous with those of member of the immunoglobulin supergene family; therefore, it may affect cellular recognition and interaction. As a member of the group of cell adhesion molecules (CAMs), CEA may be involved in tumor metastases. The experimental study by Hostetter *et al.*<sup>[10]</sup> suggested that the metastasis in the liver of nude mice was enhanced by the CEA pretreatment. The cells adhered by movement of microvilli and contact of cells. The recognition between CEA molecule and its receptor is a process depending on bivalent cation (Mg<sup>2+</sup>)<sup>[11]</sup>. Therefore, this study suggested that the anti-tumor mechanism of octreotide might involve cell adhesion molecules (CAMs). Unfortunately, we did not determine the changes in the levels of other CAMs.

In conclusion, our findings indicate that the somatostatin anal-

ogue, octreotide, can effectively inhibit the growth and development of hepatic metastases. Recently, octreotide has been employed in the treatment of advanced gastrointestinal cancer patients refractory to chemotherapy. Although the results of initial clinical trials of somatostatin analogues were controversial, we believe that somatostatin may be a useful agent in endocrinotherapy for gastrointestinal cancer.

## REFERENCES

- 1 **Liu R**, Wang BM. The application of somatostatin in digestive diseases. *Zhonghua Shiyong Zazhi* 1993; **13**: 681-683
- 2 **Schally AV**. Oncological applications of somatostatin analogues. *Cancer Res* 1988; **48**: 6977-6985 [PMID: 2903792]
- 3 **Saltz L**, Trochanowski B, Buckley M, Heffernan B, Niedzwiecki D, Tao Y, Kelsen D. Octreotide as an antineoplastic agent in the treatment of functional and nonfunctional neuroendocrine tumors. *Cancer* 1993; **72**: 244-248 [PMID: 8389666 DOI: 10.1002/10.97-0142(19930701)72:1<244::AID-CNCR2820720143>3.0.CO;2-Q]
- 4 **Qin Y**, Schally AV, Willems G. Treatment of liver metastases of human colon cancers in nude mice with somatostatin analogue RC-160. *Int J Cancer* 1992; **52**: 791-796 [PMID: 1358828 DOI: 10.1002/ijc.2910520520]
- 5 **Cascinu S**, Del Ferro E, Catalano G. A randomised trial of octreotide vs best supportive care only in advanced gastrointestinal cancer patients refractory to chemotherapy. *Br J Cancer* 1995; **71**: 97-101 [PMID: 7819058 DOI: 10.1038/bjc.1995.19]
- 6 **Goustin AS**, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. *Cancer Res* 1986; **46**: 1015-1029 [PMID: 3002607]
- 7 **van Eijk CH**, Slooter GD, Hofland LJ, Kort W, Jeekel J, Lamberts SW, Marquet RL. Somatostatin receptor-dependent growth inhibition of liver metastases by octreotide. *Br J Surg* 1994; **81**: 1333-1337 [PMID: 7953404 DOI: 10.1002/bjs.1800810925]
- 8 **Woltering EA**, Barrie R, O'Dorisio TM, Arce D, Ure T, Cramer A, Holmes D, Robertson J, Fassler J. Somatostatin analogues inhibit angiogenesis in the chick chorioallantoic membrane. *J Surg Res* 1991; **50**: 245-251 [PMID: 1705618 DOI: 10.1016/0022-4804(91)90186-P]
- 9 **Williams AF**. A year in the life of the immunoglobulin superfamily. *Immunol Today* 1987; **8**: 298-303 [PMID: 25290835 DOI: 10.1016/0167-5699(87)90016-8]
- 10 **Hostetter RB**, Augustus LB, Mankarious R, Chi KF, Fan D, Toth C, Thomas P, Jessup JM. Carcinoembryonic antigen as a selective enhancer of colorectal cancer metastasis. *J Natl Cancer Inst* 1990; **82**: 380-385 [PMID: 2304087 DOI: 10.1093/jnci/82.5.380]
- 11 **Benchimol S**, Fuks A, Jothy S, Beauchemin N, Shirota K, Stanners CP. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. *Cell* 1989; **57**: 327-334 [PMID: 2702691 DOI: 10.1016/0092-8674(89)90970-7]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Effect of faeces trogopterorum extract B<sub>1</sub> on the experimental gastric ulcer and gastric secretion in rats

Xiong-Wen Wang, Zhi-An Cheng, Qing-Ming Li, Wen-Zi Liu

Xiong-Wen Wang, Zhi-An Cheng, Qing-Ming Li, Wen-Zi Liu, Department of Traditional Chinese Medicine, Sun Yat Sen Memorial Hospital, Sun Yat Sen University of Medical Sciences, Guangzhou 510120, Guangdong Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: September 22, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the effects of extracts B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> from faeces trogopterorum on the experimental gastric ulcer in rats.

**METHODS:** Two different animal models of gastric ulcers were used in this experiment: Shay's model ( $n = 72$ ) and the reserpine-induced ulcer model ( $n = 76$ ). The total volume and the pH of the gastric juices were recorded. The lesion scores of gastric mucosa were also recorded.

**RESULTS:** The lesion scores of gastric mucosa in the Shay's model

of animals in the WLZ-B<sub>1</sub> groups treated with either 40 g/kg or 80 g/kg were  $8.6 \pm 10.8$  and  $1.6 \pm 1.9$  respectively, which were lower than that of the 0.9% NaCl control group ( $47.0 \pm 31.4$ ,  $P < 0.05$ ,  $P < 0.01$ ). The lesion scores for the 80 g/kg group was lower compared to those of the Ran group ( $20.5 \pm 16.4$ ,  $P < 0.01$ ). The pH of the gastric juices of the 80 g/kg group ( $3.425 \pm 0.143$ ) was higher than that of the 0.9% NaCl group ( $2.836 \pm 0.632$ ,  $P < 0.05$ ). In the reserpine model, the lesion score of the 40 g/kg group of the WLZ-B<sub>1</sub> ( $20.7 \pm 16.5$ ) was also lower than that of the 0.9% NaCl control group ( $76.3 \pm 50.6$ ,  $P < 0.05$ ).

**CONCLUSION:** B<sub>1</sub> is the most effective of the three sections in inhibiting gastric secretion, protecting gastric mucosa and preventing experimental ulceration.

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Faeces trogopterori/pharmacology; Gastric mucosa/drug effects; Stomach ulcer/prevention and control

Wang XW, Cheng ZA, Li QM, Liu WZ. Effect of faeces trogopterorum extract B<sub>1</sub> on the experimental gastric ulcer and gastric secretion in rats. *World J Gastroenterol* 1997; 3(2): 71 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/71.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.71>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Fos expression in catecholaminergic medullary neurons induced by chemical stimulation of stomach projecting to the paraventricular nucleus of the hypothalamus in rats

Yuan-Xiang Dong, Kang-Hui Xiong, Zhi-Ren Rao, Ji-Wu Shi

Yuan-Xiang Dong, Kang-Hui Xiong, Zhi-Ren Rao, Ji-Wu Shi, Department of Anatomy, The Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China

Dr. Yuan-Xiang Dong, male, born on Feb. 5, 1950, Jianli County, Hubei Province, graduated from Department of Medicine, The Fourth Military Medical University, engaged in neuroanatomy study on the transmission and regulation of the visceral nociceptive information, having 18 papers published.

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 39370240.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Yuan-Xiang Dong, Department of Anatomy, The Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China  
Telephone: +86-29-3231057  
Fax: +86-29-3283229

Received: September 26, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To determine whether medullary catecholaminergic neurons expressing Fos induced by chemical stimulation of the stomach project to the paraventricular nucleus of hypothalamus (PVH) in rats.

**METHODS:** Horseradish peroxidase (HRP) was introduced stereotaxically into the PVH of rats. Histochemical analysis of coronal sections through the medulla were analyzed using triple-label immunohistochemistry to identify cells that were retrogradely labeled with HRP, Fos (ABC method), and tyrosin hydroxylase (TH) (PAP method).

**RESULTS:** Seven kinds of labeled neurons were found in the nucleus tractus solitarius (NTS), the ventrolateral medulla (VLM) and the reticular formation (RF) of the medulla: Fos-like immunoreactive (FosLI) neurons, TH-like immunoreactive (TH-LI) neurons and HRP retrogradely single-labeled neurons, FosLI/HRP, FosLI/TH-LI and HRP/TH-LI double-labeled neurons, and FosLI/HRP/TH-LI triple-labeled neurons.

**CONCLUSION:** Ascending projections from the NTS, VLM and RF to the PVH might be involved in the transmitting process of visceral noxious stimulation.

**Key words:** Medulla oblongata; Paraventricular hypothalamic nucleus; Neurons; Immunocytochemistry

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Dong YX, Xiong KH, Rao ZR, Shi JW. Fos expression in catecholaminergic medullary neurons induced by chemical stimulation of stomach projecting to the paraventricular nucleus of the hypothalamus in rats. *World J Gastroenterol* 1997; 3(2): 72-74 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/72.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.72>

### INTRODUCTION

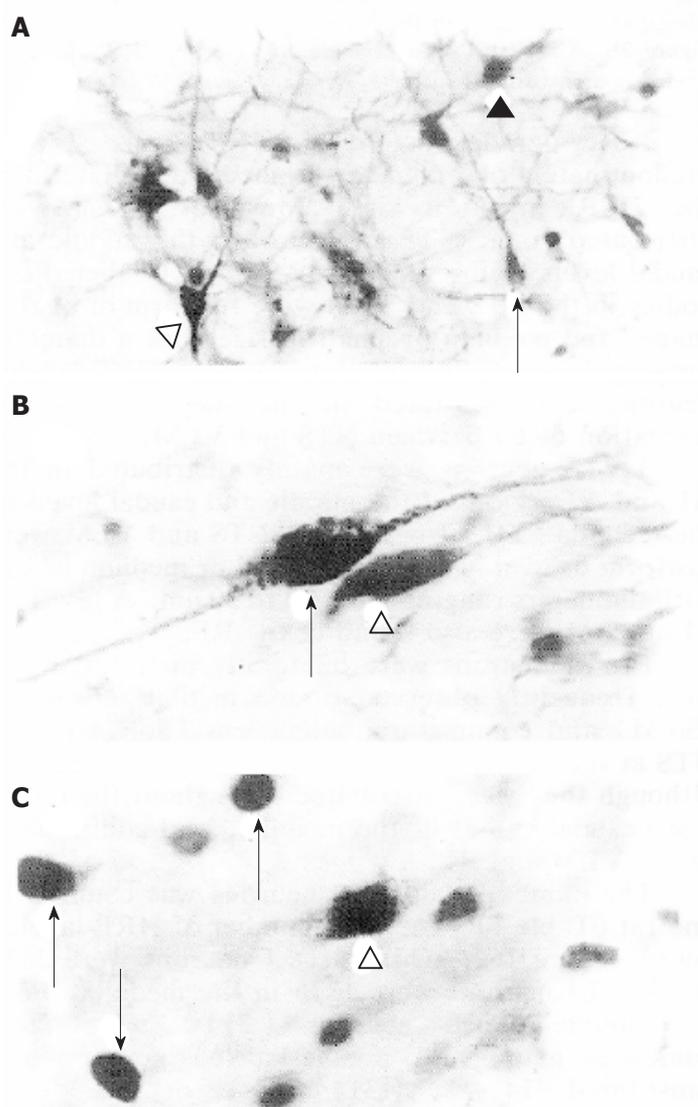
It is well known that catecholaminergic medullary neurons are mainly located in the A1 and A2 regions of the brain stem, while a few of them are distributed in the reticular formation (RF) between A1 and A2 regions<sup>[1,2]</sup>. It has been previously reported that only a few neurons in nucleus tractus solitarius (NTS) sent projective fibers to the paraventricular nucleus of hypothalamus (PVH)<sup>[3-5]</sup>; however, recent studies have demonstrated that many neurons in the NTS and ventrolateral medulla (VLM) send their axons directly to the PVH<sup>[6]</sup>, with some of them originating from A1 and A2 regions<sup>[2]</sup>. The paraventricular nucleus of hypothalamus (PVH) is an important part of the hypothalamus, and hypothalamus is known to play an important role in regulating visceral activities. Additionally, after injection of formalin into the stomach, numerous Fos-like immunoreactive (FosLI) neurons were detected within the central nervous system of the rats, including the NTS, VLM and RF<sup>[7]</sup>. Therefore, in this study, we investigated whether the catecholaminergic neurons in rat medulla projecting to the PVH responded to the gastrointestinal noxious stimulation.

### MATERIALS AND METHODS

Twelve male Sprague-Dawley rats, weighing 180-230 g, were used. The rats were kept in a dark, warm and quiet facility for 36 h before experiment. Then the rats were randomly divided into one experimental group ( $n = 6$ ) and three control groups ( $n = 2$  for each group).

#### Experimental group

The rats were anesthetized with sodium pentobarbital (40 mg/kg, *i.p.*) and stereotaxically injected unilaterally with 0.1  $\mu$ L of a 30% solution of horseradish peroxidase (HRP) dissolved in 0.9% saline into the PVH. Forty-eight hours later, the rats were anesthetized again by placing the animals into a transparent glass box containing



**Figure 1** A: Horseradish peroxidase labeled neuron (open triangle), Tyrosin hydroxylase-like immunoreactive neuron (black triangle) in the ventrolateral medulla and reticular formation of medulla,  $\times 200$ ; B: Tyrosin hydroxylase-like immunoreactive neuron (open triangle) and Horseradish peroxidase/Tyrosin hydroxylase-like immunoreactive double-labeled neuron (arrow in the nucleus tractus solitarii of medulla,  $\times 400$ ; C: Fos-like immunoreactive neuron (arrows) and Fos-like immunoreactive/Horseradish peroxidase double-labeled neuron (open triangle) in the nucleus tractus solitarii of medulla,  $\times 400$ .

methoxyflurane gas and then administered with 2 mL of 1% formalin directly into the stomach through a plastic tube. Within 3-5 min after the injection, the animals awoke. Two hours later, the rats were re-anesthetized with an overdose of sodium pentobarbital, perfused through the ascending aorta with 150 mL of 0.9% saline, followed by 500 mL of a fixative containing 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH7.4), and 200 mL of 10% sucrose in PB. The brains were removed and stored in 30% sucrose in 0.1 mol/L PB overnight at 4 °C. The brains were cut through the medulla into coronal sections 40  $\mu\text{m}$  thick on a freezing microtome.

The sections were processed with a triple labeling technique<sup>[8]</sup>. For the histochemical demonstration of injected and transported HRP, tetramethylbenzidine (TMB) was used as a chromogen and sodium tungstate as a stabilizer. The HRP reaction products were intensified with diaminobenzidine (DAB) and  $\text{CoCl}_2$ . Then, the sections were used for immunohistochemical staining for Fos according to the avidin biotin peroxidase complex (ABC) method. The sections were successively incubated with sheep anti Fos protein antibody (1:4000; Cambridge Biochemical Research, United Kingdom) for 48 h at 4 °C, followed by biotinylated rabbit anti sheep IgG (1:300; Vector) for 4 h at room temperature (RT) and finally streptavidin HRP complex (1:300; Vector) for 1 h at RT. Then, the sections were treated using the glucose oxidase DAB nickel method, the reactions usually lasted between 30-50 min at RT.

Finally, the sections were washed in 0.01 M phosphate buffered saline (PBS), and were then used for immunocytochemical staining for tyrosine hydroxylase (TH) by the peroxidase anti peroxidase (PAP)

method. The sections were successively incubated with a monoclonal antibody against TH (1:300; Biochringer Mannbein Biochemical) for 48 h at 4 °C, followed by goat anti mouse IgG (1:100; Sigma) and finally a mouse PAP complex (1:300; Sigma), each for 24 h at 4 °C. The sections were then treated with 0.05% DAB and 0.03%  $\text{H}_2\text{O}_2$  in 0.05 mol/L Tris HCl buffer (pH7.6) for 10-20 min at RT.

### Control groups

The control experiments (groups 1 to 3) were performed in six rats as follows: Two rats were reared as described above without any treatment; two rats were only anesthetized by methoxyflurane gas without any formalin injected into the stomach and two received HRP injection into the PVH without any additional treatment. After survival for 36 h, 2 h and 48 h, respectively, the rats were anesthetized with sodium pentobarbital (40 mg/kg, *i.p.*) and transcardially perfused as described above. The sections obtained from these six control rats were processed for immunohistochemical staining for Fos only.

Some of the sections obtained from the experimental rats were selected for the primary antibody substitution test (control group 4), in which the anti-Fos sera and monoclonal antibody against TH were replaced by 0.01 mol/L PBS (1:50-100).

## RESULTS

### Experimental group

Under light microscope, seven kinds of labeled cells (*i.e.* HRP, TH-LI and FosLI single-labeled neurons; hRP/TH-LI, FosLI/TH-LI and FosLI/HRP double-labeled neurons; hRP/FosLI/TH-LI triple-labeled neurons) were observed in the medulla.

HRP reaction products were seen as black punctate granules in the cytoplasm (Figure 1A), while FosLI neurons appeared with a dark, round or ovoid nuclei and exhibited no staining in the cytoplasm (Figure 1C and Figure 2A). The cytoplasm of TH-LI neurons were homogeneously brown (Figure 1A, 1B and Figure 2B). Thus, HRP/TH-LI double-labeled neurons were identified by the presence of black punctate granules on a homogeneous brown background (Figure 1B); FosLI/TH-LI double-labeled neurons were characterized by the presence of a dark nucleus with a homogeneous brown cytoplasm (Figure 1A and 2A). Numerous black punctate granules surrounding a dark nucleus were seen in FosLI/HRP double-labeled neurons (Figure 1C). HRP/FosLI/TH-LI triple-labeled neurons contained a dark nucleus and black punctate granules on homogeneous brown background of the cytoplasm (Figure 2B). As the three different reaction products were clear and definite, the differently labeled neurons could easily be distinguished.

Retrogradely labeled HRP neurons were predominately observed in the medulla ipsilateral to the HRP injection site. Most of them were distributed in the NTS and VLM at the middle and caudal levels of the medulla. Most HRP labeled cell bodies in the NTS and VLM were fusiform or oval in shape, and medium or small in size with a diameter ranging from 15 to 30  $\mu\text{m}$ . A few HRP labeled neurons were scattered in the medulla reticular formation (RF) between the NTS and VLM.

TH-LI neurons were mainly distributed in the A1 and A2 regions at the middle and caudal levels of the medulla. TH-LI neurons in NTS and VLM were fusiform or oval in shape and small or medium in size with diameters ranging from 20 to 30  $\mu\text{m}$ . A few TH-LI neurons were also found in the RF.

FosLI neurons were bilaterally distributed and were frequently observed in the medial subnucleus (SoIM) and commissural subnucleus (SoIC) of the NTS at the middle and caudal levels of the medulla, although they were distributed throughout the entire rostro-caudal extent of the medulla, and additionally in the VLM and RF.

The number of labeled neurons was counted in one rat (Table 1). The total number of HRP labeled neurons was 162, while TH-LI neurons was 263, and FosLI neurons was 1275 in the medulla. FosLI/TH-LI double-labeled neurons (314) were most numerous among all double-labeled neurons and constituted 14.4% (314/2185) of the total population of labeled neurons. On the other hand, HRP/TH-LI and FosLI/HRP double-labeled neurons were much less frequent than FosLI/TH-LI double-labeled neurons, with 77 HRP/TH-LI

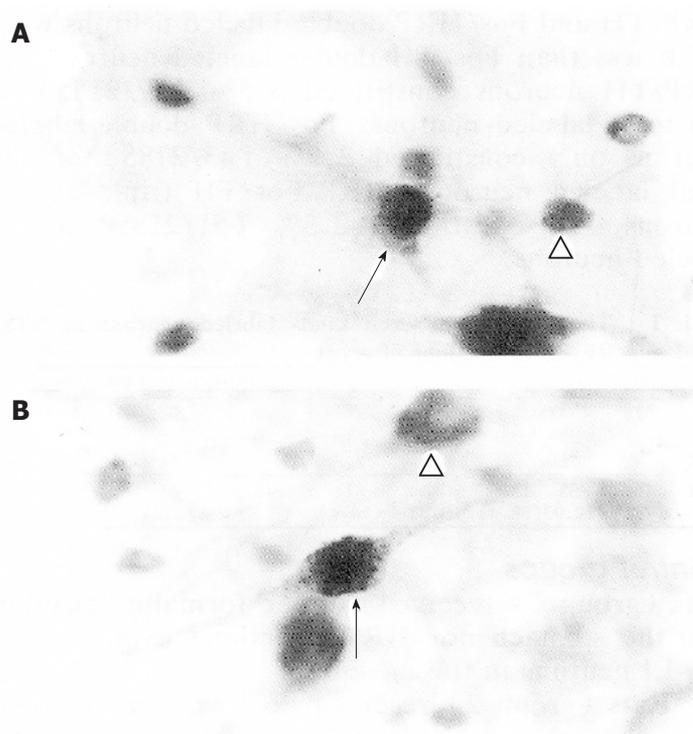


Figure 2 A: Fos-like immunoreactive neurons (open triangle) and Fos-like immunoreactive/Tyrosin hydroxylase-like immunoreactive double-labeled neuron (arrow) in the nucleus tractus solitarius of medulla; B: Tyrosin hydroxylase-like immunoreactive (open triangle) and Fos-like immunoreactive/Horseradish peroxidase/Tyrosin hydroxylase-like immunoreactive triple-labeled neuron (arrow) in the nucleus tractus solitarius of medulla, × 400.

Table 1 Number of seven kinds of labeled neurons in nucleus tractus solitarius, ventrolateral medulla and reticular formation of the medulla in the sections of a rat

	HRP	TH-LI	FosLI	HRP/TH-LI	FosLI/TH-LI	FosLI/HRP	HRP/FosLI/TH-LI	Total
NTS	119	153	937	41	77	28	33	1388
RF	11	32	204	7	19	6	2	281
VLM	32	78	134	29	218	9	16	516
Total (%)	162 (7.4)	263 (12.0)	1275 (58.4)	77 (3.5)	314 (14.4)	43 (2.0)	51 (2.3)	2185 (100)

HRP: Horseradish peroxidase; TH-LI: Tyrosin hydroxylase-like immunoreactive; FosLI: Fos-like immunoreactive; NTS: Nucleus tractus solitarius; VLM: ventrolateral medulla; RF: Reticular formation.

neurons constituting 3.5% (77/2185) of the total labeled neurons. FosLI/HRP double-labeled neurons only constituted 2.0% (43/2185) of the total labeled neurons. HRP/FosLI/TH-LI triple-labeled neurons only constituted 2.3% (51/2185) of all labeled neurons.

### Control groups

Rats that did not receive either the formalin injection into the stomach or the HRP injection (group 1) did not express any FosLI neurons in the medulla.

Rats that did not receive a formalin injection into their stomachs, but did inhale the methoxyflurane gas anesthesia (group 2) did express a minimal amount of FosLI neurons in the medial septal nucleus and the PVH at 2 h after inhalation.

Rats subjected to HRP injection into the PVH and sacrificed 48 h later (group 3) did not express FosLI neurons in the medulla.

The sections that were used to verify the antibodies using the substitution test (group 4) were negative, with neither FosLI nor TH-LI detected in the medulla of these sections.

## DISCUSSION

A previous study had demonstrated that after a tritiated amino acid tracer was injected into the NTS of rats, the longest distance of the labeled ascending fibers and terminals was observed at the level of the pons<sup>[4]</sup>. A different study found that the injection of HRP into the PVH led to a significant number of HRP labeled neurons in the VLM<sup>[3]</sup>. Other more recent studies have confirmed that neurons in the NTS and VLM send axons to the PVH<sup>[3,5,6,9]</sup>. Our results have proved that the projection fibers to the PVH originated mainly from the SolM and SolC and NTS and the VLM, with fewer projections from RF at the middle and caudal segments of medulla. However, the transmitters, modulators and function of these projecting neurons were not known. Phaseolus vulgaris leucoagglutinin (PHA-L) anterograde tracing study has demonstrated that the neurons in the A1 and A2 regions project to the PVH<sup>[2]</sup>. In this study, we found that FosLI neurons were observed within the NTS, VLM and RF of the rat following gastrointestinal (visceral) noxious stimulation<sup>[7]</sup>.

Our results show that 77% (1683/2185) of the total population of labeled neurons in the NTS, VLM and RF responded to visceral noxious stimulation, and were Fos positive. About 15% (333/2185) of all seven kinds of labeled neurons in the NTS, VLM and RF sent their axons to the PVH, about 5.9% of the neurons projected to the PVH exhibited TH-LI, and some of these TH-LI neurons were Fos positive. These results suggest that TH-LI neurons in the NTS, VLM and RF might be involved in the transmission of nociceptive information from the stomach to the PVH.

## ACKNOWLEDGMENTS

The authors are grateful to Prof. Li YQ for his critical reading of the manuscript and helpful suggestions.

## REFERENCES

- Mantyh PW, Hunt SP. Neuropeptides are present in projection neurons at all levels in visceral and taste pathways: from periphery to sensory cortex. *Brain Res* 1984; **299**: 297-312 [PMID: 6733452 DOI: 10.1016/0006-8993(84)90711-X]
- Cunningham ET, Sawchenko PE. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol* 1988; **274**: 60-76 [PMID: 2458397 DOI: 10.1002/cne.902740107]
- Ciriello J, Caverson MM. Ventrolateral medullary neurons relay cardiovascular inputs to the paraventricular nucleus. *Am J Physiol* 1984; **246**: R968-R978 [PMID: 6204539]
- Norgren R. Projections from the nucleus of the solitary tract in the rat. *Neuroscience* 1978; **3**: 207-218 [PMID: 733004 DOI: 10.1016/0306-4522(78)90102-1]
- Ciriello J, Caverson MM, Polosa C. Function of the ventrolateral medulla in the control of the circulation. *Brain Res* 1986; **396**: 359-391 [PMID: 3542115 DOI: 10.1016/0165-0173(86)90005-6]
- Ricardo JA, Koh ET. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res* 1978; **153**: 1-26 [PMID: 679038 DOI: 10.1016/0006-8993(78)91125-3]
- Chen LW, Jin GR, Rao ZR, Shi JW. C-fos expression within central nervous system of the rat following gastrointestinal visceral noxious stimulation. *Shenjing Jiepo Xue Zazhi* 1993; **9**: 185-191
- Rao ZR, Chen LW, Jin GR, Han ZA, Dong YX, Xiong KH. Simultaneous demonstration of Fos protein, tyrosine hydroxylase (TH) and HRP retrograde labeling in same neurons-A triple labeling technique. *Acta Anatomica Sinica* 1994; **25**: 219-223
- Kannan H, Yamashita H. Connections of neurons in the region of the nucleus tractus solitarius with the hypothalamic paraventricular nucleus: their possible involvement in neural control of the cardiovascular system in rats. *Brain Res* 1985; **329**: 205-212 [PMID: 3978442 DOI: 10.1016/0006-8993(85)90526-8]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Characteristics of DNA repair induced by DNA polymerase $\beta$ in hepatoma cells after $\gamma$ -ray irradiation

Jian-Ming Cai, Xiu-Long Zheng, Cheng Luo, Jian-Guo Gao, Tian-Min Cheng

Jian-Ming Cai, Xiu-Long Zheng, Jian-Guo Gao, Department of Radiation Medicine, Second Military Medical University, Shanghai 200433, China

Cheng Luo, Tian-Min Cheng, Third Military Medical University, Chongqing 630038, China

Jian-Ming Cai, Associate Professor of Radiobiology, Vice Director of the Department of Radiation Medicine, having 26 papers published.

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 39370230.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Jian-Ming Cai, Associate Professor, Department of Radiation Medicine, Second Military Medical University, Shanghai 200433, China  
Telephone: +86-21-65347018-71499

Received: September 14, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the effects of DNA repair induced by DNA polymerase  $\beta$  in hepatoma cells after  $\gamma$ -ray irradiation.

**METHODS:** Cell nuclei were prepared from mouse model (SMMC LTNM), in which human hepatoma cells are transplanted on nude mice. The nuclei were then irradiated with  $^{60}\text{Co}$ - $\gamma$  rays at different dose levels or dose rates. A selective inhibitor test was then used to detect the effects of the radiation on DNA repair using N-ethylmaleimide (NEM) and ddTTP as selective inhibitors to DNA polymerases  $\gamma$  and  $\beta$  respectively.

**RESULTS:**  $^3\text{H}$ -TTP incorporation into irradiated nuclei or calf thymus DNA was significantly higher than that the rate at which it is incorporated into non-irradiated nuclei when either DNA polymerase  $\alpha$  or  $\gamma$  was inhibited. When both NEM and ddTTP are present, the  $^3\text{H}$ -TTP incorporation in irradiated DNA was not significantly different from the non-irradiated nuclei. Furthermore,  $^3\text{H}$ -TTP incorporation into DNA of SMMC-LTNM hepatoma nuclei was higher than that of normal hepatocyte nuclei ( $P < 0.01$ ). This suggests that DNA repair induced by DNA polymerase  $\beta$  was more active in hepatoma cell nuclei than in normal hepatocyte nuclei.

**CONCLUSION:** DNA polymerase  $\beta$  may be more responsive to

DNA damage in some tumor cells than that in normal cells, which may facilitate the cells to repair DNA damages from radiation more efficiently.

**Key words:** DNA polymerases; DNA repair  $\gamma$ -rays; Liver neoplasms; Liver/radiation effects

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Cai JM, Zheng XL, Luo CJ, Gao JG, Cheng TM. Characteristics of DNA repair induced by DNA polymerase  $\beta$  in hepatoma cells after  $\gamma$ -rays irradiation. *World J Gastroenterol* 1997; 3(2): 75-77 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/75.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.75>

### INTRODUCTION

DNA polymerase  $\beta$  (pol  $\beta$ ), which is the smallest and simplest polymerase, exists in most vertebrate cells. The exact cellular roles of pol  $\beta$  are still obscure, especially in the repair of DNA damaged from  $\gamma$ -ray exposure<sup>[1-3]</sup>. However, recent studies showed that pol  $\beta$  could participate in DNA repair synthesis after  $^{60}\text{Co}$ - $\gamma$  ray irradiation. In the present study, the differential characteristics of pol  $\beta$  in radiation-induced DNA damage in DNA repair between hepatoma cells and normal liver cells was investigated.

### MATERIALS AND METHODS

#### Nuclei preparations

BALB/c or NC nude mice bearing SMMC-LTNM hepatomas were killed and the livers and SMMC-LTNM hepatomas were harvested. The tissues were washed with Hanks solution, cut into pieces, homogenized and filtered through a nylon net. The cells were collected by centrifuge and the nuclei were isolated as described previously<sup>[4]</sup>.

#### Sample irradiation

Cell nuclei suspensions or calf thymus DNA solutions (for the controls; Sigma Co.) were put in 1.5 mL sterile Eppendorf tubes in an ice bath before being transferred into a field of  $^{60}\text{Co}$ - $\gamma$  rays ( $1.85 \times 10^{15}$  Bq) and irradiated with 1-40 Gy at 1-10 Gy/min dose rates. The samples were maintained in an ice bath throughout the procedures. The DNA repair synthesis test was carried out immediately after irradiation.

#### Assay for DNA repair synthesis

DNA repair synthesis was measured in the following reaction

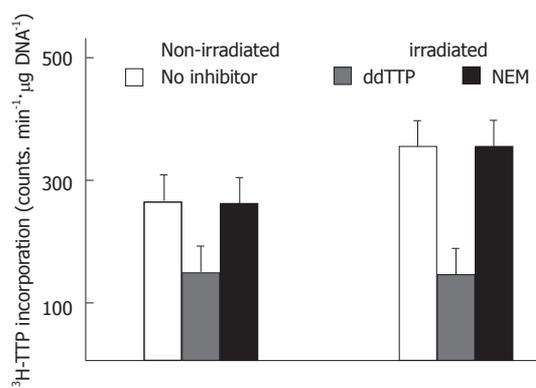


Figure 1 Effects of selective inhibitors on DNA repair synthesis regulated by DNA polymerase  $\beta$ .

Table 1 Effect of DNA polymerase  $\beta$  on the repair of  $\gamma$ -rays irradiated nuclei DNA at different dose rates ( $\bar{x} \pm s$ )

Dose rate (Gy·min <sup>-1</sup> )	<sup>3</sup> H-TTP incorporation (counts · min <sup>-1</sup> · μg DNA <sup>-1</sup> )	
	Hepatoma nuclei	Hepatocyte nuclei
1	281 ± 50	207 ± 24
5	282 ± 27	204 ± 18
10	284 ± 37	208 ± 22
Control	236 ± 35 <sup>a</sup>	179 ± 17 <sup>a</sup>

$n = 7$ , <sup>a</sup> $P < 0.05$  compared with irradiated group.

mixture. Each reaction mixture contained 0.1 mL sample solution (nuclei or calf thymus DNA) and 0.1 mL DNA repair synthesis reaction solution. The reaction solution contained, at a final concentration, 18 mmol/L Tris-HCl (pH8.5), 2 mmol/L dithiothreitol, 40 mmol/L MgCl<sub>2</sub>, 4 mmol/L ATP, 2 mmol/L dCTP, dGTP, dATP, and 3.7 × 10.4 Bq <sup>3</sup>H-TTP. Selective inhibitors (NEM or ddTTP) were used as required. For the calf thymus DNA samples, rat DNA polymerase  $\beta$  (a gift from Dr. Akio Matsukage) was also put in the reaction mixtures. After incubation at 37 °C for 120 min with shaking, 1 mL reaction stopping solution containing 1 mol/L perchloric acid and 20 mmol/L sodium pyrophosphate was added to each reaction mixture. The radioactive DNA product was collected on a piece of cellulose paper, and the incorporation of <sup>3</sup>H-TTP was measured using a scintillation counter.

## RESULTS

### Effect of pol $\beta$ in DNA repair synthesis

In order to evaluate the role of pol  $\beta$  in DNA radiation damage repair, we first examined the effects of radiation on DNA repair by comparing the amount of <sup>3</sup>H-TTP incorporation in irradiated and non-irradiated calf thymus DNA (10 Gy, 5 Gy/min). The <sup>3</sup>H-TTP incorporation was much higher ( $P < 0.01$ ) in irradiated DNA than in non irradiated DNA (Figure 1). This increase was also present in DNA treated with NEM. However, when ddTTP was added to the reaction mixture to inhibit pol  $\beta$ , the amount of <sup>3</sup>H-TTP incorporation was similar in the irradiated and non-irradiated DNA (Figure 1).

Next, we examined DNA repair in hepatoma and normal liver nuclei. As seen with the calf-thymus DNA, the incorporation of <sup>3</sup>H-TTP into both irradiated SMMC-LTNM hepatoma nuclei and normal liver nuclei was higher ( $P < 0.05$ ) than that in non irradiated nuclei (Table 1). To investigate whether pol  $\beta$  is responsible for the DNA repair synthesis in irradiated nuclei, a subset of irradiated hepatoma and normal liver nuclei were treated with the pol  $\beta$  selective inhibitor ddTTP. As illustrated in Figure 2, the incorporation of <sup>3</sup>H-TTP did not significantly increase in nuclei treated with ddTTP; however, nuclei from both hepatoma and normal liver that were not treated with ddTTP had a significant increase in the incorporation of <sup>3</sup>H-TTP ( $P < 0.01$ ).

### Characteristics of DNA repair synthesis induced by pol $\beta$ in hepatoma

The characteristics of DNA repair synthesis induced by pol  $\beta$  in SMMC-LTNM hepatoma nuclei were compared with that in normal hepatocyte nuclei after different doses of  $\gamma$ -ray exposure. We found that the increment of <sup>3</sup>H-TTP incorporation (<sup>3</sup>H-TTP incorporation of

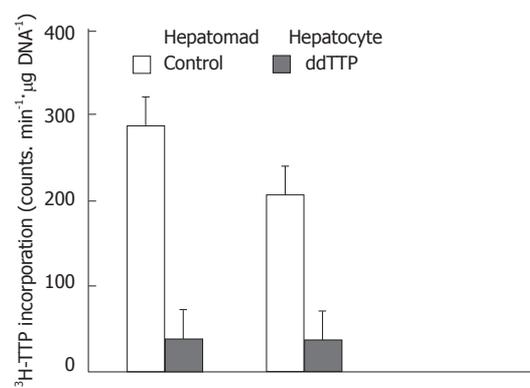


Figure 2 Inhibition effect of ddTTP on the DNA repair synthesis regulated by polymerases in nuclei of hepatomas and hepatocytes.

Table 2 Comparison of the increment of <sup>3</sup>H-TTP incorporation of irradiated hepatoma nuclei with that of hepatocyte nuclei (counts · min<sup>-1</sup> · μg DNA<sup>-1</sup>,  $\bar{x} \pm s$ )

Dose rate (Gy·min <sup>-1</sup> )	Increment of <sup>3</sup> H-TTP incorporation	
	Hepatoma nuclei	Hepatocyte nuclei
1	45 ± 8.0 <sup>b</sup>	28 ± 2.0
5	46 ± 4.4 <sup>b</sup>	29 ± 4.1
10	48 ± 6.3 <sup>b</sup>	32 ± 5.1

$n = 7$ , <sup>b</sup> $P < 0.01$  compared with hepatocyte nuclei group.

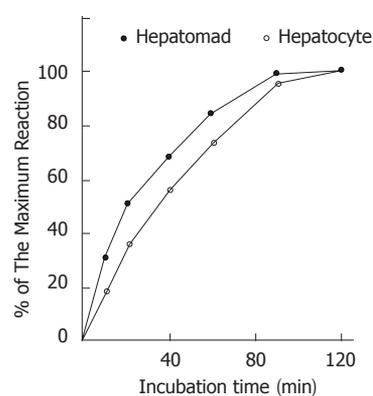


Figure 3 Extent of <sup>3</sup>H-TTP incorporating into irradiated nuclei of hepatoma and hepatocyte at different incubation time induced by DNA polymerase  $\beta$ .

irradiated nuclei-<sup>3</sup>H-TTP incorporation of non irradiated nuclei) of hepatoma nuclei were much higher than that in normal hepatocyte nuclei ( $P < 0.01$ ) at all dose rate groups under the same reaction conditions and same absorbed dose (10 Gy) (Table 2).

Further investigation revealed that there was a significant difference in the reaction speed of DNA repair synthesis induced by pol  $\beta$  between the two kinds of nuclei. The reaction induced by pol  $\beta$  in hepatoma nuclei was faster than that in hepatocyte nuclei. The incubation time, which the <sup>3</sup>H-TTP incorporation increased to 50% of the maximum, was about 18 min in hepatoma nuclei and about 32 min in normal hepatocyte nuclei (Figure 3).

## DISCUSSION

DNA damage caused by ionization can be partly repaired under proper conditions<sup>[5]</sup>. However, the mechanism of DNA repair is not clearly understood<sup>[6,7]</sup>. The role of pol  $\beta$  in DNA repair after ionization radiation exposure is one of the problems remaining to be solved. For example, which polymerase is responsible for DNA repair synthesis after  $\gamma$ -ray exposure has been a controversy in radiobiology. The evidence obtained in this study further demonstrates that pol  $\beta$  does participate in DNA repair synthesis and it is an important enzyme in the process of DNA repair after  $\gamma$ -ray exposure.

This study also found some intriguing differences in the DNA repair induced by pol  $\beta$  between SMMC-LTNM hepatoma cells and normal hepatocytes. We found that SMMC-LTNM hepatoma cells had a faster DNA repair synthesis reaction, a higher incorporation of <sup>3</sup>H-TTP after radiation damage, and a lower dose of radiation

required to reach the maximum  $^3\text{H-TTP}$  incorporation. All these characteristics suggest that the DNA repair synthesis induced by pol  $\beta$  in hepatoma cells was strong. In other experiments we found that the gene of pol  $\beta$  was overexpressed and the enzyme activity was high in hepatomas. These findings are meaningful in the radiotherapy of tumors. Cell death in radiotherapy is closely related to the extent of DNA damage and DNA repair. Cells in which their pol  $\beta$  gene is overexpressed and which have high enzymatic activity have a stronger ability for DNA repair synthesis, and therefore, more cells could theoretically survive the killing effects of radiation. The resistance to ionization radiation of some tumor cells may be related with the effects of pol  $\beta$ . It is highly necessary to investigate the radiobiologic characteristics of pol  $\beta$  in more tumors, especially in those tumors which are resistant to radiation, and to study the radiosensitizing effects by the way of selectively inhibiting the pol  $\beta$  in tumor cells.

## REFERENCES

- 1 **Davies JF**, Almassy RJ, Hostomska Z, Ferre RA, Hostomsky Z. 2.3 A crystal structure of the catalytic domain of DNA polymerase beta. *Cell* 1994; **76**: 1123-1133 [PMID: 8137427 DOI: 10.1016/0092-8674(94)90388-3]
- 2 **Nowak R**, Siedlecki JA. Effect of busulphan treatment and elevated temperature on the expression of the beta-pol gene in rat testis. *Mol Biol Rep* 1991; **15**: 25-31 [PMID: 1678854 DOI: 10.1007/BF00369897]
- 3 **Cai JM**, Zheng XL, Luo CJ, Gao JG, Chen TM, Yang RJ. The roles of DNA polymerase Y on repair of DNA irradiated by Y-rays. *J Med Coll PLA* 1995; **10**: 75-78
- 4 **Jiang YF**, Zheng XL. Effect of DNA polymerase-Y on repair of Y-ray irradiated hepatoma (Ha22) cell nuclei. *J Radiant Res Radiant Process* 1990; **8**: 150-154
- 5 **Price A**. The repair of ionising radiation-induced damage to DNA. *Semin Cancer Biol* 1993; **4**: 61-71 [PMID: 8513149]
- 6 **Friedberg EC**. DNA repair. San Francisco: W.H. Freeman and Company 1985: 75-220
- 7 **Ohnishi T**, Yuba S, Date T, Utsumi H, Matsukage A. Rat DNA polymerase beta gene can join in excision repair of Escherichia coli. *Nucleic Acids Res* 1990; **18**: 5673-5676 [PMID: 2216761 DOI: 10.1093/nar/18.19.5673]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Short- and long-term effect of interferon therapy for chronic hepatitis C

Zhen-Ya Tang, Jun-Ying Qi, Han-Xing Shen, Dong-Liang Yang, Lian-Jie Hao

Zhen-Ya Tang, Jun-Ying Qi, Han-Xing Shen, Dong-Liang Yang, Lian-Jie Hao, Clinical Immunology Research Unit, Department of Infectious Diseases, Tongji Hospital, Tongji Medical University, Wuhan 430030, Hubei Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: September 14, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the short and long-term effect of interferon therapy for chronic hepatitis C.

**METHODS:** Sixty-eight patients with chronic hepatitis C were treated with interferon ( $3 \times 10^6$  IU, im/2 d, for a course of three months) with 1 to 5 courses of treatment and followed for 1.5 to 3 years after the therapy.

**RESULTS:** According to antiviral effect of interferon, 76.5% (52/68)

of the cases had a complete response by the end of the first therapy course, while 20.6% (14/68) and 2.9% (2/68) had a partial response or non-response. Over a half of the patients with a complete response (27/52, 51.9%) relapsed within 6 to 10 mo after the first course. Of the original cohort, nineteen patients received two courses of therapy, while one patient received three and another three received five courses of therapy. The follow-up for these patients was between 1.5 to 3 years, at which time 29 (42.7%) of the patients sustained a complete response, with four of them having HCV RNA positive serum, while the others had either a partial (37/68, 2.9%) or non-response.

**CONCLUSION:** Interferon therapy had a high short-term complete response but a low long-term complete response in the treatment of chronic hepatitis C.

**Key words:** Hepatitis C/therapy; Interferons/therapeutic use

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Tang ZY, Qi JY, Shen HX, Yang DL, Hao LJ. Short- and long-term effect of interferon therapy for chronic hepatitis C. *World J Gastroenterol* 1997; 3(2): 77 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/77.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.77>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Protective effect of vitamin E on age-related alterations of Kupffer cell energy metabolism

Wen-Bing Sun, Rui-Liang Ma, Zhi-Ming Peng, Kun Li, Heng-Chun Duan, Ben-Li Han

Wen-Bing Sun, Rui-Liang Ma, Zhi-Ming Peng, Kun Li, Heng-Chun Duan, Ben-Li Han, Hepatobiliary Surgery Center, Southwest Hospital, the Third Military Medical University, Chongqing 630038, China

Wen-Bing Sun, PhD, Associate Professor of Surgery, Vice Surgeon in Chief, having 40 papers published.

Author contributions: All authors contributed equally to the work.

Granted by The Chinese PLA "8<sup>th</sup> Five year Plan" Research Funds, No. 91C093-0199.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Wen-Bing Sun, PhD, Associate Professor, Hepatobiliary Surgery Center, Southwest Hospital, the Third Military Medical University, Chongqing 630038, China  
Telephone: +86-811-8754232

Received: December 21, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

### Abstract

**AIM:** To investigate the mechanism of age-related reduction of Kupffer cell (KC) phagocytic capacity and the protective management.

**METHODS:** Using rhodamine 123 fluorescence density and rate of glucose utilization as parameters, we measured the mitochondrial energy metabolism status *in vitro* and the glucose utilization capacity of isolated rat liver Kupffer cells (KCs) from rats of various ages (6 mo, 12 mo, 18 mo and 24 mo) and the effect of vitamin E (VE) pretreatment (500 mg/kg/wk × 13 wk).

**RESULTS:** The rate of KC glucose utilization and the rhodamine fluorescence density of KC mitochondria of 18 mo-old untreated rats (NVEG) were significantly lower than that of 6 mo-old NVEG by 19.3% ( $4.0 \text{ nmol}\cdot\text{h}^{-1} \pm 0.4 \text{ nmol}\cdot\text{h}^{-1} \cdot 10^6 \text{ cells}^{-1}$  vs  $5.7 \text{ nmol}\cdot\text{h}^{-1} \pm 0.6 \text{ nmol}\cdot\text{h}^{-1} \cdot 10^6 \text{ cells}^{-1}$ ,  $P < 0.05$ ) and 19.5% ( $80.5 \pm 6.3$  vs  $100.0 \pm 4.7$ ,  $P < 0.01$ ) respectively; Rate of KC glucose utilization and the rhodamine fluorescence density of KC mitochondria of 6 mo-old rats were also lower than the 24 mo-old NVEG by 35.1% ( $3.7 \text{ nmol}\cdot\text{h}^{-1} \pm 0.6 \text{ nmol}\cdot\text{h}^{-1} \cdot 10^6 \text{ cells}^{-1}$  vs  $5.7 \text{ nmol}\cdot\text{h}^{-1} \pm 0.6 \text{ nmol}\cdot\text{h}^{-1} \cdot 10^6 \text{ cells}^{-1}$ ,  $P < 0.01$ ) and 32.1% ( $67.9 \pm 7.4$  vs  $100.0 \pm 4.7$ ,  $P < 0.01$ ) respectively. The two parameters of 18 mo-old VE pretreated rats (VEG) were significantly higher than those of 18 mo-old NVEG, and statistically comparable to those of 6 mo-old VEG. The two parameters of the 24 mo-old VEG were significantly higher in comparison with those of 24 mo-old NVEG, but still significantly lower than those of 6 mo-old

VEG.

**CONCLUSION:** Aging has a significantly negative effect on KC energy metabolism, which can be alleviated by VE pretreatment.

**Key words:** Aging; Kupffer cells; Energy metabolism; Glucose metabolism; Vitamin E; Liver metabolism

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Sun WB, Ma RL, Peng ZM, Li K, Duan HC, Han BL. Protective effect of vitamin E on age-related alterations of Kupffer cell energy metabolism. *World J Gastroenterol* 1997; 3(2): 78-80 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/78.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.78>

### INTRODUCTION

Aged Kupffer cells (KCs) have a decreased phagocytic capacity<sup>[1]</sup>. A recent study found that alterations of cellular energy metabolism is one of the main causes responsible for the disordered cellular function<sup>[2]</sup>. The present study investigated the possible protective effect of vitamin E on the decrease in KC energy metabolism from aging in order to further study the mechanism of age-related suppressed phagocytosis, and a possible clinical intervention.

### MATERIALS AND METHODS

#### Animal and groups

Forty healthy Wistar rats of both sexes and different ages were obtained from the Chinese Herb Research Institute of Sichuan Province. Rats were housed in climatized rooms on a 12 h light/dark cycle, with water and food *ad libitum* and were studied at 6, 12, 18 and 24 mo of age. Ten rats for each age group were divided at random into two groups, five treated with VE (VEG) and five that were not treated (NVEG), for a total of eight treatment groups with  $n = 5$ . Animals in VEG received an intraperitoneal injection of 5% VE solution for 3 mo (500 mg/kg-wk, 2 injections/wk) prior to the experiment. Animals in the NVEG groups received normal saline as control.

#### Isolation of KC

KCs were isolated using a collagenase perfusion method as previously described<sup>[1]</sup>. Briefly, after anesthesia (30 mg of barbital/kg body wt., intraperitoneally), the liver was perfused *in situ* with  $\text{Ca}^{2+}$  free Hanks balanced salt solution at 37 °C for 3 min. Then 0.05% collagenase (Type IV; Sigma) was added and the liver was perfused for an additional 4 min with Hanks balanced salt solution. The liver

**Table 1** Rhodamine 123 fluorescence density of mitochondria and rate of glucose utilization of Kupffer cells of different age groups ( $n = 5$ ,  $\bar{x} \pm s$ )

Group	Rhodamine 123 fluorescence density utilization of mitochondrion		Rate of glucose ( $\text{nmol}\cdot\text{h}^{-1}\cdot 10^6 \text{ cells}^{-1}$ )	
	NVEG	VEG	NVEG	VEG
6	100.0 $\pm$ 4.7	100.0 $\pm$ 5.3	5.7 $\pm$ 0.6	5.6 $\pm$ 0.8
12	97.5 $\pm$ 7.1	99.4 $\pm$ 8.6	5.9 $\pm$ 0.8	6.0 $\pm$ 0.7
18	80.5 $\pm$ 6.3 <sup>b</sup>	97.3 $\pm$ 6.8 <sup>cd</sup>	4.0 $\pm$ 0.4 <sup>e</sup>	5.4 $\pm$ 0.5 <sup>d</sup>
24	67.9 $\pm$ 7.4 <sup>b</sup>	84.2 $\pm$ 8.7 <sup>bd</sup>	3.7 $\pm$ 0.6 <sup>b</sup>	4.5 $\pm$ 0.6 <sup>bd</sup>

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs 6 mo group; <sup>d</sup> $P < 0.01$  vs non-vitamin E group (NVEG). VEG: Vitamin E pretreated group.

was then removed and placed in buffer. Under a tissue culture hood, the liver was gently teased with a smooth forceps to release the cells and filtered through a 60  $\mu\text{m}$  mesh. The cells were then centrifuged twice at  $50 \times g$  for 3 min to remove parenchymal cells (sediment). The supernatant was then sedimented at  $300 \times g$  for 10 min. The pellet of nonparenchymal cells was resuspended and cultured with RPMI 1640 medium (containing 15 mmol/L HEPES, 0.05 U/mL insulin, 15 mmol/L L-glutamine, 100 U/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin) supplemented with 10% newborn calf serum. After 30 min, the non-adherent cells were removed. The viability of KCs was greater than 90% as determined by trypan blue exclusion.

#### Measurement of mitochondrial energy metabolism

The technique used to measure KC mitochondrial energy metabolism was a modification of that described previously<sup>[3]</sup>. Briefly, KCs were seeded on collagen-coated glass coverslips in 6-well plates at a density of  $4 \times 10^5$  mL/well. After overnight culture, the wells were replenished with fresh medium, and supplemented with rhodamine 123 (Sigma) at a final concentration of 0.8  $\mu\text{mol}/\text{L}$ . Cells were incubated at 37  $^{\circ}\text{C}$  for 15 min and then washed 3 times with RPMI 1640 medium. The coverslips were viewed with a fluorescence microscope (Olympus VANOX AHB-LB) using a 40 X objective. Excitation light for fluorescence was provided by a 100 watt lamp set at 490 nm. The fluorescence images were collected using a Panasonic CL320 videotape lens put into a computer image analyzing system (CMIAS007) and quantitative assessment of fluorescence was made using the computer software. Since rhodamine actively accumulates in mitochondria in response to changes in transmembrane potential, the amount of intracellular accumulation of this dye can be used to reflect the mitochondrial energy metabolism status.

#### Measurement of glucose utilization

Glucose utilization was measured using a modified method reported by Spolarics<sup>[4]</sup>. Briefly, KCs were seeded into a 24-well culture plate. The culture medium contained 20 mmol/L HEPES, 4.2 mmol/L  $\text{NaHCO}_3$ , 120 mmol/L NaCl, 5.4 mmol/L KCl, 1.8 mmol/L  $\text{CaCl}_2$ , 1.2 mmol/L  $\text{MgSO}_4$ , 5 mmol/L glucose and 1% albumin with the pH adjusted to 7.4. After 24 h of culture, the supernatant was collected and stored at -20  $^{\circ}\text{C}$  until analysis. Glucose concentration was assayed enzymatically using oxidase and peroxidase.

#### Statistical analysis

Data were analyzed using variance analysis (ANOVA). When an ANOVA indicated a significant difference ( $P < 0.05$ ), post hoc analysis was performed using Student-Newman-Keuls test.

## RESULTS

The rate of KC glucose utilization and the rhodamine 123 fluorescence density of KC mitochondrion decreased with age (Table 1). These two factors were significantly lower in both 18 and 24 mo-old NVEG animals as compared to 6 mo-old NVEG rats. The rates of KC glucose utilization and the rhodamine fluorescence densities of KC mitochondria of 18 mo-old VEG were significantly higher than those of 18 mo-old NVEG, and statistically comparable to those of 6 mo-old VEG. These two factors were also significantly higher in 24 mo-old VEG animals when comparison with those of 24 mo-old NVEG rats, but still significantly lower than those of the 6 mo-old VEG animals.

## DISCUSSION

Several studies<sup>[4]</sup> have demonstrated that macrophages readily oxidize glucose, and that glycolysis is an important source of energy in these cells. In physiological conditions, KCs are activated by endotoxins from the gut, and to some degree are a prerequisite for the regulation of some of the important functions of hepatocyte<sup>[5]</sup>. Therefore, a normal glucose utilization by KCs may be necessary to support liver's numerous metabolic and immunological functions. Therefore, it is of both theoretical and clinical significance to study the KC glucose utilization ability and mitochondrial energy metabolism status.

So far, very few reports are available on the KC mitochondrial energy metabolism. The reasons for this may include the complexity of the isolation process of KC, the limited number of KC recovered, and the difficulty of the mitochondria isolation, making it impossible to investigate KC mitochondrial respiratory function by the polarographic method usually used for hepatocytes' mitochondrial study. In this study, we examined the energy metabolism status of KC with rhodamine 123, as reported by Lemasters<sup>[3]</sup> originally used to investigate the energy metabolism of hepatocytes.

Rhodamine 123 uptake in living cells can be used as an indicator of mitochondrial membrane potential, since it has been reported that this fluorescent dye actively accumulates in mitochondria in response to changes in transmembrane potential<sup>[6]</sup>. In the absence of inhibition of the mitochondrial ATPase, the mitochondrial membrane potential should, more or less, be proportional to the phosphorylation potential, which is the free energy change of ATP synthesis. The present study demonstrated that this method was also a qualified one for the study of the mitochondrial energy metabolism of KC.

Oxidants are a major contributor to the aging process<sup>[7]</sup>. VE is the most effective chain breaking lipid-soluble antioxidant in the biological membrane, where it contributes to membrane stability. It protects critical cellular structures against damage from oxygen free radicals (an atom or group of atoms containing an unpaired electron) and reactive products of lipid peroxidation<sup>[8]</sup>. We used the VE supplementation dosage for the prevention of acute tissue injury reported elsewhere to determine the dosage used in the present study for the prevention or the treatment of ageing induced KC functional disorders.

The results of our study found that the rate of KC glucose utilization and the rhodamine 123 fluorescence density of KC mitochondrion decreased with age. These two factors were significantly lower in 18 and 24 mo-old untreated rats (NVEGs) than that of 6 mo-old NVEG. These data are further evidence of the aging theory of energy metabolism disorders. At the same time, these results also indicate that in the evolving course of Wistar rats, the KC age-related energy metabolism disorders occurred between the ages of 12 and 18 mo, a period equivalent to that of the ages of 40 and 55 years-old for human beings. These results also showed that the rates of KC glucose utilization and the rhodamine fluorescence densities of KC mitochondria of 18 mo old VEG rats were significantly higher than those of 18 mo-old NVEG rats and statistically comparable to those of 6 mo-old VEG. Additionally, those factors were significantly higher in 24 mo-old VEG rats as compared with those of 24 mo-old NVEG rats; however, they were still significantly lower than those of 6 mo-old VEG. From the data above we might as well infer that VE is effective in preventing the KC mitochondrial decay due to ageing, however it can only alleviate the already formed damage to some degree, indicating that the principle of "Prevention First" should be followed by the human being for the management of aging, which is in conformity with the principle of the "fight against aging should begin from childhood" in traditional Chinese medicine.

## REFERENCES

- 1 Praaning-Van Dalen DP, Knook DL. Quantitative determination of *in vivo* endocytosis by rat liver Kupffer and endothelial cells facilitated by an improved cell isolation method. *FEBS Lett* 1982; **141**: 229-232 [PMID: 7095151 DOI: 10.1016/0014-5793(82)80054-9]
- 2 Castelluccio C, Baracca A, Fato R, Pallotti F, Maranesi M, Barzanti V, Gorini A, Villa RF, Parenti Castelli G, Marchetti M. Mitochondrial activities of rat

- heart during ageing. *Mech Ageing Dev* 1994; **76**: 73-88 [PMID: 7885068 DOI: 10.1016/0047-6374(94)91583-0]
- 3 **Lemasters JJ**, DiGuseppi J, Nieminen AL, Herman B. Blebbing, free Ca<sup>2+</sup> and mitochondrial membrane potential preceding cell death in hepatocytes. *Nature* 1987; **325**: 78-81 [PMID: 3099216 DOI: 10.1038/325078a0]
- 4 **Spolarics Z**, Lang CH, Bagby GJ, Spitzer JJ. Glutamine and fatty acid oxidation are the main sources of energy for Kupffer and endothelial cells. *Am J Physiol* 1991; **261**: G185-G190 [PMID: 1872392]
- 5 **Sun WB**, Han BL, Ma RL, Duan HC, Li K, Chen J. The role of Kupffer cells during the course of hepatocyte ageing and its mechanism. *China Natl J New Gastroenterol*, in press.
- 6 **Richelmi P**, Mirabelli F, Salis A, Finardi G, Berte F, Bellomo G. On the role of mitochondria in cell injury caused by vanadate-induced Ca<sup>2+</sup> overload. *Toxicology* 1989; **57**: 29-44 [PMID: 2749742 DOI: 10.1016/0300-483X(89)90032-2]
- 7 **Ames BN**, Shigenaga MK. Oxidants are a major contributor to aging. *Ann N Y Acad Sci* 1992; **663**: 85-96 [PMID: 1482105]
- 8 **Meydani M**. Vitamin E. *Lancet* 1995; **345**: 170-175 [PMID: 7823675 DOI: 10.1016/S0140-6736(95)90172-8]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Alterations in p53 expression and cell proliferation in esophageal epithelia among patients from geographical areas with high or low incidence of esophageal cancer

Li-Dong Wang, Qi Zhou, Yu-Chun Zhang, Xiao-Fang Li, Wen-Ping Wang, Ling He, Shan-Shan Gao, Young-Xin Li

Li-Dong Wang, Qi Zhou, Xiao-Fang Li, Shan-Shan Gao, Young-Xin Li, Laboratory for Cancer Research, Henan Medical University, Zhengzhou 450052, Henan Province, China

Yu-Chun Zhang, Wen-Ping Wang, Ling He, Fanxian People's Hospital, Fanxian County

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: December 21, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

### Abstract

**AIM:** To study changes in p53 expression and cell proliferation in esophageal epithelia of subjects from high or low esophageal cancer incidence areas in Henan Province to understand their molecular basis.

**METHODS:** Esophageal endoscopic mucosa biopsies were acquired and histopathological examinations were performed on 220 subjects from high esophageal cancer incidence areas and 50 subjects from low incidence areas in Henan Province. Esophageal epithelia were diagnosed as normal, basal cell hyperplasia or dysplasia based on cell morphology and tissue structure. Immunohistochemistry avidin biotin

peroxidase complex (ABC method) was performed to analyze alterations in p53 and proliferating cell nuclear antigen (PCNA) expression in normal epithelia and epithelia with different lesion severities. The numbers of p53-positive and PCNA-positive cells were counted.

**RESULTS:** p53- and PCNA-positive nuclei were present in esophageal epithelia from subjects from both high and low incidence areas. The number of PCNA-positive cells gradually increased with lesion severity for both the high and low incidence areas. The number of p53-positive cells was higher in high incidence areas compared to low incidence areas, and rapidly increased with lesion severity. p53 expression positively correlated with PCNA expression.

**CONCLUSION:** The number of both p53- and PCNA-positive cells increased with lesion severity. p53 expression was higher in subjects from high esophageal cancer incidence areas compared to those from low incidence areas. These results may shed light into the molecular basis for the geographical distribution of esophageal cancer.

**Key words:** Esophageal neoplasms/pathology; Genes; p53; Esophagus/pathology

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Wang LD, Zhou Q, Zhang YC, Li XF, Wang WP, He L, Gao SS, Li YX. Alterations in p53 expression and cell proliferation in esophageal epithelia among patients from geographical areas with high or low incidence of esophageal cancer. *World J Gastroenterol* 1997; 3(2): 80 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/80.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.80>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Protective effect of rhubarb on the intestinal mucosal barrier

De-Chang Chen, Xin-Yi Yang, Xiang-Yu Zhang, Xie-Yun Chen

De-Chang Chen, Xin-Yi Yang, Xiang-Yu Zhang, Xie-Yun Chen, Emergency and Critical Care Center, Shanghai Chang Zheng Hospital, Shanghai 200003, China

De-Chang Chen, born in 1963 in Shanghai and graduated from the Second Military Medical University in 1986. De Chang is an attending doctor, holds a Master's degree in critical care medicine, and is engaged in studies on gut failure in critical illness and has published 15 papers

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 39370666.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. De-Chang Chen, Emergency and Critical Care Center, Shanghai Chang Zheng Hospital, Shanghai 200003, China  
Telephone: +86-21-63275997-432

Received: November 30, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the mechanism of rhubarb protection of the gut barrier.

**METHODS:** The gut barrier damage models caused by hemorrhagic shock and intraperitoneal endotoxin were used to study the protective effect of rhubarb on the intestinal mucosal barrier. Rats were randomly divided into four groups, as follows: treatment (rhubarb) group; Positive control group; Negative control group; Placebo treatment group. Plasma endotoxin, tissue superoxidodismutase (SOD) and lipoperoxide (LPO) concentrations were measured and histological analysis was performed. Rhubarb was observed to have a protective effect on the gut.

**RESULTS:** Rhubarb decreased intestinal permeability, attenuated endotoxin absorption (endotoxin serum levels: shock group 0.557 EU/mL  $\pm$  0.069 EU/mL *vs* rhubarb group 0.345 EU/mL  $\pm$  0.055 EU/mL), and decreased tissue SOD and tissue LPO levels (SOD serum, intestine and liver levels: endotoxin group 122.92 NU/mL  $\pm$  43.19 NU/mL, 292.24 NU/mL  $\pm$  88.76 NU/mL, 272.70 NU/mL  $\pm$  85.79 NU/mL *vs* rhubarb group 312.23 NU/mL  $\pm$  54.93 NU/mL, 391.09 NU/mg  $\pm$  98.16 NU/mg, 542.86 NU/mg  $\pm$  119.93 NU/mg; LPO content in the intestine and liver: endotoxin group 8.57  $\mu$ mol/L  $\pm$  2.58  $\mu$ mol/L, 86.97  $\mu$ mol/L  $\pm$  46.54  $\mu$ mol/L *vs* rhubarb group 3.05  $\mu$ mol/L  $\pm$  1.13  $\mu$ mol/L, 13.18  $\mu$ mol/L  $\pm$  19.64  $\mu$ mol/L). Gut histopathology revealed that rhubarb promoted goblet cell proliferation, increased mucus secretion and protected intestinal mucosa in the hemorrhagic shock

model.

**CONCLUSION:** Rhubarb may protect the gut barrier by decreasing intestinal permeability, scavenging oxygen free radicals, and promoting goblet cell proliferation within the intestinal mucosa.

**Key words:** Rhubarb; Shock; Hemorrhagic; Endotoxin; Intestinal mucosa; Free radicals

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen DC, Yang XY, Zhang XY, Chen XY. Protective effect of rhubarb on the intestinal mucosal barrier. *World J Gastroenterol* 1997; 3(2): 81-83 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/81.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.81>

### INTRODUCTION

Gut-derived infection is the primary route of intensive care unit (ICU)-acquired infection<sup>[1]</sup> and is an initial pathophysiological response in multiple organ dysfunction syndrome (MODS) to trauma, shock and infection. Prevention of gut barrier damage can prevent fatal complications after severe trauma. Recent studies have shown that rhubarb has therapeutic effect on intestinal mucosal barrier damage caused by hemorrhagic shock and intraperitoneal endotoxins<sup>[2,3]</sup>. The aim of this study was to examine the mechanism of rhubarb protection of the gut barrier.

### MATERIALS AND METHODS

#### Animal models

**Hemorrhagic shock model** The hemorrhagic shock model was generated as described previously, with some modifications<sup>[4]</sup>. Briefly, Sprague-Dawley rats (350 g-450 g) were fasted overnight, then anesthetized with intraperitoneal 50 mg/kg sodium pentobarbital. The right carotid arteries were cannulated under sterile conditions, and the arterial blood pressure was measured. The animals were bled 30 min later, and the mean arterial blood pressure was reduced to 5.32 kPa and maintained for 60 min by withdrawing or reinfusing shed blood as needed. The shed blood was kept at 37 °C. The animals were resuscitated at the end of the shock period by reinfusing all of the shed blood. Catheters were sealed with heparin caps. The animals were given 2 mL saline at 2, 8, and 12 h after shock resuscitation *via* the heparin cap. Sham shock rats were anesthetized and their carotid arteries were cannulated, but no blood was withdrawn or infused. They received 2 mL saline at 2, 8, and 12 h after the operation. Blood samples were withdrawn from the carotid artery before shock and 24 h after shock resuscitation for endotoxin measurement.

**Table 1** The effect of rhubarb on plasma endotoxin (EU/mL,  $\bar{x} \pm s$ )

Group	n	Pre shock	24 h after resuscitation
Shock	16	0.163 ± 0.042	0.557 ± 0.069 <sup>b</sup>
Rhubarb	14	0.173 ± 0.039	0.345 ± 0.055 <sup>d</sup>
Placebo	5	0.209 ± 0.034	0.625 ± 0.049
Sham shock	5	0.198 ± 0.034	0.166 ± 0.043

The data were expressed as endotoxin unit (EU) per milliliter of plasma. <sup>b</sup>*P* < 0.01 vs sham-shocked rats. <sup>d</sup>*P* < 0.01 versus the shocked rats and the placebo treated rats.

**Table 2** The effect of rhubarb on the content of tissue superoxidizedismutase (NU/mL or mg,  $\bar{x} \pm s$ )

Group	n	Plasma	Small intestine	Liver
Endotoxin	17	122.92 ± 43.19	292.24 ± 88.76	272.70 ± 85.79
Rhubarb	8	312.23 ± 54.93 <sup>b</sup>	391.09 ± 98.16 <sup>a</sup>	542.86 ± 119.93 <sup>b</sup>
Placebo	4	149.71 ± 19.45	257.16 ± 73.78	213.86 ± 22.53

The data were expressed as nitrate unit per milliliter or microgram. <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, vs endotoxin and placebo group.

**Table 3** The effect of rhubarb on the content of tissue lipoperoxide ( $\mu\text{mol/L}$ ,  $\bar{x} \pm s$ )

Group	n	Small intestine	Liver
Endotoxin	17	8.57 ± 2.58	86.97 ± 46.54
Rhubarb	8	3.05 ± 1.13 <sup>a</sup>	13.18 ± 19.64 <sup>a</sup>
Placebo	4	5.97 ± 1.18	74.88 ± 16.42

The data were expressed as nanomole per milliliter of 5% tissue homogenate. <sup>a</sup>*P* < 0.01 vs endotoxin and placebo group.

**Endotoxin model** ICR mice (20-30 g) were intraperitoneally administered 0.4 mg endotoxin, then were fasted overnight with free access to water. Mice were sacrificed 24 h after endotoxin administration. Livers, small intestine and plasma were collected, and the livers and small intestine were weighed, homogenized and diluted into 5% homogenate. Plasma was centrifuged at 3000 r/min for 10 min, and the supernatant was collected for superoxidizedismutase (SOD) and lipoperoxide (LPO) measurements.

#### Experimental design

**Hemorrhagic shock model** Rats were randomly divided into the following four groups: Shock group (*n* = 26), sham shock group (*n* = 5), rhubarb group, and placebo group (*n* = 8). The rhubarb (powder, 50 mg/kg; Rhubarb Lab, Xiang Shan Traditional Chinese Medicine Hospital, Shanghai) was administered through an oral gastric tube before shock, and 4 and 12 h after shock resuscitation. The placebo group was given 1 mL saline at the same time points.

**Endotoxin model** The mice were divided into the following four groups: Endotoxin group (*n* = 17, endotoxin given intraperitoneally), control group (*n* = 5, intraperitoneal saline), rhubarb group (*n* = 8, 10 mg rhubarb administered orally 4 and 12 h after endotoxin administration), and placebo group (*n* = 4, treated with saline in place of rhubarb).

**Endotoxin measurements** Blood was withdrawn from the carotid artery, and 20 U heparin sodium was added to each sample. The samples were centrifuged at 3000 r/min for 10 min to isolate the plasma. Each sample was diluted 1:4 with pyrogen-free water and heated at 100 °C for 6 min to remove any inhibitory or activating factors that can interfere with the limulus assay. The samples were stored at -40 °C until use. Plasma endotoxin was measured using the limulus assay according to the manufacturer's instructions. The limulus kits were provided by the Shanghai Clinical Medical Laboratory Center.

#### Measurements

**SOD measurements** SOD kits were provided by the Department

of Pathophysiology of Nanjing Railway Medical College. Measurements were acquired according to the manufacturer's protocol. SOD concentrations were expressed as nitrate units per milliliter.

**LPO measurements** Thiobarbituric acid chromatography was used to measure LPO levels<sup>[5]</sup>.

**Morphological analysis** The proximal ileum was isolated after the animal was sacrificed. Three samples per group were analyzed by light microscopy. Tissues were fixed in 5% formaldehyde. The tissues were then dehydrated to 95% ethanol and embedded in paraffin. Semi-thin (2-3  $\mu\text{m}$ ) sections were cut and stained with 1% eosin.

#### Statistical analysis

The results are expressed as  $\bar{x} \pm s$ . Variance analysis was used to compare quantitative data. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Hemorrhagic shock model

Hemorrhagic shock can increase intestinal permeability. The concentration endotoxin in the plasma of shocked rats markedly increased 24 h after shock resuscitation, while it did not change 24 h after operation in sham shocked rats. Rhubarb administration decreased intestinal permeability of rats subjected to hemorrhagic shock, as they had lower plasma endotoxin levels than rats in the shock group or placebo group (Table 1).

We performed histological analysis on three rats from each group to determine the effect of rhubarb on ileal mucosa structure. The villi of animals in the shock group and placebo group showed submucosal edema and mucosal necrosis at villar tips. We also observed inflammatory cell infiltration and hemorrhage in the submucosa. However, villi in the rhubarb group showed mild submucosal edema but no mucosal necrosis. We also observed increased goblet cell proliferation in the intestinal villi.

### Endotoxin model

Tissue SOD concentrations were much lower in the endotoxin group and placebo group compared to the rhubarb group, and these differences were statistically significant. These data suggest that rhubarb attenuates tissue SOD consumption (Table 2).

The tissue LPO concentration was much higher in the control groups compared to the rhubarb group, suggesting that rhubarb can markedly decrease tissue LPO formation (Table 3).

According to our data, rhubarb may function in scavenging oxygen free radicals. We performed histological analysis of three mice from each group. Ileal mucosal epithelial cells were necrotic and disordered in the endotoxin and placebo groups, while the ileum only showed mild submucosal edema with no evidence of hemorrhage or necrosis in the rhubarb group.

## DISCUSSION

The gastrointestinal (GI) tract is traditionally considered a nutritional organ, as its main function is to transport and process nutritional substrates. It was recently shown that the GI tract is a key organ affected by pathophysiological processes in critical illnesses. Upon gastrointestinal failure, gut barrier function is lost, the GI tract becomes a reservoir for endotoxins and pathogenic bacteria that can translocate into systemic organs and circulation, inducing systemic inflammatory response syndrome (SIRS). Our study shows that hemorrhagic shock can increase intestinal permeability, resulting in endotoxin absorption within the GI tract. Rhubarb may decrease the shock-induced increase in intestinal permeability and attenuate endotoxin absorption by the GI tract. Our previous work has also shown that rhubarb inhibited bacterial translocation<sup>[2]</sup>. As a traditional Chinese herb, rhubarb can be used to protect gastrointestinal function, particularly gut barrier. Our findings suggest that rhubarb can be used to prevent and treat gastrointestinal failure due to critical illness.

Many studies in various fields have examined the mechanism

of hemorrhagic shock- and endotoxin-induced gut barrier, including clinical research, animal models, ultrastructural analysis, and biochemistry. It is currently thought that intestinal mucosal hypoperfusion, oxygen free radicals and cytokines are three primary pathogenic factors<sup>[6-8]</sup> that induce gut barrier damage, especially oxygen free radical injury. Because the intestinal mucosa contains a large amount of xanthine oxidase, oxygen free radicals are overproduced upon ischemia reperfusion, leading to gut mucosa injury<sup>[9]</sup>. The current study revealed that rhubarb alleviated intraperitoneal endotoxin-induced gut mucosa injury and scavenged oxygen free radicals. Our previous study also demonstrated that oxygen free radical scavenging prevented hemorrhagic shock-induced gut mucosa damage<sup>[10]</sup>. As discussed above, oxygen free radicals play an important role in gut mucosa injury, which is consistent with previous studies<sup>[11]</sup>. Importantly, rhubarb can act as an oxygen free radical scavenger to protect the gut mucosa in patients suffering from critical illnesses.

Morphological analysis revealed that rhubarb promoted goblet cell proliferation within the intestinal mucosa, which secrete large amounts of mucus. The mucus can prevent endotoxin absorption, inhibit bacteria adherence to intestinal epithelial cells and bacterial translocation. Several studies have shown that rhubarb can promote endotoxin excretion within the gut, prevent bacterial overproduction and maintain the balance of the bacteria flora<sup>[12,13]</sup>. Rhubarb can also promote bile excretion, which can bind endotoxin to inhibit endotoxin absorption. Some studies have demonstrated that rhubarb increased the PGI<sub>2</sub>/TXA<sub>2</sub> ratio in tissues. Other possible mechanisms of rhubarb function in the gut require further studies.

## REFERENCES

- 1 **Marshall JC**, Christou NV, Meakins JL. The gastrointestinal tract. The "undrained abscess" of multiple organ failure. *Ann Surg* 1993; **218**: 111-119 [PMID: 8342990 DOI: 10.1097/0000658-199308000-00001]
- 2 **Chen DC**, Jin BW. The effect of rhubarb on gut derived infection. *Zhongguo Jijiu Yixue* 1993; **13**: 7-9
- 3 **Chen DC**, Jin BW, Chen JD. The effect of rhubarb on gut derived infection caused by endotoxin. *Zhonghua Yixue Zazhi* 1994; **3**: 84-86
- 4 **Nakayama S**, Kramer GC, Carlsen RC, Holcroft JW. Infusion of very hypertonic saline to bled rats: membrane potentials and fluid shifts. *J Surg Res* 1985; **38**: 180-186 [PMID: 3968876 DOI: 10.1016/0022-4804(85)90025-3]
- 5 **Chen SZ**. Comparison of three methodology of lipoperoxide TAB color reaction. *J Clin Lab* 1984; **2**: 8-10
- 6 **Morris SE**. Decreased mesenteric blood flow independently promotes bacterial translocation in chronically instrumented sheep. *Surg Forum* 1989; **40**: 88-94
- 7 **Parks DA**, Bulkley GB, Granger DN, Hamilton SR, McCord JM. Ischemic injury in the cat small intestine: role of superoxide radicals. *Gastroenterology* 1982; **82**: 9-15 [PMID: 6273253]
- 8 **van Lanschoot JJ**, Mealy K, Wilmore DW. The effects of tumor necrosis factor on intestinal structure and metabolism. *Ann Surg* 1990; **212**: 663-670 [PMID: 2256757 DOI: 10.1097/0000658-199012000-00003]
- 9 **Zheng QC**. The effect of oxygen free radicals on acute ischemic injury of small intestine. *Zhonghua Waikexue Zazhi* 1989; **6**: 68-71
- 10 **Ma L**. Studies on the mechanism of pathogenesis of gut derived infection caused by endotoxin. *Zhonghua Zhengxing Waikexue Zazhi* 1996; **6**: 164-166
- 11 **Chen DC**, Jin BW. The effect of the vitamin C on protection of gut barrier. *Zhongguo Jijiu Yixue* 1994; **14**: 8-10
- 12 **Sheng DC**, Feng H. The effect of rhubarb on fever caused by endotoxin and the concentration of cAMP of spinal fluid. *Zhonghua Bingli Shengli Zazhi* 1989; **5**: 77-79
- 13 **Chen CM**, Wang WF. Selection of Chinese traditional herb resistant to anaerobic bacteria *in vitro*. *Dier Junyi Daxue Xuebao* 1989; **16**: 399-400

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Exploration of the immunoreactivity of the Traditional Chinese medicine Shenrouyangzhentang to vasoactive intestinal polypeptide

Yu-Chun Gu, De-Zhen Chen

Yu-Chun Gu, De-Zhen Chen, Department of Traditional Chinese Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: All authors contributed equally to the work.

Original title: China National Journal of New Gastroenterology (1995-1997) renamed World Journal of Gastroenterology (1998-).

Received: July 4, 1996  
Revised: November 14, 1996  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the immunoreactivity of the Chinese medicine Shenrouyangzhentang to vasoactive intestinal polypeptide (VIP) and its therapeutic mechanism.

**METHODS:** The immunoreactivity of the Chinese medicine Shenrouyangzhentang to VIP was detected in the plasma of 20 normal people and 20 patients with Piyinxu (Spleen Yin deficiency) using the radioimmunoassay (RIA) method.

**RESULTS:** The maximum binding rate  $B_0/T$  was 53.29%, the non-specific binding rate  $N_0/T$  was 1.170%, and the VIP standard curve was  $Y = 0.81983 + 0.44319X - 0.28927X^2$ ,  $R^2 = 0.990$ . The VIP content in Shenrouyangzhentang was  $106.6 \text{ ng/L} \pm 20 \text{ ng/L}$ , while it was  $90.16 \text{ ng/L} \pm 15 \text{ ng/L}$  in normal human plasma and  $63.25 \text{ ng/L} \pm 11 \text{ ng/L}$  in the plasma of Pixinxu patients. The difference between normal plasma and Pixinxu patient plasma was statistically significant ( $P < 0.05$ ).

**CONCLUSION:** The Chinese medicine Shenrouyangzhentang demonstrated VIP immunoreactivity similar to that of normal plasma. The (vasoactive intestinal polypeptide) VIP content in Pixinxu patient plasma was lower than that in healthy subjects ( $P < 0.05$ ).

**Key words:** Qi-reinforcing agents/analysis; Radix Paeoniae Alba/analysis; Pseudostellaria heterophylla/analysis; Poria cocos/analysis; Vasoactive intestinal peptide/analysis; Composite

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Gu YC, Chen DZ. Exploration of the immunoreactivity of the Traditional Chinese medicine Shenrouyangzhentang to vasoactive intestinal polypeptide. World J Gastroenterol 1997; 3(2): 83 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/83.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.83>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Immunohistochemical study of gastrin in colorectal carcinoma tissues and adjacent mucosa

Chun-Ping Jiang, Yu-Quan Chen, Jian-Wei Zhu, Hong-Xun Shen, Xiu Yu

Chun-Ping Jiang, Yu-Quan Chen, Jian-Wei Zhu, Hong-Xun Shen, Xiu Yu, Department of General Surgery, Affiliated Hospital, Nantong Medical College, Nantong 226001, Jiangsu Province, China

Chun-Ping Jiang has published 12 papers

Author contributions: All authors contributed equally to the work.

Supported by The Youth Science Foundation of Jiangsu Province, China, No. Q9202.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Chun-Ping Jiang, Department of General Surgery, Affiliated Hospital, Nantong Medical College, Nantong 226001, Jiangsu Province, China  
Telephone: +86-513-5513161-352

Received: July 4, 1996  
Revised: November 14, 1996  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study gastrin expression in colorectal carcinoma tissues and adjacent mucosa and discuss the function of gastrin in colorectal carcinoma.

**METHODS:** Gastrin expression in colorectal carcinoma tissues and adjacent mucosa was examined in 58 cases using immunohistochemical and immunoelectron microscopy.

**RESULTS:** A total of 35.1% of colorectal carcinoma transitional mucosa (TM), 48.3% of nontypical dysplasia mucosa and 60.3% of carcinoma tissue were positive for gastrin expression ( $P < 0.05$ ). Immunoelectron microscopy revealed that protein A gold (PAG) granules localized to different electron-dense secretory granules in carcinoma cells, the intercellular spaces, and the microvillar membrane surface.

**CONCLUSION:** Gastrin expression in colorectal carcinoma tissue and adjacent mucosa, and the release of gastrin by carcinoma may be an initiating factor in carcinoma occurrence and development. Positive gastrin expression in colorectal carcinoma tissues can serve as a differentiation marker.

**Key words:** Colorectal neoplasms; Gastrin; Immunohistochemistry; microscopy; Electron

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Jiang CP, Chen YQ, Zhu JW, Shen HX, Yu X. Immunohistochemical study of gastrin in colorectal carcinoma tissues and adjacent mucosa. *World J Gastroenterol* 1997; 3(2): 84-86 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/84.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.84>

### INTRODUCTION

Recent studies have shown that exogenous gastrin can promote human colorectal carcinoma cell strain growth, that colorectal carcinoma cells express gastrin mRNA, and that gastrin-like material can be detected in the culture medium<sup>[1-3]</sup>. We examined gastrin expression in 58 cases of human colorectal carcinoma tissue and adjacent mucosa by immunohistochemical methods and immunomicroscopy to determine the function of gastrin and clinical significance of its expression in colorectal carcinoma occurrence and development.

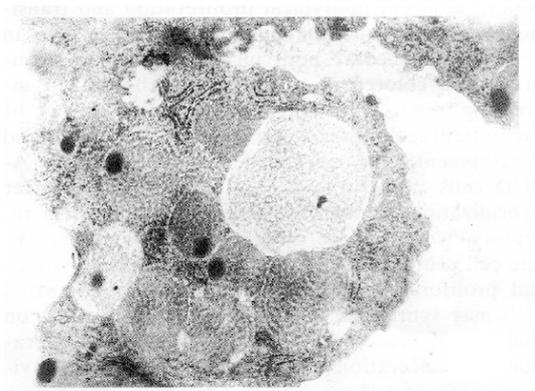
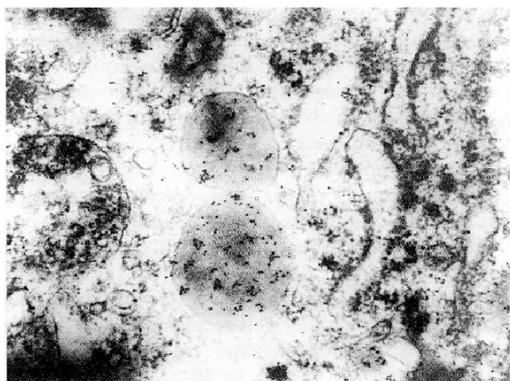
### MATERIALS AND METHODS

#### Light microscopy

Surgically resected specimens were from 58 colorectal cancer cases, including 25 colon cancer cases and 33 rectal cancer cases. Carcinoma tissues and their adjacent mucosal tissues were resected separately, fixed in 10% neutral formalin, embedded in paraffin and sectioned into three slices. Hematoxylin and eosin (HE), high iron diamine-alcian blue (HID-AB), and immunohistochemical staining was performed. (1) HID-AB staining. Sections were dewaxed with dimethylbenzene, dehydrated in alcohol, stained in high iron diamine for 6 h, washed with distilled water, soaked with 1% Alcian blue, 3% acetic aqueous solution for 15 min, dehydrated with anhydrous alcohol, and fixed in xylene. The Filipe and Greaves<sup>[4]</sup> standard was used for scoring transitional mucosa (TM). (2) Immunohistochemical staining of gastrin by the ABC method. A rabbit anti-human gastrin antibody and all ABC agents were purchased from DAKO Company, and the staining was performed according to the manufacturer's protocol. Normal gastroantrum mucosa served as a positive control. PBS and normal rabbit serum served as negative controls. The positive judging standard was used as follows: Weak positive (+) tissue had less than 20% of cells that were weakly brown in color with individual brown cells, positive (+++) tissue contained more than 60% of cells stained brown or dark brown with a few weakly stained brown cells, and moderately positive (++) tissue stained tissue in a range between the weak positive and strong positive tissues.

**Table 1** Gastrin expression in colorectal adenocarcinoma tissue and adjacent mucosa

Group	Colorectal carcinoma		Atypical dysplasia		Transitional mucosa	
	<i>n</i>	Positive (%)	<i>n</i>	Positive (%)	<i>n</i>	Positive (%)
Histological type						
Tubular	47	29 (61.5)	24	12 (50.0)	45	16 (35.6)
Nipple	5	3 (60.0)	2	1 (50.0)	5	2 (40.0)
Mucinous	6	3 (50.0)	3	1 (33.3)	6	2 (33.3)
Differentiation						
Mild	12	11 (91.7)	7	3 (42.8)	12	5 (41.7)
Moderate	34	18 (52.9)	17	9 (52.9)	32	11 (34.4)
Low	6	3 (50.0)	2	1 (50.0)	6	2 (33.3)
Ducke's stages						
A	27	16 (59.2)	13	6 (46.2)	26	10 (38.5)
B	10	6 (60.0)	4	2 (50.0)	10	4 (40.0)
C	21	13 (61.9)	12	6 (50.0)	20	6 (30.0)
Total	58	35 (60.3)	29	14 (48.3)	56	20 (35.1)

**Figure 1** Protein A gold-positive granules in highly differentiated cancer primarily localized to type A secretory granules. Protein A gold  $\times 16000$ .**Figure 2** Protein A gold granules in low differentiated cancer primarily localized to type B secretory granules.  $\times 50000$ .

### Electron microscopy

In the five specimens observed by electron microscopy, three of the cases were considered immunohistochemically strong positive (+++) for gastrin expression, one was moderately positive (++) and one was weakly positive (+). According to Ducke's tumor stages, three cases were stage B and two were stage C. Specimens were acquired from fresh material within one minute of removal and were prefixed in 2.5% glutaraldehyde. Specimens were then fixed with osmic acid, washed with 0.1 mol/L PBS, and embedded in Epon 812. For immuno-gold staining (IGS), the staphylococcus Protein A gold (PAG) method was used as follows: Tissues were incubated in 1% ovalbumin for ten minutes, followed by incubation with a rabbit anti human gastrin antibody (1:100) for two hours. Samples were washed with 0.05 mol/L TBS, and 0.02 mol/L TBS for five minutes each, followed by incubation in 1% ovalbumin for ten minutes, PAG (1:7) for 2 h, 0.02 mol/L-TBS for five minutes, and 0.05 mol/L TBS. Samples were transiently stained with uranium acetate and plumbum citrate, followed by observation on a JEM-100s electron microscope. Normal gastroantrum mucosa served as a positive control. TBS and normal rabbit immunoserum served as negative controls.

### Statistical analysis

A  $\chi^2$  test and Ridit analysis were performed, with 95%CI,  $\alpha = 0.050$ .

## RESULTS

### HE and HID-AB staining

We detected non-adenoma and atypical hyperplasia in 29 adjacent mucosa from the 58 colorectal carcinoma cases. Fifty-six out of 58 cases were positive for salivary mucin (blue). The transitional mucosa limits were 0.5-6.7 cm, with a mean of 2.12 cm. We acquired 28 specimens from the distal remnant adjacent to the cancer more than 15 cm away from the neoplasm border. Light microscopic examination was normal, and sulfomucin staining by HID-AB reaction (dark brown) looked comparable to normal mucosa.

### Gastrin immunohistochemical staining

Table 1 demonstrates the relationship between the rate of positive gastrin staining in colorectal carcinoma tissue and tumor differentiation, but gastrin expression did not correlate to histological type or Ducke's tumor stage ( $P > 0.5$ ). There was no relationship between gastrin expression in the atypical proliferative mucosa and the transitional mucosa, and tumor differentiation, pathological stage or tumor type ( $P > 0.5$ ). The relationship between gastrin expression in the adjacent transitional mucosa, atypical hyperplasia and colorectal carcinoma tissues was statistically significant ( $P < 0.05$ ). A total of 28 normal colorectal mucosal cases were negative for gastrin expression.

### Immunoelectron microscopy

Colorectal cancer cells were polygonal in shape, with unusual variably sized nucleoli concentrated on the edge. The cytoplasm contained many vacuolated mitochondria, and the cells contained secretion granules 400-1500 nm in diameter with a clear membrane border. There were two types of granular appearance: Type A was largest in bulk size, with well distributed low electro density, and the granular core appeared loose; type B was smaller in bulk size, with well distributed high electro density, and the nucleus was usually compact. PAG-positive granules partially localized to secretory granules. PAG-positive granules in highly differentiated cancers primarily localized to type A secretory granules (Figure 1). PAG-positive granules in low differentiated cancer primarily localized to type B secretory granules (Figure 2). PAG granules partially localized to the inside and outside of the cancer cell membrane and microvillus membrane.

## DISCUSSION

Studies have shown that colorectal cancer cells synthesize and secrete gastrin<sup>[5,6]</sup>, and our study further confirmed gastrin was expressed in the mucosa of adjacent atypical proliferative and transitional mucosa, and the rate of gastrin-expressing cells in transitional mucosa, atypical proliferation membrane and colorectal cancer tissues showed an increasing trend, suggesting that the gastrin expression is one of the initial factors regulating colorectal cancer occurrence and development. It has been hypothesized that APUD cells synthesize and secrete gastrin after hybridization, allowing for gastrin receptor activity on the cell surface membrane to stimulate cell proliferation, leading to the development of transitional mucosa, atypical proliferation, and ultimately cancer development. The cancer cells can synthesize and secrete gastrin to activate gastrin receptors and stimulate cell proliferation and cancer formation in a positive feedback loop. Inhibition of the positive feedback loop could inhibit neoplasm growth. This may open a way for the treatment of colorectal carcinoma by using endocrine therapy. Kaneyma *et al*<sup>[7]</sup> treated colon carcinoma metastasis to the liver by using gastrin receptor antagonists and achieved good results.

Immunoelectron microscopy revealed that there were gastrin-containing secretory granules in colorectal carcinoma cells, and the gastrin granules were adsorbed into the cancer cell membrane and the inside and outside of the microvillus, and to the carcinoma interstice. These findings provide a morphological basis for colorectal cancer cell production of gastrin, and a theoretical basis for endocrine treatment. The authors examined serum gastrin levels in 30 colorectal cancer cases and found that in some patients, serum gas-

trin levels were increased, especially in the cases of well-differentiated cancer<sup>[8]</sup>. However, the mechanism is not yet clear. Our study indicated that gastrin localized to type A granules in highly differentiated adenocarcinoma and to type B granules in poorly differentiated adenocarcinomas. G17-positive granules were of type A, and G34-positive granules were of type B. G17 had higher bioactivity than G34, consistent with the above mentioned mechanism.

Gastrin was expressed in 91.7% of highly differentiated cancers, which was higher than the middle and low differentiated adenocarcinoma cases ( $p < 0.05$ ). Middle and low differentiated adenocarcinomas have been shown to produce mutated gastrin mRNA, as described by Singh, which leads to insufficient levels of mature gastrin<sup>[9]</sup>. Thus, the positive expression of gastrin in colorectal carcinoma tissues may serve as a marker of well differentiated cancer. Cases with positive expression in colorectal carcinoma tissues and its adjacent mucosa may be good candidates for treatment with a gastrin antagonist.

## REFERENCES

- 1 **Baldwin GS**, Zhang QX. Measurement of gastrin and transforming growth factor alpha messenger RNA levels in colonic carcinoma cell lines by quantitative polymerase chain reaction. *Cancer Res* 1992; **52**: 2261-2267 [PMID: 1559230]
- 2 **Van Solinge WW**, Nielsen FC, Friis-Hansen L, Falkmer UG, Rehfeld JF. Expression but incomplete maturation of progastrin in colorectal carcinomas. *Gastroenterology* 1993; **104**: 1099-1107 [PMID: 8462798]
- 3 **Xu Z**, Dai B, Dhruva B, Singh P. Gastrin gene expression in human colon cancer cells measured by a simple competitive PCR method. *Life Sci* 1994; **54**: 671-678 [PMID: 7509020 DOI: 10.1016/0024-3205(94)00550-8]
- 4 **Wang Q**, Kao H, Wang Y, Chen YL, He J. The immunohistochemical study of carcino-embryonic antigen in the transitional mucosa adjacent to colorectal cancer. *Zhonghua Xiaohua Zazhi* 1990; **10**: 25-27
- 5 **Finley GG**, Koski RA, Melhem MF, Pipas JM, Meisler AI. Expression of the gastrin gene in the normal human colon and colorectal adenocarcinoma. *Cancer Res* 1993; **53**: 2919-2926 [PMID: 8504433]
- 6 **Zhu JW**, Chen YQ. Gastrin and neoplasms. *Zhongguo Putong Waike Zazhi* 1996; **11**: 7-10
- 7 **Kameyama M**, Nakamori S, Imaoka S, Yasuda T, Nakano H, Ohigashi H, Hiratsuka M, Sasaki Y, Kabuto T, Ishikawa O. [Adjuvant chemo-endocrine chemotherapy with gastrin antagonist after resection of liver metastasis in colorectal cancer]. *Gan To Kagaku Ryoho* 1994; **21**: 2169-2171 [PMID: 7944431]
- 8 **Jiang CP**, Shen HX, Chen YQ, Chen RX. The changes of serum gastrin level in 60 cases with gastrointestinal tumors. *Jiangsu Yiyao* 1994; **20**: 73-74
- 9 **Singh P**, Xu Z, Dai B, Rajaraman S, Rubin N, Dhruva B. Incomplete processing of progastrin expressed by human colon cancer cells: role of noncarboxyamidated gastrins. *Am J Physiol* 1994; **266**: G459-G468 [PMID: 8166285]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Ultrastructural cytochemical study of enzymes expressed by signet ring cells in gastric cancer

Guang-Lin Yang, Yu-Ming Dong, Wei-Dong Du, Ying-Hao Su, Hong Zhang, Ji-Fen Wu, Dao-Bin Wang, Ai-Lian Xu

Guang-Lin Yang, Yu-Ming Dong, Wei-Dong Du, Ying-Hao Su, Hong Zhang, Ji-Fen Wu, Dao-Bin Wang, Ai-Lian Xu, Department of Pathology, Anhui Medical University, Hefei 230032, Anhui Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: September 28, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the ultrastructural localization of five marker enzymes (ALPase, ACPase, G6Pase, TPPase and CCOase) in gastric cancer signet ring cells to demonstrate their biologic behaviors.

**METHODS:** Five marker enzymes were examined in signet ring cells of seven gastric cancer patients by ultrastructural enzyme cytochemi-

cal techniques.

**RESULTS:** The number of corresponding organelles and the activities of marker enzymes, especially ACPase and TPPase, increased, leading to stronger mucus synthesis, secretion and digestion in gastric cancer signet ring cells. There was a lack of collagenous fibers in the stroma around the cancer nests.

**CONCLUSION:** Signet ring cell carcinoma is very invasive with metastasis rates due to the secretion of proteolytic enzymes.

**Key words:** Stomach neoplasms/enzymology; Carcinoma, signet ring cell/enzymology; Alkaline phosphatase/analysis; Acid phosphatase/analysis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Yang GL, Dong YM, Du WD, Su YH, Zhang H, Wu JF, Wang DB, Xu AL. Ultrastructural cytochemical study of enzymes expressed by signet ring cells in gastric cancer. *World J Gastroenterol* 1997; 3(2): 86 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/86.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.86>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Changes in *p53* and *Waf1p21* expression and cell proliferation in esophageal carcinogenesis

Li-Dong Wang, Wan-Cai Yang, Qi Zhou, Ying Xing, Yun-Ying Jia, Xin Zhao

Li-Dong Wang, Wan-Cai Yang, Qi Zhou, Ying Xing, Xin Zhao, Laboratory for Cancer Research, Henan Medical University, Zhengzhou 450052, Henan Province, China

Yun-Ying Jia, Department of Gastroenterology, The People's Hospital of Puyang City, Puyang 457000, Henan Province, China

Author contributions: All authors contributed equally to the work.

Supported by The Henan Scientific Foundation for Outstanding Young Scientists and National Natural Science Foundation of China, No.39670290, 39419711156 and U.S. NCI Grant CA 65871.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Li-Dong Wang, MD, Laboratory for Cancer Research, Henan Medical University, Zhengzhou 450052, Henan Province, China  
Telephone: +86-371-6970165

Received: September 28, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the correlation between changes in *p53* and *Waf1p21* expression and cell proliferation, determined by proliferating cell nuclear antigen (PCNA), at different stages of human esophageal carcinogenesis.

**METHODS:** Biopsied and resected esophageal tissues from a high risk population of esophageal cancer in northern China were used in this study. All specimens were fixed in 85% alcohol and processed for routine histology. The avidin biotin peroxidase complex (ABC) method was used to detect *p53*, *Waf1p21* and PCNA.

**RESULTS:** Strong nuclear staining of *p53*, *Waf1p21* and PCNA was observed in normal esophageal epithelium and epithelia with different lesion severities. As the lesions progressed to dysplasia (DYS) and to esophageal squamous cell carcinoma (SCC), the *Waf1p21* immunoreactivity percentage decreased. The number of *Waf1p21*-positive cells slightly increased from normal to basal cell hyperplasia (BCH), but did not further increase in *DYS* and *SCC*. The total number of *Waf1p21*-positive cells was lower than the number of *p53*-positive cells in normal and *BCH* esophageal epithelia and much lower in *DYS* and *SCC*. *Waf1p21*-positive cells were located in the third and fourth cell layers in half of the samples examined, which was 2-4 cell layers higher than the cells expressing PCNA and *p53* in the same

histological categories of normal, *BCH* and *DYS*.

**CONCLUSION:** Low *Waf1p21* levels at the *DYS* stage may be related to a functional loss of *p53*. Other mechanisms may also be responsible for the decreased *Waf1p21* expression in *DYS* and *SCC*.

**Key words:** Esophageal neoplasms; Precancerous conditions; *p53* genes; *Waf1p21*; Genes suppressor; Tumor

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang LD, Yang WC, Zhou Q, Xing Y, Jia YY, Zhao X. Changes in *p53* and *Waf1p21* expression and cell proliferation in esophageal carcinogenesis. *World J Gastroenterol* 1997; 3(2): 87-89 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/87.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.87>

### INTRODUCTION

Esophageal carcinoma (EC) is a widely occurring disease in Huixian and Linxian of the Henan Province in northern China and remains a leading cause of cancer-related deaths<sup>[1]</sup>. The development of human esophageal squamous cell carcinoma (SCC) is a progressive multistage process<sup>[2-5]</sup>. An early indicator of abnormality in patients predisposed to EC is increased esophageal epithelial cell proliferation, which is morphologically manifested as basal cell hyperplasia (BCH), dysplasia (DYS) or carcinoma *in situ* (CIS), which are considered precancerous EC lesions. Changes in cell proliferation and cell death may be key factors that contribute to the rate of neoplastic progression and tumor growth. Although previous studies on subjects from these high incidence areas have suggested the fundamental importance of epithelial cell hyperproliferation in human esophageal carcinogenesis, little information is available on the molecular basis of cell proliferation in this disease.

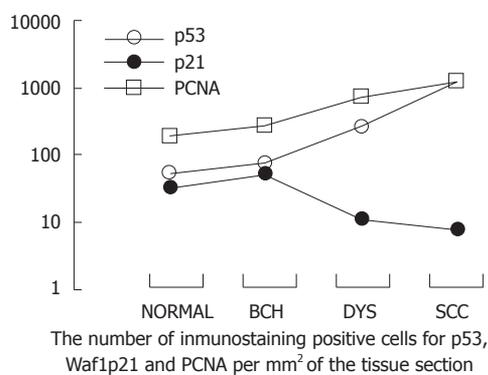
The tumor suppressor transcription factor *p53* has been shown to function in the cellular DNA damage response, resulting in either G1 arrest or apoptosis<sup>[6]</sup>. The *Waf1/CIP1* gene encodes protein *p21*, which is transcriptionally regulated by *p53*<sup>[7]</sup>. Upon DNA damage, *p53* upregulates *Waf1p21* expression to induce G1 arrest to allow time for damaged DNA to be repaired, or it triggers apoptosis to eliminate genetically damaged cells<sup>[8]</sup>. As *Waf1p21* is upregulated by wild type *P53*, the levels of *Waf1p21* protein may reflect the functional status of *p53* in carcinogenesis.

Our previous studies indicated that *P53* protein accumulated in early human esophagus lesions and even in histopathologically normal epithelium<sup>[9,10]</sup>; *p53* gene mutations were observed in some of these samples<sup>[11-13]</sup>. The results suggested that *P53* protein accumulation and gene mutation were early events in esophageal

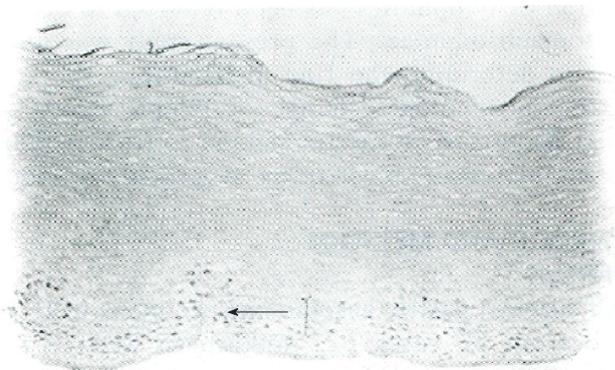
**Table 1** Changes in p53, Waf1p21 and Proliferating Cell Nuclear Antigen expression in esophageal cancer patients from Henan, China ( $\bar{x} \pm s$ ).

Histology	p53 immunostaining		Waf1p21 immunostaining		Proliferating Cell Nuclear Antigen immunostaining		
	Positive %	Positive cells/mm <sup>2</sup> (n/n)	Positive %	Positive cells/mm <sup>2</sup> (n/n)	Samples examined	Positive cells/mm <sup>2</sup>	
Normal	74.2	(23/31)	53 ± 78	39.3 (11/28)	34 ± 72	31	200 ± 113
BCH	87.2	(102/117)	74 ± 66	37.8 (14/37)	53 ± 10	106	286 ± 150
DYS <sup>b</sup>	100	(31/31)	262 ± 341	27.3 (3/11)	11 ± 20.2	31	719 ± 389
SCC	88.5	(23/26)	1297 ± 1110	14.3 (1/7)	8 ± 22	26	1261 ± 545

<sup>b</sup>P < 0.01, *vs* BCH and SCC, by Wilcoxon rank\_sum test. SCC: Squamous cell carcinoma; DYS: Dysplasia; BCH: Basal cell hyperplasia.



**Figure 1** Changes in p53 and Waf1p21 expression in normal esophageal epithelia and epithelia with different degrees of lesions. As the lesions progressed from BCH, DYS to SCC, the number of p53- and PCNA-positive cells significantly increased. In contrast, the number of Waf1p21-positive cells slightly increased from normal to BCH, but there was no further increase in DYS and in SCC. SCC: Squamous cell carcinoma; DYS: Dysplasia; BCH: Basal cell hyperplasia.



**Figure 2** Waf1p21 immunostaining in biopsy samples of esophageal BCH. Waf1p21-positive cells localized to the third and fourth cell epithelial layers (arrow). Scale bar, 2.4 μm. BCH: Basal cell hyperplasia.

carcinogenesis.

Quantitative analysis of both p53 and Waf1p21 expression can provide important insights into cancer development, but such analysis has not been performed at different stages of human esophageal carcinogenesis. In this study, we investigated the expression of p53, Waf1p21 and PCNA in human esophageal epithelia with different severities of precancerous and cancerous lesions from subjects in Henan, China.

## MATERIALS AND METHODS

### Tissue collection and processing

Esophageal tissues were biopsied from 241 symptom-free subjects, and surgically resected EC specimens were collected from 38 patients in Huixian County and Linxian County, China. Of these 279 subjects, 160 were males (20-71 years of age with a mean ± SD of 48 ± 14 years) and 119 females (20-79 years of age with a mean ± SD of 47 ± 16 years). None of the cancer patients received chemotherapy or radiotherapy treatment before the operation. All specimens were fixed in 85% alcohol, embedded in paraffin, and serially sectioned at 5 μm. The sections were mounted onto histostick-coated slides. Three or four adjacent ribbons were collected for

histopathological analysis (hematoxylin and eosin stain) and immunohistochemical staining.

### Histopathology analysis

Histopathological diagnoses for esophageal epithelia were made according to cellular morphological changes and tissue architecture using previously established criteria<sup>[14]</sup>. In brief, the normal esophageal epithelium contained 1.3 proliferating basal cell layers, and the papillae were confined to the lower half of the epithelium. In BCH, the proliferating basal cells increased to more than three cell layers and less than half of the entire epithelial thickness. DYS was characterized by partial loss of cell polarity and nuclear atypia. SCC was characterized by confluent and invasive sheets of cohesive, polymorphous cells with hyperchromatic nuclei.

### p53 and Waf1p21 immunohistochemical staining

The avidin biotin peroxidase complex (ABC) method was used for p53, Waf1p21 and PCNA antigen detection (Ongogene Science, Inc., Manhasset, NY, United States). After dewaxing, inactivating endogenous peroxidase activity, and blocking crossreactivity with normal serum, the sections were incubated overnight at 4°C in a diluted solution of primary antibodies (1:1000 for p53, 1:20 for Waf1p21 and 1:200 for PCNA). Primary antibody detection was achieved by subsequent application of a biotinylated anti primary antibody, an avidin biotin complex conjugated to horseradish peroxidase, and diaminobenzidine (Vectastain Elite Kit; Vector Laboratories, Burlingame, CA, United States). Normal serum and absence of primary antibody were used as negative controls.

### Quantitative analysis of immunostaining results

Quantitative analysis of nuclear immunostaining results were recorded as the number of positive cells per mm<sup>2</sup> of tissue section as described previously<sup>[10]</sup>. All positively stained cells were counted in the entire tissue section under a microscope at × 400 magnification. A total of 24 fields were typically counted for biopsied tissues and 50 fields for surgically resected specimens.

### Statistical analysis

The mean ± SE of the p53-, Waf1p21- and PCNA-positive cell number/mm<sup>2</sup> in the esophageal biopsy and surgically resected EC samples in each histologic category were calculated by using univariate analysis, and comparisons were made using the Wilcoxon rank sum test for unpaired data and correlation by using Pearson's correlations. The  $\chi^2$  test was used for the statistical analysis of the percentage of positively stained samples ( $p < 0.05$  was considered significant).

## RESULTS

### p53, Waf1p21 and PCNA immunoreactivity in different stages of esophageal carcinogenesis

We observed intense p53 protein and PCNA nuclear immunostaining of tissues with different lesions, which was consistent with our previous observations. In brief, we observed similar immunostaining patterns for PCNA and p53, but the number of PCNA-positive cells was higher than that of p53 in the same histological category of normal, BCH and DYS (3-4 fold, Table 1). As the esophageal tissue progressed from BCH, DYS to SCC, the number of PCNA- and p53-positive cells significantly increased and expanded upwards in the epithelium.

The percentage of samples with Waf1p21-positive immunostaining was 39 and 38 in normal and BCH esophageal epithelia, respectively. Waf1p21 immunostaining was confined to the cell nucleus. As the lesions progressed to DYS and SCC, the percentage of Waf1p21 immunoreactivity decreased. The number of Waf1p21-positive cells slightly increased from normal to BCH, but there was no further increase in DYS and SCC. The total number of Waf1p21-positive cells was lower than that of p53 in normal and BCH esophageal epithelia, much lower in DYS (24-fold) and markedly lower in SCC (162-fold) (Table 1, Figure 1). Waf1p21-positive cells localized to the third and fourth cell layers in half of the samples, which was 2-4 cell

layers higher than PCNA-positive and p53-positive cells in the same histological categories of normal, BCH and DYS (Figure 2).

## DISCUSSION

Interestingly, we found that the distribution of Waf1p21-positive cells was distinct from that of p53- and PCNA-positive cells such that Waf1p21-positive cells localized to the more differentiated middle third of the epithelium, which was approximately 2-4 layers of basal cells higher than p53- and PCNA-positive cells, suggesting that Waf1p21 expression may be related to cell differentiation. The small number of Waf1p21-positive cells may be due to functional loss of p53 by mutations.

Dysplasia has been considered a severe precancerous lesion of esophageal squamous cell carcinoma. The current study indicated that the levels of p53 and Waf1p21 was similar in normal and BCH lesions, but there was a clear segregation of high p53 and low Waf1p21 expression, from dysplasia to SCC, suggesting that dysplasia may be an important target for molecular insult in esophageal carcinogenesis. The molecular mechanism for the development of dysplasia remains to be further characterized.

## REFERENCES

- 1 **Yang CS.** Research on esophageal cancer in China: a review. *Cancer Res* 1980; **40**: 2633-2644 [PMID: 6992989]
- 2 **Correa P.** Precursors of gastric and esophageal cancer. *Cancer* 1982; **50**: 2554-2565 [PMID: 7139550]
- 3 **Mundle SD,** Gao XZ, Khan S, Gregory SA, Preisler HD, Raza A. Two *in situ* labeling techniques reveal different patterns of DNA fragmentation during spontaneous apoptosis *in vivo* and induced apoptosis *in vitro*. *Anticancer Res* 1995; **15**: 1895-1904 [PMID: 8572575]
- 4 **Qiu SL,** Yang GR. Precursor lesions of esophageal cancer in high-risk populations in Henan Province, China. *Cancer* 1988; **62**: 551-557 [PMID: 3390795 DOI: 10.1002/1097-0142(19880801)62:3<551::AID-CNCR2820620319>3.0.CO;2-Y]
- 5 **Wang LD,** Lipkin M, Qiu SL, Yang GR, Yang CS, Newmark HL. Labeling index and labeling distribution of cells in esophageal epithelium of individuals at increased risk for esophageal cancer in Huixian, China. *Cancer Res* 1990; **50**: 2651-2653 [PMID: 2328490]
- 6 **Shimamura A,** Fisher DE. p53 in life and death. *Clin Cancer Res* 1996; **2**: 435-440 [PMID: 9816188]
- 7 **Hartwell LH,** Kastan MB. Cell cycle control and cancer. *Science* 1994; **266**: 1821-1828 [PMID: 7997877 DOI: 10.1126/science.7997877]
- 8 **Kuerbitz SJ,** Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc Natl Acad Sci United States* 1992; **89**: 7491-7495 [PMID: 1323840 DOI: 10.1073/pnas.89.16.7491]
- 9 **Wang LD,** Hong JY, Qiu SL, Gao H, Yang CS. Accumulation of p53 protein in human esophageal precancerous lesions: a possible early biomarker for carcinogenesis. *Cancer Res* 1993; **53**: 1783-1787 [PMID: 8467496]
- 10 **Wang LD,** Shi ST, Zhou Q, Goldstein S, Hong JY, Shao P, Qiu SL, Yang CS. Changes in p53 and cyclin D1 protein levels and cell proliferation in different stages of human esophageal and gastric-cardia carcinogenesis. *Int J Cancer* 1994; **59**: 514-519 [PMID: 7960222 DOI: 10.1002/ijc.2910590414]
- 11 **Gao H,** Wang LD, Zhou Q, Hong JY, Huang TY, Yang CS. p53 tumor suppressor gene mutation in early esophageal precancerous lesions and carcinoma among high-risk populations in Henan, China. *Cancer Res* 1994; **54**: 4342-4346 [PMID: 8044781]
- 12 **Wang LD,** Zhou Q, Hong JY, Qiu SL, Yang CS. p53 protein accumulation and gene mutations in multifocal esophageal precancerous lesions from symptom free subjects in a high incidence area for esophageal carcinoma in Henan, China. *Cancer* 1996; **77**: 1244-1249 [PMID: 8608498 DOI: 10.1002/(SICI)1097-0142(19960401)77:7<1244::AID-CNCR3>3.0.CO;2-I]
- 13 **Shi ST,** Feng B, Yang GY, Wang LD, Yang CS. Immunohistochemical sequencing (IHSS) of p53 tumor suppressor gene in human oesophageal precancerous lesions. *Carcinogenesis* 1996; **17**: 2131-2136 [PMID: 8895479]
- 14 **Wang LD,** Qiu SL, Yang GR, Lipkin M, Newmark HL, Yang CS. A randomized double-blind intervention study on the effect of calcium supplementation on esophageal precancerous lesions in a high-risk population in China. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 71-78 [PMID: 8420615]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Morphological analysis of lymph vessels and capillaries in gastric carcinoma

Xin-Bo Liao, Wei-Ping Tang, Qin-Ming Zhang, Zhi-Gang Fu, Ying-Hai Zhao

Xin-Bo Liao, Wei-Ping Tang, Qin-Ming Zhang, Ying-Hai Zhao, Department of Pathology, Guangdong Medical College, Zhanjiang 524023, Guangdong Province, China

Zhi-Gang Fu, Department of Foreign Language Teaching, Guangdong Medical College, Zhanjiang 524023, Guangdong Province, China

Xin-Bo Liao, male, born on March 3, 1963 in Puqi City, Hubei Province, graduated from the Department of Medicine of Hubei Medical College, Master and Lecturer, specialized in the study of tumor metastatic mechanisms, with nine published papers

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 39470296.

Presented at The 10<sup>th</sup> International Conference of Histochemistry and Cytochemistry, Kyoto, 18<sup>th</sup> August, 1996

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Xin-Bo Liao, Department of Pathology, Guangdong Medical College, Zhanjiang 524023, Guangdong Province, China  
Telephone: +86-759-2281544-3037

Received: September 27, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the morphology of lymph vessel capillaries in both human gastric carcinomas and their peritumoral tissues, as well as the relation of this morphology to lymphatic metastasis.

**METHODS:** The morphology and fine distribution of both lymph vessels and capillaries in and around the primary foci of gastric carcinoma were studied using the 5'Nase Alpace double staining method. The total amount of opened vessels and the opening rate of lymph vessels and capillaries were counted using a light microscope (100 × magnification), and the maximal luminal area, perimeter and diameter were measured using an image analysis technique.

**RESULTS:** Lymph vessels and capillaries displayed strong 5'Nase-positive staining (brown and dark brown), while blood vessels and capillaries revealed significant Alpace activity (blue). There were many lymph vessels, capillaries and solid strip-like tissues found in the gastric carcinoma samples analyzed. The total amount of lymphatics in the metastatic group (gastric carcinoma vs peritumoral tissue) and non-metastatic group was  $26.9 \pm 14.2$  vs  $10.4 \pm 4.0$ ,  $11.4 \pm 3.4$  and  $9.7 \pm 3.2$ ,  $P < 0.01$ , respectively. Their opening rates were

$21.2$  vs  $47.5$  and  $40.4$  vs  $46.0$ ,  $P < 0.01$ , respectively. Their maximal luminal areas were  $1502.98 \pm 1236.91$  vs  $5526.80 \pm 4853.42$ ;  $1918.14 \pm 2299.24$  vs  $3836.16 \pm 3549.16$ ;  $5526.80 \pm 4853.42$  vs  $3836.16 \pm 3549.16$ ,  $P < 0.05$ , their perimeters were  $220.33 \pm 130.25$  vs  $441.43 \pm 276.51$ ;  $241.79 \pm 171.13$  vs  $333.80 \pm 199.66$ ;  $441.43 \pm 276.51$  vs  $333.80 \pm 199.60$ ,  $P < 0.05$ , and their diameters were  $28.80 \pm 14.98$  vs  $59.39 \pm 28.53$ ;  $25.37 \pm 15.79$  vs  $46.22 \pm 20.85$ ;  $59.39 \pm 28.53$  vs  $46.22 \pm 20.85$ ,  $P < 0.05$ , respectively. In summary, the lymphatics found in gastric carcinoma samples from the metastatic group were significantly lower than those of the other groups.

**CONCLUSION:** There are newly formed lymph capillaries found in gastric carcinoma. Dilation of lymph capillaries may be related to edema found in peritumoral connective tissues. The observed lymph node metastases from gastric carcinoma occur through mature lymph capillaries that invade in and around primary gastric carcinoma foci.

**Key words:** Stomach neoplasms; Lymphatic metastasis; Lymphatic system/pathology

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Liao XB, Tang WP, Zhang QM, Fu ZG, Zhao YH. Morphological analysis of lymph vessels and capillaries in gastric carcinoma. *World J Gastroenterol* 1997; 3(2): 90-92 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/90.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.90>

### INTRODUCTION

Lymph node metastases from human gastric carcinoma typically occur through lymphatic channels, however its mechanism remains unclear. Some researchers<sup>[1]</sup> believe that the probability of lymph node metastasis increases with an accompanying increase in the number of lymph vessels and capillaries in the invaded peritumoral tissues. Nevertheless, others<sup>[2]</sup> think that the opening of lymph vessels and capillaries due to edema in peritumoral connective tissues facilitates cancer cell metastasis. Previous reports have suggested that the stomachs of mice exhibit a fine distribution of lymphatics. This study was performed in order to both profile the distribution, quantity and morphologic changes of lymph vessels and capillaries in gastric carcinoma, as well as to further illuminate the metastatic mechanism underlying gastric cancer.

### MATERIALS AND METHODS

#### Materials

Fresh, surgically resected gastric carcinoma specimens were collected from 32 cases (22 metastatic and ten non-metastatic) and carefully examined. All light microscopy specimens were fixed in 10% forma-

**Table 1** Lymph vessel and capillary counts ( $\bar{x} \pm s$ )

Group	Total	Opened No.	Opening rate (%)
Metastatic			
Gastric carcinoma	26.9 ± 14.2	5.7 ± 5.0	21.2
Peritumoral tissue	10.4 ± 4.0	4.9 ± 2.5	47.5
Non metastatic			
Gastric carcinoma	11.4 ± 3.4	4.6 ± 3.2	40.5
Peritumoral tissue	9.7 ± 3.2	4.5 ± 2.3	46

**Table 2** Morphological quantitative analysis of lymphatics ( $\bar{x} \pm s$ )

Group	<i>n</i>	Lumen area ( $\mu\text{m}^2$ )	Perimeter ( $\mu\text{m}$ )	Diameter ( $\mu\text{m}$ )
Metastatic				
Gastric carcinoma	43	1502.98 ± 1236.91	220.33 ± 130.25	28.80 ± 14.98
Peritumoral tissue	61	5526.80 ± 4853.42	441.43 ± 276.51	59.39 ± 28.53
Non metastatic				
Gastric carcinoma	47	1918.14 ± 2299.24	241.79 ± 171.13	25.37 ± 15.79
Peritumoral tissue	58	3836.16 ± 3549.16	333.80 ± 199.66	46.22 ± 20.85

lin and paraffin-embedded. Sections were stained with hematoxylin and eosin., Gastric carcinoma and their peritumoral tissues were simultaneously frozen at  $-20\text{ }^\circ\text{C}$  and sectioned using a cryostat. All frozen sections with a  $6\text{ }\mu\text{m}$  thickness were fixed in preparation for 5' NaseAlpase double staining. 5'adenosine monophosphate (AMP, No. A-1752, Sigma), naphthol AS-MX phosphate (No. N-5000, Sigma), fast blue BB (No. F-3578, Sigma), Tetramisol (No. T-1512, Sigma).

### 5'-Nase Alpase double staining<sup>[3-8]</sup>

The Alpase reaction to label blood vessels was followed by 5' Nase staining to label the lymphatics. Following incubation for 60 min at  $37\text{ }^\circ\text{C}$ , the slides with treated with standard medium containing Wachstein, incubated for two minutes. at room temperature in yellow ammomium sulfide, and incubated for 15 min. at room temperature in standard medium with Burston. Control slides were incubated without substrate (AMP or naphthol AS-MX phosphated).

### Quantification of lymph vessels and capillaries

The total number of opened vessels and the opening rate (percentage = the opened/the total) of both lymph vessels and capillaries in the submucosa of peritumoral and carcinoma tissues were studied under a light microscope ( $\times 100$  magnification). Lymph vessels and capillaries in tissue cross sections were counted, while those in longitudinal and oblique sections were excluded.

### Morphological quantitative analysis of lymph vessels and capillaries

The maximal luminal area, perimeter and diameter of both lymph vessels and capillaries containing opened lumens were measured using an image analysis device. This device included both light and fluorescent microscopes (Nikon, Japan), a digital brand, a computer, and a morphologic program (stereology, United States).

## RESULTS

### Swelling and metastatic lymph nodes

There were 15 lymph nodes within the lesser curvature of the stomach, 23 within the greater curvature of the stomach and 10 at other sites, and all these lacked signs of lymph node metastases. Additionally, there were 39 lymph nodes within the lesser curvature, 41 within the greater curvature, and 10 at other sites that accounted for 52 metastases (59.4%).

### Enzyme histochemistry

There were significant differences detected between the lymphatic and blood vessel staining. Using 5'Nase Alpase double staining,  $\text{Mg}^{2+}$  as an activator and lead as a capture agent, the reaction product of 5'Nase activity was detected as a brown or dark brown precipitate of lead sulfide on lymph vessel and capillary walls following incubation in 5'Nase standard medium. When tissue sections were then incubated in azo dye reaction medium for Alpase activity, the coloration of the blood vessels and capillaries changed to blue. The lymph vessels were morphologically irregular, with large lumens and thin-walled vessels, while the blood vessels were regular and

thick-walled. The slides demonstrated 5'Nase activity of both lymph vessels and capillaries. The slides only showed Alpase-positive staining of blood vessels and capillaries, with no sign of Alpase-positive lymph vessels and capillaries. The control slides that were incubated without 5' AMP or naphthol AS MX monophatate demonstrated no lymph, blood vessel, or capillary staining.

### Lymph vessel and capillary distribution and morphology

Many lymph capillaries were identified in the mucosal layer of peritumoral tissues, mostly between the bottom of the gastric gland and mucosal muscularis. Some lymph capillaries extended into the connective tissues within the gastric gland, while others extended into the mucosal muscularis or submucosa. There were many lymph vessels and capillaries in the submucosa, with the majority of lymph capillaries found near the mucosal muscularis and the majority of lymph vessels found near the muscularis. Many lymph vessels and capillaries were found in the muscularis, and many more between the oblique, circular and longitudinal muscles. The lymph vessels and capillaries found in the serosa were also seen in connective tissues near the longitudinal muscles, which were also surrounded by large lymph vessels.

All of the lymph vessels and capillaries in the gastric carcinoma tissues exhibited irregular morphologic shapes and branches. Lymph capillaries were partially opened with small lumens and had either lobular or triangle shapes. Some appeared strip-like with enlarged light brown endothelial cells that were irregularly arranged. It is possible that these strip-like tissues are in fact newborn lymph capillaries. A few strip-like tissues that stained positive for 5'Nase were seen in peritumoral tissues, which could be collapsed lymph capillaries. In and around the primary foci of gastric carcinoma, the carcinoma cells appeared to destroy the lymph capillary walls. These capillaries contained small and regularly shaped 5'Nase-positive endothelial cells that invaded the lumen. The lumens were large with irregular morphologies. These lymph capillaries may therefore be mature lymph capillaries.

### Quantification of lymph vessels and capillaries

In the metastatic group, the total number of lymph vessels and capillaries in the primary foci of gastric carcinoma were significantly higher ( $P < 0.01$ ) and their opening rates were significantly lower ( $P < 0.01$ ) than that of the peritumoral tissues of this same group. Additionally, the total numbers in the gastric carcinoma and peritumoral tissues of the metastatic group were significantly higher than those of the non-metastatic group ( $P < 0.01$ ), whereas the differences in the number of openings were not significant between the two groups ( $P > 0.05$ ) (Table 1).

### Morphological quantitative analysis of lymph vessels and capillaries

There were significant differences ( $P < 0.05$ ) in the maximal luminal area, perimeter and diameter of lymph vessels and capillaries between gastric carcinoma and their peritumoral tissues in metastatic group, gastric carcinoma and their peritumoral tissues in non metastatic group, and between peritumoral tissues in metastatic and non metastatic group, whereas the difference of the maximal luminal area, perimeter and diameter ( $P < 0.05$ ) between gastric carcinoma in metastatic group and in non metastatic group was not significant. Statistically the maximal lumen area, perimeter and diameter of lymph vessels and capillaries in carcinoma tissues in metastatic group were significantly smaller than those in other groups (Table 2).

## DISCUSSION

While lymph capillaries were observed to be thin-walled vessels with only a single layer of endothelium, the newborn capillaries were lined only by a single endothelial layer and thin basement membrane that lacked smooth muscle. This therefore made it considerably challenging to distinguish between the lymph capillaries and newborn capillaries. Using a light microscope, the composition of the lumen and the morphological characteristics of vessels were usually used for analysis. The lymph vessels were thin-walled with large

lumens that lacked red blood cells and contained irregularly projected endothelial nuclei. The lumens of these vessels also contained a few proteins and white blood cells. Using the 5'Nase Albase double staining method, lymph vessels and capillaries appeared brown in color, while blood vessels and capillaries appeared blue. Therefore, compared with traditional lymphatic injection, glass mounting and H and E staining of slides, the 5'Nase Albase double staining method accurately demonstrates the fine distribution of lymph vessels and capillaries. This provides an experimental method and morphological basis for performing research on the morphological shapes of lymph vessels and capillaries in human gastric carcinoma, as well as its relation to lymph node metastasis of gastric carcinoma.

Previous research postulated that the absence of lymphatic metastasis in early gastric carcinoma is due to the lack of lymphatics in superficial layers of the mucosa. With the discovery of tumor infiltration, we now believe that the increase in lymph vessels and capillaries around the primary foci of the tumor may be one of the critical factors that elevate the rate of lymph node metastasis<sup>[9]</sup>. Lubach *et al.*<sup>[11]</sup> determined that melanoma invasiveness significantly increased the number of lymph vessels and capillaries in surrounding tissues as well as metastatic activities in lymph vessels. These results suggest that lymph node metastasis in carcinomas are related to the increase in the number of lymph vessels and capillaries found in peritumoral tissues, which is due to the distribution of lymph vessels and capillaries that are not newborn. This study shows that the total number of lymph vessels and capillaries in gastric carcinoma is larger than those of their peritumoral tissues. Some capillaries, either partially opened or solid strip-like with enlarged, brown and irregularly arranged endothelial cells, may be newborn lymph capillaries. Upon comparing the morphologic characteristics of lymph capillaries in carcinoma and peritumoral tissues, it appears that lymph capillaries are not induced by the opening of collapsed lymph capillaries, an event caused by edema in connective tissues. Ryan<sup>[10]</sup> believes that angiogenesis should encompass not only blood vessels but also lymphatics. It remains unclear whether lymph capillary proliferation is induced by active secretions from tumor angiogenesis or by the host's reaction to tissue damage caused by the tumor.

Under normal conditions, most lymphatics exist in a collapsed state because only a portion of the entire lymphatic system is actively involved in the transportation process. The majority of lymphatics are therefore inactive, functioning as a potential reservoir for increased lymph flow<sup>[11]</sup>. Rapid tumor and edema growth among peritumoral tissues increased the pressure within the tissue, which dilates most lymph capillaries. During each stage of human and animal tumor invasion, severe edema was observed in peritumoral tissues and favored tumor cell infiltration. As a result, many tumor

cells accumulated in the afferent lymphatic vessels of the lymph node, particularly at drainage areas.

Among the several factors that induce edema in peritumoral tissues, the most important one may be the increase of vascular permeability and ineffective circulation of the lymphatic fluid within the tumor area<sup>[2]</sup>. High fluid pressure was induced due to a large amount of fluid accumulation within the tumor stroma and this fluid was transported from the center to the edge of the tumor. The edema in the peritumoral tissue widened and opened the lumen between the connective tissue bundles. Furthermore, the tissue fluid pushed the tumor cells to lymph capillaries, and both the tumor cells and tissue fluid entered into the lumen of the lymph capillaries. Lymph capillaries in gastric carcinoma showed light staining, low opening rates and small lumens, which indicated that lymph node metastases occur by invading through mature lymph capillaries that are located in and around the primary foci of human gastric carcinomas.

## REFERENCES

- 1 Lubach D, Berens von Rautenfeld D, Kaiser HE. The possible role of the initial lymph vessels of the skin during metastasis of malignant tumors. *In Vivo* 1992; **6**: 443-450 [PMID: 1520846]
- 2 Boucher Y, Jain RK. Microvascular pressure is the principal driving force for interstitial hypertension in solid tumors: implications for vascular collapse. *Cancer Res* 1992; **52**: 5110-5114 [PMID: 1516068]
- 3 Liao XB, Tang WP, Zhao YH, Li FH, Mo MY. Fine distribution of lymph vessels in gastric carcinoma. Fourth Congress of Asia Pacific Association of Societies of Pathologist, Beijing 1995: 162-163
- 4 Liao XB, Tang WP, Zhao YH, Li FH, Cai QZ. Research on the distribution of the lymph vessels in gastric carcinoma and peritumoral tissues. *Zhongguo Zuzhi Huaxue Yu Xibao Huaxue Zazhi* 1996; **5**: 67-71
- 5 Liao XB, Tang WP, Zhao YH, Li FH. A study of 5'-nucleotidase-alkaline phosphatase double staining on lymph capillaries. *Zhongguo Zuzhi Huaxue Yu Xibao Huaxue Zazhi* 1996; **5**: 184-187
- 6 Kato S, Miyauchi R. Enzyme-histochemical visualization of lymphatic capillaries in the mouse tongue: light and electron microscopic study. *Okajimas Folia Anat Jpn* 1989; **65**: 391-403 [PMID: 2546113 DOI: 10.2535/ofaj1936.65.6\_391]
- 7 Kato S. Histochemical localization of 5'-nucleotidase in the lymphatic endothelium. *Acta Histochem Cytochem* 1990; **23**: 613-620 [DOI: 10.1267/ahc.23.613]
- 8 Kato S. Enzyme-histochemical identification of lymphatic vessels by light and back-scattered image scanning electron microscopy. *Stain Technol* 1990; **65**: 131-137 [PMID: 2378010]
- 9 Lehnert T, Erlandson RA, Decosse JJ. Lymph and blood capillaries of the human gastric mucosa. A morphologic basis for metastasis in early gastric carcinoma. *Gastroenterology* 1985; **89**: 939-950 [PMID: 4043674]
- 10 Ryan TJ. Distinguishing lymphatics from blood vessels in normal and diseased skin. *Lymphology* 1987; **20**: 179-181 [PMID: 2451093]
- 11 Werner JA, Schünke M, Rudert H, Tillmann B. Description and clinical importance of the lymphatics of the vocal fold. *Otolaryngol Head Neck Surg* 1990; **102**: 13-19 [PMID: 2106112]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Pathogenetic effects of salted pork in an area of China with high-risk for stomach cancer

Yuan Yuan, Hui-Zhi Lin, Yin-Chang Zhang, Xuan-Jie Wang, Yie-Qiu Wu, Hua Gao, Lan Wang, Yan-Hou Liu, Fang Lu, Su-Qing Lou

Yuan Yuan, Hui-Zhi Lin, Yin-Chang Zhang, Yie-Qiu Wu, Hua Gao, Lan Wang, Cancer Institute, China Medical University, Shenyang 110001, Liaoning Province, China

Xuan-Jie Wang, Traditional Chinese Medicine Hospital of Zhuanghe City

Yan-Hou Liu, Fang Lu, Su-Qing Lou, The People's Hospital of Zhuanghe City

Yuan Yuan, female, born on October 28<sup>th</sup>, 1956 in Jinzhou, Liaoning Province, graduated from the Faculty of Medicine, China Medical University in 1982. Associate Professor, engaged in the etiologic analysis and early diagnosis of gastric cancer, with more than 40 published papers

Author contributions: All authors contributed equally to the work.

Supported by The "Eighth 5 year plan" National Science and Technology Foundation (85-914-01-10).

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Yuan Yuan, MD, Cancer Institute, China Medical University, Shenyang 110001 Liaoning Province, China  
Telephone: +86-24-3866766-6351

Received: November 17, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

### Abstract

**AIM:** To study the pathogenetic effects of salted pork (SP) (a special food in Zhuanghe City, a region of northern China that is a high-risk area for stomach cancer) on stomach cancer, and provide scientific basis for the primary prevention of stomach cancer in this high-risk region.

**METHODS:** This study consisted of three distinct parts. The first part involved a study of SP mutagenicity and employed both the Ames test and micronuclei assay using *V79* cells. The second part included a study of SP's effect on the gastric mucosa of residents in the Zhuanghe area who had consumed SP for more than ten years. Additionally, these studies involved an analysis of the dose effect relationship between SP and pathological changes in gastric mucosa, with a total of 300 cases analyzed. The third part of this study involved an observation of the mucosal lesions from experimental dogs by both gastroscopy and mucosal biopsy. Six healthy male dogs were selected, three were fed with SP, and the others served as controls.

**RESULTS:** This study revealed that SP extract could mutate *Salmonella typhimurium* TA<sub>98</sub> and induce an increase in both the micro

nuclei rate (MNR) and micro nuclei cell rate (MNCR) of *V79* at a dose range of 20-80  $\mu$ L/mL. There were significant dose-effect relations between SP and either MNR or MNCR. Pathological changes in the gastric mucosa of local residents who had consumed SP were significantly different from those of the control group. In people who had consumed SP for ten years, mucosal lesions were found that contained evidence of necrosis and erosion; In those who consumed SP for ten-20 years, both hyperplasia and dysplasia were seen in addition to the above lesions. In individuals who had consumed SP for 20-30 years, severe dysplasia and malignant changes were found. Furthermore, SP had damaging effect on the gastric mucosa of dogs that were fed SP. The mucosal lesions became more severe with increased feeding time.

**CONCLUSION:** SP is a strong mutagen and long-term SP exposure may result in repeated gastric mucosal damage and repair, ultimately leading to severe dysplasia and malignancy.

**Key words:** Stomach neoplasms/etiology; Meat; Mutagenicity; Gastric mucosa/pathology

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Yuan Y, Lin HZ, Zhang YC, Wang XJ, Wu YQ, Gao H, Wang L, Liu YH, Lu F, Lou SQ. Pathogenetic effects of salted pork in an area of China with high-risk for stomach cancer. *World J Gastroenterol* 1997; 3(2): 93-94 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/93.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.93>

### INTRODUCTION

Zhuanghe City is an area in northern China with an elevated occurrence of stomach cancer. The mortality of stomach cancer is 49.6/100000 for males and 22.2/100000 for females. Epidemiological investigations found that there was a close relationship between the high mortality of stomach cancer and the dietary habits of local residents. Salted pork (SP) is a special food consumed in this area<sup>[1]</sup>. In order to understand the pathogenetic effect of SP on stomach cancer, we studied three distinct aspects of SP: Its mutagenicity defined as the effect of SP on the gastric mucosa of local Zhuanghe residents who had consumed SP for more than ten years, the dose-effect relationship between SP and pathological changes of the gastric mucosa and the mucosal lesions of experimental dogs fed with SP.

### MATERIALS AND METHODS

#### Test of salted pork mutagenicity

SP samples were collected from residents in Mingyang town,

**Table 1 Ames test on salted pork**

Samples	Mutagenicity (rev/g TA <sub>98</sub> )	
	-S <sub>g</sub>	+S <sub>g</sub>
Salted pork		
-NaNO <sub>2</sub>	8640	13000
+NaNO <sub>2</sub>	8810	14900

**Table 2 Micronuclei test of salted pork-induced V<sub>79</sub> cells**

Samples	-S <sub>g</sub>		+S <sub>g</sub>	
	MNR	MNCR	MNR	MNCR
Extract of salted pork (0 mL/L)	13	12	9.0	8.0
Extract of salted pork (20 mL/L)	19	17	5.0	5.0
Extract of salted pork (40 mL/L)	19	17	8.5	8.5
Extract of salted pork (80 mL/L)	27	20	6.0	5.0
Methyl nitrate nitrosoguanidine (5 mg/L)	96	86		
Aflatoxin B1 (0.01 mg/L)			19.0	18.0

MNR: Micro nuclei rate; MNCR: Micro nuclei cell rate.

Zhuanghe City. After the nitrosation treatment and filtering of SP, the Ames test and micro nuclei assay using V<sub>79</sub> cells were conducted. The experimental methods were used based on previous reports<sup>[2]</sup>.

#### **Histopathological examination of the gastric mucosa of local residents who did or did not consume SP**

Among 12000 local residents in the Zhuanghe area who had consumed SP for many years, 150 residents who consumed SP four times a week (about 1000 g) were selected as subjects for the study, including 40 who had consumed SP continuously for ten years, 60 for 20 years, and 50 for 30 years. Another 150 residents who were matched in both sex and age but had not consumed SP were selected as controls. A retrospective investigation and comparative analysis of the living habits and dietary structure during the past 30 years in the two groups were conducted, which involved gastroscopies and mucosal biopsies. Biopsies covered the antrum, gastric angle, and anterior and posterior body of the stomach, respectively. The 1200 samples of mucosal biopsies were fixed with formalin, and routine paraffin sections, H and E staining, AB/PAS, as well as HID/AB staining were performed.

#### **Histopathological examination of the gastric mucosa from experimental dogs fed with SP**

Six two-month old healthy male dogs from the same strain that each weighed 4.5 kg were chosen for the experiment. The dogs were randomly divided into test and control groups (three dogs per group). The dogs in the test group were fed three times a day with SP that had been mixed with maize gruel (150 g). The dogs in the control group were fed with fresh pork mixed with maize gruel and vegetables. Any additional feeding conditions were the same for the experimental and control groups. During pre- and inter-testing, gastroscopies and mucosal biopsies were performed on the anterior and posterior wall of both the dog's antrum and body of its stomach, respectively. At the end of the test (12 mo later), an autopsy was performed and the stomachs were dissected and opened along its greater curvature. The specimens were then fixed in formalin. Blocks of gastric wall were isolated from the cardia to the pylorus, including the anterior and posterior body, antrum, esophagus and duodenum. After routine sectioning and staining, histopathological examinations were made on the gastric mucosa samples.

## **RESULTS**

#### **Ames test of SP mutagenicity**

In this study, whether or not SP was treated with nitrosation or S<sub>g</sub> and mixed with SP, the reverted number of salmonella typhimurium TA<sub>98</sub> all increased significantly. These findings indicate that SP extract is a strong mutagen to strain TA<sub>98</sub> (Table 1).

#### **Micronuclei test of SP-induced V<sub>79</sub> cells**

The SP extract could increase both the micronuclei rate (MNR) and micronuclei cell rate (MNCR) of V<sub>79</sub> cells at a dose range between 20 mL/L-80 mL/L. There were dose-effect relations between SP and either MNR or MNCR. Up to an 80 μL dose of MNR was two times higher in the test group when compared with that of the control group (Table 2).

#### **Histopathological examination of the gastric mucosa from local residents**

Necrosis and erosion foci (55/150, 36.6%), hyperplasia and dysplasia (31/150, 20.6%), as well as metaplasia (9/150, 6%) were detected in the gastric mucosa of 150 cases within the test group; one case of severe dysplasia was suspected of having cancer and poorly differentiated carcinoma in two stomachs were detected. Owing to different time spans of consuming SP, the degree of mucosal changes revealed significant differences. The pathological changes e.g. erosion and dysplasia became more severe with increased SP consumption over the years.

Among 150 cases from the control group, only a few cases were detected as having superficial gastritis, metaplasia and atrophic gastritis.

#### **Histopathological examination of the gastric mucosa from experimental dogs**

In this study, gastroscopic and histopathological examinations were done on three experimental dogs and three control dogs at the time of pre-, inter- (feeding SP for eight months) and post-experiment (12 mo later). Compared with the control group, the gastric mucosa from the test group showed various changes, which included erosion, necrosis, hyperplasia, inflammation, etc. These mucosal lesions became more severe with increased time of SP feeding.

## **DISCUSSION**

Although a few reports have been made on the pathogenesis of food in areas at high-risk for stomach cancer, studies on SP pathogenesis have not proven fruitful. In this study, we verified that SP from Zhuanghe City, an area in northern China at high-risk for stomach cancer, showed a strong occurrence of mutagenicity and demonstrated the damaging effect of SP on the gastric mucosa of dogs. Pathological changes of the gastric mucosa from local residents who had consumed SP showed significant differences from those of the control group. In people who had consumed SP for ten years, gastric mucosal lesions, which included signatures of erosion, necrosis and inflammatory change, were seen; in those for ten-20 years, hyperplasia and dysplasia were found in addition to the above specified lesions, and both severe dysplasia and malignant changes were observed in those who had consumed SP for 20-30 years.

There were significant differences in the mucosal changes observed between the test and control groups. Within the test group, the degree of mucosal changes, particularly erosion, metaplasia, dysplasia and tumorigenesis, had a positive relationship with the duration of SP consumption. This indicated that long-term exposure to SP may result in repeated gastric mucosal damage and repair, ultimately leading to severe dysplasia and malignancy. The results of this study may provide an important scientific basis for carrying out "primary prevention" by changing dietary habits in order to decrease the incidence of stomach cancer in the Zhuanghe area.

## **REFERENCES**

- 1 Shun ZX, Bai XW, Lin HZ, Wan JH, Yu XS, Li H. Epidemiological investigation of natural population in high and low risk area of stomach cancer. *Zhongguo Yixue Kexueyuan Xuebao* 1988; **17**: 23-26
- 2 Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. *Mutat Res* 1983; **113**: 173-215 [PMID: 6341825 DOI: 10.1016/0165-1161(83)90010-9]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Evaluation of preoperative staging in advanced gastric cancer with MRI

Guang-Yu Tang, Qing-Lu Guo, Ping-Ping Xin

Guang-Yu Tang, Qing-Lu Guo, Ping-Ping Xin, Section of MRI, the Second Affiliated Hospital, Jiangxi Medical College, Nanchang 330006, Jiangxi Province, China

Guang-Yu Tang, Associate Professor of Radiology (has six manuscript publications and one published book.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Guang-Yu Tang, Associate Professor, Section of MRI, the Second Affiliated Hospital, Jiangxi Medical College, Nanchang 330006, Jiangxi Province, China  
Telephone: +86-791-6266558-313

Received: September 11, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To evaluate the application of magnetic resonance imaging (MRI) to the preoperative staging of advanced gastric cancer.

**METHODS:** An MRI (SE sequence) was preoperatively performed on 34 patients with advanced gastric cancer. The tumors were located at the cardia fundus in 11 patients, the corpus in 14, the antrum in ten and throughout the entire stomach in two. The images were analyzed and staged on the basis of the criteria proposed by Matsushita M. The results were compared with the corresponding histopathologic findings to analyze the rate of diagnostic accordance.

**RESULTS:** The diagnostic rate accuracy by MRI was 77.8% (seven out of nine) for T<sub>2</sub> tumors; 77.3% (17 out of 22) for T<sub>3</sub> tumors and 100% (three out of three) for T<sub>4</sub> tumors, with the overall accuracy equaling 79.4%. When grades T<sub>3</sub> and T<sub>4</sub> tumors were considered as a single group to determine the presence or absence of extraserosal invasion using MRI technology, the diagnostic accuracy was 88.3%. Statistically, MRI staging showed a significant correlation with the corresponding histopathologic staging using the Spearman correlation test ( $r_s' = 0.743, P < 0.01$ ). When the concordance between MRI and histopathologic staging results were studied according to tumor location, the staging accuracy was highest (90.9%) in tumors located in gastric cardia fundus.

**CONCLUSION:** MR imaging is moderately valuable when staging advanced gastric cancer, especially for tumors located in gastric cardia fundus.

**Key words:** Stomach neoplasms/diagnosis; Magnetic resonance

imaging; Neoplasm staging

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Tang GY, Guo QL, Xin PP. Evaluation of preoperative staging in advanced gastric cancer with MRI. *World J Gastroenterol* 1997; 3(2): 95-97 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/95.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.95>

### INTRODUCTION

Endoscopy and barium studies are two main methods for clinical diagnosis of gastric cancer. Nevertheless, computed tomography and ultrasonography are used for diagnosis in patients with known gastric cancer as well as in complementary studies for the evaluation of extraserosal invasion, metastasis to lymph nodes and other organs<sup>[1-3]</sup>. However, there have only been a few studies that have utilized magnetic resonance imaging (MRI) for gastric cancer. The purpose of this study was to evaluate the utility of MR imaging in the preoperative staging of gastric cancer by analyzing the MR images from 34 patients with advanced gastric cancer.

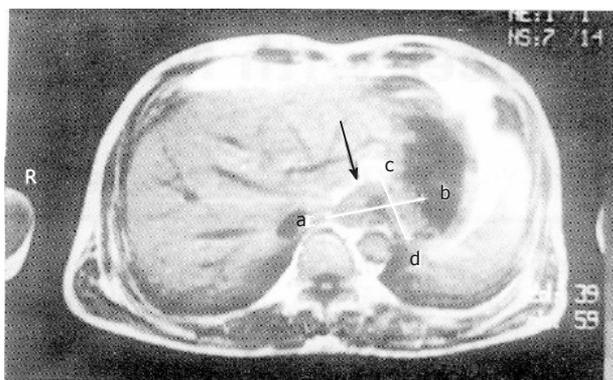
### MATERIALS AND METHODS

#### Patients

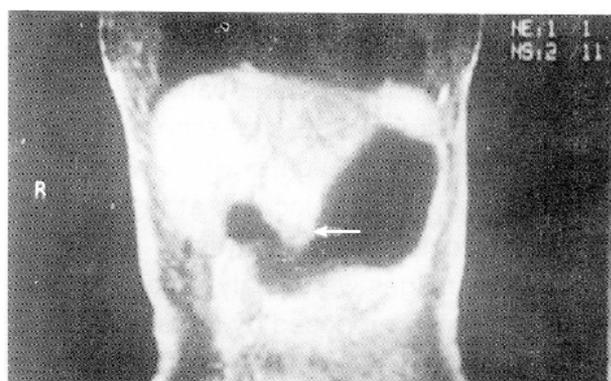
MR imaging was performed on 34 patients (26 men and eight women; mean age 55.6 years) with advanced gastric cancer. The lesions of all the patients were diagnosed pathologically following surgery. The interval between MRI and surgery ranged from three to 21 d. Gastrectomy was performed on 29 patients and exploratory laparotomy was performed on five patients. The tumors were located at the cardia fundus in 11 patients, the corpus in 14, the antrum in ten and the entire stomach in two. The degree of tumor invasiveness was evaluated according to the criteria<sup>[4]</sup> of the Tumor Node Metastasis (TNM) classification formulated by the China National Cooperative Group of Gastric Cancer. Nine patients had stage T<sub>2</sub> cancer, 19 had stage T<sub>3</sub>, and six had stage T<sub>4</sub>.

#### Imaging technique

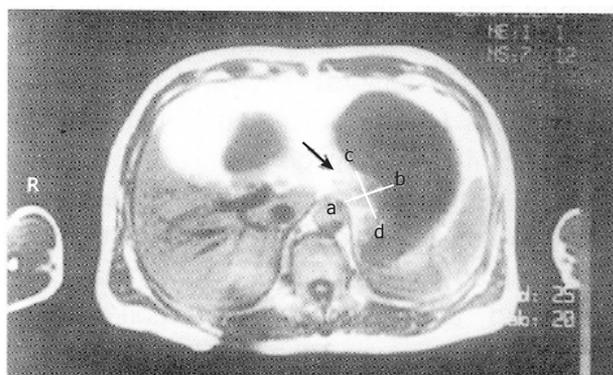
The patients fasted for 10-12 h. Before undergoing the MRI examination, the patients drank 600-1000 mL of water and were administered 10 mg of 654-2 intramuscularly to relax their stomachs. All patients were imaged in the supine position. MR imaging was performed on a 0.15 tesla MRI unit (ASM-015P Analogic Scientific Inc.) using body coil, a T<sub>1</sub>-weighted spin echo (SE) sequence (TR 700 ms/TE 30 ms), and a T<sub>2</sub>-weighted SE sequence (TR 1800 ms/TE 90 ms). The slice thickness was 8-10 mm. Axial and coronal images were routinely obtained. When necessary, sagittal imaging was also conducted.



**Figure 1** Grade MRT<sub>2</sub> tumor, T<sub>1</sub>WI. Axial images show a smooth low signal intensity band around the lesion (↑).



**Figure 2** Grade MRT<sub>2</sub> tumor, T<sub>1</sub>WI. Coronal images show a smooth low signal intensity band around the lesion (↑).



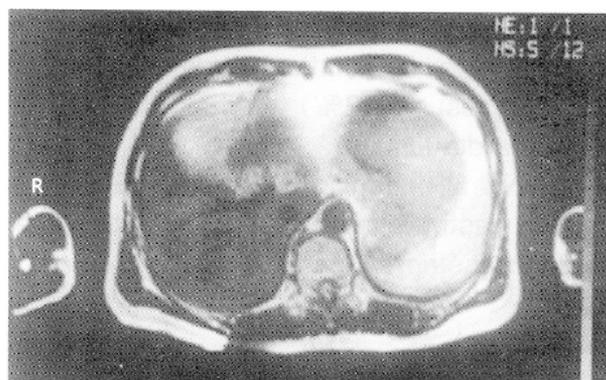
**Figure 3** Grade MRT<sub>3</sub> tumor, T<sub>1</sub>WI. Axial image shows an obscure hypointense band and blurring fat plane outside of the tumor (↑).

### Image analysis

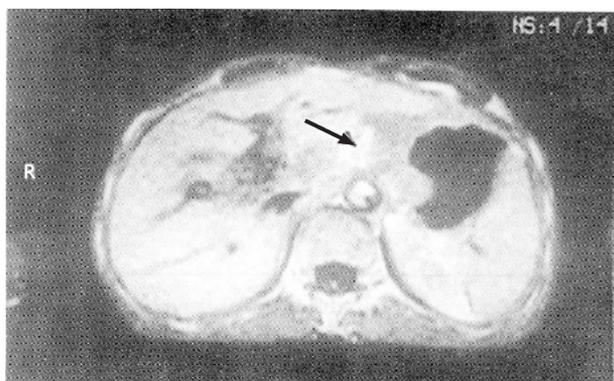
The MR images were analyzed by two radiologists before patients underwent surgery. The locations, appearances, sizes as well as the relation of the tumors to adjacent organs were evaluated. Location was classified according to four areas: Cardia fundus, corpus, antrum and entire stomach. A gastric wall > 1 cm thick was considered abnormal<sup>[1,5,6]</sup>. According to the criteria<sup>[5]</sup> proposed by Matsushita M, the degree of serosal invasion observed by MR imaging was classified as MRT-1 (no abnormal findings), MRT-2 (presence of a clear hypointense band or clear fat plane surrounding the lesion) (Figures 1 and 2), MRT-3 (presence of irregular margin or fat signal blurring around the lesion) (Figures 3 and 4), or MRT-4 (presence of contiguous extension of lesion signal to adjacent organs or definite involvement of adjacent structures), (Figure 5). The evaluation of both the extent and depth of gastric cancer relied primarily on the observation of images on the T-1WI<sup>[6]</sup>. This was due to the excellent contrast between the hypointensity of water signal in the stomach lumen, the isointensity of the gastric wall, and the hyperintensity of the fat plane outside the stomach.

## RESULTS

The findings of a thickened wall that was characterized as a lesion



**Figure 4** Same patient as in Figure 3, T<sub>2</sub>WI. The tumor was not as easily identifiable as that in Figure 3.



**Figure 5** Grade MRT<sub>4</sub> tumor, T<sub>1</sub>WI. Fat interval between stomach and hepatic left lobe disappeared and was occupied by a isointense tumor.

with MRI corresponded to the gastric cancer lesion observed during surgery. On the T-1WI, thickened gastric wall ranged from 1.3-3.9 cm (mean 2.29 cm). The stomach was sometimes deformed and stenosed. In patients with advanced gastric cancer, the moderate low signal of the tumor extended to contiguous organs through hyperintense fat intervals. On the T-2WI, moderate high tumor signal did not contrast sharply with the high signal of inside water and outside fat, so the tumor margin could not be easily identified. MRI results were compared with findings from pathologic examination (Table 1).

From the Table, we can see that the MR diagnostic accuracy was 77.8% (seven out of nine) for T<sub>2</sub>, 77.3% (17 out of 22) for T<sub>3</sub>, and 100% (three out of three) for T<sub>4</sub> tumors, with the overall accuracy equaling 79.4%. Moreover, the accuracy in determining the degree of serosal invasion was 88.2% (27 out of 34) when T<sub>3</sub> and T<sub>4</sub> lesions were regarded as a single group. Statistically, MRI staging showed a significant correlation with histopathologic staging using the Spearman correlation test ( $r_s' = 0.743, P < 0.1$ ). However, seven of 34 cases were staged incorrectly using MR imaging. Two of the stage T<sub>3</sub> tumors were understaged as stage MRT-2 because there were no hyperintense signals of fat plane in emaciated patients. Two of the stage T<sub>2</sub> tumors were overstaged as stage MRT-3 due to motion artifacts. Three of the stage T<sub>4</sub> tumors were understaged as stage MRT-3 because peritoneal and omentum invasion were not detected. Analysis of the concordance between MRI and histologic staging results based on tumor location (Table 2), revealed that the staging accuracy was highest in gastric cardia fundus tumors.

## DISCUSSION

Preoperative staging in advanced gastric cancer is very important for surgeons to judge the probability of gastrectomy, select the appropriate operative approach and evaluate prognosis<sup>[7]</sup>. It is also helpful for patients who have to undergo radiotherapy<sup>[8]</sup>. There have been many reports about preoperative staging in advanced gastric cancer with CT<sup>[1,7,9]</sup> and endoscopic US<sup>[2,3]</sup>. The staging accuracy by CT and US is 42%-69% and 81%-92%, respectively. Anatomically, the stomach is obliquely in contact with the left lobe of the liver, which interferes with the accuracy of determining hepatic invasion

**Table 1 Relationship between magnetic resonance imaging and histopathologic diagnosis of invasive depth ( $n = 34$ ).**

Magnetic Resonance Imaging diagnosis	Histologic diagnosis		
	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
MRT <sub>2</sub>	7	2	
MRT <sub>3</sub>	2	17	3
MRT <sub>4</sub>			3

**Table 2 Concordant rate between magnetic resonance imaging and histologic staging based on the location of tumors (%).**

Location	Cases	Concordant number	Concordant rate
Cardia fundus	11	10	90.9
Corpus	14	11	78.6
Antrum	7	5	71.4
Entire stomach	2	1	

by CT due to its partial volume effect. Furthermore, the limited spatial resolution often misses tiny metastases<sup>[1]</sup>. Cho JS suggested that dynamic CT be used to improve the diagnostic accuracy in the preoperative staging of gastric cancer<sup>[9]</sup>. As endoscopic US is invasive and its performance requires special skills<sup>[1]</sup>, it is difficult to perform on patients with advanced gastric cancer. For MRI, however, no special preparation is required. MRI not only provides axial images, but also coronal, sagittal, and oblique images. The shape, size, tumor location and its relation with adjacent structures can be found easily by concurrently analyzing various images. Oral water administration can both distend the gastric wall as well as wash out the gastric juice attached to the gastric wall so that the advanced cancer wall area cannot be distended. As a result, it appears in an MRI as a thickened area and can be easily differentiated from the normal wall. Water also acts as a negative contrast agent. On T<sub>1</sub> WI, the gastric wall appears very well due to the sharp contrast between the inside hypointense water and the outside hyperintense fat plane. Slight extraserosal invasion, which is difficult to visualize with CT, causes blurring of the fat layer on an MRI and so it is easily identified. It is thus very helpful for staging gastric cancer. Our study shows that there is a significant correlation between preoperative MRIs and pathologic staging of gastric cancer. The staging accuracy was somewhat superior to the previously published results that used CT. The 88% accuracy in determining extraserosal invasion indicated

that MRI staging was more valuable for advanced gastric cancer. The higher staging accuracy found for gastric cardia fundus tumors was likely due to the relative stability of the cardia region, resulting in minimal motion artifacts and enhanced image clarity<sup>[6]</sup>.

In our study, preoperative staging with MRI was incorrect in seven cases. Most of them were due to obvious motion artifacts or the emaciation of patients. For extremely emaciated patient, there is a minimal fat interval between the stomach and nearby organs, which might result in inaccurate staging<sup>[8]</sup>. In two cases, the metastases to the peritoneal and omentum were undetected, possibly due to the limited MR resolution of low field strength and the relatively small size of the lesions.

In conclusion, preoperative staging in advanced gastric cancer with MRI is particularly valuable for cardia fundus lesions. The evaluation of both the extent and depth of tumors relies primarily on the observation of images on the T<sub>1</sub>WI.

## REFERENCES

- 1 **Minami M**, Kawauchi N, Itai Y, Niki T, Sasaki Y. Gastric tumors: radiologic-pathologic correlation and accuracy of T staging with dynamic CT. *Radiology* 1992; **185**: 173-178 [PMID: 1523303 DOI: 10.1148/radiology.185.1.1523303]
- 2 **Botet JF**, Lightdale CJ, Zauber AG, Gerdes H, Winawer SJ, Urmacher C, Brennan MF. Preoperative staging of gastric cancer: comparison of endoscopic US and dynamic CT. *Radiology* 1991; **181**: 426-432 [PMID: 1924784 DOI: 10.1148/radiology.181.2.1924784]
- 3 **Akahoshi K**, Misawa T, Fujishima H, Chijiwa Y, Maruoka A, Ohkubo A, Nawata H. Preoperative evaluation of gastric cancer by endoscopic ultrasound. *Gut* 1991; **32**: 479-482 [PMID: 2040468]
- 4 **China National Cooperative Group of Gastric Cancer**. [Surgical treatment of gastric cancer in China: an analysis of 11, 734 cases]. *Zhonghua WaiKe Zazhi* 1982; **20**: 577-580 [PMID: 7151595]
- 5 **Matsushita M**, Oi H, Murakami T, Takata N, Kim T, Kishimoto H, Nakamura H, Okamoto S, Okamura J. Extraserosal invasion in advanced gastric cancer: evaluation with MR imaging. *Radiology* 1994; **192**: 87-91 [PMID: 8208971 DOI: 10.1148/radiology.192.1.8208971]
- 6 **Gao YG**, Chai YQ, Chai ZL (compile). *Diagnosis of MRI*. Beijing: The People's Military Doctor Publication 1993: 528-529
- 7 **Zhang WS**, Xu JX, Ming SL, Zhang J. The evaluation of CT examination on gastric and esophageal tumor. *Zhonghua Shiyong WaiKe Zazhi* 1989; **9**: 527-528
- 8 **Lee KR**, Levine E, Moffat RE, Bigongiari LR, Hermreck AS. Computed tomographic staging of malignant gastric neoplasms. *Radiology* 1979; **133**: 151-155 [PMID: 472284 DOI: 10.1148/133.1.151]
- 9 **Cho JS**, Kim JK, Rho SM, Lee HY, Jeong HY, Lee CS. Preoperative assessment of gastric carcinoma: value of two-phase dynamic CT with mechanical iv. injection of contrast material. *AJR Am J Roentgenol* 1994; **163**: 69-75 [PMID: 8010251 DOI: 10.2214/ajr.163.1.8010251]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Clinical significance of PCR in *Helicobacter pylori* DNA detection in human gastric disorders

Guo-Ming Xu, Xu-Huai Ji, Zhao-Shen Li, Xiao-Hua Man, Hong-Fu Zhang

Guo-Ming Xu, Xu-Huai Ji, Zhao-Shen Li, Xiao-Hua Man, Hong-Fu Zhang, Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

Guo-Ming Xu, male, was born on March 19, 1939 in Wuxi, Jiangsu Province, graduated from Department of Medicine, Shanghai Medical University in 1962, physician in chief, Professor, engaged in the research of gastrointestinal disorders, published over 80 papers and 4 books

Author contributions: All authors contributed equally to the work.

Supported by A grant for the molecular epidemiology of *H. pylori* infections from the National Natural Science Foundation of China

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Guo-Ming Xu, Professor, Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China  
Telephone: +86-21-65560684  
Fax: +86-21-65560684

Received: September 11, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the clinical significance of the PCR assay in the diagnosis of gastric *Helicobacter pylori* (*Hp*) infection.

**METHODS:** *Hp* infection in gastric antral biopsied specimens was identified by using the polymerase chain reaction (PCR) to amplify the specific *Hp* urease gene fragments (PCR-*Hp*-DNA) in 154 patients with gastrointestinal disorders. *Hp* urease gene oligonucleotide primers specific for *Hp* (16s rRNA) were used. Urease test and enzyme-linked immunosorbent assay (ELISA) for anti *Hp*-IgG serum were also used as controls.

**RESULTS:** PCR-*Hp*-DNA was detected in 140 (91%) of the 154 patients, where patients 114 and 125 were found infected with *Hp* by urease test and ELISA *Hp* IgG, respectively. There was a marked difference in the *Hp*-positive rate between the PCR-*Hp*-DNA and the urease test or ELISA-*Hp*-IgG ( $P < 0.05$ ). The *Hp* infection rate increased with age, although a minority of infected people developed signs and symptoms of gastric disorders. *Hp* infection is closely related to adenocarcinoma in both the gastric antrum as well as the down body of the stomach.

**CONCLUSION:** PCR is a sensitive and specific method for the detec-

tion of *Hp* in human gastric tissues. Detection of *Hp* DNA *in vivo* using this approach might improve the clinical diagnosis and epidemiological research related to *H. pylori* infection.

**Key words:** Peptic ulcer; Gastritis; Stomach neoplasms; *Helicobacter pylori*; *Helicobacter* infections; Polymerase chain reaction (PCR)

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Xu GM, Ji XH, Li ZS, Man XH, Zhang HF. Clinical significance of PCR in *Helicobacter pylori* DNA detection in human gastric disorders. *World J Gastroenterol* 1997; 3(2): 98-100 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/98.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.98>

### INTRODUCTION

*Helicobacter pylori* (*Hp*) is now recognized as the major etiologic agent of chronic active gastritis, peptic ulceration and a risk factor for the development of adenocarcinoma of the distal stomach<sup>[1]</sup>. Currently, the phenotypic characteristics known to differ among strains include the production of *VacA* and the presence of *CagA*<sup>[2]</sup>. Mucosal and systemic immunologic recognition of *Hp*-infected individual is associated with peptic ulcer disease. However, expression of *Hp* virulence factors *in vitro* may not accurately reflect the expression profiles in host tissues. *In vivo* detection of the *Hp* gene may improve the accuracy of clinical diagnosis<sup>[3]</sup>.

We employed the polymerase chain reaction (PCR) assay using primers specific for *Hp* 16s rRNA to detect *Hp* infection. We then compared our results with those from the rapid urease test and enzyme-linked immunosorbent assay (ELISA) performed on serum *Hp* IgG and evaluated the sensitivity and specificity of PCR for the detection of *Hp* infection in clinical practice.

### MATERIALS AND METHODS

#### Clinical specimens

A total of 154 patients undergoing gastric endoscopy from the Department of Gastroenterology of the Shanghai Changhai Hospital were studied retrospectively. Patients were excluded if they had a history of gastric surgery, receiving steroids or other immunomodulating drugs, abusing alcohol or illicit drugs, or were HBsAg-positive. Among the 154 patients (95 male and 59 female; mean age 51 years, range between 18-63 years) with gastric disorders, 40 had chronic superficial gastritis (CSG), 12 had chronic atrophic gastritis (CAG), 44 had duodenal ulcers, 16 had gastric ulcers and 42 had gastric carcinoma, which was determined based on histological examination.

**Table 1 Comparison of urease test, enzyme-linked immunosorbent assay and polymerase chain reaction for detection of *Helicobacter Pylori* in gastric mucosa**

Diagnosis	No. of patients	Rapid urease test	ELISA-Hp-IgG	PCR-Hp
		No. (%)	No. (%)	No. (%)
Chronic superficial gastritis	40	28 (70)	34 (85)	37 (93)
Chronic atrophic gastritis	12	7 (58)	8 (67)	10 (83)
GU	16	13 (81)	13 (81)	14 (88)
DU	44	37 (84)	37 (84)	42 (96)
GC	42	24 (68)	33 (78)	37 (88)
Total	154	114 (74)	125 (81)	140 (91)

ELISA-Hp-IgG: Enzyme-linked immunosorbent assay for anti *Helicobacter pylori*-IgG; PCR-Hp: Polymerase chain reaction to amplify the specific *Helicobacter pylori* urease gene fragments.

**Table 2 The age distribution of gastric mucosal *Helicobacter Pylori* infection with polymerase chain reaction**

Age	No. studied	Positive number	Positive rate (%)
10-	5	3	60
20-	12	9	75
30-	19	15	79
40-	50	48	96
50-	42	40	95
≥ 60	26	25	96
Total	154	140	91

Gastric biopsy specimens from gastric antrum (15 μg) were placed immediately in normal saline at 4 °C and were coarsely homogenized in 400 μL TE buffer (10 mmol/L Tris-HCl, 0.1 mmol/L EDTA, pH7.8) with a tissue grinder. In an Eppendorf, (50 μL) of homogenized mucus was then mixed with 50 μL of lytic solution and proteinase K was added to reach a final concentration of 200 mg/L. The mixture was incubated at 50 °C for 30 min until the tissue pellets were completely digested, and they were then boiled for 10 min. The samples were centrifuged at 20000 × g for 1 min at 4 °C and the supernatants were stored in sterile vials at -75 °C until they were used as PCR templates.

Peripheral blood samples were obtained to measure the immunoglobulin G (IgG) response to *Hp* with ELISA.

**PCR amplification**

PCR primers were designed based on published sequences of *Hp* 16s rRNA. The primers used were as follows: Primer 1: 5'-GCGCAA-TCAGCGTCAGGTAATG-3' Primer 2: 5'-GCTAAAGAGATCAGCCTA-TGTCC-3'. These primers were synthesized using the automated phosphoramidite coupling method. Amplification of *Hp* genomic DNA sequences was carried out in 25 μL PCR buffer [50 mmol/L KCl, 10 mmol/L Tri HCl (pH8.3)], 1.5 mmol/L MgCl<sub>2</sub>, 200 μmol/L deoxynucleotides, and 1 μL of boiled *Hp* supernatant as DNA template as well as the control. The 16s rRNA primers were each used at a final concentration of 0.5 μmol/L. Each reaction was amplified for 36 cycles as follows: 1 min at 94 °C for denaturation, 45 s at 60 °C for annealing and 90 s at 72 °C for extension. PCR of cDNA from gastric biopsied specimens was performed exactly as described above. Agarose gel electrophoresis with ethidium bromide staining was performed from each PCR mixture. Negative and positive comparisons were made for each experiment.

**Enzyme linked immunosorbent assay (ELISA)**

The purified *Hp* crude preparation of urease (CPU) antigen was diluted in 0.1 mol/L carbonate buffer (pH9.6) to a final concentration of 0.5 mg/L. Polystyrene microtest plates were coated with 100 μL/well of antigen solution and incubated overnight at 4 °C. 100 μL of each serum sample was added to wells and incubated at 37 °C for 2 h after the plates were washed. After three washings, 100 μL/well of a substrate solution was added to each well. Each plate contained a positive and a negative control serum.

**Rapid urease test**

A 2-mm pinch biopsy was taken from the prepyloric mucosa (within 5 cm of the pylorus, at an angle of about ten o'clock), and the tissue was pushed beneath the surface of the reactive solution. In positive

**Table 3 Stratified analysis of *Helicobacter Pylori* infection with respect to tumor location**

Site of tumors	No. studied	PCR- <i>Hp</i> positive	
		No.	(%)
Gastric antral	15	14	-93
Gastric corpus	17	16	-94
Gastric cardia	5	4	-80
Esophageal	5	3	-60
Total	42	37	-88

PCR-Hp: Polymerase chain reaction to amplify the specific *Helicobacter pylori* urease gene fragments.

**Table 4 Stratified analysis of *Helicobacter Pylori* infection with respect to tumor histological type**

Tumors	No. studied	<i>Helicobacter pylori</i> positive	
		No.	(%)
Adenocarcinoma	30	29	-97
Myxoeithelioma	2	1	-50
Undifferentiated cancer	3	2	-67
Myoangiosarcoma	2	1	-50
Benign tumors	5	4	-80
Total	42	37	-88

cases a red tinge developed around the biopsy at one minute. There was no color changes if *Hp* was absent.

**RESULTS**

There were marked difference in positive rates between various methods in the determination of *Hp* infection (Table 1).

*Hp* was detected in 114 (74%) of 154 patients using rapid urease test, and all of these samples showed positive PCR results in gastric mucosa.

Thirty of 40 rapid urease test-negative cases were PCR positive. Out of 125 (81%) of ELISA-positive cases, 123 were PCR positive and 2 were negative (a CSG and a CAG, respectively). However, 16 of 29 ELISA-negative cases had positive PCR results. Among the 154 patients with antral gastritis, peptic ulcer or gastric carcinoma, *Hp* was found in 140 (91%), 114 (74%) and 125 (81%) by *Hp* PCR, rapid urease test and ELISA-Hp IgG, respectively, which were significantly different from each other (*P* < 0.05). There was, however, a positive correlation among these three methods. The *Hp*-PCR was the most sensitive and specific. There was no difference between the males (89, 93%) and the females (51, 87%) in *Hp*-PCR positive results. The age distribution of gastric mucosal *Hp* detected with PCR is shown in Table 2. The positive rates in various age groups with gastric disorders by PCR method are higher than those by other methods. *Hp* infections occur earlier and more frequently at all ages in the Chinese population compared to Western populations.

These studies of *Hp* infection in patients with gastric carcinoma indicated that there was a relationship between *Hp* infection and tumor location. *Hp* infection was found in 35 of 42 (83%) patients with gastric carcinoma using PCR. Gastric carcinoma patients were more likely to be infected by *Hp* than normal controls. In a stratified analysis of tumor location within the upper gastrointestinal tract (Table 3, Table 4), *Hp* infection in noncardia tumors was significantly increased (29/32, 91%). Twenty eight of 30 *Hp* positive gastric carcinoma cases (93%) had intestinal adenocarcinoma.

**DISCUSSION**

*Hp* infection has been implicated in the pathogenesis of active chronic gastritis and peptic ulcer. Recently, it has also been identified as a risk factor for gastric cancer. The diagnosis of *Hp* infection is usually based on invasive methods such as biopsy with histological examination, culture and urease test of the gastric biopsy specimens, as well as noninvasive methods such as the <sup>13</sup>Curea and <sup>14</sup>Curea breath tests. Due to the fastidious growth of *Hp* and the prolonged incubation period required, several alternative approaches have been developed for the accurate and rapid detection of *Hp* in gastric mucosa<sup>[4-6]</sup>. The urease test appeared to have a low sensitivity in detecting *Hp* when compared with other diagnostic tech-

niques<sup>[5]</sup>. The sensitivity and specificity of the urease test ranged from 60%-93% and 96%, respectively, but with false positive and false negative results<sup>[6]</sup>. The sensitivity and specificity of the ELISA are 81%/97% and 78%/97%, respectively<sup>[7]</sup>.

Current diagnostic tests for *Hp* infection, however, still involve invasive gastric endoscopy and detection of the organism in gastric biopsy specimens<sup>[1,5]</sup>. Molecular biological techniques have been applied to the diagnosis of *Hp* infection and both a genomic *Hp* DNA probe and an oligonucleotide probe developed for an *Hp*-specific 16s rRNA sequence have been found to detect as few as 10<sup>4</sup> *Hp* cells. These nucleic and hybridization methods, however, require the use of <sup>32</sup>P radioactivity and take up three days to provide autoradiographic results. PCR can selectively amplify the copy number of a target gene more than 10<sup>6</sup>-fold. PCR, therefore, has great potential to improve the ability to diagnose infectious diseases caused by fastidious or slow-growing microorganisms.

A rapid, sensitive and specific test for *Hp* would be of great value because of the clinical importance of this pathogen and the large number of laboratory identifications being routinely undertaken around the world. The reference "gold standard" used for the comparative detection of *Hp* in clinical biopsy samples was bacteriological culturing<sup>[3]</sup>. PCR-detected *Hp* in all biopsy samples was found to contain culturable *Hp*. The fact that some samples were negative by rapid urease test and ELISA for *Hp*-IgG and positive by PCR may reflect the high sensitivity of this method. PCR, however, detected *Hp* in two of the patients found not to have *Hp* by ELISA for *Hp*-IgG. The results may represent either false positive PCR results or the time difference between the eradication of *Hp* in gastric mucosa and the turnover of antibodies against *Hp* in serum. PCR analysis of clinical biopsy samples by the boiling DNA extraction method is convenient and sensitive for routine use. Currently, the phenotypic characteristics among strains include both the production of a *vacuolating cytotoxin* (*VacA*) and the presence of a high molecular weight protein encoded by *CagA* gene (*CagA*)<sup>[8,9]</sup>. Although present in most cytotoxic strains, *CagA* is not necessary for *VacA* expression. Expression of these virulence factors *in vitro* may not accurately reflect the expression profile in host tissues. Therefore, *in vivo* detection of the *Hp CagA* or *VacA* genes and their expression levels may improve the diagnosis of toxic *Hp* strain infection<sup>[2]</sup>. PCR of genomic DNA for the *Hp CagA* gene in the corresponding bacterial isolates was closely correlated with either *CagA* or *VacA* gene expression in gastric tissue. In the near future, PCR for the *CagA* or *VacA* genes in gastric mucosa would prove very useful in both routine diagnosis of *Hp* as well as in *Hp*-related molecular epidemiology research.

Another main finding in this study was the significantly increased prevalence of *Hp* infection among patients with noncardia gastric cancer compared with other patients. This difference could not be explained by dietary or socioeconomic factors. Our findings were in agreement with previous results of studies on association between *Hp* infection and gastric cancer<sup>[10]</sup>. In patients with carcinoma located at the gastric cardia, we found no significant association with *Hp* infection based on *Hp* previous epidemiological and clinical studies. It has been hypothesized that the pathogenesis of the intestinal

type of gastric carcinoma may be linked with environmental factors. As chronic atrophic gastritis (type B) has mainly been linked to the intestinal type of gastric carcinoma, *Hp* infection would be expected to show the same relationship. In this study, *Hp* infection was found to be associated with an increased risk of gastric intestinal adenocarcinoma, and it seems to be an independent risk factor for gastric carcinoma. There are several possible mechanisms by which *Hp* infection may be involved in gastric carcinogenesis. *Hp* adversely affects the chemical and physical properties of the mucous layer, which may make the mucosa susceptible to carcinogenic factors. Moreover, *Hp* seems to promote the progression from a normal to a metaplastic epithelium, possibly by inducing a hyperproliferative state in the inflamed gastric mucosa. There has been more evidence that *Hp* is a major risk factor for human gastric adenocarcinomas and all low-grade B cell gastric lymphomas. The International Agency for Research on Cancer categorized *Hp* as a carcinogen<sup>[11]</sup>.

In conclusion, PCR is a highly sensitive and specific method for the detection of the presence of *Hp* in human gastric tissues. Detection of *Hp* DNA *in vivo* by this approach may improve the clinical diagnosis and molecular epidemiological research of *Hp* infection.

## REFERENCES

- 1 **Tompkins LS**, Falkow S. The new path to preventing ulcers. *Science* 1995; **267**: 1621-1622 [PMID: 7886448 DOI: 10.1126/science.7886448]
- 2 **Telford JL**, Ghiara P, Dell'Orco M, Comanducci M, Burroni D, Bugnoli M, Tecce MF, Censini S, Covacci A, Xiang Z. Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. *J Exp Med* 1994; **179**: 1653-1658 [PMID: 8163943 DOI: 10.1084/jem.179.5.1653]
- 3 **Peek RM**, Miller GG, Tham KT, Pérez-Pérez GI, Cover TL, Atherton JC, Dunn GD, Blaser MJ. Detection of *Helicobacter pylori* gene expression in human gastric mucosa. *J Clin Microbiol* 1995; **33**: 28-32 [PMID: 7699060]
- 4 **Xiang Z**, Bugnoli M, Ponzetto A, Morgando A, Figura N, Covacci A, Petracca R, Penatini C, Censini S, Armellini D. Detection in an enzyme immunoassay of an immune response to a recombinant fragment of the 128 kilodalton protein (*CagA*) of *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 1993; **12**: 739-745 [PMID: 8307041 DOI: 10.1007/BF02098460]
- 5 **Foxall PA**, Hu LT, Mobley HL. Use of polymerase chain reaction-amplified *Helicobacter pylori* urease structural genes for differentiation of isolates. *J Clin Microbiol* 1992; **30**: 739-741 [PMID: 1313051]
- 6 **Marshall BJ**, Warren JR, Francis GJ, Langton SR, Goodwin CS, Blincow Editor. Rapid urease test in the management of *Campylobacter pyloridis*-associated gastritis. *Am J Gastroenterol* 1987; **82**: 200-210 [PMID: 3548326]
- 7 **Xiang Z**, Censini S, Bayeli PF, Telford JL, Figura N, Rappuoli R, Covacci A. Analysis of expression of *CagA* and *VacA* virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that *CagA* is not necessary for expression of the *vacuolating cytotoxin*. *Infect Immun* 1995; **63**: 94-98 [PMID: 7806390]
- 8 **Marchetti M**, Aricò B, Burroni D, Figura N, Rappuoli R, Ghiara P. Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. *Science* 1995; **267**: 1655-1658 [PMID: 7886456 DOI: 10.1126/science.7886456]
- 9 **Crabtree JE**, Farmery SM, Lindley IJ, Figura N, Peichl P, Tompkins DS. *CagA*/cytotoxic strains of *Helicobacter pylori* and interleukin-8 in gastric epithelial cell lines. *J Clin Pathol* 1994; **47**: 945-950 [PMID: 7962609 DOI: 10.1136/jcp.47.10.945]
- 10 **Hansson LE**, Engstrand L, Nyrén O, Evans DJ, Lindgren A, Bergström R, Andersson B, Athlin L, Bendtsen O, Tracz P. *Helicobacter pylori* infection: independent risk indicator of gastric adenocarcinoma. *Gastroenterology* 1993; **105**: 1098-1103 [PMID: 8405854]
- 11 **Graham DY**, Go MF, Genta RM. *Helicobacter pylori*, duodenal ulcer, gastric cancer: tunnel vision or blinders? *Ann Med* 1995; **27**: 589-594 [PMID: 8541037 DOI: 10.3109/07853899509002474]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Immunohistochemical detection of proliferating cell nuclear antigen in hepatocellular carcinoma

Dong Wang, Jing-Quan Shi, Feng-Xuan Liu

Dong Wang, MD, Department of Clinical Pathology, Daping Hospital, Third Military Medical University, Chongqing 630042, China

Jing-Quan Shi, Department of Pathology, Third Military Medical University, Chongqing 630042, China

Feng-Xuan Liu, Department of Clinical Pathology, Southwest Hospital, Third Military University, Chongqing 630042, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Dong Wang, MD, Associate Professor, Department of Clinical Pathology, Daping Hospital, Third Military Medical University, Chongqing 630042, China

Received: September 13, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the expression levels of proliferating cell nuclear antigen (PCNA) and its correlation with the mitotic count, histological grade, and metastasis of human hepatocellular carcinoma (HCC).

**METHODS:** PCNA expression was detected immunohistochemically with PC10 monoclonal antibody for 80 cases of HCC. The PCNA labeling index (LI) was assessed by counting the positively-stained nuclei per 500 cells.

**RESULTS:** PCNA LI ranged from 1.8% to 91.4% (mean = 33.9%) in cancerous tissues, and was significantly related to the tumor size, histological grade, mitotic count and the metastases found in HCC samples.

**CONCLUSION:** HCC Proliferation activity, as defined by PCNA immunohistochemical analysis, is strongly correlated with tumor invasiveness. These findings hold potential prognostic value for HCC patients.

**Key words:** liver neoplasms; Carcinoma; Hepatocellular; Immunohistochemistry; Antigens; Neoplasm

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang D, Shi JQ, Liu FX. Immunohistochemical detection of proliferating cell nuclear antigen in hepatocellular carcinoma. *World J Gastroenterol* 1997; 3(2):

101-103 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/101.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.101>

### INTRODUCTION

Proliferating cell nuclear antigen (PCNA), also known as cyclin, is a 36 kDa nuclear protein and has been recognized as an auxiliary protein for DNA polymerase delta<sup>[1]</sup>. PCNA is believed to play an essential role in regulating both DNA synthesis and cell proliferation<sup>[2]</sup>. PCNA expression peaks at G1/S of interphase, and then drops during mitosis to a level that is too low to be detected using the immunohistochemical method. Thus, PCNA serves as an endogenous histologic marker for the G1/S phases of the cell cycle. In recent years, studies have revealed a significant relationship between PCNA expression and both the mitotic activity and grading in solid tumors<sup>[3]</sup>. Proliferation activity, defined by PCNA immunohistochemistry, is a useful signature in a variety of malignant tumors, including gastric carcinomas<sup>[4]</sup>, gastrointestinal lymphomas<sup>[5]</sup> and colorectal cancers<sup>[6]</sup>. The purpose of this study is to measure the PCNA expression levels in 80 cases of human hepatocellular carcinoma (HCC) to determine whether there is a correlation between PCNA expression and HCC histological grade, mitotic count and metastasis.

### MATERIALS AND METHODS

#### Subjects

We obtained cancerous and juxta cancerous tissues, which were surgically resected from 80 HCC patients at the Southwest Hospital of our university from 1988 and 1993. All specimens were fixed in 10% formaldehyde solution and embedded in paraffin. Sections 5  $\mu$ m thick stained with H and E and antibodies. Tumors were divided into three groups according to size: (1) 11 cases of single tumors with a diameter  $\leq$  3 cm; (2) 19 cases of single tumors with a diameter between 3 cm-5 cm; and (3) 50 cases of single tumors with a diameter  $\geq$  5 cm or with multiple tumors. Intrahepatic and extrahepatic metastases were discovered during surgery in 29 cases and one case, respectively. According to the WHO classification, 23 cases were of the trabecular type, 31 of the pseudoglandular type, 22 of the compact type and four of the sclerosing type. The differentiation degree was categorized into four groups according to Edmondson and Steiner's grading: Grade I in four cases (5.0%), grade II in 33 (41.3%), grade III in 27 (33.8%) and grade IV in 16 (20.0%). The number of mitotic cancer or liver cells in ten high power fields (lens objective,  $\times$  40) were randomly counted three times. Among them, the mitotic numbers for the most mitotically active areas was referred to as the mitotic count (MC). The result was divided into three groups: 0-4, 5-9, and 10 and over.

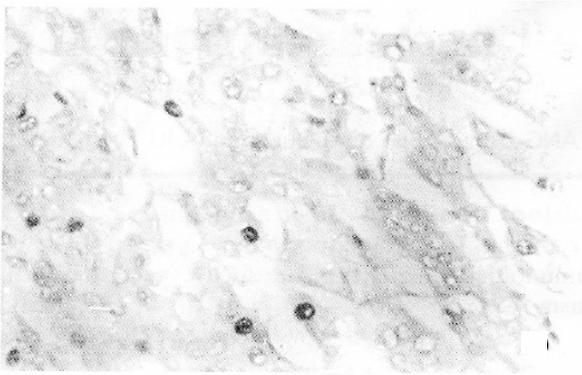


Figure 1 Proliferating cell nuclear antigen immunostaining of grade I hepatocellular carcinoma.

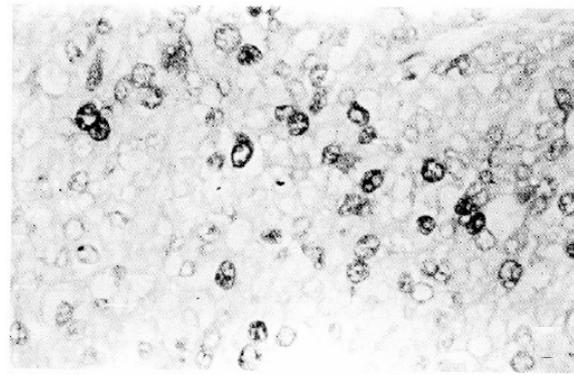


Figure 3 Proliferating cell nuclear antigen immunostaining of grade III hepatocellular carcinoma.

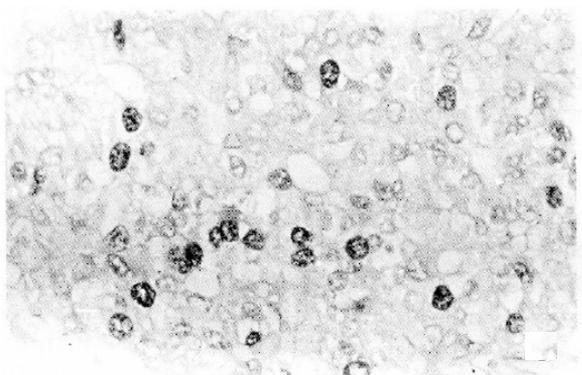


Figure 2 Proliferating cell nuclear antigen immunostaining of grade II hepatocellular carcinoma.

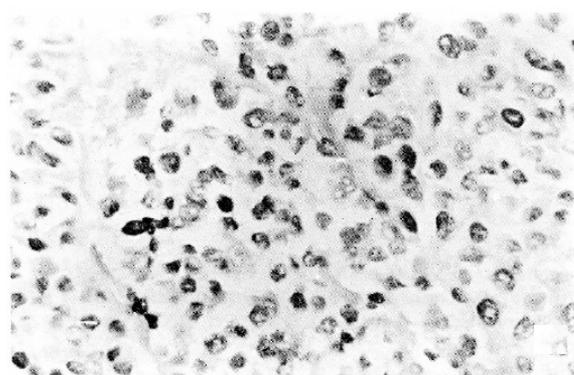


Figure 4 Proliferating cell nuclear antigen immunostaining of grade IV hepatocellular carcinoma.

**PCNA immunohistochemical staining**

The PC10 monoclonal antibody to PCNA, a donation from Dr. D.P. Lane, Dundee, United Kingdom, was used at a dilution of 1:100 and immunohistochemical staining was performed using the ABC method. Negative control sections were incubated with PBS instead of the primary antibody.

**PCNA labeling index (LI)**

PCNA staining was confined to the nuclei. Although the nuclear staining varied in intensity, all identifiable staining was considered positive. Sections were counted at high magnification ( $\times 400$ ), and 5.8 fields were randomly assessed for tumor or nontumor liver cells. Five hundred cell nuclei of each cell type were found and the PCNA labeling index (LI, %) was determined.

**Statistical analysis**

The significant differences between the distinct PCNA LI groups was assessed using the PDA-2 statistical package provided by our university.  $P < 0.05$  was considered statistically significant.

**RESULTS**

**PCNA expression and distribution**

PCNA antigen was detected exclusively in the nuclei of liver and cancer cells, however no staining was detected in the cytoplasm. In non-cancerous tissues, including chronic hepatitis and nodular cirrhosis, a few PCNA-positive cells were also found and distributed in a single or focal pattern, however no reaction was observed in the normal liver tissue adjacent to the tumor. The PCNA LI was 0%-12.3% in the surrounding cancer tissues, and 1.8%-91.4% (mean 33.9%) in the cancerous tissues. The distribution of PCNA immunohistochemical reactivity within tumors appeared scattered, patchy, and diffuse. The number of PCNA-positive cells varied with the degree of HCC, with a higher tumor grading correlating with a higher PCNA LI (Figures 1-4). Meanwhile, PCNA-positive nuclei were numerous in the tumor cells adjacent to the surrounding cancer tissues, tumor thrombi and areas of extracapsular tumor growth.

**Table 1 Proliferating cell nuclear antigen labeling index correlation with histologic grading in hepatocellular carcinoma**

Histologic grading of hepatocellular carcinoma	Number of cases	Positive ( $\bar{x} \pm s$ )	Range (%)
Edmondson I	4	4.3 $\pm$ 2.6	1.8-8.0
Edmondson II	33	20.4 $\pm$ 10.2	10.7-48.3
Edmondson III	27	42.1 $\pm$ 14.6	18.4-63.7
Edmondson IV	16	55.1 $\pm$ 17.3	34.6-91.9

**The relationship between PCNA LI and tumor pathological features**

The relationship between PCNA LI and the pathological tumor features of PCNA LI was 22.5% in HCC with a diameter  $< 3$  cm, 30.9% in HCC with diameters between 3 cm and 5 cm, and 37.4% in HCC with a diameter  $> 5$  cm. There was significant difference between PCNA LI in HCC with a diameter  $< 3$  cm and PCNA LI in HCC with a diameter  $> 5$  cm. The histologic grading of HCC in PCNA LI is shown in Table 1. The differences between PCNA positivity and tumor histological grade were statistically significant.

PCNA LI was 18.6%, 42.4% and 55.1% in the three MC groups, respectively, and the differences were significant ( $P < 0.01$ ). This indicated that there was a close relationship between PCNA LI and MC.

PCNA LI was 44.8% in 30 cases of metastatic HCC, and 29.4% in 50 cases of HCC without metastasis ( $P < 0.05$ ). There was a significant correlation between PCNA LI and the rate of tumor metastasis, while no significant correlation was found between PCNA LI and HBV infection, nodular cirrhosis, or tumor classification.

**DISCUSSION**

The proliferative activity of tumor cells depends primarily on the malignant severity of the host tumor. We employed several *in situ* methods, including morphometric image analysis and immunohistochemical staining for both BrdU and argyrophil to determine cell proliferation levels, which is the "histologic gold standard". Heratake *et al*<sup>(7)</sup> studied 140 HCC cases with hepatic resection, and noted that, among various clinicopathologic factors, the mitotic indices were well correlated with prognosis. This suggested that the mitotic count was accurate and reliable in predicting prognosis. Unfortunately, mitotic

count can be affected by tissue fixation, the microscopy field size as well as other factors, and can therefore lead to irreproducibility and inaccurate conclusions.

Compared with the mitotic counts, PCNA immunohistochemical staining has proven to be more effective and reliable. In general, cell proliferation significantly contributed to the degree of malignancy where the less differentiated cells showed a stronger ability to proliferate, thus enhancing tumor malignancy. These studies reveal that PCNA expression is significantly related to the degree of HCC differentiation and that numerous PCNA-positive nuclei were particularly observed in tumor thrombi and areas of extracapsular tumor growth. A significant correlation was also established between PCNA LI and tumor size, histological grade, tumor metastasis, and mitotic count, which is consistent with results previously reported by Taniai *et al.*<sup>[8]</sup>. Additionally, Ng *et al.*<sup>[9]</sup> studied 72 patients with surgically resected HCC and found that those with a PCNA less than or equal to 200 showed significantly greater disease-free survival rates than those with scores greater than 200, therefore confirming the important role of PCNA immunohistochemical staining in predicting HCC prognosis. In conclusion, these data suggest that PCNA staining is a reliable metric of HCC tumor proliferation. This, in turn, establishes a very valuable method for not only evaluating tumor invasion and metastasis, but also predicting HCC patient prognosis.

## REFERENCES

- 1 **Bravo R**, Frank R, Blundell PA, Macdonald-Bravo H. Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature* 1987; **326**: 515-517 [PMID: 2882423 DOI: 10.1038/326515a0]
- 2 **Takasaki Y**, Deng JS, Tan EM. A nuclear antigen associated with cell proliferation and blast transformation. *J Exp Med* 1981; **154**: 1899-1909 [PMID: 6172535 DOI: 10.1084/jem.154.6.1899]
- 3 **Robbins BA**, de la Vega D, Ogata K, Tan EM, Nakamura RM. Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. *Arch Pathol Lab Med* 1987; **111**: 841-845 [PMID: 2888448]
- 4 **Jain S**, Filipe MI, Hall PA, Waseem N, Lane DP, Levison DA. Prognostic value of proliferating cell nuclear antigen in gastric carcinoma. *J Clin Pathol* 1991; **44**: 655-659 [PMID: 1679766 DOI: 10.1136/jcp.44.8.655]
- 5 **Woods AL**, Hall PA, Shepherd NA, Hanby AM, Waseem NH, Lane DP, Levison DA. The assessment of proliferating cell nuclear antigen (PCNA) immunostaining in primary gastrointestinal lymphomas and its relationship to histological grade, S+G2+M phase fraction (flow cytometric analysis) and prognosis. *Histopathology* 1991; **19**: 21-27 [PMID: 1680784 DOI: 10.1111/j.1365-2559.1991.tb00890.x]
- 6 **al-Sheneber IF**, Shibata HR, Sampalis J, Jothy S. Prognostic significance of proliferating cell nuclear antigen expression in colorectal cancer. *Cancer* 1993; **71**: 1954-1959 [PMID: 8095176 DOI: 10.1002/1097-0142(19930315)71:6<1954::AID-CNCR2820710605>3.0.CO;2-#]
- 7 **Haratake J**, Takeda S, Kasai T, Nakano S, Tokui N. Predictable factors for estimating prognosis of patients after resection of hepatocellular carcinoma. *Cancer* 1993; **72**: 1178-1183 [PMID: 7687921 DOI: 10.1002/1097-0142(19930815)72:4<1178::AID-CNCR2820720408>3.0.CO;2-Q]
- 8 **Taniai M**, Tomimatsu M, Okuda H, Saito A, Obata H. Immunohistochemical detection of proliferating cell nuclear antigen in hepatocellular carcinoma: relationship to histological grade. *J Gastroenterol Hepatol* 1993; **8**: 420-425 [PMID: 8105998 DOI: 10.1111/j.1440-1746.1993.tb01541.x]
- 9 **Ng IO**, Lai EC, Fan ST, Ng M, Chan AS, So MK. Prognostic significance of proliferating cell nuclear antigen expression in hepatocellular carcinoma. *Cancer* 1994; **73**: 2268-2274 [PMID: 7513246 DOI: 10.1002/1097-0142(19940501)73:9<2268::AID-CNCR2820730906>3.0.CO;2-O]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Effect of amygdalin on proliferation of rat hepatic fat-storing cells and collagen production *in vitro*

Lie-Ming Xu, Cheng Liu, Ping Liu

Lie-Ming Xu, Cheng Liu, Ping Liu, Liver Diseases Research Center, Shanghai Academy of Traditional Chinese Medicine, Shanghai 200032, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: August 25, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the anti-fibrotic mechanism of amygdalin, a component of *Semen Persicae*, on fat-storing cells (FSC).

**METHODS:** Livers of normal adult rats were perfused with Pronase E and collagenase *in situ*. FSC were isolated by centrifugation with 11% Metrizamide. The subcultured FSC were incubated with  $10^{-4}$ - $10^{-8}$  mol/L amygdalin for 72 h, and then FSC proliferation and collagen

production were assayed, respectively.

**RESULTS:** Low doses of amygdalin reduced incorporation of L-[ $^3$ H]-thymidine into FSC and L-[ $^3$ H]-proline into secreted collagenase-sensitive proteins and cell layer-associated collagenase-sensitive proteins. The strongest effects were seen for the  $10^{-8}$  mol/L dose of amygdalin, which inhibited the proliferation of FSC by 25.0%, and decreased collagen production in medium and cell layer by 24.2% and 26.8%, respectively.

**CONCLUSION:** An anti-fibrotic mechanism of amygdalin is to inhibit the proliferation and collagen production of active FSC.

**Key words:** Amygdalin; Pharmacology; Liver; Drug effects; Cirrhosis; Drug therapy; Collagen; Biosynthesis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Xu LM, Liu C, Liu P. Effect of amygdalin on proliferation of rat hepatic fat-storing cells and collagen production *in vitro*. *World J Gastroenterol* 1997; 3(2): 103 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/103.htm>  
DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.103>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Hepatic arterial infusion chemotherapy and embolization in the treatment of primary hepatic carcinoma

Chuan-Sheng Zheng, Gan-Sheng Feng, Ru-Ming Zhou, Bo Liang, Hui-Min Liang, Jun Zhen, Jian-Ming Yu, Hui Liu

Chuan-Sheng Zheng, Gan-Sheng Feng, Ru-Ming Zhou, Bo Liang, Hui-Min Liang, Jun Zhen, Jian-Ming Yu, Hui Liu, Department of Radiology, Union Hospital of Tongji Medical University, Wuhan 430022, Hubei Province, China

Chuan-Sheng Zheng, male, born on August 23, 1966, chief resident of Department of Radiology, having 12 papers published

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Chuan-Sheng Zheng, MD, Department of Radiology, Union Hospital of Tongji Medical University, Wuhan 430022, Hubei Province, China  
Telephone: +86-27-5807711-598

Received: August 25, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the effects of hepatic arterial infusion chemotherapy (HAI) or embolization (HAE) in the treatment of primary hepatic carcinoma (PHC) and the factors influencing these effects.

**METHODS:** A retrospective analysis of 188 patients (166 males and 22 females) with PHC treated with HAI ( $n = 82$ ) or HAE ( $n = 106$ ) was conducted.

**RESULTS:** In the group as a whole, the percentage of patients experiencing therapeutic outcomes was as follows: Symptomatic relief (59.6%); Tumor shrinkage (55%); A decrease in blood alpha fetoprotein (AFP) (37.8%) and overall survival rates at 0.5, 1.0, 2.0, and 3.0 years of 75.4%, 46%, 23.5% and 14.7%, respectively. The mean survival time in the entire group was 12.2 mo, and the longest survival period was 50 mo. In the HAI group the survival rates at 0.5, 1.0, 2.0 and 3.0 years of follow-up were 61.0%, 25.4%, 4.5% and 0%, respectively, with a mean survival time of 7.7 mo. In the HAE group the corresponding survival rates were 86.8%, 61.7%, 37.8% and 26.1%, respectively, with a mean survival time of 15.7 mo. Eighteen patients received secondary surgery. Factors that had a favorable effect on therapeutic outcomes were presence of no or only mild liver cirrhosis, presence of stage I or Stage II disease, presence of only a single tumor, a tumor size less than 10 cm in diameter, an absence cancerous thrombus within the portal vein or hepatic arterio venous fistula, and HAE treatment.

**CONCLUSION:** This study helped identify the effects of HAI and

HAE in patients with PHC, and identified some important factors which influence such treatment.

**Key words:** Liver neoplasms; Chemoembolization; Therapeutic

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Zheng CS, Feng GS, Zhou RM, Liang B, Liang HM, Zhen J, Yu JM, Liu H. Hepatic arterial infusion chemotherapy and embolization in the treatment of primary hepatic carcinoma. *World J Gastroenterol* 1997; 3(2): 104-107 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/104.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.104>

### INTRODUCTION

Treatment options for patients with primary hepatic carcinoma (PHC) include hepatic arterial infusion chemotherapy (HAI) or embolization (HAE). In this study, we investigated outcomes in PHC patients treated with HAI and/or HAE. We also investigated factors that influence the efficacy of HAI and HAI in the treatment PHC patients.

### MATERIALS AND METHODS

#### Patients

Between May 1990 and February 1994, we recruited 388 patients with PHC who were not fit for surgical resection and we instead treated with HAI and HAE. Of these 388 patients, follow-up information was available for 188 patients, and these 188 patients formed the patient group for this study. One hundred and eighty eight patients with PHC were clinically diagnosed. They included 166 males and 22 females. Their ages ranged from 21 to 70 years with a mean age of 47 years. The sizes of the tumors ranged from 4 to 25 cm in diameter with a mean diameter of 11.2 cm. 170 patients had liver cirrhosis. After interventional treatment, 18 patients obtained the chance to receive the secondary surgery, the resected specimens were confirmed to be hepatocellular carcinoma pathologically. At the first visit to a doctor, no patient had indication for surgical operation and they were treated with HAI or HAE.

#### Treatment

After puncturing the femoral artery percutaneously by Seldinger's method, celiac or common hepatic arteriography was initially performed in each patient. After angiography, the catheter was superselectively inserted into the blood supplying artery of the tumor for HAI or HAE (the success rate was 87%). Common chemotherapeutic drugs that were infused included 500 mg of carboplatin (CAP), 1000 mg of 5-Fu and 10-20 mg of mitomycin C (MMC). The emul-

**Table 1 Findings on hepatic arteriography in 188 cases of primary hepatic carcinoma**

Findings	Cases	%
Tumor stain	186	98.9
Tumor vessels	180	95.7
Displacement and distortion of arteries	128	68.1
Pool or lake-like collections	114	60.6
Vascular encasement	106	56.4
Tumor thrombus within portal vein	96	51.1
Extrahepatic metastases	32	17.0
Arteriovenous fistula	24	12.8

**Table 2 Changes in tumor size in the hepatic arterial infusion chemotherapy and hepatic arterial embolization groups**

Group	n	Tumor shrinking rate			No change	Enlargement
		≥ 50%	25%-50%	< 25%		
HAI	80	2	8	59	7	4
HAE	102	78	12	3	5	4

Chi-square test value = 110.1,  $P < 0.01$ . HAI: Hepatic arterial infusion chemotherapy; HAE: Hepatic arterial embolization.

**Table 3 Effects of different methods of treatment**

Methods of treatment	n	Survival rates (%)				Mean survival time (mo)
		6 mo	12 mo	24 mo	36 mo	
HAI	82	61.0	25.4	4.5	0	7.7
HAE	106	86.8 <sup>a</sup>	61.7	37.8	26.1	15.7
HAE-Lp-GS	50	80.0	48.9	31.1	0	11.1
HAE-Lp-BS	56	92.9 <sup>b</sup>	81.9	44.9 <sup>c</sup>	33.6	19.8

Note: <sup>b</sup> $P > 0.05$ , <sup>a,c</sup> $P < 0.05$ , others  $P < 0.01$ . HAI: Hepatic arterial infusion chemotherapy; HAE: Hepatic arterial embolization.

sion of 10-20 mL 40% Lipiodol (Lip) mixed with MMC was used for peripheral angioembolization. Gelatin sponges (GS) or Bletilla Striata (BS) powders (in sizes of 1-2 mm<sup>3</sup>), which were mixed with 5 mL of 60% Meglumini Diatrizoate composita (a contrast medium) before injection, was used for proximal angioembolization. The BS powders were a self prepared embolizing agent<sup>[1]</sup>.

### Follow-up observations

After HAI or HAE treatment, changes in symptoms and signs, hepatic and renal function, and blood alpha fetoprotein (AFP) were observed. Liver ultrasonography (USG), computed tomography (CT), angiography, and other necessary examinations were performed regularly during follow-up.

### Assessment of efficacy

Symptomatic relief was assessed in terms of improvement in appetite and body weight lasting for more than 2 mo. Tumor shrinkage was defined as a reduction in tumor size of more than 25% that lasted for at least two months, as measured by USG, CT, or angiography. A decrease on AFP decrease was defined as a reduction by more than 25%, or a normal AFP level, which lasted more than two months. After treatment, the survival rates at 0.5, 1, 2 and 3 years, as well as the mean survival time, were calculated according to the life table method<sup>[2]</sup>. Factors potentially influencing HAI and HAE treatment effects were assessed with Mann Whitney U test or Chi square test<sup>[3]</sup>.

## RESULTS

Of the 188 patients included in the study, 166 were males and 22 were females. Their ages ranged from 21 to 70 years with a mean age of 47 years. Tumors size ranged from 4 to 25 cm in diameter with a mean diameter of 11.2 cm. A total of 170 patients had liver cirrhosis. After HAI or HAE treatment, 18 patients obtained the chance to receive the secondary surgery, the resected specimens were confirmed to be hepatocellular carcinoma pathologically.

### Appearances of PHC on hepatic arteriography

The findings of the angiography for PHC are shown in Table 1. The

**Table 4 Factors influencing survival rate**

Factors	n	Survival rate (%)			
		6 mo	12 mo	24 mo	36 mo
Clinical stages					
II	112	88	60	33	19
III	68	53	17	0	
Pathological types					
Nodulous	28	83	53	24	19
Massive	128	77	48	27	15
Diffusive	32	56	23	0	
Mass diameter (cm)					
< 5	10	100	100	62	54
5-10	78	85	61	32	18
≥ 10	100	66	29	11	0
Treatment time					
1	58	45	0		
2	48	79	48	23	0
> 2	82	95	78	39	26
Tumor thrombi					
(+)	96	65	28	3	0
(-)	92	87	65	39	23
Liver cirrhosis					
None or mild	109	87	68	39	24
Moderate or severe	79	39	18	4	0

Note: Between the nodulous and the massive type,  $P > 0.05$ . Others,  $P < 0.05$  or 0.01.

majority of PHC cases showed a considerable number of tumor vessels and a substantial degree of tumor stain. The nodulous type of PHC primarily showed increased tumor vessels and stain, while the massive type of PHC was marked by increased tumor vessels, displacement of the arteries, and vascular encasement. The diffusive type of PHC primarily showed tumor stain, pool or lake-like collections of contrast medium, and only rarely showed displacement of the arteries.

### Changes in tumor size after treatment

In the entire patient group, 33.5% had tumor shrinking rates of 75% or more, 44% of the patients had a tumor shrinking rate of 50% or more, 55% of patients had a shrinkage rate of 25% or more, and six patients showed no evidence of In 12 patients, tumor size did not change after treatment, and tumor size increased in eight patients post-treatment. The tumor shrinking rate was significantly higher in the HAE group than in the HAI group (Table 2) ( $P < 0.01$ ).

### Efficacy

After treatment, there was symptomatic relief in 59.6% of the patients) and a decrease in blood AFP in 37.8% of patients. The survival rates at 0.5, 1, 2, and 3 years were 75.4%, 46%, 23.5% and 14.7%, respectively, with a mean survival time of 12.2 mo and a maximum survival period of 50 mo.

The survival rates were higher in the HAE group than those in the HAI group (Table 3). The mean survival time was also longer in the HAE group, of which the HAE-Lp-BS group showed better effects than the HAE-Lp-GS group.

Survival rates according to various factors are shown in Table 4. Survival rates in patients with stage 1 PHC were high. There were no significant differences in survival rates between the nodulous and the massive types of PHC ( $P > 0.05$ ). However, among patients with the nodulous type of PHC, survival rates in patients with multinodulous type PHC were lower compared to survival rates for patients with massive type of PHC ( $P < 0.01$ ).

Additionally, PHC with tumor thrombus within the portal vein or the PHC associated with liver cirrhosis had poor prognosis (Table 4). The factor having the biggest effect on outcomes was treatment method (Figures 1-4).

### Secondary surgical resection

After HAE, 18 patients with obvious tumor shrinkage and favorable physical conditions were eligible for secondary surgical resections. Among them, 15 patients are still alive today. Pathologically, most of the resected specimens were defined clearly, with integrated encapsulation in 12 tumors. In the areas of the tumors, the tumor cells

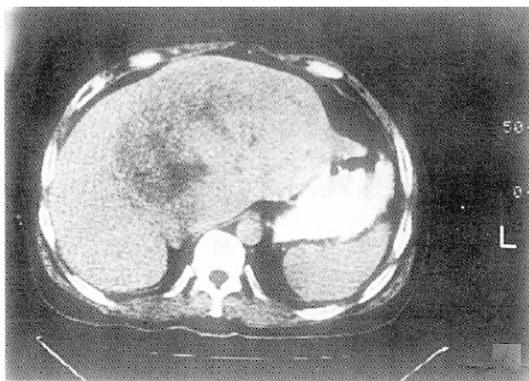


Figure 1 Before treatment, the computed tomography scan of the massive type of primary hepatic carcinoma showing local low density in the right and left hepatic lobar regions with 13 cm × 12 cm in size.

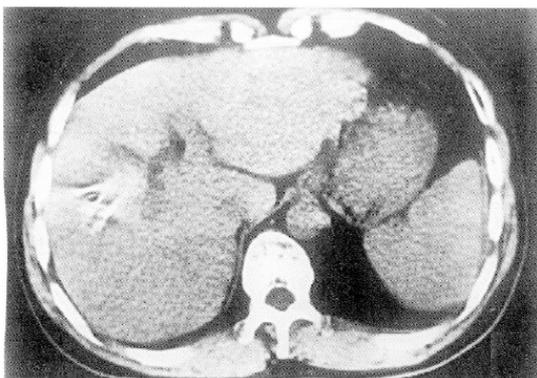


Figure 2 Before treatment, angiography of the patient showing a massive hypervascular tumor with its blood supply from the right and the left hepatic arteries. HAI combined with HAE\*Lp-BS treatment being performed. HAI: Hepatic arterial infusion chemotherapy; HAE: Hepatic arterial embolization.

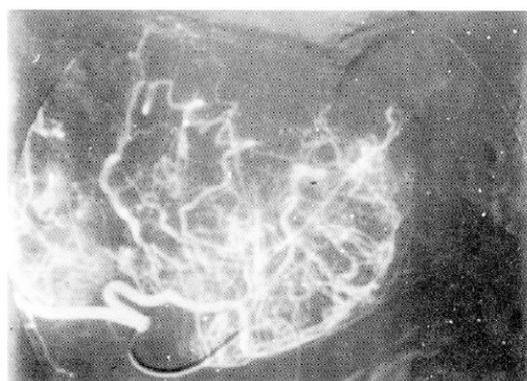


Figure 3 Eight months after treatment with HAI combined with HAE\*Lp-BS, the computed tomography scan of the patient showed obvious tumor shrinking with 5 cm × 2.5 cm in size and complete intratumor retention of lipiodol. HAI: Hepatic arterial infusion chemotherapy; HAE: Hepatic arterial embolization.



Figure 4 Eight months after treatment, angiography of the patient showed obvious tumor shrinking and complete retention of Lipiodol in the massive tumor and in the areas surrounding both small tumor nodules, without tumor vessels and collateral circulation. There was no recanalization of the main trunk of the hepatic artery.

had complete or partial coagulative necrosis, with a few inflammatory cells and some extent of proliferation of fibrous tissue, and some amount of remaining tumor cells at the surrounding tumor sites.

### Complications

After HAI or HAE, common symptoms were nausea, vomiting, liver site pain, and fever. The majority of patients developed fever of 38-39 °C, and a few patients had fever of 39.5-40 °C. The fever lasted 5-20 d and patients recovered in a mean period of two weeks. Other symptoms disappeared gradually, 3-7 d after treatment. Complications include the occurrence of hematoma at the punctured site (5 cases), gastric ulcer (2 cases) and acute failure of liver function (2 cases).

## DISCUSSION

### Efficacy of HAI and HAE

Generally, the natural survival time of patients with PHC is 1-4 mo and the majority of PHC patients may not be fit for surgical resection<sup>[4]</sup>. HAI and HAE may prolong the survival of patients with PHC and make it possible for some patients to become fit for secondary surgical resection.<sup>[5]</sup> The effect of HAI is short, but HAE can produce a more substantial necrosis of tumors, thereby leading to a more favorable outcome. Nishufu and Soge reported that one, two and three years survival rates in 523 PHC patients treated with HAE-Lp-GS were 60.4%, 42.9% and 28%, respectively<sup>[6]</sup>, whereas in the Okuda and Tang's study the corresponding rates for PHC patients treated with HAI were 8.8%-22%, 0.0%-8.9% and 0%, respectively<sup>[7]</sup>.

The reason why the efficacy was better in this study may have been that many patients were in early or intermediate stage PHC (63.8%) and were treated with the superselective embolization of the hepatic segmental arteries. Additionally, in this study, the indications for treatment of HAI and HAE were strict. The following conditions are considered as contraindications. (1) serum AST and/or ALT levels more than twice of the normal upper limit value, with total

serum bilirubin more than 25.7 μmol. (2) serum AST and/or ALT levels greater than four times of the normal upper limit value. (3) Total serum bilirubin levels more than 51.3 μmol. (4) A complete or partial blockage of the, but HAI might be still performed. And (5) the presence of obvious ascites.

### Factors influencing the effects of treatment

We found several factors influenced the effects of treatment. First the prognoses of the single nodulous and the massive types of PHC were better than those of the multi nodulous and the diffusive types. This might be due to the fact that the former two types of PHC had plenty of tumor vessels, encapsulations, less diffusion and obvious tumor necrosis after HAI or HAE. In contrast, the latter two types of PHC were often complicated with serious liver cirrhosis and poor liver function, had tumor thrombus within the portal vein, often had extrahepatic metastasis,, and were not suitable to be treated with HAE<sup>[8]</sup>. Second we also found that stages I and II PHCs had better treatment effects than stage III PHCs, especially with respect to long-term effects. Recently, it was believed that in stage I PHCs, surgical resection might be the first choice of treatment, for the interventional treatment could hardly raise the survival rate of stage I PHC in view of the fact that HAI and HAE could injure the normal liver parenchyma and thereby counteract their anticarcinogenic effects<sup>[7]</sup>. Stages II and III PHCs were considered fit for interventional treatment, but most patients with stage III-PHC were not sufficiently fit for HAE treatment and thus, the therapeutic effects were poor. Third, the effects of PHCs with no or mild liver cirrhosis were better than those with moderate or serious liver cirrhosis. PHC was often complicated with liver cirrhosis, which gradually worsened along with PHC progression. Because serious liver cirrhosis influenced surgical resection, HAI, and HAE, the PHCs with serious liver cirrhosis after any of the treatments only yielded low survival rates. Liver transplantation<sup>[7]</sup> might better suit these patients.

Fourth, tumor thrombus within the portal vein or arterio venous fistula unfavorably influenced the prognosis of PHC. On the one

hand, the tumor thrombus easily produced intrahepatic diffusion and/or extrahepatic metastasis; on the other hand, it blocked the portal vein and produced portal hypertension which in the long run could cause the patient's death from a massive hemorrhage of the upper digestive tract. When the tumor thrombus blocks the portal vein by more than 60%, or a large arterio venous fistula occurs, HAE should not be performed in order to avoid liver failure and pulmonary infarction<sup>[8]</sup>.

Currently, three methods of HAI and HAE are commonly used: (1) single HAI, (2) HAI combined with HAE-Lp or HAE-GS, and (3) HAI combined with HAE-Lp-GS. Prior literature and the results of this study reveal that among the three above-mentioned methods, HAI combined with HAE-Lp-GS yields the best effect in treatment of PHC. This method not only kept a local higher concentration of anti-carcinogen to kill the tumor cells in the tumor area, but also blocked the peripheral and proximal blood supply of the arteries of the tumor to promote its ischemic necrosis<sup>[5]</sup>. In the HAE group in this study, treatment with HAE-Lp-BS in 5-6 patients showed the best therapeutic effects, in which the Bletilla Striata powder, a traditional Chinese medicine, was used. This is a new type of drug prepared by us for intravascular embolization. It has coagulant, antibiotic, and antineoplastic function, which can accomplish a persistent embolization of the main trunk of the arterial blood supply of the tumor, so as to cause long term ischemic necrosis of the tumor and less collateral circulation<sup>[9]</sup>. This prolongs the intermission of the patient's treatment, and lessened symptoms and complications of the patients. As

such, in patients with PHC, treatment with HAE-Lp-BS may be preferable to treatment with HAE-Lp-GS.

## REFERENCES

- 1 **Feng GS**, Yan XQ, Wang LY. Animal experiment and clinical application of Bletilla Striata as an embolizing agent. *Zhonghua Fangshe Yixue Zazhi* 1985; **19**: 193-196
- 2 **Geng GY**. Epidemiology. 2nd Editor. Beijing: The People's Health Publishing House 1984: 203-206
- 3 **Lin QF**, Liu XX, Liang HC. Environmental medical statistics. 1st Editor. Beijing: The People's Health Publishing House 1989: 147-153
- 4 **Bartolozzi C**, Lencioni R, Caramella D, Vignali C, Cioni R, Mazzeo S, Carrai M, Maltinti G, Capria A, Conte PF. Treatment of large HCC: transcatheter arterial chemoembolization combined with percutaneous ethanol injection versus repeated transcatheter arterial chemoembolization. *Radiology* 1995; **197**: 812-818 [PMID: 7480761 DOI: 10.1148/radiology.197.3.7480761]
- 5 **Chang JM**, Tzeng WS, Pan HB, Yang CF, Lai KH. Transcatheter arterial embolization with or without cisplatin treatment of hepatocellular carcinoma. A randomized controlled study. *Cancer* 1994; **74**: 2449-2453 [PMID: 7922999 DOI: 10.1002/1097-0142(19941101)74:9<2449::AID-CNCR2820740910>3.0.CO;2-4]
- 6 **Nishifu U**, Soge Y. Recent status of interventional radiology. *J Clin Surg (in Japanese)* 1987; **42**: 1623-1625
- 7 **Okuda K**, Tang ZY. Neoplasms of the liver. 1st Editor. Tokyo: Springer Pub. Co. 1987: 32-34, 327-329 [DOI: 10.1007/978-4-431-68349-0]
- 8 **Chung JW**, Park JH, Han JK, Choi BI, Han MC, Lee HS, Kim CY. Hepatic tumors: predisposing factors for complications of transcatheter oily chemoembolization. *Radiology* 1996; **198**: 33-40 [PMID: 8539401 DOI: 10.1148/radiology.198.1.8539401]
- 9 **Zheng C**, Feng G, Zhou R. [New use of Bletilla striata as embolizing agent in the intervention treatment of hepatic carcinoma]. *Zhonghua Zhongliu Zazhi* 1996; **18**: 305-307 [PMID: 9387329]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Hepatitis C virus RNA detection in serum and peripheral blood mononuclear cells of patients with hepatitis C

Ping Zhou, Qing Cai, You-Chun Chen, Mu-Sen Zhang, Jian Guan, Xiao-Juan Li

Ping Zhou, Qing Cai, You-Chun Chen, Mu-Sen Zhang, Jian Guan, Xiao-Juan Li, Department of Infectious Diseases, Chinese Air Force General Hospital, Beijing 100036, China

Author contributions: All authors contributed equally to the work.

Presented at International Medical Conference, Shenzhen, China, 6-10 June, 1996

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Ping Zhou, Department of Infectious Diseases, Chinese Air Force General Hospital, Beijing 100036, China  
Telephone: +86-10-68410099-8619

Received: September 25, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To investigate the existence and clinical significance of hepatitis C virus (HCV) RNA in the serum and peripheral blood mononuclear cells (PBMC) of patients with hepatitis C.

**METHODS:** HCV RNA was detected by nested polymerase chain reaction (Nested PCR) in serum and in PBMC of 46 patients with acute hepatitis C (AHC) and in 42 patients with chronic hepatitis C (CHC).

**RESULTS:** The positive rate of HCV RNA in PBMC of patients with CHC was markedly higher than that of patients with AHC ( $P < 0.01$ ). The positive rates of HCV RNA in serum of patients with AHC and CHC and in PBMC of patients with CHC were significantly higher than those of anti-HCV positive patients with normal alanine aminotransferase (ALT) levels ( $P < 0.01$ ). HCV RNA was negative in the serum of two patients, but could be detected in PBMC. In 12 patients, anti HCV was negative while HCV RNA was positive in serum.

**CONCLUSION:** (1) detection of serum HCV RNA by nested PCR might be helpful in the early diagnosis of anti-HCV negative hepatitis C; (2) liver damage in patients with hepatitis C might be correlated with HCV-viremia; (3) infection of PBMC by HCV might play an important role in chronic liver damage in patients with HCV and in the chronicity of its clinical course; and (4) PBMC might be considered as a "reservoir" for HCV.

**Key words:** Hepatitis C; RNA; Viral analysis; Monocytes; Polymerase

chain reaction

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhou P, Cai Q, Chen YC, Zhang MS, Guan J, Li XJ. Hepatitis C virus RNA detection in serum and peripheral blood mononuclear cells of patients with hepatitis C. *World J Gastroenterol* 1997; 3(2): 108-110 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/108.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.108>

### INTRODUCTION

In recent years, detection of hepatitis C virus (HCV) RNA by polymerase chain reaction (PCR) in peripheral blood mononuclear cells (PBMC) of patients with chronic hepatitis C has been reported abroad<sup>[1-3]</sup>. To investigate HCV RNA in PBMC of patients with hepatitis C in our country, 88 cases of acute or chronic hepatitis C have been examined by nested PCR. HCV RNA in serum, anti-HCV, and alanine aminotransferase (ALT) were assayed as well.

### MATERIALS AND METHODS

#### Patients

Eighty eight patients with hepatitis C were selected from December 1992 to June 1995, 46 of them had acute hepatitis C (AHC) (36 with post-transfusive hepatitis C (PTH C) and 10 with sporadic hepatitis C (SHC)) and 42 had chronic hepatitis C (CHC). All of the patients had elevated serum alanine ALT with antibody to HCV or positive HCV RNA in serum. Hepatitis A, B, D, and E virus infection were excluded in all. Eighteen patients had positive anti HCV only, but with normal ALT, and 10 healthy blood donors served as control.

#### Isolation of PBMC and extraction of HCV RNA

PBMC was isolated from 5 mL heparinized venous blood with Ficoll Hypaque density gradient centrifugation and washed 5 times with Hank's balanced salt solution, and then stored at  $-70^{\circ}\text{C}$ . Each specimen each from positive and negative serum HCV RNA was compared with positive and negative PCR amplification. HCV RNA in serum and PBMC was extracted as described by Chomczynski<sup>[4]</sup>.

#### Nested-PCR

Two pairs of oligonucleotide primers deduced from the 5' terminal noncoding region of HCV were synthesized on a 391 DNA synthesizer (Applied Biosystems, United States). Reverse transcription was carried out at  $42^{\circ}\text{C}$  for 45 min in  $10\ \mu\text{L}$  of Tris HCl buffer (50 mmol/L, pH8.4) containing 8 mmol/L  $\text{MgCl}_2$ , 30 mmol/L KCl, 1 mmol/L dithiothreitol, 50 pmol of an antisense primer, 10 mmol/L each of

**Table 1** Detection of serum anti-hepatitis C virus and hepatitis C virus RNA in serum and peripheral blood mononuclear cells of patients with hepatitis C

Groups	Cases	Anti-Hepatitis C virus (+)	Serum Hepatitis C virus RNA (+)	Peripheral blood mononuclear cells-Hepatitis C virus RNA (+)
		cases (%)	cases (%)	cases (%)
Acute hepatitis C	46	36 (78.3)	40 (86.9) <sup>d</sup>	15 (32.6)
Post-transfusive hepatitis C	36	31 (86.1) <sup>f</sup>	32 (88.9)	14 (38.9)
Shronic hepatitis C	10	5 (50.0)	8 (80.0)	1 (10.0)
Chronic hepatitis C	42	40 (95.7) <sup>a</sup>	34 (80.95) <sup>d</sup>	33 (78.57) <sup>bd</sup>
Anti-hepatitis C virus (+) only	18	18 (100)	5 (27.7)	2 (11.1)
Total	106	94 (88.7)	79 (74.5)	50 (47.2)

<sup>a</sup>*P* < 0.05 vs Acute hepatitis C; <sup>b</sup>*P* < 0.01 vs Acute hepatitis C; <sup>d</sup>*P* < 0.01 vs Anti-hepatitis C virus (+) only; <sup>f</sup>*P* < 0.01 vs Shronic hepatitis C.

**Table 2** Relationship of anti-hepatitis C virus and hepatitis C virus RNA in serum and peripheral blood mononuclear cells

Clinical type	Number (%)
Anti-HCV+ (S+, PBMC+)	46 (52.3)
Anti-HCV+ (S-, PBMC-)	12 (13.6)
Anti-HCV+ (S+, PBMC-)	16 (18.2)
Anti-HCV+ (S-, PBMC+)	2 (2.3)
Anti-HCV- (S+, PBMC+)	0
Anti-HCV- (S-, PBMC-)	0
Anti-HCV- (S+, PBMC-)	12 (13.6)

HCV: Hepatitis C virus S: serum; PBMC: Peripheral blood mononuclear cells

the four deoxyribonucleoside triphosphate, 10 units of RNase inhibitor, and 10 units of avian myeloblastosis virus reverse transcriptase. The first stage of PCR was performed for 35 cycles with one pair of outer primer in a DNA thermal cycler (Perkin Elmer Cetus, United States). Each reaction cycle underwent denaturation at 94 °C for 1 min, and primer annealing and extension at 60 °C for 1.5 min. Approximately 1/10 of the products were subjected to a second PCR for 35 cycles using one pair of inner primer with a reaction cycle as described above. The products of the second PCR were mixed with 3.5 µL of sample buffer, separated on 2% agarose gel, stained with ethidium bromide, and observed under ultraviolet light. The length of the second PCR amplified products was 145 bp.

### Other reagents

Second generation anti HCV and HBsAg/anti HBs, HBeAg/anti HBe and anti HBc kits were supplied by the Beijing Si Huan Biological Engineering Products Company. Anti HAV-IgM and anti HEV-IgM kits were supplied by Beijing Ke Wei Clinical Diagnostic Regents Factory, and HDAg, anti HD and anti HD-IgM kits were supplied by Beijing Tian Yuan Biological Medical Scientific and Technological Developing Company.

## RESULTS

### Relationship between clinical classification of infection and anti HCV, HCV RNA in serum and PBMC

Of the 88 patients with acute or chronic hepatitis, 76 (86.4%) were anti-HCV positive, and 74 (84.1%) and 48 (54.6%) were HCV RNA positive in serum and PBMC, respectively (Table 1). The positive rates of anti HCV and HCV RNA in PBMC of CHC patients were much higher than those in AHC patients (*P* < 0.05 and *P* < 0.01, respectively), while there was no significant difference in the positive rate of HCV RNA in serum (*P* > 0.05) (Table 1). The positive rates of HCV RNA in serum of patients with AHC and CHC and in PBMC of patients with CHC were significantly higher than those in anti-HCV positive patients with normal ALT levels (*P* < 0.01). The positive rate of anti HCV in serum of patients infected *via* transfusion was markedly higher than that of sporadic ones (*P* < 0.01). Anti-HCV and HCV RNA in serum and PBMC of 10 adult healthy donors were all negative (Table 1).

### Relationship between anti-HCV in serum and HCV RNA in serum and PBMC

As indicated in Table 2, positive HCV RNA in PBMC occurred only in anti-HCV positive patients. None of the 12 anti-HCV negative patients were found to have HCV RNA in PBMC. Two patients were HCV RNA negative in serum but positive in PBMC.

## DISCUSSION

Some scholars abroad have found that the HCV infection rate of PBMC in patients with CHC was as high as 70%-100%<sup>[1-3]</sup> and the structure and function of PBMC also changed markedly<sup>[5,6]</sup>. These scholars suggested that HCV infection in PBMC might play an important role in the pathogenesis of hepatitis C. In this study, we used nested PCR to detect HCV RNA in PBMC of 46 patients with AHC and 42 patients with CHC. The results showed that the positive rate of HCV RNA in PBMC of patients with CHC was much higher than that of patients with AHC. This suggests that HCV infection in PBMC might be related to the chronicity of hepatitis C. The positive rates of HCV RNA in serum of patients with AHC and CHC and in PBMC of patients with CHC were significantly higher than in patients with positive anti-HCV only. But the positive rate of HCV RNA in PBMC of patients with positive anti-HCV was not significantly different compared with that of patients with AHC. This suggests that liver damage of patients with AHC was associated with HCV RNA viremia. Apart from HCV RNA viremia, liver damage of patients with CHC might also be related to HCV infection in PBMC as well. These observations are consistent with other reports<sup>[7-9]</sup>, suggesting HCV might take part directly in the process of liver damage in acute infection, while immune function disorders might be the major cause of liver lesions in chronic liver disease.

Nested PCR is highly sensitive. A positive finding of HCV RNA in PBMC may be due to contamination from HCV RNA present in serum. To investigate whether this contamination can be eliminated, the following study has been carried out. While tested by PCR, HCV RNA in the fifth time Hank's wash solution of 10 specimens from patients with positive HCV RNA were all negative, indicating that the contamination could be eliminated after washing PBMC for five times. Twenty-eight patients were HCV RNA positive in serum, but negative in PBMC while 2 patients were HCV RNA positive in PBMC, but negative in serum. These results also suggested that the influences caused by HCV RNA in serum could be excluded.

Positivity of HCV RNA in serum is a direct marker of underlying HCV infection and it is important for the diagnosis of hepatitis C. The positive rates of HCV RNA in serum of AHC and CHC were all over 80% with no significant differences between the two. The positive rates of anti-HCV in CHC and acute PTHC patients were markedly higher than those in AHC and acute SHC patients, respectively. Two patients with CHC and 10 with AHC were anti-HCV negative, but positive for HCV RNA in serum. Anti-HCV seems to be a more reliable marker for diagnosing CHC and acute PTHC as compared to AHC and acute SHC. Determination of HCV RNA in serum by nested PCR might be helpful for the early diagnosis of hepatitis C, especially acute SHC and CHC with negative anti-HCV.

In addition, we found that the positivity of HCV RNA in PBMC occurred only in anti HCV positive patients and could occur without positivity of HCV RNA in serum. This result suggests that PBMC might be an extra hepatic site for HCV storage and replication. PBMC up-take of HCV might be either by phagocytosis or direct infection. Investigations of HCV infection in PBMC might be helpful for elucidating the distribution, incubation, and replication site of HCV. It might also be useful for shedding light on, repeated infections, targets for treatment, and criteria for cure. It might also be of importance in the selection of blood donors so as to avoid transmission of HCV infection.

## REFERENCES

- 1 **Willems M**, Peerlinck K, Moshage H, Deleu I, Van den Eynde C, Vermynen J, Yap SH. Hepatitis C virus-RNAs in plasma and in peripheral blood mononuclear cells of hemophiliacs with chronic hepatitis C: evidence for viral replication in peripheral blood mononuclear cells. *J Med Virol* 1994; **42**: 272-278 [PMID: 7516421 DOI: 10.1002/jmv.1890420314]
- 2 **Yun ZB**, Sönnnerborg A, Weiland O. Hepatitis C virus replication in liver and peripheral blood mononuclear cells of interferon-alpha-treated and untreated patients with chronic hepatitis C. *Scand J Gastroenterol* 1994; **29**: 82-86 [PMID: 8128182 DOI: 10.3109/00365529409090442]
- 3 **Saleh MG**, Tibbs CJ, Koskinas J, Pereira LM, Bomford AB, Portmann BC, McFarlane IG, Williams R. Hepatic and extrahepatic hepatitis C virus replication in relation to response to interferon therapy. *Hepatology* 1994; **20**: 1399-1404 [PMID: 7982638 DOI: 10.1002/hep.1840200604]
- 4 **Chomczynski P**, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159 [PMID: 2440339 DOI: 10.1016/0003-2697(87)90021-2]
- 5 **Holzer TJ**, Heynen CA, Kennedy MM, Peterson DA. Altered lymphocyte phenotypes and proliferative responses in chimpanzees infected with hepatitis C virus. *J Med Primatol* 1991; **20**: 295-301 [PMID: 1757972]
- 6 **Castilla A**, Subirá ML, Civeira MP, Prieto J. Correlation between lymphocyte and monocyte function in patients with chronic non-A, non-B hepatitis. *Am J Gastroenterol* 1989; **84**: 978 [PMID: 2502911]
- 7 **Kurosaki M**, Enomoto N, Sato C, Sakamoto N, Hoshino Y, Haritani H, Marumo F. Correlation of plasma hepatitis C virus RNA levels with serum alanine aminotransferase in non-A, non-B chronic liver disease. *J Med Virol* 1993; **39**: 246-250 [PMID: 7682256 DOI: 10.1002/jmv.1890390313]
- 8 **Lau JY**, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, Mizokami M, Neuwald PD, Wilber JC. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet* 1993; **341**: 1501-1504 [PMID: 8099380 DOI: 10.1016/0140-6736(93)90635-T]
- 9 **Jeffers LJ**, Dailey PJ, Goelho-Little E, De Medina M, Scott C, LaRue S. Correlation of HCV RNA quantitation in sera and liver tissue of patients with chronic hepatitis C. *Gastroenterology* 1993; **104**: A923

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Expression and analysis of McAbs antigen against human hepatocellular carcinoma

Li Mi, Zhi-Nan Chen

Li Mi, Zhi-Nan Chen, Department of Pathology, 4<sup>th</sup> Military Medical University, Xi'an 710032, Shaanxi Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: September 20, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To analyze the cause of tumor antigen heterogeneity and solve the problem of targeting diagnosis and therapy.

**METHODS:** Using flow cytometry, the expression of McAbs antigen against human hepatocellular carcinoma (HAb18, E<sub>5</sub>, F<sub>11</sub> and HAb27) was investigated. Analyses of the antigen sites were made quantitatively on the human hepatoma cell lines (SMMC-7721; QGY-7701; BEL-7402; HHCC and 9204). In particular, expression of human hepatoma, its association with antigen HAg18, and its relations with cell cycle in four human hepatoma cell lines using the methods of indirect immunofluorescence and dual-parameter DNA dyeing were studied.

**RESULTS:** The corresponding antigen of McAbs HAb18, HAb27, E<sub>5</sub> were expressed on the five hepatoma cell lines and F<sub>11</sub> was expressed only on the 7721 and 7701 hepatoma cell lines, but their mean fluorescence intensity showed different values on each cell line. HAb18 and HAb27 showed a relatively high level of expression,

while E<sub>5</sub> and F<sub>11</sub> showed a lower level of expression. The value of AI (additivity index) was 136% for HAb18 and E<sub>5</sub>, 108% for HAb18 and HAb27, and 118.6% for E<sub>5</sub> and H27As AI < 30% shows that both antibodies sites are the same, AI > 40% shows that both anti bodies sites are different, so the HAb18, HAb27 or E<sub>5</sub> McAbs were combined in pairs, showing that their antigen sites were different. Furthermore, HAg18 antigen was expressed very highly and the positive rate of HAg18 was 100% in all the four human hepatoma cell lines. The was a mean intensity fluorescence was 8.237 ± 1.168 for SMMC-7721; 5.627 ± 1.678 for QGY-7701; 4.378 ± 1.525 for BEL-7402 is 4.378 ± 1.525 and 7.38 ± 1.919 for HHCC. However, in the normal human liver cell (QZG), HAg18 antigen showed low expression (0.534 ± 0.018) and its positive rate was only 9%. The relationship between human hepatoma associated antigen HAb18 and the cell cycle was expressed at the lowest level in G<sub>0</sub>-G<sub>1</sub> stages, a higher level in S stage and the highest level in G<sub>2</sub>-M stage.

**CONCLUSION:** Analysis of the anti-hepatoma McAbs corresponding to antigen may provide the basis for targeted diagnosis and therapy. The expression heterogeneity of human hepatoma-associated antigen HAg18 is related to the stage of cell cycle in the same cell lines, but not related to the stage of cell cycle in different cell lines.

**Key words:** Liver; Carcinoma, hepatocellular; Antigens, neoplasms; Flow cytometry; Antibodies, monoclonal

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Mi L, Chen ZN. Expression and analysis of corresponding antigen of McAbs against human hepatocellular carcinoma. *World J Gastroenterol* 1997; 3(2): 110 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/110.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.110>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## A prospective study of vertical transmission of hepatitis C virus

De-Gui Sun, Cai-Yun Liu, Zong-Da Meng, Yong-De Sun, Shu-Cong Wang, Yu-Qi Yang, Zheng-Lun Liang, Hui Zhuang

De-Gui Sun, Cai-Yun Liu, Shu-Cong Wang, Yu-Qi Yang, Guan County Sanitary and Antiepidemic Station, Guan County 065500, Hebei Province, China

Zong-Da Meng, Yong-De Sun, Hebei Provincial Sanitary and Antiepidemic Station, Guan County 065500, Hebei Province, China

Zheng-Lun Liang, Hui Zhuang, Beijing Medical University, Beijing 100000, China

De-Gui Sun, male, born on June 1, 1953, in Guan County, Hebei Province, graduated from Langfang Health School, Associate Professor, specialized in the prevention of viral hepatitis, with 27 papers published

Author contributions: All authors contributed equally to the work.

Supported by The Hebei Scientific Foundation, No. 91021701, and the National "8<sup>th</sup> Five Year" Research Project.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. De-Gui Sun, Guan County Sanitary and Antiepidemic Station, Guan County 065500, Hebei Province, China  
Telephone: +86-316-6362937

Received: September 20, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To prospectively study the mechanism of mother to infant transmission of hepatitis C virus (HCV).

**METHODS:** Using a nested PCR for detection of HCV RNA and the second generation ELISA for detection of anti-HCV, 13 pregnant women who suffered from post transfusion hepatitis C (PT-HCV) and their 15 babies were studied to evaluate mother to infant transmission of HCV.

**RESULTS:** The total infection rate of HCV was 86.7% in the babies, including one case of clinical HCV (7.7%), three subclinical cases of HCV (23.1%), and nine inapparent cases of HCV (69.2%). The positive rates of anti-HCV and HCV RNA declined with the age of the babies, to 7.7% for anti-HCV and 15.4% for HCV RNA at the age of three years.

**CONCLUSION:** Babies born to mothers infected with HCV were vertically infected with HCV at a high rate, but the consequences were not serious. Four fetuses born, born through induced labor to mothers positive for anti-HCV and HCV, were all infected by HCV, suggesting that the mother to infant transmission of HCV mainly occurred in the uterus.

**Key words:** Hepatitis C virus; RNA; Viral; Disease transmission; Vertical

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Sun DG, Liu CY, Meng ZD, Sun YD, Wang SC, Yang YQ, Liang ZL, Zhuang H. A prospective study of vertical transmission of hepatitis C virus. *World J Gastroenterol* 1997; 3(2): 111-113 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/111.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.111>

### INTRODUCTION

It is well known that mother to infant transmission of hepatitis B virus (HBV) is the main cause of chronic carrier HBV, and the vertical transmission rate of human immunodeficiency virus (HIV) is also high with grave consequences. However, the existence and extent of vertical transmission of hepatitis C virus (HCV) are still largely unclear. Serological markers (anti-HCV) indicate various rates of vertical transmission of HCV<sup>[1-3]</sup>. Recently, the polymerase chain reaction (PCR) has been developed, which has enabled more accurate and reliable studies of vertical transmission of HCV<sup>[4,5]</sup>. In this study, we prospectively followed 15 infants born to 13 mothers with post transfusion HCV (PT-HCV). The results suggest that the rate of vertical transmission of HCV is high, but that the consequences in the infants are not serious, and that the transmission occurs mainly in the uterus.

### MATERIALS AND METHODS

#### *Mother to infant transmission*

Pregnant women with PT-HCV and their offspring were selected (13 mo and 15 offspring). Mothers were interviewed, and their sera were collected at three, seven and nine months during pregnancy, and at one month after delivery. Sera of infants was collected at the ages of one, three, and six months, and every six months thereafter. Parents provided informed consent for the infants.

#### *Infection in utero*

Five fetuses from five anti-HCV-positive women with induced labor and 97 fetuses from 97 anti-HCV-negative women with induced labor were studied. Liver tissue and sera of the fetuses were collected and frozen.

#### *Serologic tests and gene amplification*

Anti-HCV antibody was tested by second generation ELISA kits provided by YesBiotech Lab (C, Ns3, Ns4, Ns5) and Shenyang Huimin, Co.

**Table 1 Sequences of hepatitis C virus test and genotype primers**

Purpose	Code	Position	n	Direction	Sequences (5'-)	Target geno bp type
Test	F1	5'NC	153	Sense	GGCGACTCCACCATGAATC	
	R1		154	Antisense	GGTGCACGGTCTACGAGACCT324	
	F3		204	Sense	ATGAGTGTCTGTCAGCCTCCA111	
Type	R3	C	203	Antisense	GTTTATCCAAGAAAGGACCCG	
	A		256	Sense	CGCGCGACTAGGAAGACTTC	
	B		186	Antisense	ATGTACCCCATGAGGTGGGC272	
	C		104	Sense	AGGAAGACTTCCGAGCGGTC	
	D1		132	Antisense	TGCTTGGGGATAGGCTGAC57	
	D2		133	Antisense	GAGCCATCCTGCCCTCCCA144	
	D3		134	Antisense	CCAAGAGGGACGGGAACCTC174	
	D4		135	Antisense	ACCCTCGTTCCGTACAGAG123	
					I	
					II	
					III	
					IV	

**Table 2 Temporal sequential results of hepatitis C virus RNA, anti-hepatitis C virus, and alanine aminotransferase of mothers**

Code	Age	Disease onset date	Conception date	Pregnancy				1 month after delivery			
				C	R	A	S	C	R	A	S
1	26	Jun-90	Sep-90	+	+	+	+	+	+	-	+
1-2			Feb-93	+	-	-	-	+	-	-	-
2	29	Oct-88	Nov-91	+	+	+	+	+	+	-	-
2-2			Apr-93	+	+	-	-	+	+	-	-
3	32	Jul-89	Apr-90	+	-	-	-	+	-	-	-
4	25	Jun-90	Jul-90	+	+	+	+	+	+	-	+
5	40	Sep-90	Apr-91	+	+	+	+	+	+	-	-
6	29	May-90	May-91	+	+	-	-	+	+	-	-
7	29	Jul-90	Dec-91	+	+	-	-	+	+	-	-
8	29	May-90	Nov-92	+	+	-	-	+	+	-	-
9	31	Nov-90	Dec-92	+	+	-	-	+	+	-	-
10	31	Aug-91	Jul-93	+	+	+	-	+	+	-	-
11	22	Jun-90	Jul-93	+	+	-	-	+	-	-	-
12	22	Sep-91	Sep-92	+	+	-	+	+	+	-	-
13	27	Mar-89	Oct-92	+	-	-	-	N	N	N	N

C: Anti Hepatitis C virus; R: Hepatitis C virus RNA; A: Alanine aminotransferase; S: with clinical symptoms and signs; N: ND

**Table 3 Serological markers and clinical feature of infants**

Code	Mothers infected with hepatitis C virus during pregnancy			Months from conception to disease onset	Age (months) of infants																				
	C	R	A		1		3		6		9		12		18		24		36						
					C	R	A	C	R	A	C	R	A	C	R	A	C	R	A	C	R	A			
1	+	+	+ <sup>1</sup>	3	+	+	+ <sup>1</sup>	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
1-2	+	-	-	32	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
2	+	+	+ <sup>1</sup>	37	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
2-2	+	+	-	54	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+ <sup>1</sup>	1	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
5	+	+	+ <sup>1</sup>	7	+	+	-	+	+	-	+	+	-	N	N	N	-	+	-	-	-	-	-	-	-
6	+	+	-	12	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
7	+	+	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	-	30	+	+	-	N	N	N	+	+	-	N	N	N	-	-	-	-	-	-	-	-	-
9	+	+	-	25	+	+	-	+	+	-	+	+	-	+	+	-	N	N	N	-	-	-	-	-	-
10	+	+	+	23	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	-	37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	- <sup>1</sup>	12	+	+	+	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	-	-	29	+	+	-	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-	-	-

C: Anti Hepatitis C virus; R: Hepatitis C virus RNA; A: Alanine aminotransferase; N: ND; <sup>1</sup>: Symptom and sign of hepatitis.

RT-nested PCR<sup>[6]</sup> was used for the HCV RNA PCR test, and the primers were from the 5' untranslated region of the HCV genome. For the genotype PCR, the primers were from the core region<sup>[7]</sup> (Table 1).

HBsAg screening kits (RPHA) were provided by the Institute of Beijing Biological Products, and the confirmative reagents were provided by the Beijing Sihuan Factory.

**Definition of hepatitis and HCV infection**

Clinical cases of HCV. were defined as patients who were positive for anti HCV, who had elevated alanine aminotransferase (ALT) level, and who had symptoms and signs of hepatitis. Subclinical HCV cases were defined as patients who were positive both for HCV RNA and anti HCV and who had elevated ALT, but who did not have symptoms and signs of hepatitis. Inapparent cases of HCV were defined as patients positive both for HCV RNA and anti HCV, but who had normal ALT levels. Anti-HCV antibody passive transfer cases were defined as newborns who were positive for anti HCV antibody initially, but subsequently anti HCV negative. Infection with HCV in utero was defined as the detection of HCV RNA in the liver tissue or heart blood of a fetus.

**RESULTS**

**Infection of mothers**

Between 1988 and 1990, we recruited 13 mo aged 22 to 40 years

(mean: 28.6 ± 4.6 years) who suffered from PT-HCV. None of these mothers were infected with HBV. Table 2 shows the mothers' sera markers of HCV. During pregnancy, five cases had clinical HCV, one had subclinical HCV, six had unapparent HCV, and three were only anti HCV positive. All mothers had normal ALT levels at one month after delivery, but two cases still had symptoms and signs of HCV.

**Infection of infants**

Of the 15 infants (5 male, 9 female), seven were followed up for three years, six for one year, and two for nine months. The infection rate of HCV was 86.7% (13/15), and two infants (13.3%) were negative both for anti-HCV antibody and HCV RNA. Among the 13 infected infants, one (7.7%) had clinical HCV, 2 (23.1%) subclinical infection, and 9 (69.2%) inapparent HCV infection (Table 3). Table 4 summarizes the characteristics of anti-HCV antibody and HCV RNA during follow-up. The seroconversion rate was 100% before three months, with some starting to turn negative at six months, and the rate decreasing to 33.3% at 18 mo. Anti-HCV was persistently positive in one infant over 36 mo (16.7%). The detection rates of HCV RNA were the same as that of anti-HCV antibody before 9 mo, but were 66.7% at 18 mo and 33.3% at 36 mo. We did not find cases of anti-HCV antibody passive transfer only, or re-positive cases after negative conversion.

The genotypes of 10 HCV RNA positive infants born to nine HCV RNA positive mothers were all of genotype II.

Table 4 Serological profiles of infants during follow-up

Month(s)	Number	Anti hepatitis C virus antibody +		hepatitis C virus RNA +	
		Number	%	Number	%
1	12 (13)	12 (13)	100.0 (100.0)	12 (13)	100.0 (100.0)
3	9 (11)	9 (11)	100.0 (100.0)	9 (11)	100.0 (100.0)
6	10 (11)	9 (10)	90.0 (90.9)	9 (10)	90.0 (90.9)
9	11 (13)	6 (6)	54.5 (46.2)	5 (5)	45.5 (38.5)
12	12 (13)	3 (3)	25.0 (23.1)	5 (5)	41.7 (38.5)
18	6 (13)	2 (2)	33.3 (15.4)	4 (4)	66.7 (30.8)
24	6 (13)	1 (1)	16.7 (7.7)	2 (2)	33.4 (15.4)
36	6 (13)	1 (1)	16.7 (7.7)	2 (2)	33.3 (15.4)

### Infection of HCV in uterine

Four fetuses from women who underwent induced labor were positive for both anti-HCV antibody and HCV RNA in cord and heart sera, and their liver tissues were HCV RNA positive as well. However, a fetus from a woman who was positive only for anti-HCV was uninfected with HCV, and the sera and liver tissues were all negative for anti-HCV and HCV RNA.

## DISCUSSION

In this prospective study, we used a nested PCR for HCV RNA detection and a second generation ELISA for anti-HCV detection. The results suggest that the vertical transmission rate of HCV is high (86.7%). This vertical transmission can lead to clinical HCV, subclinical HCV, and inapparent infection. These results are consistent with the results reported by Thaler<sup>[4]</sup>. Our results also showed that the types of clinical manifestations and the duration of HCV RNA was related to the conditions of mothers in pregnancy.

Infants born to mothers with clinical HCV had a high rate of clinical HCV. Their HCV RNA and anti-HCV antibody could persist for a long time-as much as three years in 2. Furthermore, the clinical manifestations types of infants were related to the clinical manifestations of their mothers. One baby born to a mother who had acute HCV during pregnancy had clinical HCVs, with persistent viremia up to three years, but three babies whose mothers were had chronic or inapparent HCV during pregnancy showed inapparent infection and their HCV RNA and anti-HCV disappeared in six to nine months.

The results suggest is indicated that mothers with acute hepatitis C transmit HCV to their babies at a high rate.

The grave consequences of vertical transmission of HBV are well known. The rate of vertical transmission of HCV is higher in our study, but mainly (69.2%) resulted in inapparent HCV, the virus was cleared in one year in most cases, and only a few (15.4%) were persistently positive for 3 years. These results suggested the consequence of HCV vertical transmission are not as serious as those associated with vertical HBV transmission. Unlike the transmission of HBV, the transmission of HCV mainly occurs in uterus. (1) The infants were all positive for anti-HCV antibody and HCV RNA at one month after delivery. (2) The detection rates of anti-HCV and HCV RNA decreased with with age. And (3) All of fetuses from For mothers with induced labor who were positive for anti HCV and HCV RNA, all of their fetuses were positive for anti-HCV and HCV RNA in heart sera, and one of those fetuses was HCV RNA positive in liver tissue.

## REFERENCES

- 1 **Kuroki T**, Nishiguchi S, Fukuda K, Shiomi S, Monna T, Murata R, Isshiki G, Hayashi N, Shikata T, Kobayashi K. Mother-to-child transmission of hepatitis C virus. *J Infect Dis* 1991; **164**: 427-429 [PMID: 1649878 DOI: 10.1093/infdis/164.2.427]
- 2 **Reesink HW**, Wong VC, Ip HM, van der Poel CL, van Exel-Oehlers PJ, Lelie PN. Mother-to-infant transmission and hepatitis C virus. *Lancet* 1990; **335**: 1216-1217 [PMID: 1971054 DOI: 10.1016/0140-6736(90)92734-Y]
- 3 **Chen DS**, Lin HH, Chang MH. Mother to child transmission of hepatitis C virus: reply. *J Infect Dis* 1991; **164**: 428-431 [DOI: 10.1093/infdis/164.2.428]
- 4 **Thaler MM**, Park CK, Landers DV, Wara DW, Houghton M, Veereman-Wauters G, Sweet RL, Han JH. Vertical transmission of hepatitis C virus. *Lancet* 1991; **338**: 17-18 [PMID: 1676085 DOI: 10.1016/0140-6736(91)90006-B]
- 5 **Novati R**, Thiers V, Monforte AD, Maisonneuve P, Principi N, Conti M, Lazzarin A, Brechot C. Mother-to-child transmission of hepatitis C virus detected by nested polymerase chain reaction. *J Infect Dis* 1992; **165**: 720-723 [PMID: 1313070 DOI: 10.1093/infdis/165.4.720]
- 6 **Garson JA**, Tedder RS, Briggs M, Tuke P, Glazebrook JA, Trute A, Parker D, Barbara JA, Contreras M, Aloysius S. Detection of hepatitis C viral sequences in blood donations by "nested" polymerase chain reaction and prediction of infectivity. *Lancet* 1990; **335**: 1419-1422 [PMID: 1972209 DOI: 10.1016/0140-6736(90)91446-H]
- 7 **Okamoto H**, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol* 1992; **73** (Pt 3): 673-679 [PMID: 1312125 DOI: 10.1099/0022-1317-73-3-673]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Study on clinical pathology and immunohistochemistry of chronic erosive gastritis

Chang-Shu Ke, Dao-Fen Li, Wei Wang, Yi-Ming Wang

Chang-Shu Ke, Dao-Fen Li, Wei Wang, Yi-Ming Wang, Department of Pathology and Gastroenterology, Wuhan General Hospital of Chinese PLA, Guangzhou Command Area, Wuhan 430070, Hubei Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: November 10, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the clinical pathology of chronic erosive gastritis (CEG) and determine the expression of epithelial tumor markers, oncoprotein p21 and carcinoembryonic antigen (CEA), and G cells by immunohistochemistry.

**METHODS:** Gastric mucosal biopsies from 40 CEG cases were examined. Histopathology and *Helicobacter pylori* (*Hp*) infection were determined by light microscopy. Thirty-one biopsies from CEG cases were immunostained with antibodies against p21, CEA, proliferation cell nuclear antigen (PCNA), and gastrin using the labeled streptavidin-biotin (LSAB) method.

**RESULTS:** A total of 35/40 (87.5%) CEG lesions showed antral location; 75% of the lesions were associated with different degrees of atrophic change. Twenty percent presented with mild and moderate atypia of mucosal epithelia and 27.5% showed intestinal metaplasia. Acute inflammatory changes were observed in 25% of the cases. *Hp* was identified in 40.0% of the specimens. Immunohistochemistry studies showed that 67.7% of the CEG mucosal epithelial samples expressed oncoprotein p21 and 29.0% expressed CEA, rates significantly higher than those observed in control samples from a chronic superficial gastritis group. However, PCNA and gastrin expression in mucosal G cells was not significantly different between CEG samples and samples from the control group ( $P > 0.05$ ).

**CONCLUSION:** CEG is a chronic gastric mucosal proliferative lesion that expresses higher levels of p21 and CEA than control samples. Our observations suggest that antral location of the lesion and *Hp* infection do not participate in the pathological process of CEG.

**Key words:** Gastritis/pathology; Chronic disease

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Ke CS, Li DF, Wang W, Wang YM. Study on clinical pathology and immunohistochemistry of chronic erosive gastritis. *World J Gastroenterol* 1997; 3(2): 113 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/113.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.113>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Preparation and application of monoclonal antibodies against hepatitis C virus nonstructural proteins

Jian-En Gao, Qi-Min Tao, Jian-Ping Guo, He-Ping Ji, Zheng-Wei Lang, Ying Ji, Bai-Fang Feng

Jian-En Gao, Qi-Min Tao, Jian-Ping Guo, He-Ping Ji, Zheng-Wei Lang, Ying Ji, Bai-Fang Feng, Hepatology Institute of People's Hospital of Beijing Medical University, Beijing 100044, China

Jian-En Gao, male, born on July 1<sup>st</sup>, 1964 in Beijing, graduated from Department of Biology, Beijing University in 1990, Assistant Researcher, engaged in the molecular biology study on viral hepatitis, having 4 papers published.

Author contributions: All authors contributed equally to the work.

Supported by The Eighth Five year Plan of Ministry of Health, No. 859160103.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Jian-En Gao, Hepatology Institute of People's Hospital of Beijing Medical University, Beijing 100044, China.  
Telephone: +86-10-68314422-5726

Received: November 10, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To prepare hybridoma cell lines that secrete monoclonal antibodies against hepatitis C virus (HCV) recombinant proteins NS3 and NS5 and to evaluate their use in the study of HCV NS3 and NS5 antigen distribution in human liver tissue.

**METHODS:** Hybridoma cell lines were generated using spleen cells from BALB/C mice immunized with recombinant NS3 and NS5 proteins, following conventional protocols. Antibody-secreting cells were screened by solid phase ELISA and cloned by limited dilution. The specificity of the monoclonal antibodies was determined by testing hybridoma culture supernatants by Western blots of *E. coli* expressing the recombinant HCV proteins and ELISA with HCV core and hepatitis B virus (HBV) antigens. The monoclonal antibodies were employed in immunohistochemistry studies to determine the distribution of HCV NS5 and NS3 antigens in 51 paraffin embedded human liver tissue samples.

**RESULTS:** Eight hybridoma cell lines secreting monoclonal antibodies against HCV NS3 and NS5 proteins were generated and named 2B6, 2F3, 3D8, 3D9, 8B2, 6F11, 4C6 and 7D9. Only one of them, 2B6 (secreting antibodies against NS3 protein), cross-reacted with the C7 polypeptide, a different recombinant NS3 polypeptide. The rest of the cell lines showed no cross-reactivity with HCV core or HBV antigens. In addition, monoclonal antibodies against NS3 antigens did not cross-react with NS5 antigens, and vice versa. In immuno-

histochemistry studies, these monoclonal antibodies did not detect HCV antigens in specimens from patients infected only with HBV ( $n = 20$ ). In HCV-infected specimens ( $n = 31$ ), the rates of positive detection of NS3 and NS5 antigens were 51.6% (16/31) and 54.9% (17/31), respectively. Six of these 31 specimens were from patients infected only with HCV and half of them were positive for HCV NS3 and NS5 antigens. In specimens from patients co-infected with HBV and HCV ( $n = 25$ ), the rates of NS3 and NS5 antigen positive detection were 52% (13/25) and 56% (14/25), respectively, which are similar to those obtained in samples from patients infected only with HCV. In specimens from chronic active cirrhosis patients, the rates of HCV NS3 and NS5 antigen detection were 70.6% (12/17) and 76.5% (13/17), respectively.

**CONCLUSION:** We successfully prepared monoclonal antibodies that are specific against recombinant HCV NS3 and NS5 proteins and could be useful for clinical immunohistochemistry diagnosis.

**Key words:** Hepatitis C virus; Antibodies; Monoclonal; Viral proteins; Antigens; Viral

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Gao JE, Tao QM, Guo JP, Ji HP, Lang ZW, Ji Y, Feng BF. Preparation and application of monoclonal antibodies against hepatitis C virus nonstructural proteins. *World J Gastroenterol* 1997; 3(2): 114-116 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/114.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.114>

### INTRODUCTION

Hepatitis C virus (HCV) is a major causative agent of non-A, non-B hepatitis that is associated with the development of cirrhosis and hepatocellular carcinoma (HCC)<sup>[1]</sup>. Although the mechanism of liver injury by HCV infection is still unknown, replication of HCV plays a key role. The diagnosis of HCV infection and the assessment of the effectiveness of interferon (IFN) therapy for its treatment are mainly performed by serological assays. These assays include the detection of anti-HCV IgG antibodies in serum and of HCV RNA by RT-PCR, which can only reflect the presence of actively replicating virus<sup>[2]</sup>. Tsutsumi *et al*<sup>[3]</sup> (1994) reported that although HCV RNA in serum was negative after IFN therapy, HCV NS5 antigen was still present in liver cells from IFN-treated patients.

NS3 and NS5 are important genes involved in virus replication and assembly. NS3 encodes a putative helicase and NS5 encodes a putative RNA-dependent RNA polymerase<sup>[4]</sup>. Monoclonal antibodies against these two proteins would be useful for the determination of HCV state

**Table 1** Immunochemistry analysis of liver disease samples (*n* = 51)

Diagnosis	HCV-infected					Co-infected with HCV and HBV					HBV-infected				
	No.	HBsAg	HBcAg	NS3Ag	NS5Ag	No.	HBsAg	HBcAg	NS3Ag	NS5Ag	No.	HBsAg	HBcAg	NS3Ag	NS5Ag
CAC	3	0	0	2	2	14	13	8	10	11	9	9	6	0	0
SAFH	1	0	0	0	0	1	0	0	0	0	2	1	1	0	0
CFH	2	0	0	1	1	5	4	3	2	2	5	4	2	0	0
HCC	0	0	0	0	0	5	3	1	1	1	4	3	1	0	0
Total	6	0	0	3	3	25	20	12	13	14	20	17	10	0	0

HCV: Hepatitis C virus; HBV: Hepatitis B virus; CAC: Chronic active cirrhosis; SAFH: Sub-acute fulminant hepatitis; CFH: Chronic fulminant hepatitis; HCC: Hepatocellular carcinoma.

**Table 2** Anti-NS3 monoclonal antibody cross-reactivity with other antigens

Monoclonal Antibodie	CP9	CP10	C11	C7	HBsAg	NS3Ag
2B6	0.075	0.081	0.124	1.591	0.079	2.378
2F3	0.074	0.085	0.096	0.089	0.161	2.233
3D8	0.091	0.082	0.086	0.078	0.136	2.139
3D9	0.076	0.081	0.081	0.079	0.113	2.350

**Table 3** Anti-NS3 epitope analysis

Monoclonal Antibodie	Inhibition rate (%)			
	2B6	2F3	3D8	3D9
2B6	100.0	0	0	0
2F3	0	100.0	97.9	101.9
3D8	30.0	103.6	100.0	110.0
3D9	36.0	93.5	93.0	0

in liver cells and for immunochemistry studies on type C hepatitis pathogenesis. In addition, they will greatly help in the development of methods to determine the success of IFN therapy. Therefore, we generated monoclonal antibodies against recombinant HCV NS3 and NS5 proteins and tested them in immunohistochemistry studies using paraffin embedded liver tissue sections from HCV-positive patients.

## MATERIALS AND METHODS

### Patients

Fifty-one autopsy specimens of human liver from patients with type C and type B hepatitis were obtained. Among them, 31 were HCV-positive and 20 were hepatitis B virus-positive (HBV-positive). Twenty-six of these cases had active cirrhosis, four showed sub-acute fulminant hepatitis, 12 had chronic fulminant hepatitis and nine showed hepatocarcinoma (Table 1). All specimens were stained with hematoxylin-eosin, HBV antigens (HBsAg and HBcAg), and HCV antigens (NS3Ag and NS5Ag), separately. Three liver biopsies from non-liver disease patients served as controls. In order to confirm specificity we replaced first antibody with mouse serum, human serum, or PBS in control assays.

### Monoclonal antibody preparation

BALB/c mice were immunized 3 times at a 2-wk interval with recombinant NS3 (30 kDa) and NS5 (27 kDa) polypeptides expressed in *E. coli*. Three days after the last immunization, spleen cells were obtained and fused with Sp2/0 myeloma cells, following conventional methods for the generation of monoclonal antibodies (McAbs). Antibody-secreting cells were screened by solid phase ELISA and cloned by limited dilution<sup>[5]</sup>.

### Monoclonal antibody characterization

The immunoglobulin subclasses of McAbs were determined following conventional methods. The specificity of McAbs was established by testing cross-reactivity of culture supernatants from hybridoma cells in Western blots of membranes containing HBsAg, CP9, CP10, C7, and C11 antigens<sup>[6]</sup>. McAb epitopes were analyzed by an inhibition assay.

## RESULTS

The fusion rates after cell fusion were 100%; 30%-40% of hybridoma cells were antibody-secreting. Four hybridoma cell lines secreting antibodies against NS3Ag (2B6, 2F3, 3D8, and 3D9) and four

**Table 4** Anti-NS5 monoclonal antibody cross-reactivity with other antigens

Monoclonal Antibodie	CP9	CP10	C11	C7	HBsAg	NS3Ag	NS5Ag
8B2	0.065	0.054	0.078	0.088	0.098	0.098	2.325
6F11	0.055	0.053	0.076	0.078	0.097	0.088	1.987
4C6	0.065	0.067	0.078	0.090	0.085	0.102	2.165
7D9	0.070	0.072	0.085	0.092	0.070	0.103	2.100

hybridoma cell lines secreting antibodies against NS5Ag (8B2, 6F11, 4C6, and 7D9) were cloned and further characterized. Hybridoma cell line 2B6 was the only cell line that cross-reacted with the C7 polypeptide, a different recombinant NS3 polypeptide, the rest of the hybridoma cell lines showed no cross-reactivity with other antigens (Table 2).

Anti-NS3Ag McAbs reacted against two different NS3 epitopes (Table 3); anti-NS5Ag McAbs reacted against the same NS5 epitope (Table 4).

The McAbs generated against HCV NS3Ag and NS5Ag were tested by Western blot using membranes prepared by SDS-PAGE of *E. coli* expressing recombinant NS3, recombinant NS5, and non-recombinant plasmid. A single band with a molecular weight of 30 kDa was observed for recombinant NS3 plasmid, whereas two bands with molecular weights of 27 kDa and 23.5 kDa were observed in the *E. coli* expressing recombinant NS5 plasmid.

Using these McAbs in immunohistochemistry studies, HCV NS3Ag and NS5Ag were visualized only in liver sections from patients positive for HCV but not in sections from patients with only HBV infection (Table 1). We identified three types of staining pattern in hepatocytes: Diffuse, clustered, and patchy. In samples from HCV-infected patients, the rates of positive HCV NS3Ag and NS5Ag were 51.6% (16/31) and 54.9% (17/31), respectively. In samples from patients infected only with HCV, 50% (3/6) were positive for NS3Ag and NS5Ag. Out of the 25 specimens from patients co-infected with HCV and HBV, 13 specimens were positive for NS3Ag and 14 specimens were positive for NS5Ag. The rate of samples positive for HCV antigens was not significantly different between these two groups ( $P > 0.1$ ).

## DISCUSSION

NS3 and NS5 are important HCV genes encoding non-structural proteins of the virus (a putative helicase and an RNA-dependent RNA polymerase, respectively)<sup>[4]</sup>. Since HCV is a single positive stranded RNA virus distantly related to flaviviruses<sup>[7]</sup>, a helicase and an RNA-dependent RNA polymerase may be necessary for HCV replication. The presence of HCV NS3Ag and NS5Ag in liver tissue from patients may reflect the replication state of the virus. In this study, we used recombinant NS3 and NS5 polypeptides (with molecular weights of 30 kDa and 27 kDa, respectively) expressed in *E. coli* to immunize mice and generate several strains of McAb-secreting hybridoma cells. These McAbs were proved stable by *in vitro* transmission for three months.

By Western blot analysis, we demonstrated that the anti-HCV NS3 and anti-HCV NS5 McAbs were specific against the antigens they were raised against. The detection of two bands with molecular weights of 27 kDa and 23.5 kDa in Western blots of NS5-expressing bacteria may indicate the presence of a partially degraded polypeptide in addition to the full-length one.

Epitope analysis of anti-NS3 McAbs identified at least two different epitopes for this molecule, one of them being the same as for C7, which is a different NS3 recombinant polypeptide.

By immunohistochemistry studies, we observed nuclear staining of NS5Ag in one case. As this observation differs from the results of

Tsutsumi *et al.*<sup>[3]</sup>, further analyses are needed. The rate of samples positive for HCV antigen was similar between samples from patients infected only with HCV and samples from patients co-infected with HCV and HBV, indicating that the McAbs we generated are specific to HCV antigens. The rate of samples positive for HBV antigens is higher than that of HCV antigens. This observation may be explained by the fact that HBV antigens (HBsAg and HBcAg) reflect the presence of the virus, whereas HCV antigens (NS3Ag and NS5Ag) reflect the replication state of the virus.

In conclusion, we have generated McAbs that are specific against HCV NS3Ag and NS5Ag and have good prospects for application in HCV immunochemistry studies. As the presence of these two antigens reflects the replication state of HCV, they may become useful markers for HCV-infection diagnosis and treatment.

## REFERENCES

- 1 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562 DOI: 10.1126/science.2523562]
- 2 Miyamura T, Saito I, Katayama T, Kikuchi S, Tateda A, Houghton M, Choo QL, Kuo G. Detection of antibody against antigen expressed by molecularly cloned hepatitis C virus cDNA: application to diagnosis and blood screening for posttransfusion hepatitis. *Proc Natl Acad Sci United States* 1990; **87**: 983-987 [PMID: 2105505 DOI: 10.1073/pnas.87.3.983]
- 3 Tsutsumi M, Urashima S, Takada A, Date T, Tanaka Y. Detection of antigens related to hepatitis C virus RNA encoding the NS5 region in the livers of patients with chronic type C hepatitis. *Hepatology* 1994; **19**: 265-272 [PMID: 7507461 DOI: 10.1002/hep.1840190202]
- 4 Chung RT, Kaplan LM. Isolation and characterization of an HCV-specific RNA-dependent RNA polymerase activity from extracts of infected liver tissue. First Annual Meeting, Venice, 1992 July, Abstract A 15, P21
- 5 John GR, Hurrell (Editor). Monoclonal hybridoma antibodies: techniques and applications. CRC Press Inc. 1982: 1-57
- 6 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. Cold Spring Harbor La 1989
- 7 Miller RH, Purcell RH. Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups. *Proc Natl Acad Sci United States* 1990; **87**: 2057-2061 [PMID: 2156259 DOI: 10.1073/pnas.87.6.2057]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Clinical and experimental study on therapeutic effect of Weixibaonizhuanwan on gastric precancerous lesions

Xu-Chen Zhang, Rui-Feng Gao, Bing-Qing Li, Lian-Sheng Ma, Li-Xin Mei, Yu Zhen Wu, Feng Qin Liu, Zhen Lin Liao

Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College

Rui-Feng Gao, Bing-Qing Li, Yu Zhen Wu, Feng Qin Liu, Zhen Lin Liao, Department of Gastroenterology, Affiliated Hospital of Chengde Medical College, Chengde 067000, Hebei Province, China

Lian-Sheng Ma, Research Center of Gastroenterology of Taiyuan, Taiyuan 030001, Shanxi Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: August 8, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the therapeutic effect of Weixibaonizhuanwan on gastric precancerous lesions.

**METHODS:** Thirty-six patients with gastric precancerous lesions were treated with Weixibaonizhuanwan for 3 mo. Thirteen (36.1%) patients presented with mild atrophic gastritis, 14 (38.9%) with moderate atrophic gastritis, and nine (25.0%) with severe atrophic gastritis. Twenty-two (61.1%) and 27 (75.0%) of the cases were accompanied by intestinal metaplasia (IM) and dysplasia (DYS), respectively. Twenty of the 36 patients were men and 16 were women, ranging from 30 to 67 years in age, with 61.1% of the patients being 40-59 years old. The duration of the disease in these patients ranged from 3 mo to 21 years, with 20 (55.6%) patients experiencing durations of the disease between 5 and 10 years. The clinical manifestations of the disease in these patients included fullness of the abdomen (31 cases), abdominalgia (27 cases), anorexia (30 cases), eructation (26 cases), pantothenic acid (6 cases), and loose stool (9 cases). Patients were treated with Weixibaonizhuanwan and symptom improvement, level of atrophy of the gastric mucosa, and IM and DYS progression were analyzed.

**RESULTS:** After a 3-mo treatment with Weixibaonizhuanwan, seven patients experienced recovery. The treatment was effective in 11 cases, improved symptoms in 13 cases, and was ineffective in five

cases. The overall efficacy rate was 86.1%. In patients with mild atrophic gastritis ( $n = 13$ ), 11 improved into superficial gastritis and two experienced no improvement. In 14 cases of moderate gastritis, four cases improved into superficial gastritis and seven turned into mild atrophic gastritis, with three patients experiencing no improvement. Among severe atrophic gastritis patients ( $n = 9$ ), five improved into moderate atrophic gastritis after treatment and four experienced no improvement. The overall efficacy rate in chronic atrophic gastritis patients was 77.8%. Among 9 patients with IM, IM disappeared in six cases, whereas three cases showed no improvement after treatment. In cases with moderate IM ( $n = 10$ ), IM disappeared in two, turned into mild IM in five, and showed no change in three. Out of four cases with IM, one case turned into moderate IM and three showed no change. The overall efficacy rate in IM patients was 63.6%. Out of 16 cases of mild DYS, DYS disappeared in 11, whereas five cases showed no change. Out of nine cases of moderate DYS, DYS disappeared in two and turned into mild DYS in five cases, with two patients experiencing no change after treatment. No improvement was observed in the two cases of severe DYS after treatment. The overall efficacy rate in DYS patients was 66.7%. After treatment, expression of carcinoembryonic antigen (CEA) and proliferating cell nuclear antigen (PCNA) in gastric mucosa significantly decreased ( $P < 0.01$ ). Before treatment, cancer staging of these patients by positive CEA expression was I, II, III, and IV in 13, 12, 9, and 2 cases, respectively. After treatment, the number of cases per stage changed to 25, 7, 3, and 1, respectively. Similarly, before treatment, staging by positivity of PCNA expression was I, II, III, and IV in 16, 11, 10, and 4 cases, respectively, and changed to 21, 9, 5, and 1, respectively, after treatment.

**CONCLUSION:** The use of Weixibaonizhuanwan in the treatment of gastric precancerous lesions showed promising therapeutic effects in patients after 3-mo treatments.

**Key words:** Weixibaonizhuanwan; Stomach neoplasms/TCM therapy; Precancerous conditions; Gastric mucosa; Chinese herbal

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Zhang XC, Gao RF, Li BQ, Ma LS, Mei LX, Wu YZ, Liu FQ, Liao ZL. Clinical and experimental study on therapeutic effect of Weixibaonizhuanwan on gastric precancerous lesions. *World J Gastroenterol* 1997; 3(2): 116 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/116.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.116>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Expression of insulin-like growth factor II and its receptor in liver cells from patients with chronic liver diseases

Dong-Hua Yang, Chong Xiu, Bo Yang, Jian-Ren Gu, Lian-Fang Qian, Shu-Ming Qu

Dong-Hua Yang, Chong Xiu, Bo Yang, Department of Gastroenterology, Zhujiang Hospital, The First Military Medical University, Guangzhou 510282, Guangdong Province, China

Jian-Ren Gu, Lian-Fang Qian, Shu-Ming Qu, National Research Center for Oncogenes and Related Genes, Shanghai Cancer Institute, Shanghai 200032, China

Dong-Hua Yang, Professor of Internal Medicine, MD, Director of Department of Gastroenterology, Zhujiang Hospital, The First Military Medical University, having 45 papers and 2 books published

Author contributions: All authors contributed equally to the work.

Supported by The National Science Foundation of China, No. 39470774.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dong-Hua Yang, MD, Professor, Department of Gastroenterology, Zhujiang Hospital, The First Military Medical University, Guangzhou 510282, Guangdong Province, China  
Telephone: +86-20-84339888-88174  
Telephone: +86-21-4047029

Received: August 8, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the relationship between insulin-like growth factor II (IGF-II), IGF-II receptor, and chronic liver diseases and to investigate the clinical mechanisms of human hepatocellular carcinoma (HCC) development.

**METHODS:** We analyzed IGF-II and IGF-II receptor poly (A)+ mRNA in dysplasia liver cell (DLC;  $n = 10$ ), liver cirrhosis (LC;  $n = 9$ ), and chronic active hepatitis (CAH;  $n = 9$ ) specimens by Northern blot using human IGF-II and IGF-II receptor DNA probes labeled with  $^{32}\text{P}$  through nick translation.

**RESULTS:** Expression of IGF-II in DLC samples (10/10, 100%) was higher than in CAH (3/9, 33%) and LC samples (3/9, 33%) ( $P < 0.01$ ). Expression of IGF-II receptor in DLC samples (7/10, 70%) was significantly higher than in CAH (2/9, 22%) and LC samples (3/9, 33%). Data on hepatitis B virus (HBV) infection status from different chronic liver disease samples were also analyzed.

**CONCLUSION:** Overexpression of IGF-II and IGF-II receptor in DLC samples was associated with a preceding step to malignant phe-

notype hepatocyte transformation and may be of diagnostic value for early detection of hepatocellular carcinoma (HCC). Persistent HBV infection was strongly associated with abnormal IGF-II and IGF-II receptor mRNA expression, suggesting that an autocrine or paracrine mechanism is involved in the regulation of growth in liver cell carcinogenesis.

**Key words:** Insulin like growth factor II; Receptors; Carcinoma; Hepatocellular; Hepatitis; Liver neoplasms; Liver cirrhosis; Liver diseases

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Yang DH, Xiu C, Yang B, Gu JR, Qian LF, Qu SM. Expression of insulin-like growth factor II and its receptor in liver cells of chronic liver diseases. *World J Gastroenterol* 1997; 3(2): 117-118 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/117.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.117>

### INTRODUCTION

The role of insulin-like growth factor II (IGF-II) and IGF-II receptor in liver cell carcinoma progression was investigated by examining the transcriptional expression of IGF-II and IGF-II receptor in chronic active hepatitis (CAH), liver cirrhosis (LC), and dysplasia liver cell (DLC) samples by Northern blot. In addition, data on hepatitis B virus (HBV) infection status from the different groups of chronic liver disease samples were analyzed. Our results suggest that detection of IGF-II and IGF-II receptor mRNA expression may be useful for early diagnosis and in gene therapy studies of hepatocellular carcinoma (HCC).

### MATERIALS AND METHODS

#### Tissue specimens

Specimens from 10 patients with DLC (7 men and 3 women; mean age: 45 years) were surgically obtained. Specimens from 9 patients with CAH (5 men and 4 women; mean age: 38 years) and from 9 cases with LC (8 men and 1 woman; mean age: 42 years) were obtained by liver tissue biopsy. Normal human liver specimens were obtained from two accidental death cases. Specimens were immediately frozen and stored at  $-80\text{ }^{\circ}\text{C}$  until analysis.

#### mRNA extraction and DNA probe preparation

Recombinant IGF-II and recombinant IGF-II receptor plasmid DNA were generated as previously described and digested with restriction endonucleases *Pst* I and *Eco*R I to isolate IGF-II (0.7 kb) and IGF-II receptor (5.1 kb) DNA fragments<sup>[1]</sup>. These fragments were

**Table 1** Hepatitis B virus markers in serum from different liver disease cases

Groups	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
Dysplasia liver cell	10	0	0	2	8
Chronic active hepatitis	9	1	1	1	6
Liver cirrhosis	9	0	1	1	7
Total	28	1	2	4	21

labeled with  $\alpha$ -<sup>32</sup>P-dATP by nick translation. The specific activities of the labeled IGF-II and IGF-II receptor DNA probes were  $4.2 \times 10^7$  cpm/ $\mu$ g and  $5.6 \times 10^7$  cpm/ $\mu$ g, respectively. mRNA extracted from the different liver disease specimens was loaded (10  $\mu$ g) into 1% agarose gels, electrophoresed, and blotted into membranes. Membranes were hybridized with radiolabeled probes and autoradiographed after 48 h at -70 °C.

**Detection of HBV infection markers**

HBV infection markers in serum from the three groups of chronic liver disease cases were examined by ELISA.

**RESULTS**

**IGF-II expression**

IGF-II was differentially expressed at the mRNA level in specimens from the three different groups. In CAH, 2 cases showed moderate and 1 case showed mild IGF-II mRNA expression (3/9, 33%); in LC cases, 3/9 (33%) showed moderate IGF-II mRNA expression; in DLC, 6 cases presented high and 4 cases showed moderate IGF-II mRNA expression (10/10, 100%). In 1 of the samples from normal liver tissue a mild signal of IGF-II mRNA hybridization could be detected.

**IGF-II receptor expression**

IGF-II receptor mRNA level was moderate in 2/9 (22%) CAH cases. In LC, 2 cases showed moderate and 1 case showed mild IGF-II receptor mRNA expression (3/9, 33%) and in DLC cases, high expression was observed in 5 cases and mild expression in 2 cases (7/10, 70%). No distinct signal of IGF-II receptor mRNA hybridization was detected in normal liver tissue samples.

**HBV infection status**

We analyzed HBV infection status of the different chronic liver disease cases by detection of HBV markers in serum (Table 1).

**DISCUSSION**

Overexpression of IGF-II and IGF-II receptor mRNA was observed in human HCC cancer cells and surrounding liver tissue, suggesting that liver cancer cells might produce IGF-II factors in large amounts and, through an autocrine or paracrine mechanism, stimulate IGF-II receptor expression in cancer cells or neighboring liver cells, therefore accelerating or amplifying the growth of liver cancer cells<sup>[2-4]</sup>.

Expression of a fetal isoform of IGF-II mRNA was remarkably higher in pericancerous liver tissues than in liver cancer tissues. This observation reflects the abnormal activation of a second promoter controlling *IGF-II* gene expression in precancerous hepatocytes and could be responsible for the persistent proliferation of hepatocytes, leading to the development of liver cancer.

We showed moderate and high expression of the IGF-II mRNA in DLC samples, suggesting that liver cell dysplasia may characterize cells with potential cancerous activity and ability to transform into a malignant phenotype. Persistent overexpression of the *IGF-II* gene could indicate the preceding steps to the malignant transformation of liver cells. Therefore, the study of IGF-II expression may be important for early stage diagnosis of HCC. IGF-II receptor is a transmembranous glycoprotein present in the cytoplasm and Golgi apparatus of hepatocytes. It binds to IGF-II with high affinity, which might promote the proliferation of liver cancer cells. IGF-II binds not only to the IGF-II receptor but also to the IGF-I receptor, which belongs to the tyrosine kinase family of receptors and plays a role in promoting the division and growth of cells. The DNA sequence of the IGF-II receptor gene is 99.4% homogenous with that of the cation-independent mannose 6 phosphate receptor gene, which is associated with autocrine growth of cancer cells. The binding of IGF-I to IGF-I and -II receptors suggests a multi-pathway mechanism for the transmission of signals stimulating cell growth, which would accelerate the transformation of precancerous liver cells into their malignant counterparts. Our observation that 7/10 DLC cases express mild to high IGF-II receptor mRNA levels suggests that the IGF-II receptor is involved in the dysplasia of liver cells.

HBV infection is closely associated with the development of human HCC. Serum HBsAg was positive in samples from the three different chronic liver disease groups, which overexpressed IGF-II and its receptor to different extents. These results suggest that HBV infection is involved in the degeneration, necrosis, or cirrhosis of liver cells, which leads to the abnormal activation of the *IGF-II* gene and its receptor, stimulating aberrant division and unlimited growth of liver cells via the autocrine or paracrine system.

We suggest testing the use of IGF-II RNA in gene therapy studies for the treatment of human HCC. Introduced sense or antisense IGF-II mRNA may potentially reduce abnormal activation of HBV, suppress the biological activation of IGF-II and its receptor, and retard the progress of malignant transformation in liver cells.

**REFERENCES**

- 1 **Sambrook J**, Fritsch EF, Maniatis T. Molecular cloning, a laboratory manual, 2ed, New York: Cold Spring Harbor Laboratory press 1989: 1.21-1.51, 7.3-7.52
- 2 **Yang DH**, Liu WW, Gu JR. The expression of the products of IGF-II, IGF-II receptors and CSF-1 receptors in human primary hepatocellular carcinoma and juxtacancerous liver tissue. *Zhongguo Renmin Jiefangjun Junyi Daxue Xuebao (Yingwenban)* 1993; **8**: 368-376
- 3 **Yang DH**, Liu WW, Gu JR. Localization and expression of IGF-II, IGF-II receptor and CSF-1 receptor/c-fms oncogene products in hepatocellular carcinoma and noncancerous liver tissues. *Zhonghua Xiaohua Zazhi* 1993; **13**: 189-192
- 4 **Chen SH**, Zhou XM, Li DZ. Cloning and sequencing analysis of IGF-II cDNA variation in human liver cancer tissue. *Science Communication* 1994; **39**: 664-667

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Inflammatory bowel disease in the Hubei Province of China

Bing Xia, S Shivananda, Gui-Shui Zhang, Ji-Yun Yi, JBA Crusius, AS Peka

Bing Xia, Gui-Shui Zhang, Ji-Yun Yi, Department of Gastroenterology, The Second Hospital of Hubei Medical University, Wuhan 430070, Hubei Province, China

S Shivananda, Department of Gastroenterology and Hepatology, University Hospital Maastricht, Postbus 616, 6200 MD Maastricht, the Netherlands

JBA Crusius, AS Peka, Department of Gastroenterology, Free University Hospital, Postbus 7057, 1007 MB Amsterdam, the Netherlands

Bing Xia, male, was born on Dec. 26, 1956 in Hubei Province, graduated from Department of Medicine, Hubei Medical University in 1983, Associate Professor of internal medicine and director of Department of Science and Technology of the hospital, specialized in the study on inflammatory bowel disease, having 51 papers published

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Bing Xia, Department of Gastroenterology, The Second Hospital of Hubei Medical University, Wuhan 430070, Hubei Province, China  
Telephone: +86-27-7824212-3044

Received: October 3, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To analyze clinical features and response to treatment in inflammatory bowel disease (IBD) patients from the Hubei Province of China.

**METHODS:** Clinical data was collected retrospectively from 74 patients with IBD [66 with ulcerative colitis (UC) and 8 with Crohn's disease (CD)] admitted to The Second Hospital, Hubei Medical University from 1986 to 1995.

**RESULTS:** The most common symptoms in IBD patients were abdominal pain, diarrhea, blood and mucus in stool, and constipation. Extraintestinal manifestations of IBD were not common. In these patients, inflammation was predominantly located in the sigmoid and left colon in UC cases, and in the ileum and colon in CD cases. Treatment with sulphasalazine and corticosteroids was effective in 95% of UC cases; However, about 42% of UC patients showed disease recurrence during the follow-up period of 1.11 years. Five out of eight CD patients had part of their intestine removed, whereas three were treated with anti-tuberculosis drugs or the antibiotic metronidazole. Out of four patients we followed up for 1-8 years, one died of severe complications after surgery, two experienced recur-

rence while in treatment with drugs, and one remained in remission under sulphasalazine treatment after surgery.

**CONCLUSION:** Five percent of the patients reported a family history of IBD. About 34% of the patients were smokers and 32% of the patients were alcoholic. Epidemiological studies are urgently needed in the Hubei Province of China to assess the role that genetics and environmental factors play in the pathogenesis of inflammatory bowel diseases.

**Key words:** Colitis; Ulcerative; Crohn's disease

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Xia B, Shivananda S, Zhang GS, Yi JY, Crusius JBA, Peka AS. Inflammatory bowel disease in Hubei Province of China. *World J Gastroenterol* 1997; 3(2): 119-120 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/119.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.119>

### INTRODUCTION

Inflammatory bowel disease (IBD) has traditionally been considered rare in China. Epidemiological studies have shown that the incidence of IBD in Europe and North America is about 3-14.3/100000/year for ulcerative colitis (UC) and 0.7-11.6/100,000/year for Crohn's disease (CD). The prevalence of UC and CD is 39-234/100000 and 34-106/100000, respectively<sup>[1]</sup>. The pronounced difference in the distribution of IBD in Europe/North America and China suggests that genetic factors may play a significant role in the variation among ethnicities<sup>[2]</sup>. On the other hand, environmental factors may also contribute to this difference<sup>[3]</sup>. Up to this date, the exact incidence of IBD in China is not known. However, clinical reports indicate that the number of IBD patients has surprisingly increased over the past 10 years. In the present report, we studied 74 patients with IBD from the Hubei Province (a central region of China) admitted to The Second Hospital, Hubei Medical University from 1986 to 1995. We analyzed the clinical features of these patients and followed up their response to the treatment of choice.

### CLINICAL DATA

#### Patient characteristics

A total of 74 patients with IBD, including 66 with UC and 8 with CD, were studied. UC and CD cases were diagnosed according to the Chinese Non-infectious Diarrhea Symposium criteria<sup>[4,5]</sup>. Fifty-two out of the 74 patients were male and 22 were female (ratio: 2.4:1). The mean age of the patients was 38 years (range: 16-64). Fifty-one patients lived in cities, 19 lived in towns, and four lived in the countryside. Twenty-five (34%) patients had a smoking history and

**Table 1 Clinical manifestations of ulcerative colitis and Crohn's disease in inflammatory bowel disease patients (n = 74)**

Clinical manifestation	Ulcerative colitis (n = 66)	Crohn's disease (n = 8)
Diarrhea	65 (98%)	5 (63%)
Abdominal pain	52 (79%)	8 (100%)
Blood and mucus stool	52 (79%)	2 (25%)
Constipation	9 (14%)	5 (63%)
Oral aphthous ulcer	4	3
Arthritis	4	1
Chronic gastritis	3	
Liver disease	3	
Nephropylitis	2	
Schistosomiasis	1	1
Peripheral neuritis	2	
Diabetes mellitus	1	
Tuberculosis		1
Fistula in anus	1	
Fistula in urinary bladder		1

**Table 2 Type of disease according to location of inflammation in inflammatory bowel disease patients (n = 74)**

Type of disease	Ulcerative colitis (n = 66)	Crohn's disease (n = 8)
Proctitis	12	
Sigmoiditis	26	
Left colitis	14	1
Transversal colitis	4	
Ascending colitis		1
Total colitis	10	
Ileum and cecum disease		6

**Table 3 Medical treatment of choice in inflammatory bowel disease patients (n = 74)**

	Sulphasalazine	Steroids	Antibiotics	Chinese medicine	Surgery
Ulcerative colitis (n = 66)	44	17	21	6	1
Crohn's disease (n = 8)	1		6		5

24 (32%) were alcoholic. Six patients had a history of schistosomiasis. Only four (5%) patients had a family history of IBD.

**Clinical features**

Duration of disease varied from < 1 to 38 years, with a mean interval of 3.4 years. Out of the 74 patients, 36 presented a mild version of the disease, 24 had a moderate manifestation, and 13 showed severe illness. The clinical features of the group are shown in Table 1. The most common symptoms of UC patients were abdominal pain, diarrhea, and blood and mucus in the stool. In patients with CD, abdominal pain, diarrhea, and constipation were the dominant symptoms. Extraintestinal manifestations of the disease were not common in this group of patients.

In the 66 UC patients, colonoscopic manifestations included mucosal edema, congestion, stiffness, ulceration, and polyps. Occasionally, white or yellow exudates were seen. The localization of inflammation in these patients was determined by endoscopy and X-ray barium enema (Table 2). Out of 8 CD patients, diagnosis was confirmed by surgery in 5 cases and by endoscopy and histology in 3 cases, which showed colonic stricture and segment disease.

Barium enema was carried out in 52 IBD patients; in only 18 (35%) patients the findings of this procedure were in accordance with those obtained by endoscopy, histology, or surgery.

**Treatment and follow-up**

Medical treatments of choice are shown in Table 3. Sulphasalazine (SASP), corticosteroids, and antibiotics were commonly used. In UC patients (n = 66), SASP, SASP in conjunction with steroids, or SASP in conjunction with antibiotics (metronidazole, berberine) were effective in 63 (95%) patients after short-term observation. Thirty-one UC patients were followed up for 1-11 years. Sixteen (52%) patients remained in remission, whereas 13 (42%) patients experienced disease recurrence. One patient died of bile duct carcinoma and another died of unknown causes. Out of eight CD patients, the disease progressed in five, who underwent intestinal resection or partial colectomy. Two patients were treated with anti-tuberculosis drugs and one with the antibiotic metronidazole. We followed up four CD patients for 1-8 years. One patient with partial colectomy died of severe complications after three surgical interventions. Two patients treated with anti-tuberculosis agents or metronidazole experienced disease recurrence after 5 and 8 years respectively. One patient treated with SASP after ileum resection remained in remission for one year.

**DISCUSSION**

In the past, diseases such as UC and CD were considered uncommon in the Hubei region. However, an increasing number of IBD cases has been observed in patients admitted to The Second Hospital, Hubei Medical University, as shown in the present survey (1986-1995). Five percent of the studied patients reported a family history of IBD. About 34% of the patients had a history of smoking and 32% were alcoholic. Epidemiological studies would assess the role that genetic and environmental factors may play in the pathogenesis of IBD in the Hubei region of China.

It is important to differentiate the diagnosis of IBD from that of infectious colitis and intestinal tuberculosis. The latter two can clinically, radiologically, and endoscopically mimic UC and CD.

Our data show that the most common symptoms of IBD in these patients were abdominal pain, diarrhea, blood and mucus in stool, and constipation. Extraintestinal manifestations were not common. We found that inflammation was preferentially localized to the sigmoid and left colon in UC and to the ileum and colon in CD.

Treatment of UC patients with SASP and corticosteroids was effective. However, about 42% of UC patients experienced disease recurrence during the follow-up period of 1.11 years. For CD patients undergoing surgery, the use of SASP and corticosteroids should be continued during remission.

**REFERENCES**

- 1 Pool MO. Epidemiology of inflammatory bowel disease. In: Serological and genetic markers in inflammatory bowel diseases: a contribution to the pathogenesis and diagnosis. Ph D thesis, Free University of Amsterdam Publishing 1994: 15-17
- 2 Pena AS, Crusius JBA, Pool MO, Casanova MG, Pals G, Meuwissen SGM, Giphart MJ. Genetics and epidemiology may contribute to understanding the pathogenesis of IBD: a new approach is now indicated. *Can J Gastroenterol* 1993; 7: 71-75
- 3 Shivananda S. IBD in the Asian population in the east and west: a comparison and summary. International Falk Workshop Inflammatory Bowel Disease in Asia. March 2 1996 Hong Kong: 35-38
- 4 Chinese Non-infectious Diarrhea Symposium. Criteria of diagnosis and therapy for ulcerative colitis. *Zhonghua Xiaohua Zazhi* 1993; 13: 354
- 5 Chinese Non-infectious Diarrhea Symposium. Criteria of diagnosis and therapy for Crohn's disease. *Zhonghua Xiaohua Zazhi* 1993; 13: 372

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Preliminary study on the pathological model of Piyinxu in rats

De-Zhen Chen, Mu-Xin Wei

De-Zhen Chen, Mu-Xin Wei, Department of Traditional Chinese Medicine, The First College of Clinical Medicine, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: October 25, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

### Abstract

**AIM:** To study the method of constructing a pathological model of Piyinxu (spleen-yin deficiency) using Sprague-Dawley rats and to make preliminary observations.

**METHODS:** Folium sennae (0.8 g) and tabellae thyroidei (80 mg) were given daily by stomach feeding to each rat for 12 d to establish

the pathological model of Piyinxu. The activity of the rats and the characteristics of their stool were observed.

**RESULTS:** We observed not only the symptoms of Pixu, such as diarrhea, poor appetite, abdominal distention, weight loss, but also Yinxuneire (endogenous heat), such as increase in water drinking, nervousness, and restlessness. These symptoms were all different from that of the control group of Piyangxu (spleen-yang deficiency).

**CONCLUSION:** Combining folium sennae and tabellae thyroidei is an effective method for establishing a pathological model of Piyinxu.

**Key words:** Spleen asthenia/pathology; Spleen-yin; Disease models, animal

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen DZ, Wei MX. Preliminary study on the pathological model of Piyinxu in rats. *World J Gastroenterol* 1997; 3(2): 120 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/120.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.120>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Relationship between loss of heterozygosity of *deleted in colorectal carcinoma* gene microsatellites and prognosis of colorectal adenocarcinoma

Po Zhao, Ying-Chuan Yu, De-Wen Wang, Zhi-Ping Wang, Xin-Zhao Xu, Ping-Yong Yi, Ya-Bing Gao, Guang-Hua Yang

Po Zhao, Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China

Ying-Chuan Yu, Ping-Yong Yi, Guang-Hua Yang, Department of Pathology, West China University of Medical Sciences, Chengdu 610041, Sichuan Province, China

De-Wen Wang, Zhi-Ping Wang, Ya-Bing Gao, Department of Pathology, Academy of Military Medical Sciences, Beijing 100850, China

Xin-Zhao Xu, Department of Pathology, General Hospital of Chinese PLA, Jinan Command Area

Po Zhao, MD and PhD, Associate Professor of Pathology, Head of Molecular Pathology Lab, has published 40 papers.

**Author contributions:** All authors contributed equally to the work.

**Presented at** The Third International Symposium for Pathologists, October 1994, Guangzhou.

**Supported by** The Scientific Foundation for Chinese Postdoctorates, No. 1994-3.

**Original title:** *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

**Correspondence to:** Dr. Po Zhao, MD, PhD, Associate Professor, Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China  
Telephone: +86-10-66887329-6253

Received: October 25, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

### Abstract

**AIM:** To investigate the relationship between the loss of heterozygosity (LOH) of microsatellites on the *deleted in colorectal carcinoma* (*DCC*) gene and prognosis of colorectal adenocarcinoma.

**METHODS:** A retrospective study of 58 colorectal adenocarcinoma cases with follow-up data and paired control normal mucosal tissues from 1983 to 1985 from files from the West China University of Medical Sciences Department of Pathology was carried out by PCR microsatellite analysis. Sixteen, 35, and seven cases had well-, moderately, and poorly differentiated tumors, respectively; 11, 30, and 17 cases were staged as Dukes' A, B, and C, respectively.

**RESULTS:** LOH of *DCC* microsatellites was detected in 18 cases

(31.0%). The 5-year survival rate between LOH-positive and LOH-negative patients was 44.4% and 77.5%, respectively ( $P < 0.05$ ). The results suggest that LOH of *DCC* microsatellites correlate with prognosis but not with differentiation ( $P > 0.05$ ) and Dukes' stage ( $P > 0.05$ ) in colorectal adenocarcinoma.

**CONCLUSION:** LOH of *DCC* microsatellites may be a marker of malignancy. Combined with the traditional prognostic indicators, LOH can predict prognosis of colorectal adenocarcinoma.

**Key words:** Colorectal neoplasms; Polymerase chain reaction (PCR); Oncogenes; Adenocarcinoma; Heterozygote; Prognosis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

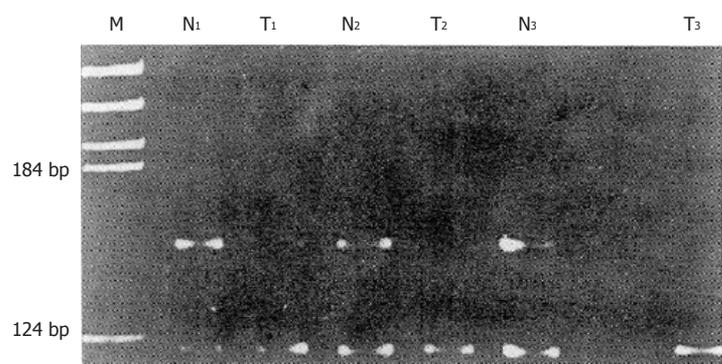
Zhao P, Hu YC, Wang DW, Wang ZP, Xu XZ, Yi PY, Gao YB, Yang GH. Relationship between loss of heterozygosity of *deleted in colorectal carcinoma* gene microsatellites and prognosis of colorectal adenocarcinoma. *World J Gastroenterol* 1997; 3(2): 121-122 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/121.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.121>

### INTRODUCTION

The *deleted in colorectal carcinoma* (*DCC*) gene is located on chromosome 18q21. In colorectal adenocarcinoma, there is an allelic deletion on the *DCC* gene and its level of expression is decreased, but few studies on the relationship between loss on the *DCC* gene and the prognosis of colorectal adenocarcinoma have been published to date. We used a PCR microsatellite method with a pair of primers flanking a dinucleotide microsatellite located within a *DCC* gene intron to study the relationship between the loss of heterozygosity (LOH) of *DCC* gene microsatellites and the prognosis of colorectal adenocarcinoma.

### MATERIALS AND METHODS

Paraffin blocks containing normal and neoplastic tissues from 71 patients with adenocarcinoma of the colon and rectum were selected for the study from files in the West China University of Medical Sciences Department of Pathology. The hematoxylin and eosin (H and E) slides from these patients were examined. Tumors were graded as well, moderately, or poorly differentiated and were assigned to Dukes' stage A, B, or C based on the standard criteria. All patients had a 5-year follow up after surgery. The PCR microsatellite method was performed as described previously<sup>[1,2]</sup> but with slight modifications, *i.e.*, ethidium bromide instead of isotope staining.



**Figure 1** Loss of heterozygosity of *deleted in colorectal carcinoma* gene microsatellites in colorectal adenocarcinoma. M: PBR322 Hae III molecular marker; N: paired normal tissues; T: tumor tissues; T<sub>2</sub>, T<sub>3</sub>: Loss of heterozygosity of *deleted in colorectal carcinoma* gene microsatellites.

## RESULTS

### Frequency of LOH

Fifty-eight amplified cases were informative for the marker of heterozygosity; 18 cases (31.0%) showed LOH. Figure 1 shows that compared to the tumor (T<sub>1</sub>) sample, the paired normal mucosa (N<sub>1</sub>) sample contained two amplified bands representing two different alleles: One paternal and another maternal, and no allele loss was scored when comparing the normal and tumor DNA. Allele loss was detected from case 2 (N<sub>2</sub>, T<sub>2</sub>) and case 3 (N<sub>3</sub>, T<sub>3</sub>), showing the loss of the top band as related to that of the normal tissue.

### Relationship between LOH and prognosis

Of the 58 colorectal carcinoma cases, 19 patients died and 39 were still alive within five years, with a 5-year survival rate of 67.2%. The 5-year survival rate in patients with and without LOH of *DCC* gene microsatellites was 44.4% (8/18) and 77.5% (31/40), respectively ( $P$

< 0.05).

### Relationship between LOH and differentiation or Dukes' stage

The positive rates of well-, moderately, and poorly differentiated colorectal adenocarcinoma were 25.0% (4/16), 31.4% (11/35), and 42.9% (3/7), respectively, in the LOH-positive cases, and 9.1% (1/11), 30.0% (9/30), and 47.1% (8/17) in Dukes' stage A, B, and C, respectively. No statistically significant correlations were found between LOH and differentiation or Dukes' stage.

## DISCUSSION

Huang *et al.*<sup>[2]</sup> reported LOH of *DCC* gene microsatellites in 33% (7/26) of colorectal adenocarcinoma cases, but they studied a small number of cases and no follow-up data. The frequency of LOH of *DCC* gene microsatellites in our study was similar to theirs, and our results were also concordant with theirs in terms of the relationship between LOH and differentiation or Dukes' stage, which could be attributed to the small sample size in both studies. According to our follow-up data, LOH of *DCC* gene microsatellites might be closely related to the 5-year survival rate of patients with colorectal adenocarcinoma. Thus, we believe that when combined with traditional prognostic indicators, LOH of *DCC* gene microsatellites might be a new predictive marker of colorectal adenocarcinoma prognosis; however, a large number of cases are needed to confirm it.

## REFERENCES

- 1 **Risinger JI**, Boyd J. Dinucleotide repeat polymorphism in the human *DCC* gene at chromosome 18q21. *Hum Mol Genet* 1992; 1: 657 [PMID: 1301182 DOI: 10.1093/hmg/1.8.657-a]
- 2 **Huang TH**, Quesenberry JT, Martin MB, Loy TS, Diaz-Arias AA. Loss of heterozygosity detected in formalin-fixed, paraffin-embedded tissue of colorectal carcinoma using a microsatellite located within the *deleted in colorectal carcinoma* gene. *Diagn Mol Pathol* 1993; 2: 90-93 [PMID: 8269282 DOI: 10.1097/00019606-199306000-00004]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Expression of the c-erbB-2 proto-oncogene product in gastric carcinoma and precancerous lesions

Jian-Qiang Mi, Shi-Qiang Yang, Ming-Chang Shen

Jian-Qiang Mi, Shi-Qiang Yang, Ming-Chang Shen, Department of Pathology, Affiliated Hospital of Luoyang Medical School, Luoyang 471003, Henan Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Received: October 25, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the relationship between the expression of the c-erbB-2 proto-oncogene product with gastric mucosal carcinogenesis and the behavior of gastric carcinoma.

**METHODS:** Specimens from nine normal gastric mucosa, 23 gastric mucosal dysplasia (10 slight, six moderate, seven severe), 18 early gastric carcinoma, and 30 advanced gastric carcinoma were marked with P<sub>185</sub> monoclonal antibody using the immunohistochemical peroxidase-avidin-biotin complex method. The relation between P<sub>185</sub> expression with histological type, size, and lymph node metastasis of gastric carcinoma were analyzed.

**RESULTS:** Normal gastric mucosa was negative for P<sub>185</sub>; Only a few cells in the neck region of the mucosal glands were very weakly positive. Relatively high positive rates were found in the slight, moderate, and severe dysplasia specimens (50%, 83.3%, and 85.7%, respectively). A 22.2% and 56.7% P<sub>185</sub>-positive rate was found in early gastric carcinoma and in advanced gastric carcinoma, respectively. Statistically, the P<sub>185</sub>-positive rates in severe dysplasia and advanced gastric carcinoma were significantly higher than that in early gastric carcinoma ( $P < 0.05$ ). The P<sub>185</sub>-positive rate in the group with lymph node metastasis was significantly higher than that of the group without lymph node metastasis (59.3% vs 23.8%,  $P < 0.05$ ), but P<sub>185</sub> expression was not related to histological type and size of gastric carcinoma.

**CONCLUSION:** The c-erbB-2 proto-oncogene might participate in gastric mucosal proliferation, repair, and carcinogenesis, and gastric carcinoma with P<sub>185</sub> expression might have a stronger potential of infiltration and metastasis.

**Key words:** Stomach neoplasms/genetics; Precancerous conditions/genetics; Proto-oncogene proteins c-erbB-2/metabolism

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Mi JQ, Yang SQ, Shen MC. The expression of c-erbB-2 proto-oncogene product in gastric carcinoma and precancerous lesions. *World J Gastroenterol* 1997; 3(2): 122 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/122.htm>  
DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.122>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Radiotherapy of 180 cases of operable esophageal carcinoma

Dong-Fu Chen, Zong-Yi Yang, Wei-Bo Yin

Dong-Fu Chen, Zong-Yi Yang, Wei-Bo Yin, Department of Radiation Oncology, Cancer Hospital, Chinese Academy of Medical Sciences, 17, Panjia Yuan, Chaoyang District, Beijing 100021, China

Dong-Fu Chen, Associate Professor of radiotherapy, has published 10 papers and has won the Young Oncologist Essay Award at the 78<sup>th</sup> annual meeting, April 1, 1996

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Dong-Fu Chen, Associate Professor, Department of Radiation Oncology, Cancer Hospital, Chinese Academy of Medical Sciences, 17, Panjia Yuan, Chaoyang District, Beijing 100021, China

Received: August 21, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To compare the validity of radiotherapy and surgery for operable esophageal carcinoma in 180 patients with pathologically proven esophageal carcinoma who had been accepted for surgery, but for various reasons were given radical radiation therapy instead.

**METHODS:** The reasons for abandoning surgery were poor cardiac function ( $n = 21$ ), poor pulmonary function ( $n = 36$ ), poor general condition ( $n = 9$ ), senility (age 69-81 years,  $n = 32$ ), and refusal by the patient ( $n = 82$ ). They were treated by the isocenter technique alone or anteroposterior plus isocenter irradiation at a total dose of 50-70 Gy/5-7 wk.

**RESULTS:** The 1-, 3-, and 5-year survival rates were 64%, 34%, and 23%, respectively. The 3- and 5-year survival rates showed that lesions in the upper third esophagus responded better than lesions in the middle and lower third ( $P < 0.05$ ). The 5-year survival rate following radiation alone (44.5%) of upper third lesions was slightly better than that following surgery. The effect on lesions following radiation to middle third lesions was slightly inferior to that of surgery, and that for lower third lesions was even poorer.

**CONCLUSION:** The results from radiation treatment alone for operable esophageal carcinoma are similar to that of surgery.

**Key words:** Esophageal neoplasms/radiation therapy; Esophageal neoplasms/surgery

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen DF, Yang ZY, Yin WB. Radiotherapy of 180 cases of operable esophageal carcinoma. *World J Gastroenterol* 1997; 3(2): 123-126 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/123.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.123>

### INTRODUCTION

Surgery and radiotherapy have always been the main treatment methods for esophageal carcinoma. In general hospitals or cancer institutes, only patients with relatively good condition; younger age; good function of the heart, lung, and other internal organs; and earlier lesions would be accepted for surgery. On the other hand, radiotherapists are more liberal in selecting patients. Only those who have perforating lesions, distant metastasis, or cachexia are not accepted. The net result is that the radiotherapy department serves more or less as a waste paper basket, accepting all of the patients not accepted by surgeons. Naturally, the result of radiotherapy for advanced cancer would be inferior to that of surgery. Could this difference in validity be ascribed to the difference in indications instead of genuine effectiveness of the treatment method? Despite the reports of Earlam and Cunha-Melo<sup>[2,3]</sup>, who compiled the result of treatment of esophageal carcinoma in the literature before 1979, the 5-year survival rate of 8489 patients in 49 institutes who had received radiation therapy before 1979 was 6% ± 6%. In contrast, the 83, 783 patients in 122 institutes operated upon in the same interval had a 5-year survival rate of 4% ± 3%. However, in the past decade or so, surgery has been reported to yield better results than radiation therapy. If either of these two modalities were used to treat similar staging and similar lesions, what would be the outcome? For this purpose, we collaborated with our thoracic surgeons and collected 180 esophageal cancer cases treated with radical radiation therapy instead of surgery since 1958. The results of surgery and radiation therapy of the three esophageal segments were compared to provide some reference for oncologists.

### MATERIALS AND METHODS

From January 1958 through 1987, 180 patients with pathologically proven esophageal carcinoma were seen at our thoracic oncologic outpatient department. The thoracic surgeons had accepted them for surgery after having evaluated their history data, including chest films and barium esophagograms. Yet, for the reasons stated in Table 1, radical radiation therapy was administered instead.

Of the 180 patients, 120 (66.7%) were male and 60 (33.3%) were female; age ranged 35-81 years, with a median of 63 years, and the male:female ratio was 2:1. According to the 1978 International Union Against Cancer (UICC) method of esophageal division, 27 patients (15%) had lesions in the upper third esophagus, 125 patients (69.4%) had middle third lesions, and 28 patients (15.6%)

**Table 1** Reasons for administering radiotherapy to 180 patients with esophageal cancer accepted for surgery

Reason	No.	%
Poor cardiac function	21	11.7
Poor pulmonary function	36	20.0
Poor general condition	9	5.0
Senility (68-81 yr)	32	17.7
Refusal by patient or spouse	82	45.6
Total	180	100.0

**Table 2** Results of radical radiation therapy for operable esophageal carcinoma

Follow-up	No. of patients	%
1-year	116/180	64
3-year	62/180	34
5-year	42/180	23

**Table 3** Comparison of efficacy of surgery with radiotherapy for esophageal carcinoma

Treatment	Author	Year	5-year exploratory survival rate		
			No.	%	
Surgery	Li <i>et al</i> <sup>[9]</sup>	1980	59/213	28	26/82 (32%) from patients who refused operation
	Zhang <i>et al</i> <sup>[17]</sup>	1994	942/3603	26	
Radiotherapy	Present series		42/180	23	

had lower third lesions. The length of the lesions from 2-9 cm; 68 (37.8%) were < 5 cm and 112 (62.2%) were > 5 cm. The histopathology showed squamous cell carcinoma in 178 cases (99%) and adenocarcinoma in one case (1%). The X-ray typing showed medullary disease in 136 cases (76%), fungating disease in 38 cases (21%), and intraluminal disease in six cases (3%).

Telecobalt or 8-MV X-ray was administered by routine three-field isocenter irradiation or anteroposterior (A-P) irradiation followed by three-field isocenter irradiation. For the latter, 40 Gy/4 wk was first administered by A-P opposing irradiation. Afterwards, the one anterior/one posterior isocenter technique was used to administer a further 10-30 Gy to bring the total dose to a radical level (50-70 Gy/5-7 wk). The radiation was administered routinely as 2 Gy/session, five sessions a week. The total dose administered was 50-59 Gy in eight patients, 60-69 Gy in 30 patients, and 70 Gy in 142 patients (79%). The width of the portal was 5-6 cm in most patients. Only in isolated cases were 4.5 cm wide portals used. The upper and lower border of the portal was set 3-4 cm beyond the margin of the lesion as seen on the simulator.

## RESULTS

All 180 patients were followed for more than five years after irradiation. Four patients lost to follow-up were counted as dead from the day they were missing. The overall 1-, 3-, and 5-year survival rates were 64% (116/180), 34% (62/180), and 23% (42/180), respectively (Table 2). Within five years of treatment, 138 patients died. The causes of death were local recurrence or uncontrolled, 60.5% ( $n = 109$ , among whom 21 succumbed to fatal hemorrhage or esophago-tracheal fistula), regional lymphatic metastasis ( $n = 9$ ), distant metastasis ( $n = 13$ ), and causes other than cancer ( $n = 7$ ).

### The necessity of using the exploratory survival rate when comparing the efficacy of surgery and radiotherapy

Surgeons usually report their treatment result as the resectional survival rate (number of survivors divided by the number of patients resected) and not as the exploratory resectional survival rate (number of survivors divided by the number of patients explored), which is commonly lower than the former. The survival rate of this series is equivalent to the exploratory survival rate of surgery. Therefore, exploratory survival rates should be used when comparing the effectiveness of surgery with other treatment methods. Our data show that surgery and radiation therapy are equally effective for esophageal carcinoma (Table 3).

**Table 4** Comparison of results of resectional 5-year survival rates with the present series

Treatment	Author	Year	Operation year	5-year survival rate	
				No.	%
Surgery	Wu <sup>[18]</sup>	1962	1940-1960	18/76	23.7
	Gu <sup>[6]</sup>	1964	1953-1957	21/91	23.1
	Wu <sup>[19]</sup>	1979	1957-1973	276/1040	26.6
	Li <sup>[10]</sup>	1979	1957-1973	164/664	24.7
	Li <sup>[9]</sup>	1980	1969-1973	59/201	29.4
	Zhang <sup>[21]</sup>	1980	1952-1978	303/1290	23.5
	Giuli <sup>[5]</sup>	1980	1970-1979	375/1870	20.1
	Shao <sup>[14]</sup>	1987	1965-1985	958/2032	47.1 <sup>1</sup>
	Jauch <sup>[8]</sup>	1992	1982-1989	17/86	19.8
	Elias <sup>[4]</sup>	1992	1982-1990	30/128	23.4
	Vigneswaran <sup>[16]</sup>	1993	1985-1991	27/131	20.6
	Zhang <sup>[20]</sup>	1994	1958-1992	942/3099	30.4
	Radiotherapy	Present series		1958-1987	42/180

<sup>1</sup>Including some very early lesions as discovered by cytology in public screening.

**Table 5** Comparison of surgery with radiotherapy for esophageal cancer in different esophageal segments

Treatment	Author	Year	5-year resection survival rates					
			Upper segment		Middle segment		Lower segment	
			No.	%	No.	%	No.	%
Surgery	Wu <i>et al</i> <sup>[18]</sup>	1962	0/4	0	5/33	15.2	13/39	33.3
	Gu <i>et al</i> <sup>[6]</sup>	1964	0/6	0	11/55	20.0	10/29	34.5
	Su <i>et al</i> <sup>[15]</sup>	1965	2/12	16.6	7/33	21.2	6/24	25.0
	HebaiMed.Univ. <i>et al</i> <sup>[12]</sup>	1973	10/84	11.9				
	Wu <i>et al</i> <sup>[19]</sup>	1979	3/28	11.7	87/327	26.6	72/220	32.7
	Li <i>et al</i> <sup>[9]</sup>	1980		26.0		30.0		34.0
	Giuli <i>et al</i> <sup>[5]</sup>	1980		14.0		15.0		24.0
	Akiyama <i>et al</i> <sup>[11]</sup>	1980			7/28	25.0	7/24	29.2
	Lin <i>et al</i> <sup>[11]</sup>	1983	9/43	20.9	89/388	17.8	80/288	27.8
	Elias <i>et al</i> <sup>[4]</sup>	1992		6.4		17.2		28.9
	Vigneswaran <i>et al</i> <sup>[17]</sup>	1994	9/49	18.4				
	Cancer Hosp. CAMS		93/311	29.9	398/1303	30.5	169/577	39.3
	Radiotherapy	Present series <sup>2</sup>		12/27	44.4	28/125	22.4	4/28

<sup>1</sup>Material of Dept. Thoracic Surgical Oncology to be published. <sup>2</sup>Exploratory 5-year survival rates.

### Comparison of resectional survival rate in the literature with the results of this series

As we were unable to obtain the resectional rate in most reports, we had to compare our results with their 5-year resectional survival rates, which was inevitably higher (Table 4). Even so, the 23% 5-year survival rate by radiotherapy may be comparable to that achieved by surgical resection, which ranged 20%-30.4%. The extraordinarily good result of Shao and associates<sup>[12]</sup> could be ascribed to the fact that some of their patients had very early lesions pathologically, *e.g.*, carcinoma *in situ* or pathologically early infiltrating carcinoma. Hence, their results cannot be considered typical esophageal cancer established in clinical practice.

### Comparison of surgery and radiotherapy on the three esophageal segments

As we were unable to obtain the resection rate of the various segments, we had to compare the lower exploratory 5-year survival of the present series with the higher resectional 5-year survival as we tried to assess the relative merits of either regimen for each esophageal segment. From Table 5, there is an obvious tendency for the survival to decline as we proceed from the upper to lower segment when surgery is considered: It is lowest in the upper segment, moderate for the middle segment, and highest in the lower segment. By contrast, the result for radiotherapy was best in the upper segment, moderate in the middle segment, and poorest in the lower segment. It can be concluded from Table 5 that radiotherapy surpasses surgery for treating upper segment esophageal cancer, according to the 13 reports published in the past 35 years, except for the pathologically very early lesions<sup>[11]</sup>. In contrast, surgery should be first considered for lower segment lesions, as radiation therapy yielded a 5-year survival rate only half of that following surgery. The same is true for middle-segment cancer, except the very early cases.

### Influence of lesion length on treatment result

Table 6 shows the influence of lesion length in the three segments on the radiotherapy results for operable esophageal cancer. Due to the limited number of patients, it appears that length does not have

**Table 6** Influence of lesion length of operable esophageal cancer on result of radiotherapy

Segment	Lesion length (cm)	5-year survival rate	
		No.	%
Upper <sup>1</sup>	< 3	4/9	44.4
	3-4.9	3/6	50.0
	> 5	5/12	41.7
Mid	< 3	2/7	28.6
	3-4.9	6/33	18.2
	> 5	18/85	21.2
Lower	< 3	1/4	25.0
	3-4.9	1/9	11.1
	> 5	2/15	13.3
Total		42/180	23.3

<sup>1</sup>Including four lesions in the cervical esophagus

any appreciable influence on the final outcome. Moreover, the crucial factor is the segment in which the lesion is found. To draw a clear conclusion, further studies are needed, preferably a strict prospective randomized trial.

#### **Influence of causes for cancelling surgery on treatment result**

Table 7 shows the influence of reasons for canceling surgery on the radiation therapy results in operable esophageal carcinoma. On the one hand, it is apparent that a good general condition is very important to ensure a satisfactory outcome, as none of our nine debilitated patients survived. On the other hand, if a patient who fits every physical aspect should refuse an intended operation, he is deemed to enjoy a similar good result, if not a better one, after radiation therapy—a 32% 5-year survival rate, which is unsurpassed by any of the surgical results reported (Table 4). This finding may further support the notion that radiation therapy may finally be proven a sound alternative to surgery for operable esophageal carcinoma.

## **DISCUSSION**

### **Comparison of surgery with radiotherapy**

The choice of treatment for esophageal cancer has always been inclined towards surgery, performed whenever possible. Radiation therapy is resorted to only when the patient is not accepted by surgeons. The principle "... for advanced cases, radiation is called forth for palliation," is presented in the textbooks and has been carried out accordingly in many hospitals and tumor centers. During the past two decades or so, surgery has indeed yielded better results than radiation therapy. However, it cannot be refuted that surgeons treat far earlier lesions than radiotherapists do. As early as 1980, Earlam *et al.*<sup>[2]</sup> and Cunha-Melo *et al.*<sup>[2,3]</sup> had expressed their doubts about the superiority of surgery, for which the better survival rates could have been due to the earlier disease. If surgeons and radiotherapists were on equal footing, what kind of result may they yield? The present series of 180 patients had originally been accepted by the surgeons for surgery after clinical work-up. However, for various reasons (Table 1), radical irradiation was administered instead. Even though this was not a randomized study, this still presented a relatively comparable basis, *e.g.*, length of lesion, absence of extraesophageal extension, and so on. The 5-year survival rates by surgery as reported in the literature range 20%-30.4%<sup>[1,4-12,14-17]</sup>. Only that by Su *et al.*<sup>[13]</sup> should be considered separately, as some of their patients had very early pathological lesions, *e.g.*, carcinoma *in situ* and early submucosal infiltrating carcinoma. Hence, a 5-year survival rate of 47.1% was reported (Table 4). The 5-year survival rate of the present series is, in fact, equivalent to the exploratory 5-year survival rate by surgery, which the surgeons would use when presenting their results. Typically, the resection rate of esophageal cancer ranges 78-85%, hence the 5-year survival rate by surgery (15-22%) is too high. Considering all factors, the radiation therapy result of the present series is comparable with any results obtained (Tables 3, 4). In the 82 patients who refused surgery, the 5-year survival rate following radiotherapy was 32% (Table 7), which is similar to the overall 5-year survival rate of 30.4% following surgery (Table 4). It should be noted that the former is the exploratory survival rate and the latter the resectional survival rate (15%-22%),

**Table 7** Influence of reasons for rejecting surgery on result of radiotherapy in operable esophageal carcinoma

Causes of rejecting surgery	5-year survival rate	
	n	%
Poor cardiac function	4/21	19
Poor pulmonary function	5/36	14
Poor general condition	0/9	0
Senility	7/32	22
Patient refusal	26/82	32
Total	42/180	23

which is too high. For genuine comparison, a prospective randomized trial carried out by both surgeons and radiotherapists is warranted and a truly objective conclusion may thus be obtained.

### **Treatment options for esophageal carcinoma in different esophageal segments**

Due to the difficulty in resecting upper segment lesions, radiotherapy used to be the preferred treatment for esophageal carcinoma. In the present series, a 44% 5-year survival rate was obtained for 27 patients with upper segment cancer. By improving the operative procedures in this segment, surgery has achieved better results in recent years, ranging 0%-26.3%<sup>[1,4-6,8-10,13-16]</sup>. In 1982, Shao *et al.*<sup>[11]</sup> reported 50% survival for upper segment esophageal cancer. According to their report, 13.4% (142/1061) was stage 0-I early pathologic lesions. In contrast, the staging of the present series showed only 4% (7/180) early lesions, designated as 3 cm in length by the barium meal esophagograms. Some of these patients may have had tumors far more advanced than what was shown on the X-ray films. The high survival in the report of Shao and colleagues may have been due to the abundance of actual early lesions in their patients. Even so, the results of upper segment lesions in the present series and that of Shao and colleagues are still comparable (44% vs 50%). For upper segment esophageal cancer, there have been only a few reports on combined treatment, reporting 5-year survival rates of 23.1%-47.6%<sup>[7,17]</sup> which are not superior to that following radiotherapy alone. Generally, it is believed that radiotherapy is slightly superior to surgery and is similar to preoperative radiation plus surgery. Hence, radiotherapy is suitable for upper segment esophageal carcinoma, especially for patients who have very short lesions, without obvious stenosis, or extraesophageal invasion, or very superficial, intraluminal, or fungating disease. Aside from the satisfactory results, radiation therapy raises very little risk of radiation injury and costs less, so it is readily acceptable to the patient. The 5-year survival rate of operable esophageal carcinoma was 44% for the upper segment, which is better than the 21% and 14% for the middle and lower segments, respectively ( $P < 0.05$ ).

The reasons for the poor 5-year survival rate of 14% for the lower segment lesions may be that the lower carcinoma locations are apt to develop lymphatic metastasis along the left gastric and epigastric vessels, which are difficult to discover clinically. Some of the patients may have already developed metastases when they received radiotherapy. Consequently, recurrence would naturally lead to failure as these involvements are easily missed by the conventional portals. The lower segment cancers usually have a 5-year survival rate of 30% following surgery<sup>[1,4-6,8-10,12-16]</sup>, which is superior to that following radiation therapy. Therefore, surgery should be indicated with priority for lower segment esophageal carcinoma. The same is true for middle segment lesions, for which surgery is also preferred. The conclusions drawn from this study are as follows:

1. When treated by radiation therapy alone, operable esophageal carcinoma yields comparable results to that treated by surgery.
2. Radiation therapy, surpassing surgery for upper segment esophageal carcinoma, is preferred for this kind of lesion.
3. Surgery, surpassing radiation therapy for lower segment esophageal carcinoma, is preferred for this kind of lesion.
4. Comparison of surgery with radiation therapy for middle segment lesions shows that the latter is less effective. Surgery is generally preferred although radiation therapy is acceptable for certain types of the disease.

## REFERENCES

- 1 **Akiyama H.** Surgery for carcinoma of the esophagus. *Curr Probl Surg* 1980; **17**: 53-120 [PMID: 6987040 DOI: 10.1016/S0011-3840(80)80025-6]
- 2 **Earlam R, Cunha-Melo JR.** Oesophageal squamous cell carcinoma: I. A critical review of surgery. *Br J Surg* 1980; **67**: 381-390 [PMID: 6155968 DOI: 10.1002/bjs.1800670602]
- 3 **Earlam R, Cunha-Melo JR.** Oesophageal squamous cell carcinomas: II. A critical view of radiotherapy. *Br J Surg* 1980; **67**: 457-461 [PMID: 6158354 DOI: 10.1002/bjs.1800670702]
- 4 **Elias D, Lasser P, Mankarios H, Cabanes PA, Escudier B, Kac J, Rougier P.** Esophageal squamous cell carcinoma: the specific limited place of surgery defined by a prospective multivariate study of prognostic factors after surgical approach. *Eur J Surg Oncol* 1992; **18**: 563-571 [PMID: 1478288]
- 5 **Giuli R, Gignoux M.** Treatment of carcinoma of the esophagus. Retrospective study of 2, 400 patients. *Ann Surg* 1980; **192**: 44-52 [PMID: 7406563 DOI: 10.1097/0000658-198007000-00008]
- 6 **Gu YZ, Wang ZH, Zhang JQ.** Long term results of surgical treatment and radiotherapy for carcinomas of esophagus and cardia. *Zhonghua Waike Zazhi* 1964; **12**: 115-120
- 7 **Huang GJ, Gu XZ, Zhang RG, Zhang LJ, Zhang DW, Miao YJ, Wang LJ, Lin H, Wang GQ, Xiao QL.** Combined preoperative irradiation and surgery in esophageal carcinoma. Report of 408 cases. *Chinese Medical Journal (English Edition)* 1981; **94**: 73-76 [PMID: 6786841]
- 8 **Li CL.** Analysis of the results of surgical treatment for 327 cases of carcinomas of esophagus and gastric cardia. *Zhonghua Zhongliu Zazhi* 1980; **2**: 116-117
- 9 **Lin XS, Chen BT, Fang KB.** Long term effects of surgical treatment on esophageal carcinoma. *Zhonghua Zhongliu Zazhi* 1983; **5**: 303-304
- 10 **No.4 Hospital, Hebei Medical University.** Surgical treatment in 2483 patients with carcinoma of esophagus and gastric cardia. *Cancer Prev Treat* 1993; **3**: 1-7
- 11 **Shao LF, Li ZC, Liu SX.** [Results of surgical treatment of 3, 155 cases of esophageal and cardiac carcinoma]. *Zhonghua Waike Zazhi* 1982; **20**: 19-22 [PMID: 7075375]
- 12 **Shao LF, Li ZC, Wang MF.** [Results of surgical treatment in 6123 cases of carcinoma of the esophagus and gastric cardia]. *Zhonghua Waike Zazhi* 1987; **25**: 452-45, 500 [PMID: 3691248]
- 13 **Su YH, Weng PG, Liu JQ.** Long term results of carcinoma of esophagus and gastric cardia after treated with resection. *Zhonghua Waike Zazhi* 1965; **13**: 232-234
- 14 **Vigneswaran WT, Trastek VF, Pairolero PC, Deschamps C, Daly RC, Allen MS.** Extended esophagectomy in the management of carcinoma of the upper thoracic esophagus. *J Thorac Cardiovasc Surg* 1994; **107**: 901-906; discussion 901-96; [PMID: 8127121]
- 15 **Wu YK, Huang GJ, Zhang W.** Long term results of squamous epithelioma treated with resection. A corpus of cancer research papers. Shanghai: Shanghai Science Technology Publishing House 1962: 155-157
- 16 **Wu YK, Huang GJ.** Experiences of surgical treatment of esophageal carcinoma. *Zhonghua Zhongliu Zazhi* 1979; **1**: 241-243
- 17 **Zhang ZX, Feng QF, Gu XZ.** Evaluation of preoperative irradiation of esophageal carcinoma: an analysis of 1021 cases. *Zhonghua Fangshe Zhongliuxue Zazhi* 1992; **1**: 169-171

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Effect of muscarinic blocker on enhancing the action of fructus aurantii immaturus on intestinal myoelectric activity in dogs

Zi-He Huang, De-Zhi Yang, Yi-Quan Wei, Yin-Hui Luo

Zi-He Huang, Yin-Hui Luo, Second Affiliated Hospital of Shantou University Medical College, Shantou 515031, Guangdong Province, China

De-Zhi Yang, Yi-Quan Wei, Physiology Department of Nanjing Railway Medical College, Shantou 515031, Guangdong Province, China

Zi-He Huang, female, born on April 14, 1956, graduated from the Medical Department, Shanghai Medical University in 1982. Lecturer of electrophysiology, engaged in clinical electrophysiological testing, has published 9 papers

This paper was presented at The National Symposium on Gastrointestinal Activity and Motility, June 1995, Beijing.

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 3870577.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Zi-He Huang, Second Affiliated Hospital of Shantou University Medical College, Shantou 515031, Guangdong Province, China  
Telephone: +86-754-8565185

Received: October 11, 1996

Revised: January 31, 1997

Accepted: March 1, 1997

Published online: June 15, 1997

### Abstract

**AIM:** To investigate the effect of fructus aurantii immaturus (FAI) on small intestinal electrical activity in dogs.

**METHODS:** The effect of FAI was observed using a computerized electrophysiological method with the migrating myoelectric complex as a criterion. Fasted, healthy, and conscious dogs were given 100% FAI concentrated solution by gastrostogavage, and as soon as the effect on small intestinal electrical activity appeared, atropine was injected intramuscularly.

**RESULTS:** The enhancing action of FAI was inhibited significantly by atropine, a cholinergic receptor antagonist. Both the number of spike bursts per cluster and the number of spikes per minute in phase II and III and the general cycle were decreased ( $P < 0.01$ ), although the duration of phase II and the general cycle was prolonged.

**CONCLUSION:** The effect of FAI might be related to the muscarinic receptors.

**Key words:** Fructus aurantii immaturus; Small intestine; Atropine; Electrophysiology

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Huang ZH, Yang DZ, Wei YQ, Luo YH. Effect of muscarinic blocker on enhancing action of fructus aurantii immaturus in the intestinal myoelectric activity in dogs. *World J Gastroenterol* 1997; 3(2): 127-128 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/127.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.127>

### INTRODUCTION

Fructus aurantii immaturus (FAI) is a Chinese herb for reestablishing vital energy. It can promote *qi* (vital energy) circulation, disperse the accumulation of evils and phlegm, relieve flatulence, and so on<sup>[1]</sup>. Based on small intestinal electrical activity as enhanced by FAI concentrated solution, we used the muscarinic blocker atropine to affect the action of FAI to reveal the pharmacological mechanism of FAI.

### MATERIALS AND METHODS

#### Drug administration

FAI concentrated solution was prepared according to the method of Bi *et al*<sup>[2]</sup>; atropine was manufactured by the Yan Cheng Pharmaceutical Factory.

#### Laboratory method

Three healthy dogs weighing 10-20 kg were anesthetized using 3% pentobarbital sodium (1 mL/kg *i.v.*). Two pairs of platinum bipolar electrodes were implanted in the subserosa of the duodenum (4 cm below the junction of the stomach and intestine) and jejunum (5 cm below the ligament of Treitz), respectively. The diameter of the electrodes was 0.3 mm and the distance between two electrodes was 1.5 mm; the electrode wires were passed through the abdominal muscle and fixed in the skin between the shoulder blades. A nylon gastrostoma tube was inserted in to the stomach, from which gastric juice was aspirated or drugs injected.

Conscious dogs fasted 12-24 h but with free water-drinking were placed in a shielding facility. All data were handled by a four-channel physiological recorder and a Zijin 2-A computer; the 150-min migrating myoelectric complex (MMC) cycle, number of spikes per minute, spike bursts per cluster, and histograms were printed immediately.

After recording one MMC cycle, 100% FAI concentrated solution (1 mL/kg) was administered by gastrostogavage in the fifth minute of phase I of the second cycle. As soon as the spike decreased, 0.5 mg atropine was administered intramuscularly and one or two MMC cycles were recorded again.

**Table 1 Comparison of the duration of each phase and the general cycle before and after administration of drugs ( $\bar{x} \pm s$ )**

No.	Migrating Myoelectric Complex				General cycle	
	Phase I	Phase II	Phase III	Phase IV		
Control	13	31.92 ± 17.21	53.46 ± 28.90	5.38 ± 1.85	11.38 ± 10.80	104.85 ± 12.85
FAI + atropine	13	26.85 ± 18.22	102.23 ± 51.41 <sup>b</sup>	4.38 ± 2.02	9.00 ± 9.18	148.38 ± 45.87 <sup>b</sup>

<sup>b</sup>*P* < 0.01 compared with control group. FAI: Fructus aurantii immaturus.

**Table 2 Comparison of the number of spikes per cluster before and after administration of drugs (number/cluster, min,  $\bar{x} \pm s$ )**

No.	Migrating Myoelectric Complex				General cycle	
	Phase I	Phase II	Phase III	Phase IV		
Control	13		3.55 ± 2.23	4.55 ± 2.40	4.55 ± 2.40	4.14 ± 2.10
FAI + atropine	13	Some cycles had no spike	2.45 ± 1.18 <sup>b</sup>	4.66 ± 2.11 <sup>b</sup>	3.92 ± 2.46	2.79 ± 1.21 <sup>b</sup>

<sup>b</sup>*P* < 0.01 compared with control group. FAI: Fructus aurantii immaturus.

**Table 3 Variation of the number of spikes per minute before and after the use of drugs (number/min,  $\bar{x} \pm s$ )**

No.	Migrating Myoelectric Complex				General cycle	
	Phase I	Phase II	Phase III	Phase IV		
Control	13	26.82 ± 17.86	117.02 ± 46.60	42.44 ± 30.78	27.58 ± 15.90	
FAI+atropine	13	Same as in Table 2	15.15 ± 9.55 <sup>b</sup>	89.40 ± 45.93 <sup>b</sup>	36.11 ± 35.97	15.35 ± 9.82 <sup>b</sup>

<sup>b</sup>*P* < 0.01 compared with control group. FAI: Fructus aurantii immaturus.

Thirteen recordings in all were made before and after the administration of FAI and atropine.

## RESULTS

The comparison of the duration of each phase and the general cycle before and after FAI and atropine administration is shown in Table 1. Atropine was injected in to the dogs as soon as the spike burst was induced by FAI. The duration of phase I shortened from 31.92 min ± 17.21 to 26.85 min ± 18.22. The duration of phase II and the general cycle was prolonged (*P* < 0.01), indicating that FAI can induce spike burst and enhance intestinal electrical activity. The shortened duration of phase III may have been related to the effect of atropine in inhibiting the spike burst, as in phase III, the spike loading slow wave encompassed over 95% of the slow wave. The reduced spike decreased the spike loading slow waves.

The comparison of the number of spikes per cluster before and after FAI and atropine administration is shown in Table 2. The mean number of spikes per cluster in phase II and III and the general cycle was decreased (*P* < 0.01). The results reveal that the spike induced by FAI was inhibited significantly by atropine.

The changes in the number of spikes per minute before and after FAI and atropine administration are shown in Table 3.

After atropine was administered, the number of spikes per min-

ute in phase II, phase III, and the general cycle was decreased (*P* < 0.05), suggesting that the spike induced by FAI was inhibited significantly by atropine.

## DISCUSSION

MMC is a well-known sensitive index for evaluating gastrointestinal motion and regularity<sup>[3]</sup>. Gastrointestinal contractile activities are identical to the MMC. Four sequential phases (I-IV) of MMC, defined in terms of action potential activity, appear to correspond to the resting (quiescence), preceding irregular, strong, and subsiding contractions<sup>[4]</sup>.

Smooth muscle cells may suddenly discharge one or more action potentials under the condition of depolarization. These spikes usually accompany the contraction of smooth muscle. In many slow waves, spikes appear continuously. Smooth muscle cells produce action potential and contract correspondingly with the periods of depolarization and hyperpolarization<sup>[5]</sup>.

In phase II, if stronger spikes continuously occur on three or four basic electric rhythms, the corresponding segment of the small intestine will undergo peristalsis. In phase III, if 95% basic electric rhythm loads high-frequency and -amplitude spikes, intense meta-meric activity takes place in that intestinal segment. There are many more spikes in each spike cluster in phase III than in phase II. It manifests in strengthened movement of the small intestine with the increase of spikes in each spike cluster<sup>[6]</sup>.

The intestinal tract is mainly governed by parasympathetic nerves<sup>[7]</sup>. The small intestine has a complex enteric nervous system. Its terminal neuron belongs to the cholinergic neurons. Its role is mainly related to motion that can be blocked by atropine.

FAI can strengthen the phasic contraction and tension of intestinal smooth muscle, shorten the duration of phase I and the general cycle, and prolong the duration of phase II. When small intestinal electrical activity began to be enhanced by FAI, atropine inhibited the enhancing effect of FAI, prolonging the duration of phase II and the general cycle; decreasing the number of spikes per cluster and the number of spikes per minute in phase II, phase III, and the general cycle; and shortening phase IV. Atropine can inhibit the spike induced by FAI, suggesting that the enhancing action of FAI on small intestinal electrical activity may be related to the muscarinic receptor.

## REFERENCES

- 1 Li YK, Jiang MY. Traditional Chinese medicine and pharmacology. Beijing: Chinese Traditional Medicine and Pharmacology Publisher 1992: 116-118 (in Chinese)
- 2 Bi QH, Yang DZ, Yin CZ, Din AL. Effect of Fructus Aurantii Immaturus on myoelectric activity of small intestine. *Acta Mechanica Sinica* 1991; **6**: 39-40 (in Chinese)
- 3 Zhang JJ. [Interdigestive myoelectric complex of gastrointestinal tract (author's transl)]. *Shengli Kexue Jinzhan* 1981; **12**: 325-329 [PMID: 7344075]
- 4 Itoh Z, Takeuchi S, Aizawa I, Takayanagi R. Characteristic motor activity of the gastrointestinal tract in fasted conscious dogs measured by implanted force transducers. *Am J Dig Dis* 1978; **23**: 229-238 [PMID: 665611 DOI: 10.1007/BF01072323]
- 5 Ke MY. Motility of the gastrointestinal tract. (in Chinese) Beijing: Science Publisher 1996: 30-32
- 6 Yang DZ, Bi QH, Yin CZ, Chen SC. Initial analyses of cluster of spike in phase II and phase III. *Nanjing Tiedao Yixueyuan Xuebao* 1988; **7**: 10-11 (in Chinese)
- 7 Tang SJ (Chief translator). Pharmacological basis of therapeutics. Beijing: People's Health Publisher 1981: 113 (in Chinese)

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX

## Effect of remedies for enhancing resistance and relieving blood stasis on metastasis in postoperative gastric cancer and ornithine decarboxylase levels

Ping Bu

Ping Bu, Department of Gastroenterology, Medical College of Yangzhou University, Yangzhou 225001, Jiangsu Province, China

Ping Bu, male, born on October 10, 1955 in Teixing County, Jiangsu Province, Assistant Professor of internal medicine, has published 72 papers and one book

Author contributions: The author solely contributed to the work.

Supported by The Administration Bureau of Traditional Chinese Medicine of Jiangsu Province, No. p513.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Ping Bu, Assistant Professor, Department of Gastroenterology, Medical College of Yangzhou University, Yangzhou 225001, Jiangsu Province, China

Telephone: +86-514-7345811  
Fax: +86-514-7341733

Received: August 25, 1996  
Revised: January 31, 1997  
Accepted: March 1, 1997  
Published online: June 15, 1997

### Abstract

**AIM:** To study the action of remedies for enhancing resistance and relieving blood stasis on metastasis in postoperative gastric cancer and its influence on ornithine decarboxylase (ODC).

**METHODS:** Sixty-three postoperative patients with gastric cancer were randomly divided into two groups. Thirty-one patients were treated with western medicine consisting of the FAP (5-fluorouracil, adriamycin, cisplatin) and CODP regimens (cyclophosphamide, vincristine, daunorubicin, prednisone), whereas 32 patients were treated with the FAP regimen and traditional Chinese medicine. Correlations were made between the ODC levels detected before and after treatment and other factors such as tumor diameter, infiltration depth, histological type, and lymph node metastasis.

**RESULTS:** The ODC levels in the gastric cancer tissue and adjacent normal gastric mucosal tissue were significantly higher in the patients than in the controls. There was an obvious correlation between increased ODC and tumor size, infiltration depth, degree of differentiation, and lymph node metastasis. Six months later, there were no significant changes in the ODC levels of the group using only Western medicine, while the ODC levels decreased markedly in the group using combined Western and traditional Chinese medicine ( $P < 0.01$ ).

**CONCLUSION:** The effects of traditional Chinese medicine remedies on metastases in postoperative gastric cancer are related to the reduction of ODC activity.

**Key words:** Stomach neoplasms/surgery; Neoplasms metastasis; Ornithine decarboxylase; Traditional Chinese medicine

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Bu P. Effect of remedies of enhancing resistance and relieving blood stasis on metastasis of post-operative gastric cancer and ornithine decarboxylase. *World J Gastroenterol* 1997; 3(2): 129-130 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i2/129.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i2.129>

### INTRODUCTION

Ornithine decarboxylase (ODC) is the rate-limiting enzyme of polyamine synthesis in cellular proliferation. It has been proven that ODC mutagenesis and carcinogenesis play an important role in gastric cancer development, metastasis, and relapse. During the period of 1992-1994, the author studied the action of traditional Chinese medicine remedies for enhancing resistance and relieving blood stasis on metastases in postoperative gastric cancer and their effect on ODC levels at the Department of Medicine of Tokyo University, Japan.

### MATERIALS AND METHODS

Sixty-three postoperative patients with gastric cancer from the Department of Medicine, the Affiliated Hospital of Tokyo University, Japan, comprising 41 men and 22 women with a mean age of 63.2 years, were studied. Twenty-three cases of early gastric cancer and 40 cases of advanced gastric cancer were diagnosed histopathologically. Forty-two patients had differentiated carcinoma, including papillary and tubular adenocarcinoma (nine were grade I, eight were grade II, 25 were grade III-IV). Twenty-one patients had undifferentiated carcinoma, including six with undifferentiated adenocarcinoma, six with undifferentiated mucinous adenocarcinoma, four with poorly differentiated adenocarcinoma, and five with signet ring cell carcinoma. Thirty-nine patients had lymph node metastasis and 24 patients did not.

The 63 postoperative patients were randomly assigned to two groups. There were no significant differences ( $P > 0.05$ ) between the groups in terms of age, sex, general condition, surgical approach, tumor size, invasion depth, histological type, and metastasis. Thirty-one patients were treated with Western medicine involv-

**Table 1 Mean ornithine decarboxylase levels in normal gastric mucosa before and after treatment (CO<sub>2</sub> nmol·mg protein<sup>-1</sup>·h<sup>-1</sup>)**

	<i>n</i>	Pre-treatment	Post-treatment	Pre-treatment minus post-treatment ( $\bar{x} \pm s$ )
Traditional and Western medicine	32	246.9	191.7	55.1 ± 6.49
Western medicine only	31	239.9	225.1	14.8 ± 2.01

**Table 2 Analysis of ornithine decarboxylase levels changes in normal gastric mucosa and related factors in study groups before and after treatment ( $\bar{x} \pm s$ )**

		Traditional Chinese medicine and Western medicine		Western medicine only	
		Before	After ( <i>n</i> )	Before	After ( <i>n</i> )
Tumor diameter (cm)	< 4.9	194.2/128.4	65.7 ± 9.03 (18) <sup>a</sup>	188.3/181.8	6.5 ± 1.23 (16)
	≥ 5.0	26908/248.8	21.1 ± 4.63 (14)	270.1/262.1	8.0 ± 1.73 (15)
Histological type	Advanced cancer	288.3/261.8	26.5 ± 4.2 (20)	269.8/255.1	14.7 ± 2.95 (20)
	Differentiated	201.1/125.8	75.3 ± 10.89 (21) <sup>a</sup>	211.3/193.4	17.9 ± 2.27 (2)
Lymph node metastasis	Undifferentiated	288.5/268.8	19.7 ± 3.58 (11)	276.8/282.5	-5.7 ± 1.29 (10)
	Negative	198.8/176.5	22.3 ± 3.98 (13)	201.1/188.5	12.6 ± 3.13 (11)
	Positive	381.3/255.3	126 ± 17.17 (19) <sup>b</sup>	273.8/365.4	8.4 ± 1.09 (20)

Comparison before and after treatment, <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01.

ing the FAP regimen (5-fluorouracil, 300 mg/m<sup>2</sup> intravenous gutta for five consecutive days; adriamycin, 40 mg/m<sup>2</sup> for the first day), or the CODP regimens (cyclophosphamide, vincristine, daunorubicin, prednisone) (60 mg/m<sup>2</sup> intravenous gutta). Thirty-two patients were treated with the FAP regimen and traditional Chinese medicine. The powder of eight Noble Ingredients (ginseng, white atractylodes rhizome, Poria, *Pinellia*, 4 g each; tangerine peel and Fructus Ziziphi Jujubae, 2 g each; liquorice, 1 g; dried ginger, 0.5 g) was administered in 1-g doses three times per day. Pills of cinnamon twig and Poria (cinnamon twig, Poria, Moutan bark, peach seed, peony root, 4 g each) were administered in 3 g doses three times per day for 6 mo as one course of treatment. ODC levels were detected in specimens from the border between normal gastric mucosa tissues and tumor tissues using the Furihata method<sup>[1-3]</sup>.

In the two treatment groups, normal gastric mucosa near the anastomosis was biopsied and examined endoscopically in patients with no relapse 6 mo after surgery (10 patients without gastric cancer served as the normal control). The specimens were frozen in liquid nitrogen immediately and stored at -80 °C for ODC detection within 2 wk. The correlation was analyzed between each specimen, where the mean ODC value was detected twice.

## RESULTS

There were significant differences in the rate of relapse between the Western medicine group (three relapses) and Western medicine plus traditional Chinese medicine group (one relapse) within 6 mo. The ODC levels (CO<sub>2</sub> nmol·mg protein<sup>-1</sup>·h<sup>-1</sup>) of the normal controls was 83.2 ± 6.9 ( $\bar{x} \pm s$ ). The mean diameter of gastric cancer was 4.98 cm. The ODC levels in tumors with diameter < 4.9 cm and > 5.0 cm were 364.2 ± 47.3 and 598.8 ± 79.4, respectively. The ODC levels of early and advanced cancer, differentiated and undifferentiated tumors, and negative and positive lymph node metastases were 378.3 ± 49.5 and 563.4 ± 69.8, 441.3 ± 36.9 and 538.1 ± 77.3, and 358.5 ± 50.5 and 698.8 ± 151.2, respectively. There were no significant pre-treatment differences between the normal control (*P* > 0.05) and Western medicine groups (*P* > 0.05). Significant differences were found pre- and post-treatment in the Western medicine plus traditional Chinese medicine group (*P* < 0.01) (Table 1). There were no significant differences between the pre- and post-treatment gastric mucosa ODC levels in the Western medicine group. Significant post-treatment differences were found between the Western medicine group and the Western medicine plus traditional Chinese medicine group. The ODC changes in the normal gastric mucosa and related factors in the two groups before and after treatment are shown in Table 2.

Table 2 shows a significant correlation between the ODC levels of normal gastric mucosa and tumor diameter, infiltration depth, histological differentiation type, and lymph node metastasis. In the group using traditional Chinese medicine and Western medicine, the ODC

level was decreased in tumors with diameter < 4.9 cm, early gastric cancer, moderate differentiation, and positive lymph node metastases. The results suggest that traditional Chinese medicine remedies for enhancing resistance and relieving blood stasis against metastases in postoperative gastric cancer are correlated with reduced ODC activity.

## DISCUSSION

It has been proven that a close relationship exists between gastric cancer and the important mechanism of blood stasis at each stage of cancer formation. Our study showed that blood stasis in postoperative gastric cancer is similar to the high blood coagulation state in modern medicine (a condition where free cancer cells embed in the small blood vessels and infiltrate other tissues to form metastases foci). While these patients manifested blood stasis to a certain degree, their resistance was reduced because of the surgical damage. We used the powder of eight Noble Ingredients and cinnamon twig and Poria pills to treat the patients and tonify their *qi* (vital energy) and blood, promote blood circulation and remove blood stasis, and to strengthen body resistance to disease. Six months later, metastatic relapse was reduced and the ODC levels of the gastric mucosa were sharply reduced.

The results showed that the earliest biochemical reaction of human cells treated with carcinogenic agents is rapid and that there is significant induction of ODC activity. Acting with early mutagens, ODC obviously promotes metastases and malignancy. The ODC levels in the gastric cancer tissue and the adjacent normal gastric mucosa tissue in our patients were significantly higher than that in the controls. The extent of ODC increase was obviously correlated with tumor size, infiltration depth, degree of differentiation, and lymph node metastases. Six months later, the ODC levels were not significantly different in the group treated with only Western medicine, while there was a significant decrease of ODC levels in the group using Western medicine and traditional Chinese medicine. This suggests that the effect of traditional Chinese medicine against metastases in postoperative gastric cancer is related to the reduced ODC levels. However, how the anti-metastasis effect of traditional Chinese medicine in postoperative gastric cancer is yielded by reducing ODC activity awaits further studies.

## REFERENCES

- 1 Furihata C, Yoshida S, Sato Y, Matsushima T. Inductions of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by glandular stomach carcinogens. *Jpn J Cancer Res* 1987; **78**: 1363-1369 [PMID: 3123439]
- 2 O'Brien TG, Simsiman RC, Boutwell RK. Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor-promoting agents. *Cancer Res* 1975; **35**: 1662-1670 [PMID: 48421]
- 3 O'Brien TG. The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. *Cancer Res* 1976; **36**: 2644-2653 [PMID: 1277170]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX



Published by **Baishideng Publishing Group Inc**  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

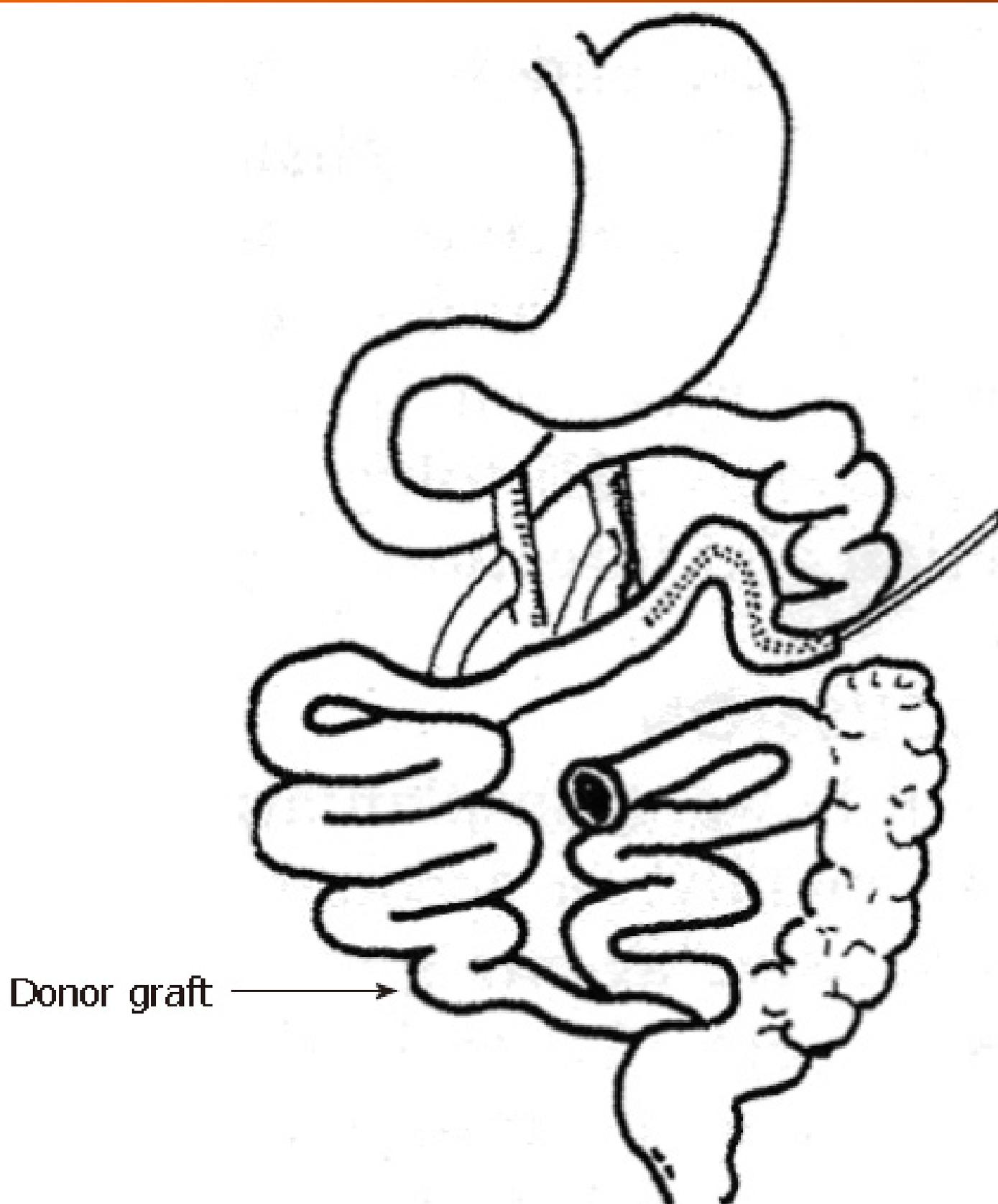


ISSN 1007-9327



# World Journal of *Gastroenterology*

*World J Gastroenterol* 1997 September 15; 3(3): 131-200



**COMMENTARY**

- 131 Evolution of surgical treatment of intrahepatic lithiasis in China  
*Huang ZQ, Huang XQ*

**ORIGINAL RESEARCH**

- 134 Ultrastructural observation of liver tissue ablation induced by high-intensity focused ultrasound  
*Cheng XQ, Zhou XD, Tang ZY, Yu Y, Bao SS, Qian DC*
- 137 Endoscopic monitoring in small bowel transplantation  
*Li YS, Li JS, Li N, Jiang ZW, Li YX, Li XH*
- 139 Construction of retroviral vectors to induce a strong expression of human class interferon gene in human hepatocellular carcinoma cells *in vitro*  
*Cao GW, Gao J, Du P, Qi ZT, Kong XT*
- 143 Hepatitis G virus infection in patients with chronic non-A-E hepatitis  
*Chang JH, Wei L, Du SC, Wang H, Sun Y, Tao QM*
- 147 Source of blood supply and embolization treatment in cavernous hemangioma and sclerosis of the liver  
*Li GW, Zhao ZR, Li BS, Liu XG, Wang ZL, Liu QF*
- 150 Survival and malignant phenotype changes of human hepatoma SMMC-7721 cell line induced by cryopreservation at -50 °C  
*Jiang SM, Xu ZH, Zhang Y, Shi XM*
- 153 Effects of Linomide on growth and metastasis of implanted human gastric cancer in nude mice  
*Tao HQ, Lin YZ, Yin HR, Gu QL, Zhu ZG, Yao M*
- 156 Loss of heterozygosity and mRNA expression at deleted in colorectal cancer gene locus in gastric cancer  
*Wang DX, Fang DC, Luo YH, Liu WW*
- 160 Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers  
*Wu GJ, Shan XN, Li MF, Shi SL, Zheng QP, Yu L, Zhao SY*
- 163 P21 and CEA expression and AgNOR counts in dimethylhydrazine-induced colon carcinoma in rats  
*Zhang ZG, Wu JY, Fu XD, Gu DK, Fang F*
- 166 Diagnostic value of occult fecal blood testing for colorectal cancer screening  
*Chen K, Jiao DA, Zheng S, Zhou L, Yu H, Yuan YC, Yao KY, Ma XY, Zhang Y*
- 169 Effects of metoclopramide on gastrointestinal myoelectric activity in rats  
*Qin XM, Li HF, Wang LD*
- 171 Characteristics of upper digestive tract diseases in Bohai Bay fishermen

Wang YB, Wang YP, Zou J, Bai BJ, Ren GC, Cai BQ

- 174 Relation between bile acids and myocardial damage in obstructive jaundice

Mu YP, Peng SY

### TRADITIONAL MEDICINE

- 177 Analysis of fibronectin, fibronectin receptor and interleukin-1 in patients with cirrhosis treated by the Yanggan Jieyu decoction

Wu H, Gao JS, Fan JZ, Huang J, Deng JW

- 180 Alterations of erythrocyte ATPase activity and oxygen consumption in patients with liver-blood deficiency syndrome

Shi LJ, Liu JF, Zhang ZQ, Lu YQ, Shu YG, Chen GL, Xin ZH, Xu JY

- 182 The effects of *Astragalus membranaceus* on oxygen consumption in the intestine

Li SZ, Tan XH

- 185 Ultrastructural observation of the gastric mucosa in chronic gastritis patients treated by traditional Chinese medicine

Zhang ZL, Bu JK, Zhao JX

- 189 Treatment of postoperative gastric cancer with the Fuzheng Huoxue anticancer prescription

Zhou AG, Huang DW, Ding YX, Jiang H, Tang ML

- 192 Effects of tetrandrine on gastric mucosa and liver in portal hypertensive rats

Mu Y, Shen YZ, Chu YF

### BRIEF REPORTS

- 159 Significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesions

Wu JF, Song YL, Yang GL, Dong YM, Wang DB, Liu MP

- 162 Clinicopathogenic studies of acute diarrhea in children

Cai LM, Zhang C, Chen H, Jiang WP, Mao WX

- 165 Double-bullet radioimmunotargeting therapy in 31 primary liver cancer patients

Wu YD, Zhou DN, Gang YQ, Hu XH, Li ZG, Song XQ, He HP, Yang KZ, Huang BY

- 168 Difference between periportal and pericentral Kupffer cells in lipopolysaccharide uptake in rats

Chen XM, Liu JC, Xu RL, Ma XH, Zhao YC, Han DW

- 176 Immunohistochemical study on endocrine-like tumor cells in colorectal carcinomas

Wang DC, Wang LD, Jia YY, Liu YQ, Feng CW, Tang FA, Zhou Q, Li ZF, Cui GL

- 179 Effects of electro-acupuncture on 5-HT, NOS and the gastric mucosa of stress rats

Zhu SL, Xu GS, Wang ZJ, Chen QZ, Jiao J

- 188 Characteristics of saliva secreted by patients with TCM-Piyinxu

Guan XZ, Wei MX, Chen DZ, Gu YC, Sun ZH, Bei SY

- 194 Cost-effectiveness study on treatment of duodenal ulcers

Chen SY, Wang JY, Chen J, Zhang XD, Zhang SS

- 195** Endoscopic ligation for benign and malignant lesions of the upper digestive tract  
*Chen YL, Chen YZ, Zou JX, Li XL*
- 196** Changes in mucosal permeability to lipopolysaccharide in the colon of chronic alcoholic rats  
*Chen XM, Xu RL, Ma XH, Zhao YC, Han DW*
- 197** Effects of Radix Rehmanniae on gastric acid secretion and gastric ulcer formation in rats  
*Wang ZL, Li L*
- 198** Detection method for peripheral venous AFP mRNA in hepatocellular carcinoma  
*Hu CJ, Yang DL*
- 199** Clinicopathological risk factors and prognostic evaluation in hepatocellular carcinoma recurrence after surgery  
*Dai YM, Chen H, Wang NJ, Ni CR, Cong WM, Zhang SP*
- 200** Endoscopic haemoclip ligation of pedunculated polyp before polypectomy  
*Wang YG, Binmoeller KF, Li ZL, Soehendra N*

**ABOUT COVER**

Li YS, Li JS, Li N, Jiang ZW, Li YX, Li XH. Endoscopic monitoring in small bowel transplantation. *World J Gastroenterol* 1997; 3(3): 137-138

**AIMS AND SCOPE**

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

**INDEXING/ABSTRACTING**

*World Journal of Gastroenterology* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central.

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Shan Hu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ze-Mao Gong*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Gastroenterology*

**ISSN**  
ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

**LAUNCH DATE**  
October 1, 1995

**FREQUENCY**  
Quarterly

**EDITORS-IN-CHIEF**  
**Bo-Rong Pan**, President of China Speciality Council of Gastrology and China Association of Huatuo Medicine, Room 12, Building 621, the Fourth Military Medical University, Xi'an 710033, Shaanxi Province, China

**Lian-Sheng Ma**, Member of the Speciality Committee of Digestive Diseases, Chinese Association of Combined Traditional Chinese and Western Medicine, Taiyuan Research and Treatment Centre for Digestive Diseases, Taiyuan 030001, Shanxi Province, China

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Gastroenterology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: bpgoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
September 15, 1997

**COPYRIGHT**  
© 1997 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_19970116143427.htm](http://www.wjgnet.com/bpg/g_info_19970116143427.htm)

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Evolution of surgical treatment of intrahepatic lithiasis in China

Zhi-Qiang Huang, Xiao-Qiang Huang

Zhi-Qiang Huang, Xiao-Qiang Huang, Department of Hepatobiliary Surgery, General Hospital of PLA, Beijing 100853, China

Zhi Qiang Huang, born in January 1922, Guangdong Province; graduated from Zhong Zheng Medical College; Professor of Surgery; engaged in hepatobiliary pancreatic surgery; having 11 books published.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Zhi-Qiang Huang, Professor, Department of Hepatobiliary Surgery, General Hospital of PLA, Beijing 100853, China  
Telephone: +86-10-66889871

Received: January 20, 1997  
Revised: March 15, 1997  
Accepted: April 20, 1997  
Published online: September 15, 1997

**Key words:** Cholelithiasis/surgery; Bile ducts, intrahepatic; Hepatectomy; Choledochostomy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Huang ZQ, Huang XQ. Evolution of surgical treatment of intrahepatic lithiasis in China. *World J Gastroenterol* 1997; 3(3): 131-133 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/131.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.131>

Cholelithiasis in China has undergone marked changes in its character since the 50 s, when the main features associated with biliary disease were recognized as biliary stones, infection, and parasitic infestation. However, at that time, cases of cholelithiasis accounted for only 40%-60% of the cases of biliary surgery, and 50% of them were associated with primary bile duct stones. This condition remained unchanged until the early 80 s. The Biliary Surgical Society of the Chinese Association of Surgery conducted a nationwide survey of 11342 surgical cases of cholelithiasis enrolled from 146 hospitals between 1983 and 1985. This survey revealed that the relative incidence was 52.8% for cholecystolithiasis, 11.0% for secondary common bile duct stones, 16.1% for intrahepatic duct stones, and 20.1% for extrahepatic bile duct stones. Thus, the relative incidence of primary bile duct stones (36.2%) had decreased. Ten years later, the survey was repeated in 1992, and the results showed a tendency of drastic decrease in the occurrence of bile duct stones, evidenced by the decrease to 5% in the relative incidence of intrahepatic bile duct stones.

However, intrahepatic bile duct stones are not completely eliminated in our country and in the Far East. A comparative survey by Nakayama (1986) revealed that the relative incidence of intrahepatic bile duct stone was 4.1% in Japan, while the relative

incidence of hepatic bile duct stone in Taiwan is reported to be the highest in the world (53.3%). However, in a retrospective review of cases of cholelithiasis from 28 hospitals in Taiwan during a 20-year period (1971-1990), Su *et al* found that intrahepatic bile duct stones accounted for 20.3% of 17182 surgical patients of cholelithiasis. This indicates that the incidence of intrahepatic lithiasis is slightly higher than that in the mainland of China.

Regional differences in the prevalence of intrahepatic bile duct stones in our country are remarkable. The incidence of intrahepatic bile duct stone among autopsy and clinical cases of cholelithiasis was 38% and 24.6%, respectively, in Chongqing; 18.7% and 31% in Chengdu and Luzhou, respectively; 43.1% in Shantou; and only 4.5% in Shanghai. It was low in the northwest and north of China (4.8% and 4.1%, respectively). A general tendency of decrease in the relative incidence of intrahepatic bile duct stones and reduction in the number of new patients diagnosed was noted. However, this change was not paralleled in different areas of the country; for example, in a 10-year survey (1981-1991) conducted in Guangxi Province, the relative incidence of gallbladder stones rose from 12.7% to 19.8% in the latter 5 years, while the incidence of bile duct stone decreased only from 55.2% to 41.8% during the same period. Therefore, intrahepatic bile duct stone was still a common disease that remains difficult to treat in many inland provinces, where the disease is not only disappearing, but also attracted more attention than before.

The basic principles of surgical treatment for intrahepatic bile duct stones are relief of obstruction, elimination of lesion, and adequate drainage. The key aspect of surgical treatment is "elimination of lesion". Intrahepatic bile duct stone is a condition with strictly intrahepatic segmental distribution; corresponding pathological changes have been reported to occur in different areas of the liver, *e.g.*, fibrosis, atrophy, and dysfunction. Huang ZQ (1957) advocated the use of planned hepatic lobectomy for treating intrahepatic bile duct stones and reported one case of left lateral hepatic lobectomy and another case of right hepatic lobectomy. Hepatic lobectomy has been gradually and widely been accepted as the treatment for intrahepatic bile duct stones, and it is now a common procedure for treating gallstones in the left lateral lobe of the liver. In a national survey on surgical therapy for intrahepatic bile duct stones occurring in 4197 patients, hepatic lobectomy was used for the treatment of 728 cases (17.3%). According to a recent report, hepatectomy rates ranged from 11% to 32% in 4 groups of surgical patients with hepatic bile duct stones, with the mean rate being 18%. The percentage of hepatectomy among the surgical procedures performed in cases of intrahepatic stones were 49.8% and 56.6% in two core hepatobiliary centers in the mainland and about 50% in Taiwan. However, as for the location of hepatectomy, 85% of the cases were of lateral left lobectomy and 95% were of left hepatic lobectomy, 10% and 17% were of right hepatic lobectomy in some hepatobiliary centers. A discrepancy was noted in the number of cases of right-sided hepatectomy between the location of hepatectomy and distribution of gallstones. Patients with intrahepatic bile duct stones often had serious

**Table 1** The surgical therapeutic outcomes for intrahepatic bile duct stones (1963~1975)

Surgery	n	A%	B%	C%	D%
Hepatic lobectomy	43	58.1	32.6	9.3	0.0
Cholangiojejunostomy	33	51.5	24.2	21.2	3.0
Cholangioduodenostomy	22	31.8	31.8	22.7	13.6
Choledocholithotomy	32	34.4	21.9	12.5	31.3
Total	130	46.2	27.7	15.4	10.8

complications before hospital admission, and the operation only provided symptom relief. Therefore, the rates of infection in the biliary tract, residual gallstones, and reoperation were high. Thanks to the development and popularization of modern imaging technology, *i.e.* ultrasound and computed tomography, early diagnosis has become possible for the patients with intrahepatic bile duct stones since the 1980s. Hepatolithiasis in early phase usually presents with mild symptoms or is asymptomatic; the symptoms of infection are also mild because of the use of antibiotics. Gallstones were often limited to 1-2 hepatic segments of the liver without involvement of extrahepatic bile duct as noted in these patients. Therefore, the concept of surgical therapy for intrahepatic lithiasis should now be modified. The surgical treatment for intrahepatic bile duct stones in its early phase should be "radical" rather than targeted at achieving symptom relief. It would be reasonable if hepatic segmental or subsegmental resection were selectively performed during the early phase of the condition. Intrahepatic bile duct stones in their early phase are often restricted to the posterior segment of the right lobe and laterosuperior segment of the left lateral lobe, without gallstones in the extrahepatic bile duct.

Stricture of hepatic bile duct is an important factor influencing the operative effect of intrahepatic bile duct stones, and its incidence is sometimes high. For example, among 3938 surgical patients with intrahepatic bile duct stones in this country, 956 (24.28%) had hepatic hilar bile duct stricture; but the corresponding rates were 41.94%, 41.76% and 40.17%, respectively, in Guangdong, Hunan and Sichuan provinces. Among patients who underwent reoperation, the incidence rate of hepatic bile duct stricture was proportional with the frequency of reoperation. The main causes of failure of surgical treatment for intrahepatic bile duct stones were incomplete correction of the stricture and presence of residual stones in the liver. Thirty-four patients in a group of 130 patients who received surgical treatment for intrahepatic stones and had been followed for an average of 8 years, did not give satisfactory results; in 82% of the cases, the failure was due to the presence of uncorrected hepatic bile duct strictures.

Strictures associated with intrahepatic bile duct stones often occur in the hepatic hilum and the left hepatic duct, which appear narrow and ring-like with dilatation of the bile duct on both ends or nearly normal appearance of the bile ducts. Therefore, the narrowing of the bile duct may be corrected by proper surgery. Necessary steps for correcting the stricture for hepatic bile duct include wide incision of the stricture site to make a new posterior wall by suturing the bile duct wall and repairing the anterior bile duct wall with a Roux en Y-jejunal loop, in addition to performing extensive cholangiotomy and cholangioenterostomy. To ensure complete removal of the hepatic lesion, hepatic lobectomy was performed simultaneously during the operation; this combined operation was called "combined surgery". If the hepatic bile duct stricture could be corrected, the surgical outcomes for intrahepatic bile duct stones could be significantly improved. For example, in a series of 107 patients with intrahepatic duct stones and hepatic duct stricture followed up for an average of 4.5 years, 87.8% of the patients obtained satisfactory results; however, two patients died at the end stage of the disease, yielding a mortality rate of 2%. Among the various surgical procedures, hepatic lobectomy achieved the best results, with the success rate being 93.0%. This indicates that the outcome of the main hilar duct stricture would not interfere with that of the intrahepatic bile duct stone if the case was managed effectively. However, if hepatic duct stricture remained untreated, the prognosis would be poor. For example, in a group of 14 cases with the stricture *in situ*, 3 died, thereby indicating a mortality rate

**Table 2** The different surgical outcomes of 80 cases intrahepatic duct stones (1975~1981)

Surgery	n	A%	B%	C%	D%
Hepatic lobectomy	23	26.1	56.5	13.0	4.3
Cholangiojejunostomy	43	27.9	48.8	18.6	4.6
Cholangioduodenostomy	7	14.3	57.1	28.6	0.0
Choledocholithotomy	7	57.1	42.8	0.0	0.0

of 21.5%.

If intrahepatic bile duct stones can be completely eliminated in patients with mild hepatic hilar duct strictures, pediculated tissues were selectively used to repair the bile duct defect after incision of the stricture. The tissues used are gallbladder wall, serosal patch of the stomach, jejunum, or round ligament flap with blood supply. The repair yielded good outcomes in the early phase, owing to the preservation of the function of Oddi's sphincter and decrease in retrograde biliary infection; however, it is still difficult to arrive at a final conclusion because the number of the patients treated in this manner was small and the follow-up period was short.

Occasionally, intrahepatic bile duct stones may be extensively distributed in the liver, and in such cases, to gain access to all the major hepatic ducts, the hilar bile duct may need to be widely incised to remove stones under the field of vision. Investigations in autopsied liver specimens and clinical experiences indicated that it is possible to make a wide incision linking the right anterior inferior hepatic bile duct, right hepatic duct, left hepatic duct, and left medial hepatic bile duct. Through this 5-8 cm-long incision, the openings of 2-3 grade intrahepatic ducts can be directly visualized. Sometimes the resection of the quadratus lobe may also be performed to further widen the operative field of the hilar biliary tract, thus making the operation easier and more perfect.

Biliary tract carcinoma complicated with gallstones is an important concern to be considered during the surgical treatment of intrahepatic bile duct stones. According to a national survey of 826 surgical cases of extrahepatic biliary carcinoma studied between 1977 and 1989, 140 (16.9%) cases were associated with gallstone. In another report on 4197 cases of intrahepatic bile duct stones, 14 (0.68%) cases were associated with biliary carcinoma. The incidence of coexisting biliary carcinoma with intrahepatic duct stones varied between 0.36% and over 10%; this variation may be attributed to the differences in the subjects involved, methods of diagnosis, and the length of follow-up. Sanes and McCallum (1942) first reported two cases of intrahepatic bile duct stones associated with biliary carcinoma. Subsequently, Koga and Chijiwa reported an incidence of 2% and 7.3% of biliary carcinoma in intrahepatic bile duct stones in Japan, respectively. A study from Taiwan indicated an incidence of 5.0%, where peripheral cholangiocarcinoma was detected during autopsy in 10% of the patients with intrahepatic bile duct stones. Cholangiocarcinoma may be discovered during surgery performed for intrahepatic bile duct stones or may develop several years after the operation; therefore, it is called delayed hepatic duct carcinoma. We investigated 6 cases of hilar cholangiocarcinoma coexisting with intrahepatic bile duct stones, accounting for 1.46% of the surgical cases of intrahepatic duct stones. Guo Hong Guang *et al* reviewed 12 cases of delayed hepatic duct carcinoma after the surgery for primary intrahepatic duct stones between 1981 and 1994, accounting for 1.5% of the operative cases of intrahepatic duct stones during the same period. Hepatic duct carcinoma was noted 3-40 years (average, 10 years) after the first biliary operation for hepatic duct stones. All the patients had history of biliary disease spanning 10-40 years, and 8 of them showed nothing remarkable in endoscopic retrograde cholangiopancreatography (ERCP) before the diagnosis of the carcinoma was established.

If the resected liver specimens for intrahepatic stones were examined, the incidence of associated hepatic duct carcinoma could be even higher. In six recently published reports in this country encompassing 661 cases of hepatic lobectomy for removal of intrahepatic duct stones, 16 (2.4%) of the cases were of cholangiocarcinoma. Chang Hai Hospital in Shanghai and the 47<sup>th</sup> Hospital of PLA reported hepatic cancer in 3.33% and 3.36%, respectively, of their resected liver specimens, while 3 (4.7%) of

**Table 3 The surgical outcomes of intrahepatic duct stones (Cai-Jing Xiu, 1983 ~ 1994)**

Surgery	n	A%	B%	C%	D%
Hepatic lobectomy	181	75.7	17.1	4.4	2.7
Hepatic lobectomy and cholangioenterostomy	192	58.3	33.0	7.3	2.0
Cholangioenterostomy	220	49.1	40.9	5.5	4.5
Choledocholithotomy	156	42.9	28.8	21.1	7.0
Total	749	56.6	30.4	8.6	4.0

63 cases of liver specimens exhibiting malignant changes at Queen Mary Hospital in Hong Kong. Chronic intrahepatic duct stones, biliary infection, and cholestasis can result in atypical hyperplasia of the epithelial mucosa of the hepatic bile duct, which may occur as part of precarcinomatous changes.

Surgical treatment of intrahepatic bile duct stones have improved over the last few decades. However, current treatment options are far from achieving complete cure, and problems such as residual stones, recurrence and progressive liver damage still await solution. At present, multiple surgical procedures for intrahepatic bile duct stones are in use, but they may be broadly classified under three main categories, namely, hepatic lobectomy, cholangioenterostomy, cholangiotomy with T-tube drainage. In fact, the above methods are often used in combination. Our experiences with various surgical techniques over 40 years of clinical practice can be chronologically arranged into three stages: 1963-1975, 1975-1981, 1983-1994. Since all the practice was centralized and carried out under a stable leadership, the stages were comparable (Tables 1-3). The success

rates (A + B) of the three stages were 73.9%, 80.1% and 87.05%, respectively. Unfortunately, only half the patients became completely asymptomatic after the operation, and the frequency of symptom recurrence might increase over a prolonged follow-up period.

The relationship between the therapeutic effect and different operative methods were affected by several factors. If the liver lesion was not removed by liver resection, the outcome of choledochoduodenostomy was usually poor, with patients frequently developing serious postoperative retrograde biliary infection.

Hepatic lobectomy has the best outcomes, with a long-term success rate of 91.16% among 439 cases. The better result of hepatectomy was confirmed by many other reports from our country. Hepatic lobectomy is usually combined with cholangioenterostomy to solve cholestasis. For example, 368 (81.3%) of 482 cases of hepatic lobectomy were associated with various cholangioenterostomies at the Chongqing Southwest Hospital. However, the patients with limited intrahepatic bile duct stones who were treated only by hepatic lobectomy gave better long term outcomes than the patients by combined cholangioenterostomy.

Although hepatic lobectomy may remove lesions in the liver, it does not eliminate the possibility of recurrent stones in the remnant portion of the liver; this was shown in the study conducted at Queen Mary Hospital in Hong Kong, which showed that 16% of the 63 cases of hepatic lobectomies had recurrent gallstones in a new area of the liver after a median follow-up period of 47 mo. Thus, the continuation of treatment even after hepatic lobectomy is necessary, and further investigations on the prevention of the recurrence of gallstones are imperative.

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Ultrastructural observation of liver tissue ablation induced by high-intensity focused ultrasound

Shu-Qun Cheng, Xin-Da Zhou, Zhao-You Tang, Yao Yu, Su-Su Bao, De-Chu Qian

Shu-Qun Cheng, Xin-Da Zhou, Zhao-You Tang, Yao Yu, Liver Cancer Institute, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032, China

Su-Su Bao, De-Chu Qian, Biomedical Ultrasound Laboratory, Shanghai Jiaotong University, Shanghai 200030, China

Shu Qun Cheng, MD and PhD, having 18 papers published, now being a medical postdoctor in the East Institute of Hepatobiliary Surgery, Second Military Medical University, China.

Author contributions: All authors contributed equally to the work.

Supported in part by the China Medical Board Grant #93-583, New York, and China Postdoctoral Science Foundation.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: **Shu-Qun Cheng, MD, PhD**, Liver Cancer Institute, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032, China  
Telephone: +86-21-65564166-72833

Received: November 1, 1996

Revised: January 8, 1997

Accepted: February 10, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To observe the ultrastructural changes of liver tissues on normal rabbit ablated by high-intensity focused ultrasound (HIFU).

**METHODS:** A single shot of 1.1 MHz focused ultrasound at an intensity of 500 W/cm<sup>2</sup> with 20-s duration of continuous exposure was applied intraoperatively in normal rabbit livers. Ultrastructural changes of the sonoablated lesion, as viewed by light and electron microscopy, were observed.

**RESULTS:** Liver cells at the center of the sonoablated lesion showed irreversible degeneration immediately after HIFU treatment; electron microscopy showed that although the liver cells appeared normal histologically, irregularly shaped cavities of about 0.3-0.5 μm in diameter were present in the cytoplasm.

**CONCLUSION:** Thermal damages may be the main mechanism of HIFU-induced ablation of liver tissues besides cavitation effect.

**Key words:** Liver/ultrastructure; Ultrasonic therapy; Eletron microscopy

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Cheng SQ, Zhou XD, Tang ZY, Yu Y, Bao SS, Qian DC. Ultrastructural observation of liver tissue ablation induced by high-intensity focused ultrasound. *World J*

*Gastroenterol* 1997; 3(3): 134-136 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/134.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.134>

### INTRODUCTION

High-intensity focused ultrasound (HIFU) is a new method of ablating non-invasively a selected volume of tissue at a certain depth within the body whilst sparing overlying tissues. This technique was originally developed as a method of selective brain tissue destruction in neurosurgical research<sup>[1]</sup>. Recently, it has been successfully applied in ophthalmology for the treatment of glaucoma<sup>[2]</sup> and is undergoing clinical trials for application in the prostate<sup>[3]</sup>. We have previously used this principle for the ablation of liver tumors in a rabbit model with encouraging results<sup>[4]</sup>. In this paper, the ultrastructural changes of the ultrasonic lesion in normal rabbit livers, as viewed by light and electron microscopy, are described.

### MATERIALS AND METHODS

#### HIFU instrument

The HIFU instrument developed by the Biomedical Ultrasound Laboratory, Shanghai Jiaotong University, is composed of the following: a signal generator, power amplifier, phase-control system, transducer motion control, microcomputer, and a transducer. The transducer had an array of 16 pistons distributed on the concave spherical surface and was 14 cm in diameter; it produced an acoustic beam with half-intensity axial and lateral dimensions of 10 mm and 8 mm, respectively, at a focal distance of 11 cm (Figure 1).

The energy was generated by the amplifier delivering 500 W/cm<sup>2</sup> at a frequency of 1.1 MHz. For experiments on the rabbit, the transducer was fixed to a motorized, three-dimensional coordinate system; this enabled a movement of 0.1 mm in accuracy. A microcomputer controlled the parameters of phase, power, emitting time, and mode.

#### HIFU treatment

Five New Zealand white rabbits, weighing 2.5 ± 0.24 kg, were anesthetized by administration of 3% pentobarbital (1 mL/kg of body weight, administered intravenously) and a subxiphoid midline abdominal incision was made. The hepatic lobe of the right center was exteriorized and exposed to the transducer hung by the motorized three-dimensional coordinate system at the focal point of the beam. A thin latex bag filled with thermostatical degassed water, which served as the acoustic coupling medium, was placed between the transducer and the liver. Then, a single shot of 20 s in the continuous wave mode was administered, followed by closure of the abdomen. The animals were allowed to recover. The surviving rabbits were killed at various intervals after HIFU treatment.

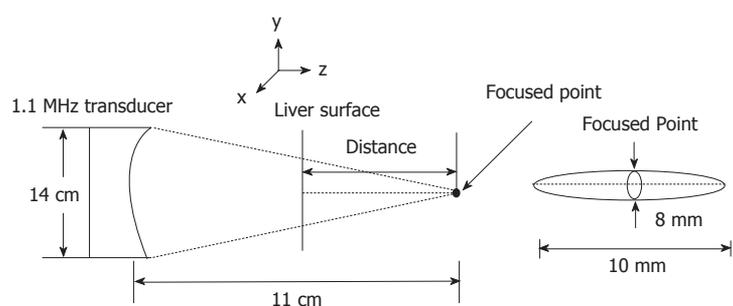


Figure 1 Beam characteristics of the 1.1 MHz high intensity focused ultrasound transducer.

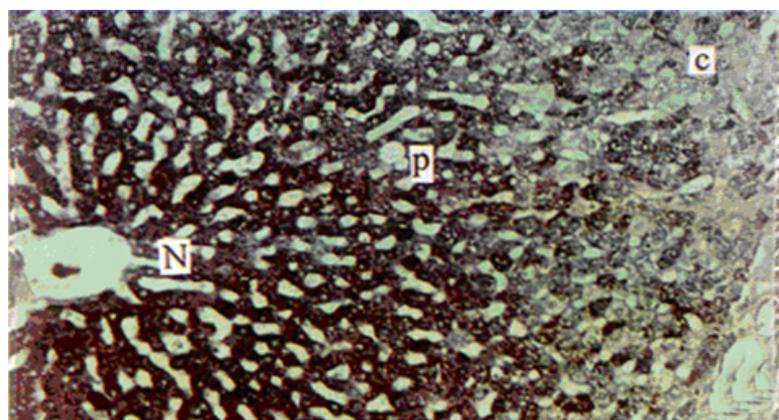


Figure 2 Light micrograph showing the cytoplasmic glycogen decreased in ablated lesion (c, p) immediately after high intensity focused ultrasound (HIFU) exposure by stained PAS. N = Normal liver; C = Lesion centre; P = Lesion edge.  $\times 66$ .

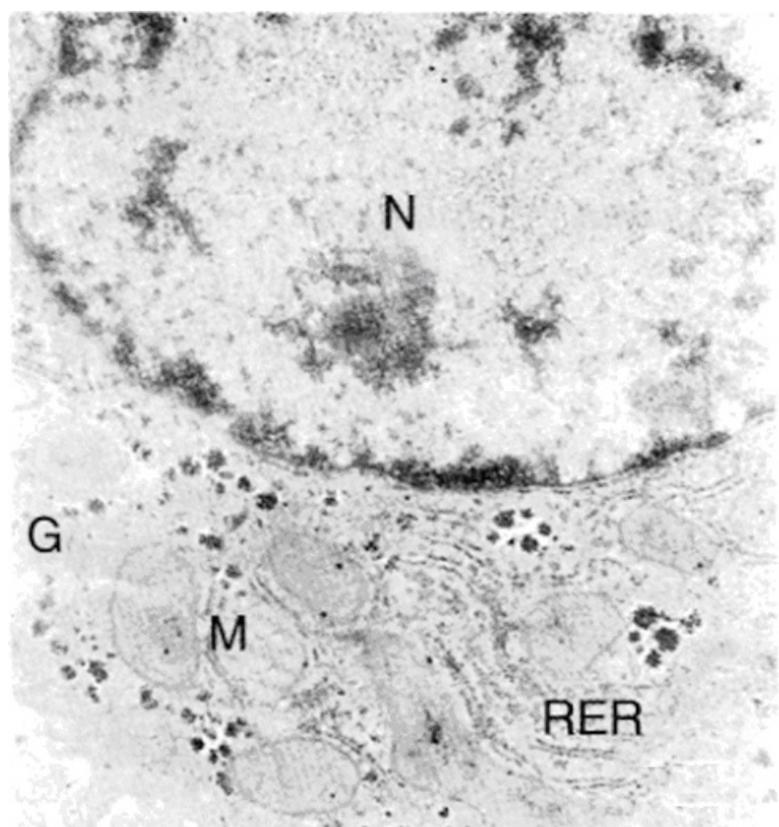


Figure 3 Electron micrograph of a normal rabbit hepatocyte. N = Nucleus; M = Mitochondria; RER = Rough endoplasmic reticulum; G = Glycogen.

#### Histological study

After the post-mortem excision of the liver, it was fixed immediately in 10% methacarn, embedded in wax, and thin sections were prepared. They were stained with hematoxylin and eosin (HE) and reticulin and periodic acid Schiff's base reaction (PAS).

#### Electron microscopy

Immediately after HIFU exposure, tissue samples in the centre and edge of the lesion (Figure 2) were placed in 2.5% glutaraldehyde, fixed overnight, and postfixed in 1% osmium tetroxide. The fixed

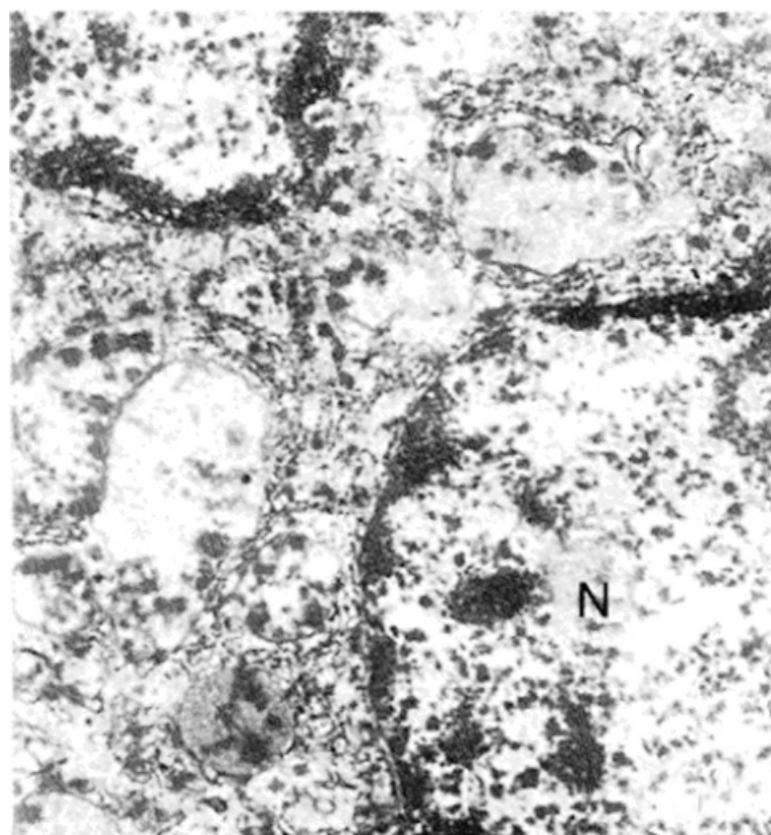


Figure 4 Electron micrograph from the edge region of the lesion immediately after HIFU treatment showing the destruction of organelles and massive vacuolization of the cytoplasm as a sign of irreversible cell death.

samples were dehydrated using a graded series of ethanol and acetone and were embedded in Epon 618 resin. Ultrathin sections were then cut, double-stained with uranyl acetate and lead citrate, and viewed under a JEM-1200EX transmission electron microscope.

## RESULTS

#### Histological findings

Immediately after HIFU exposure, a sharply defined, grey-white block with diameter 8 mm was noted in the liver. The liver cells showed enlargements and sinus showed stenosis on HE staining, but the liver plate still appeared regular and the cytoplasm and the nucleus appeared normal. PAS staining showed that the cytoplasmic glycogen had decreased but had not disappeared (Figure 2). After 24 h, the cells in this lesion died with cell debris, pyknotic nuclei, karyolysis, or karyorrhexis, and glycogen had almost disappeared completely on PAS stained lesions.

#### Electron-microscopic observation

An electron micrograph of a normal rabbit hepatocyte is shown in Figure 3, with a nucleus, mitochondria with intact cristae, glycogen, and rough endoplasmic reticulum. Immediately after insonation, the nucleus appeared irregular, but its membrane was still intact at the edge of the sonoablated lesion (Figure 4), the matrix of mitochondria was reduced and the cristae were disrupted. The ultrastructure of rough endoplasmic reticulum was disordered and some ribosome disappeared. Cavities (about 0.3-0.5  $\mu\text{m}$ ) were also found, with no glycogen and irregularly shaped holes in the cytoplasm. These cavities may be the result of mitochondria cavitation. Figure 5 shows that in the centre of the lesion, the nucleus was almost completely disrupted, and the integrity of the structure of mitochondria, the endoplasmic reticulum, and glycogen had disappeared. Again, there were 0.3-0.5  $\mu\text{m}$  cavities in the cytoplasm. The cellular ground substance was amorphous, with many electronic deposits; this indicated typical complete disruption and destruction of the cytoplasmic ultrastructure.

## DISCUSSION

In the previous study on histological changes in rabbit liver tumor

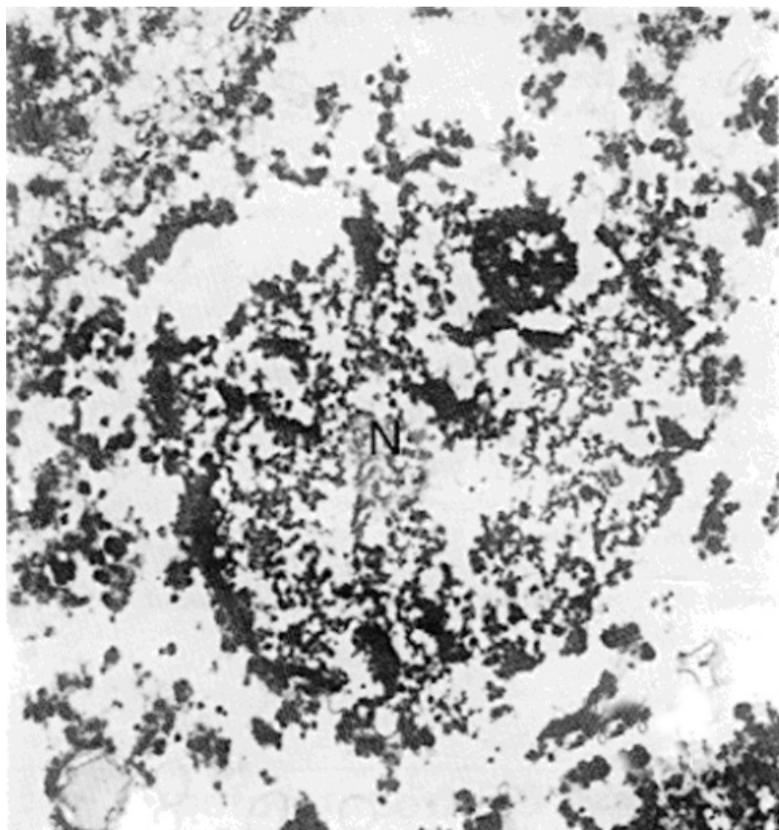


Figure 5 Electron micrography from the centre of the lesion, showing complete cellular disruption with karyorrhexis and many electronic deposits existed and cavities left.

treated with HIFU, we found that HIFU insonation at a peak intensity of 500 W/cm<sup>2</sup> for 20 s efficiently ablated the liver tumor, inducing coagulation necrosis 24 h later; however, evidence of prompt damage to the target area could not be clearly identified immediately after HIFU exposure<sup>[4]</sup>. With the same results, this study also revealed that the liver cells in the treated centre appeared histologically normal immediately after HIFU treatment administered at a similar intensity (1.1 MHz, 500 W/cm<sup>2</sup>, 20 s). However, these cells exhibited irreversible degeneration with disruption of the nuclear membrane and absence of mitochondria and endoplasmic reticulum, as observed by electron microscopy. It is indicated that coagulation necrosis of the targeted cells occurred as the last stage of the cell death, and that in fact, the liver cells were significantly damaged by HIFU insonation.

Although some recent reports have examined HIFU ablation of experimental liver tumors, few of them have focused on the resultant ultrastructural changes and the underlying mechanism, which remains to be fully determined<sup>[5]</sup>. Generally, it is believed that the two main mechanisms involved in tissue sonoablation are as follows: (1) a thermal effect (rapid elevation of local tissue temperature to over 80 °C) that promptly coagulates the cell protein and (2) a cavitation effect (generation of violently expanding and collapsing gaseous bubbles) that mechanically disrupts the cellular and tissue structure. Thermal damage is usually assumed to occur in association with long exposure

times of over 1 s and relatively low intensities (below 1000 W/cm<sup>2</sup>), while cavitation occurs with high-peak intensities and brief exposure periods. There is a range of levels of intensities and exposure periods for which both phenomena may not be distinctly identified<sup>[6,7]</sup>. In our previous study<sup>[8]</sup>, we found that the peak tissue temperature at the sonic focal region was > 90 °C, with the same energy; this thermal effect could provoke tissue destruction by coagulation necrosis. Therefore, thermal necrosis may play a dominant role in HIFU tumor ablation.

ter Haar *et al.*<sup>[9]</sup> used HIFU (1.7 MHz, 3000 W/cm<sup>2</sup>, 10 s) and performed a similar study in normal rat livers. They found that the cells in the rim of the ultrasonic lesion appeared histologically normal immediately after HIFU treatment; however, these cells were functionally impaired, for their cellular glycogen was missing. These findings are in agreement with our results. Moreover, cavities (about 4 μm) could be observed by electron microscopy, but it remains to be determined whether it is due to vaporization or acoustic cavitation. In our study, we also found many 0.3-0.5 μm cavities in the cytoplasm and deduced that it may be due to the cavitation of mitochondria or other organelles. Therefore, it is suggested that the cavitation effect may also play a significant role in inducing irreversible cell death immediately after HIFU treatment when applied under a relatively low-intensity condition.

Above all, our results show that the thermal effect and cavitation due to ablation probably occur simultaneously as the HIFU is directed at the liver tissues when the intensity is relatively low. This findings will be helpful in improving the focusing and selectiveness of liver cancer ablation extracorporeally if the cavitation could be much more enhanced, which worth investigating in the future.

## REFERENCES

- 1 FRY WJ, MOSBERG WH, BARNARD JW, FRY FJ. Production of focal destructive lesions in the central nervous system with ultrasound. *J Neurosurg* 1954; **11**: 471-478 [PMID: 13201985 DOI: 10.3171/jns.1954.11.5.0471]
- 2 Silverman RH, Vogelsang B, Rondeau MJ, Coleman DJ. Therapeutic ultrasound for the treatment of glaucoma. *Am J Ophthalmol* 1991; **111**: 327-337 [PMID: 2000903 DOI: 10.1016/S0002-9394(14)72318-9]
- 3 Foster RS, Bihrlé R, Sanghvi NT, Fry FJ, Donohue JP. High-intensity focused ultrasound in the treatment of prostatic disease. *Eur Urol* 1993; **23** Suppl 1: 29-33 [PMID: 7685694]
- 4 Cheng SQ, Zhou XD, Tang ZY, Yu Y, Wang HZ, Bao SS, Qian DC, Zhang HY. Histological changes in rabbit liver tumour treated with high-intensity focused ultrasound (in Chinese). *Chinese Journal of Experimental Surgery* 1996; **13**: 136-137
- 5 Prat F, Chapelon JY, Abou el Fadil F, Sibille A, Theillière Y, Ponchon T, Cathignol D. Focused liver ablation by cavitation in the rabbit: a potential new method of extracorporeal treatment. *Gut* 1994; **35**: 395-400 [PMID: 8150355 DOI: 10.1136/gut.35.3.395]
- 6 Yang R, Sanghvi NT, Rescorla FJ, Kopecky KK, Grosfeld JL. Liver cancer ablation with extracorporeal high-intensity focused ultrasound. *Eur Urol* 1993; **23** Suppl 1: 17-22 [PMID: 8513829]
- 7 Sibille A, Prat F, Chapelon JY, Abou el Fadil F, Henry L, Theillière Y, Ponchon T, Cathignol D. Extracorporeal ablation of liver tissue by high-intensity focused ultrasound. *Oncology* 1993; **50**: 375-379 [PMID: 8378034 DOI: 10.1159/000227213]
- 8 Cheng SQ, Zhou XD, Tang ZY, Yu Y, Wang HZ, Bao SS, Qian DC. Liver tissue ablation induced by high-intensity phased focused ultrasound: preliminary results of experimental study (in Chinese). *Chinese Journal of Ultrasound in Medicine* 1996; **12**: 1-4
- 9 ter Haar GR, Robertson D. Tissue destruction with focused ultrasound *in vivo*. *Eur Urol* 1993; **23** Suppl 1: 8-11 [PMID: 8513833]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Endoscopic monitoring in small bowel transplantation

You-Sheng Li, Jie-Shou Li, Ning Li, Zhi-Wei Jiang, Yuan-Xin Li, Xiao-Hua Li

You-Sheng Li, Jie-Shou Li, Ning Li, Zhi-Wei Jiang, Yuan-Xin Li, Xiao-Hua Li, Institute of General Surgery, PLA, Jinling Hospital, Nanjing 210002, Jiangsu Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: You-Sheng Li, MD, Surgeon in Charge, having 18 papers published, Institute of General Surgery, PLA, Jinling Hospital, Nanjing 210002, Jiangsu Province, China  
Telephone: +86-25-4403110Z

Received: August 15, 1996  
Received: October 20, 1996  
Received: November 10, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate the role of endoscopic monitoring in small bowel transplantation.

**METHODS:** This study was conducted in two parts—an initial experimental study followed by a clinical study. In the experimental study, segmental small bowel allotransplantation was performed on white outbred pigs. Stomas were created for exteriorization of the proximal and distal ends of the intestines (Thiry-Vella loop). The grafts were monitored by endoscopy *via* stomas, with or without immunosuppressive therapy. For the clinical study, the whole small-bowel allograft of a woman with short bowel syndrome was endoscopically monitored *via* distal stoma.

**RESULTS:** The most common endoscopic findings of graft rejection following small bowel allotransplantation were mucosal erythema, erosion, and ulceration. Diffuse ulceration with bleeding occurred in the late phase of rejection.

**CONCLUSION:** Endoscopic monitoring is essential to small bowel transplantation.

**Key words:** Small intestine/transplantation; Graft rejection; Endoscopy; Intestinal mucosa/pathology

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Li YS, Li JS, Li N, Jiang ZW, Li YX, Li XH. Endoscopic monitoring in small bowel transplantation. *World J Gastroenterol* 1997; 3(3): 137-138 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/137.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.137>

### INTRODUCTION

Graft rejection and infection are important causes of failure following

small bowel transplantation (SBT). Endoscopy of lesions and biopsy of graft are currently the most important modalities for the detection of signs of rejection or infections such as cytomegalovirus (CMV) infection. In this study, we investigate the role of endoscopic monitoring in SBT by a combined approach of both experimental and clinical studies.

### MATERIALS AND METHODS

Animals and experimental groups Twelve white outbred pigs weighing 18.5-22.5 kg were used in this study. General anesthesia was induced by administration of intravenous sodium pentobarbital. The animals were divided into two equal groups according to the presence or absence of immunosuppressive treatment: group I ( $n = 6$ ) with immunosuppression (cyclosporine, *Tripterygium wilfordii*, and methylprednisolone); and group II ( $n = 6$ ) without immunosuppression.

#### Operative procedure

Heterotopic segmental small bowel allotransplantation was performed as described previously<sup>[1]</sup>. The graft was perfused with and preserved in Ringer's lactate solution at 4 °C, followed by luminal perfusion with metronidazole solution at 4 °C. After a mean period of cold ischemia for 60 min, the segmental graft was vascularized. Both proximal and distal ends were exteriorized as stomas (Thiry-Vella loop) (Figure 1).

#### Endoscopic monitoring

After the transplant, the grafts were evaluated daily with endoscopic visualization and biopsy, and the biopsy specimens were examined under a standard light microscopy.

#### Clinical SBT

A woman with short bowel syndrome underwent whole small-bowel allotransplant (250 cm). The graft was orthotopically transplanted *via* end to side anastomosis of superior mesenteric artery aorta and end to side anastomosis of portal vein to superior mesenteric vein (Figure 2). Cyclosporine, *Tripterygium wilfordii*, and methylprednisolone were used as immunosuppressive agents. Biopsies guided or not guided by endoscopy were performed daily for 2 wk after the operation. From the 3<sup>rd</sup> to the 4<sup>th</sup> week, biopsies were performed every other or the third day. One month after the transplantation, endoscopy with biopsy was repeated. The biopsy specimens were examined under standard light microscopy.

### RESULTS

#### Experimental study

In group I, all animals had survived a long period of time, while those in group II had a survival period of  $17.7 \pm 7.6$  d. The cause of death in fatal cases was graft rejection. Thirty minutes after reperfusion, graft mucosal hyperemia and edema were seen. Mucosal erosion was seen on the first postoperative day (POD).

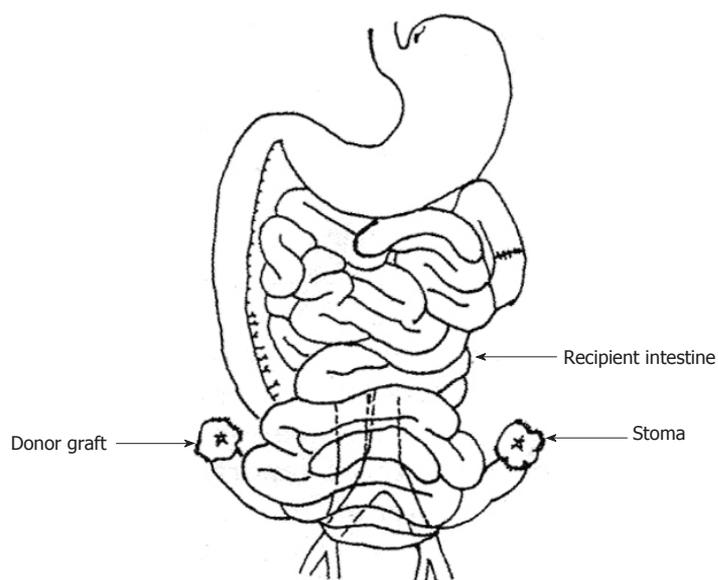


Figure 1 Heterotopic segmental small bowel allotransplantation in pigs.

Endoscopic and histologic features in the first 48 h were similar in both groups. Thereafter, in group I, graft mucosa was restored to normal appearance, except for occasional mucosal erosion and ulceration. On the other hand, group II showed punctate erythema on POD 4 or 5, local erosion or ulceration on POD 6 or 7, and diffuse ulceration with bleeding on POD 10. The histological features were necrosis and sloughing of villi tips, inflammatory cell infiltration, and intimal thickening or occlusion of vessels in submucosa.

#### Clinical study

From the 6<sup>th</sup> to the 8<sup>th</sup> month after the operation, eight endoscopic procedures with biopsies were performed. The endoscopic findings were graft mucosal hyperemia and edema on POD 6. Thereafter, endoscopic visualization showed absence of mucosal edema, glistening of the appearance of mucosa, presence of a normal vascular pattern, and increased peristalsis. Local ulceration with exudates was observed in the 3<sup>rd</sup> and the 5<sup>th</sup> month, respectively. Histological characteristics of graft rejection and infection were absent.

## DISCUSSION

Small bowel transplantation has become a clinical reality mainly because of the availability of highly effective immunosuppressive agents such as cyclosporine, FK506, and OKT3. However, control of post-transplant complications such as graft rejection and infection and other complications following SBT continues to remain challenging. Signs of rejection and infection have been traditionally monitored using the absorptive function test and evaluation of motor activity, permeability of bowel, and levels of various chemical markers. These methods, however, could not entirely replace endoscopy with biopsy, and histological evaluation of biopsy continues to remain the most reliable investigation for confirming small intestinal allograft rejection<sup>[1,2]</sup>.

In the clinical case of SBT, most of the operative procedures were similar to those in the experimental study (Figure 2). The graft distal stoma was used as the "window" for monitoring. Biopsy under naked eyes was slightly stimulative to the patients, but it was difficult to determine whether the specimens showed evidence of graft rejection. Specimens collected from sites adjacent to the stoma (< 10 cm) often showed signs of chronic inflammation, which are sometimes difficult to differentiate from those of rejection. Specimens obtained from the same site affected the diagnosis of rejection. In the clinical case of SBT, two biopsy samples taken from the colon did not show evidence of rejection, while two other biopsy samples observed under naked eyes showed characteristics suggestive of rejection histologically, but, endoscopically guided biopsies confirmed that rejection had not occurred. During the early stage of rejection, the mucosa shows local ulcer. Vascular characteristics were highly valuable in determining whether rejection

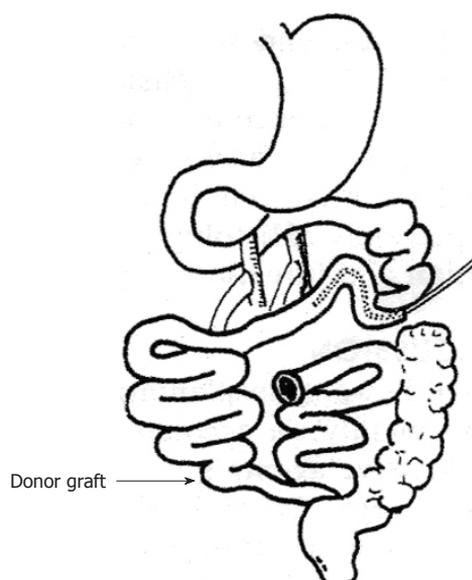


Figure 2 Whole small bowel allotransplantation in human.

has occurred. Therefore, it is important to perform full-thickness biopsies at several sites<sup>[1]</sup>.

The rate of graft rejection was 75% in the first month and 15% for the first three months<sup>[3]</sup>. Abu-Elmagd *et al*<sup>[3]</sup> recommended that endoscopy should be performed weekly during the first three postoperative months. The results of our experimental study showed that early mucosal injury was largely related to reperfusion injury and that early definitive appearances of graft rejection were present on POD 5 or POD 6. Therefore, endoscopic visualization and biopsies of graft should commence on the 5<sup>th</sup> POD. In addition to routine regular endoscopic visualization, the endoscopic procedure should be repeated if any symptoms of rejection develop, including fever, abdominal pain, nausea, vomiting, watery diarrhea, increase in stromal output, and dusking of mucosa. In the study by Abu Elmagd *et al*<sup>[3]</sup>, the endoscopic appearances during acute rejection episode were ischemia or duskiness, local ulcer, and decrease or complete loss of peristalsis. Grafts with severe rejection showed diffuse ulceration with bleeding. Chronic graft rejection was evidenced by pseudomembrane formation, thickening of mucosal folds, and chronic ulceration. Hassanein *et al*<sup>[2]</sup> evaluated more than 220 endoscopic procedures and reported that during acute rejection mucosal features were mucosal erythema (77.8%), erosion (38%), and ulceration (33%). CMV infection appeared as local ulceration. In this study, graft rejection did not occur in the clinical case of SBT, and, the results of the experimental endoscopic visualization for signs of acute graft rejection were similar to those reported by Abu Elmagd *et al*<sup>[3]</sup> and Hassanein *et al*<sup>[2]</sup>.

The findings of this study highlight the importance of endoscopic visualization with biopsy in the monitoring of cases of SBT for signs of graft rejection. However, the criteria for small intestinal graft rejection are yet to be definitively established, and the differentiation between rejection and CMV infection remains difficult. Mucosal erosion and ulceration are not characteristic of small intestinal graft rejection. However, in this study, mucosal ulceration was observed in the 3<sup>rd</sup> and 5<sup>th</sup> postoperative month in clinical SBT, but no clinical findings or histological features of graft rejection were observed. Because of the patchy distribution of the evidences of rejection, we recommend that multiple endoscopic visualization and biopsies be repeated at different sites for thorough evaluation.

## REFERENCES

- 1 Li YS, Li JS, Li N, Liao CX, Wu XH, Li NY. Histological and ultrastructural changes of segmental small bowel transplantation in the swine. *Bull Jinling Hospital* 1994; 7(1): 53-56
- 2 Hassanein T, Schade RR, Soldevilla-Pico C, Tabasco-Minguillan J, Abu-Elmagd K, Furukawa H, Kadry Z, Demetris A, Tzakis A, Todo S. Endoscopy is essential for early detection of rejection in small bowel transplant recipients. *Transplant Proc* 1994; 26: 1414-1415 [PMID: 8029962]
- 3 Abu-Elmagd KM, Tzakis A, Todo S, Reyes J, Fung J, Nakamura K, Wright H, Furukawa H, Demetris J, Van Thiel DH. Monitoring and treatment of intestinal allograft rejection in humans. *Transplant Proc* 1993; 25: 1202-1203 [PMID: 7680150]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Construction of retroviral vectors to induce a strong expression of human class interferon gene in human hepatocellular carcinoma cells *in vitro*

Guang-Wen Cao, Jun-Gao, Ping Du, Zhong-Tian Qi, Xian-Tao Kong

Guang-Wen Cao, Jun-Gao, Ping Du, Zhong-Tian Qi, Department of Microbiology, Second Military Medical University, Shanghai 200433, China

Xian-Tao Kong, Department of Experimental Medicine, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

Guang Wen Cao, Associate Professor, having 36 papers and 2 books published, Director of the Division of Tumor & Virus Gene Therapy, Department of Microbiology, Second Military Medical University, Shanghai 200433, China

Author contributions: All authors contributed equally to the work.

Supported by the National Natural Science Foundation of China (No:39500147, 39600172).

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Guang Wen Cao, Associate Professor, Department of Microbiology, Second Military Medical University, Shanghai 200433, China  
Fax:86-21-65490555

Received: February 7, 1997

Revised: April 10, 1997

Accepted: May 15, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To establish the hepatoma cell-specific expression of human interferon (IFN) gene mediated by retroviral vectors

**METHODS:** Human interferon  $\alpha$  and interferon  $\beta$  complementary DNA (IFN cDNA) were cloned into the polylinker site of pMNSM retroviral vector to construct recombinant retroviral vectors pMNSIFNA and pMNSIFNB, with the transcription of IFN gene being driven by Simian virus 40 early region promoter (SV40) early region promoter. IFN cDNAs were also cloned into pMNAIFNA, pAMNSIFNA, and pMNAIFNB, with the transcription of IFN gene being driven by SV40 early region promoter regulated by  $\alpha$ -fetoprotein enhancer. Next, the retroviral constructs were introduced into retroviral amphotropic packaging cells using the lipofectamine-mediated gene transfer procedure. The rate of plasmid transfection was  $(4-40) \times 10^3$  colonies/ $\mu$ g DNA/ $10^6$  PA317 cells. The rate of retrovirus infection was  $(5-500) \times 10^4$  colony forming units (CFU)/mL. Further, the recombinant retroviruses were used to infect human hepatoma cells, renal carcinoma cells, and melanoma cell lines in the presence of 4  $\mu$ mg/L polybrene.

**RESULTS:** Northern and Dot hybridization of total RNA from the neomycin-resistant colonies and IFN expression assay indicated that human  $\alpha$  fetoprotein enhancer induced efficient and specific transcription and expression of IFN genes driven by the promoter of

different origins in human hepatoma cells, leading to high production of  $\alpha$  fetoprotein.

**CONCLUSION:** Cis active element of  $\alpha$ -fetoprotein gene can drive specific expression of IFN genes in human hepatoma cells, which provides some valuable data for the hepatoma-specific immune gene therapy.

**Key words:** Interferon alpha; Interferon beta; Retroviridae; Carcinoma, hepatocellular; Liver neoplasms; Genes, regulatory; Gene expression; Gene therapy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Cao GW, Gao J, Du P, Qi ZT, Kong XT. Construction of retroviral vectors to induce a strong expression of human class interferon gene in human hepatocellular carcinoma cells *in vitro*. *World J Gastroenterol* 1997; 3(3): 139-142 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/139.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.139>

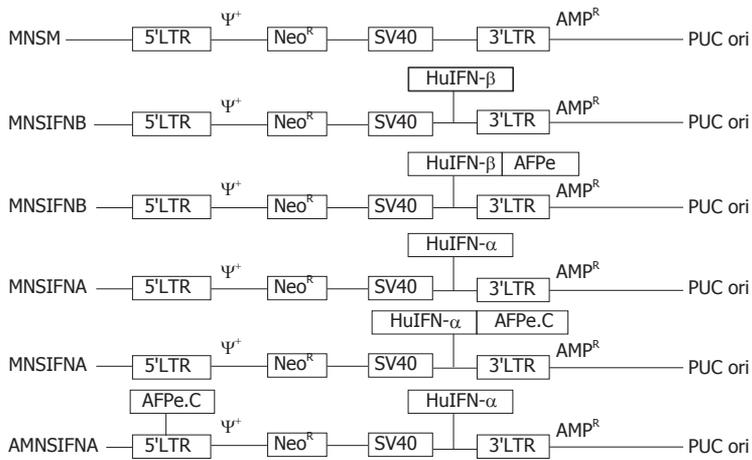
### INTRODUCTION

Class I interferon (IFN) is a potent immune-modulating factor *in vivo* that plays an important role in activating anti-tumor cytotoxic T lymphocytes and non-specific effector cells such as natural killer (NK) cells<sup>[1]</sup>. IFN genes and many other cytokine genes have been introduced into tumor cells, tumor interstitial cells, and tumor-infiltrating lymphocytes<sup>[2-4]</sup> in order to sustain an effective anti-tumor concentration of cytokine located at tumor sites. It has been shown that some cytokine-gene-modified tumor cells lost their tumorigenicity and inhibited the growth of parental tumor in animal models. Practical antitumor effects were obtained in the protocol. The morbidity of hepatoma is relatively high in China, and no effective procedure is currently available for the treatment of advanced hepatoma. In this study, hepatoma-specific IFN retroviral vectors were prepared and *in vitro* expression assays were conducted to establish an effective gene therapy against hepatoma and prevent the possible adverse effects of class I INF expression in non-tumoral tissues in addition to verifying the possibility of the enhancer of  $\alpha$ -fetoprotein (AFP) "house-keeping gene" regulating viral promoter in retroviral vector.

### MATERIALS AND METHODS

#### Plasmids

Plasmids pCGS261 containing human leukocyte interferon (IFN- $\alpha$ ) cDNA were obtained from the American Type Culture Collection (ATCC), (Rockville, United States). pSPGNHIFNB10 carrying the HuIFN- $\beta$ -cDNA was purchased from LMBP (Gent University,



**Figure 1 Structure of MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA retroviral vectors.** LTR: Retroviral long-terminal repeat sequence; Neo R: Neomycin phosphotransferase gene for G418 selection; SV40: Simian virus 40 early region promoter; HuIFN- $\beta$ -cDNA: Human interferon  $\beta$  complementary DNA; HuIFN- $\alpha$ -cDNA, Human interferon  $\alpha$  complementary DNA. AFPe: Human  $\alpha$ -fetoprotein "house-keeping gene" enhancer; AFPe.c, Human  $\alpha$ -fetoprotein "house-keeping gene" enhancer core sequence.

Belgium). Plasmid pAF5.1-CAT containing the AFP enhancer and plasmid pGEM7z-AFPe containing human AFP enhancer core sequence were kindly provided by Professor Tamaoki (Calgary University, Canada). Retroviral vector pMNSM containing the neomycin phosphotransferase and the SV40 early region promoter was a gift from Dr. Tsuchiya (Tokyo Medical and Dental University, Japan).

### Cells

Amphotropic retrovirus packaging cell line PA317, retrovirus titrating cell line NIH3T3TK, human hepatoma cell lines HepG2 and Hep3B, and IFN bioassay cell line WISH were obtained from ATCC. Retroviral packaging cell Psi2 was procured from Dr. Shigeki Kuriyama. Human hepatoma cell line SMMC7721 and human melanoma cell line M21 were obtained from Chinese Academy of Sciences. Human renal carcinoma cell line RZ94602 was established at our laboratory.

### Main reagents

TdR, dNTP, polybrene, PEG8000, guanidine isothiocyanate, PVP2500, and MTT were from Sigma. New generation liposome transfection reagent LIPOFECTAMINE<sup>TM</sup>, RPMI-1640, DMEM, FCS, random primers DNA labeling system, and G418 were from Gibco. Herring sperm DNA, restriction enzymes, T4 ligase, DNA polymerase Klenow fragment, and CIP were from Promega.  $\alpha$ -<sup>32</sup>P-dCTP was from Beijing Yuhui Biomedical Co. Nitrocellulose membrane was from Amersham.

### Construction of retroviral vectors

Plasmids extraction with alkali lysis, enzymes digestion, ligation, isolation and harvest of DNA fragments and transformation of *Escherichia coli* was carried out according to the standard methods<sup>[5]</sup>.

The procedures of construction are as follows. The plasmids pSPGNHIFNB10, pCGS261, pAF5.1-CAT, pGEM7z AFPe, and pMNSM were identified with enzyme digestion. pMNSM was linearized with *Hind* III/*Bam*HI digestion. pSPGNHIFNB10 was digested with *Hind* III/*Bam*HI to release 1.06-kb IFN- $\beta$  cDNA. IFN- $\beta$  cDNA was linked to the linearized pMNSM to construct pMNSIFNB. pAF5.1-CAT was fully digested with *Aat* II/*Spe* I/*Bam*HI to release 5.5-kb human AFP enhancer sequence and blunted with Klenow. pMNSIFNB was linearized with *Eco*R I digestion and blunted with Klenow. The enhancer was linked with the linearized pMNSIFNB to construct pMNAIFNB. pMNSIFNA and pMNAIFNA were constructed using the above construction procedure with some modifications. The human IFN- $\beta$  gene was replaced with human IFN- $\alpha$  gene, and 5.5-kb AFP enhancer sequence was replaced with 0.7-kb AFP enhancer core sequence. As for the construction of pAMNSIFNA, an 0.7-kb AFP enhancer core sequence was inserted into the long terminal repeat (LTR) region of the retroviral vector. Then, 1.1-kb human IFN- $\alpha$  gene was cloned into the polylinker site of pMNSM and under transcriptional control of SV40 early region promoter.

The identity of all the retroviral constructs were confirmed by restricted digestion.

### Lipofectamine-mediated gene transfer

Packaging cells were pretreated with 2  $\mu$ g/L TdR to induce simultaneous cell division. Psi2 cells were transfected with the retroviral constructs by using the lipofectin-mediated gene transfer procedure, which was performed as per the manufacturer's instructions. After full selection with G418, the supernatant of the modified Psi2 cells was harvested, tittered, and used to infect PA317 cells. Measurement of plasmid transfection and retrovirus infection rates was carried out according as described previously<sup>[6]</sup>.

### Expression assay of IFNs

Northern blot and dot hybridization were performed using a previously described method<sup>[5]</sup>. The IFN bioactivities in the supernatant of 10<sup>6</sup> modified cells were calculated per 24 h. IFN bioassay was performed according to the routine procedure of our laboratory.

## RESULTS

### Construction of the retroviral vectors MNSIFNB and MNAIFNB

All the retroviral constructs are outlined in Figure 1. IFN genes in pMNSIFNB and pMNSIFNA were controlled by the SV40 early region promoter. IFN genes derived by SV40 early region promoter in pMNAIFNB and pMNAIFNA were controlled by [WTBZ] AFP enhancer. Then, 0.7-kb AFP enhancer core sequence was inserted into the LTR region in order to inactivate the intrinsic enhancer element located in the region and diminish the intervention of the intrinsic enhancer on AFP enhancer.

### Preparation of recombinant retroviruses

PA317 cells with division synchronized with TdR pretreatment were infected with the supernatants of Psi2 cells containing MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA recombinant retroviruses, and the infection rate of the retroviruses in the PA317 supernatants harvested on the third day after the infection were 5.0  $\times$  10<sup>6</sup>, 6.0  $\times$  10<sup>5</sup>, 4.5  $\times$  10<sup>4</sup>, 8.0  $\times$  10<sup>6</sup>, 1.0  $\times$  10<sup>6</sup>, and 5.0  $\times$  10<sup>6</sup> CFU/mL, respectively. Psi2 cells were transfected with the retroviral constructs by using a lipofectamine-mediating procedure. The modified Psi2 cells were split 1/20 onto medium containing 400  $\mu$ g/L active G418. After 10 d selection, the corresponding transfection rates were as follows: 2.5  $\times$  10<sup>4</sup>, 8.5  $\times$  10<sup>3</sup>, 4.0  $\times$  10<sup>3</sup>, 2.3  $\times$  10<sup>4</sup>, 3.6  $\times$  10<sup>4</sup>, and 3.4  $\times$  10<sup>4</sup> CFU/ $\mu$ g DNA/10<sup>6</sup> PA317 cells. The greater the genome of the vectors, the lower the infection and transfection rates. However, all the highest retroviruses producing PA317 colonies of 6 different groups could produce 10<sup>7</sup> CFU recombinant retroviruses per 10<sup>6</sup> PA317 cells per 24 h. After being frozen in liquid nitrogen for one month, the modified PA317 did not show any significant decrease in the retrovirus production (P > 0.05)

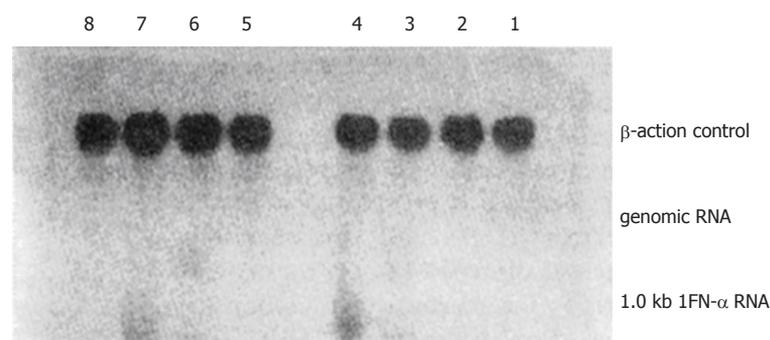
### Transcription of IFNs cDNA in the modified tumor cells

MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA retroviruses were used to infect the tumor cells *in vitro* in the presence of 4  $\mu$ g/L polybrene. The total RNA from the infected HepG2 and Hep3B hepatoma cells and M21 melanoma cells, after being fully selected with G418, were extracted by the guanidine isothiocyanate single-step methods. Total RNAs from MNSM, MNSIFNA, MNAIFNA, and AMNSIFNA modified tumor cells were electrophoresed on a 1% agarose/2.2 M formaldehyde gel and transferred onto nitrocellulose. Blots were hybridized with <sup>32</sup>P-labeled HuIFN- $\alpha$ -cDNA probe. Total RNAs from MNSM, MNSIFNB, and MNAIFNB modified tumor cells were subjected to 5-fold serial dilution from 10  $\mu$ g, transferred onto the nitrocellulose membrane, and probed with IFN- $\beta$ -cDNA. The result is shown in Figures 2 and 3. In the hepatoma cell line HepG2, which produces high levels of AFP, the transcription level of IFNs cDNA, after infection with MNAIFNB, AMNSIFNA, and MNAIFNA retroviruses, was significantly higher than that of the cells infected with MNSIFNB and MNSIFNA retroviruses. In the Hep3B cells, which produce

**Table 1** Human interferon gene expression in supernatants of gene modified human tumor cells at 20<sup>th</sup> day after infection with recombinant retroviruses ( $\bar{x} \pm s$ )

Cell lines	Origin	AFP <sup>1</sup>	Genetic alteration	G418 resistant	Interferon bioactivity (unit/10 <sup>6</sup> cells·24 h)
HepG2 (n = 5)	Human hepatocyte	2044	Parental <sup>2</sup>	-	0
			MNSM	+	0
			MNSIFNB	+	218 ± 102
			MNAIFNB	+	818 ± 35
			MNSIFNA	+	128 ± 36
			MNAIFNA	+	808 ± 180
			AMNSIFNA	+	1200 ± 36
			Parental	-	0
Hep3B (n = 4)	Human hepatocyte	311	Parental	-	0
			MNSM	+	0
			MNSIFNB	+	156 ± 48
			MNAIFNB	+	462 ± 104
			MNSIFNA	+	136 ± 42
			MNAIFNA	+	548 ± 120
			AMNSIFNA	+	465 ± 204
			Parental	-	0
SMMC7721 (n = 5)	Human hepatocyte	ND	Parental	-	0
			MNSM	+	0
			MNSIFNB	+	280 ± 80
			MNAIFNB	+	580 ± 56
			MNSIFNA	+	242 ± 120
			MNAIFNA	+	468 ± 84
			AMNSIFNA	+	524 ± 132
			Parental	-	0
M21 (n = 5)	Human melanocyte	ND	Parental	-	0
			MNSM	+	0
			MNSIFNB	+	324 ± 66
			MNAIFNB	+	21 ± 10
			MNSIFNA	+	226 ± 128
			MNAIFNA	+	42 ± 36
			AMNSIFNA	+	0 ± 2.4
			Parental	-	0
RZ94602 (n = 4)	Human renal cell	ND	Parental	-	0
			MNSM	+	0
			MNSIFNB	+	280 ± 64
			MNAIFNB	+	18 ± 8.4
			MNSIFNA	+	320 ± 120
			MNAIFNA	+	24 ± 48
			AMNSIFNA	+	0 ± 2.4
			Parental	-	0

<sup>1</sup>Expressed as ng secreted per mg of cell protein per 4 d, based on historical data<sup>[8]</sup>; <sup>2</sup>Unmodified parental tumor cell lines; ND: Undetermined

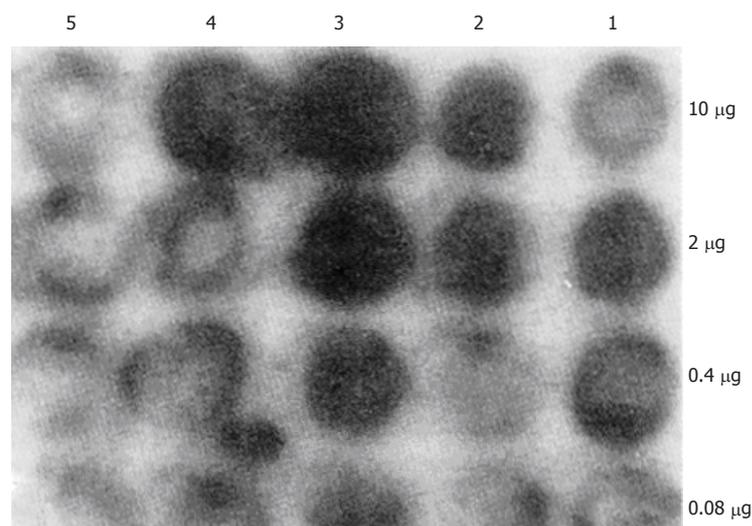


**Figure 2** Northern blot of total RNA from the tumor cells modified with human interferon- $\alpha$  gene, probed with <sup>32</sup>P labeled human interferon- $\alpha$  cDNA. Lane 1: Unmodified HepG2 cells; Lane 2: MNSM modified HepG2 cells; Lane 3: MNSIFNA modified HepG2 cells; Lane 4: MNAIFNA modified HepG2 cells; Lane 5: MNSIFNB modified HepG2 cells; Lane 6: MNSIFNA modified HepG2 cells; Lane 7: AMNSIFNA modified HepG2 cells; Lane 8: MNAIFNB modified HepG2 cells.

intermediate levels of AFP, the difference in the IFN- $\beta$ -cDNA transcription level between the two infection groups was not so significant as that in the HepG2 cells, indicating that the enhancing effect of the AFP enhancer on the SV40 early region promoter in the genome of the hepatoma cells correlated with the level of AFP produced in the hepatoma cells.

#### Expression of interferon in the gene-modified tumor cells

HepG2, Hep3B, SMMC7721, RZ94602, and M21 tumor cells were infected with MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA retroviruses, respectively, and fully selected with G418. The bioactivity of IFNs secreted by 10<sup>6</sup> gene-modified tumor cells per 24 h was precisely determined with MTT measurement and CpEI<sub>50</sub> method. The results are shown in Table 1. In the human hepatoma cell line HepG2, which produces high levels of AFP, an AFP enhancer significantly augmented the expression of IFNs gene induced by SV40 early region promoter. Specific expression of IFNs gene in the Hep3B cell line, which produces intermediate AFP levels, was not as significant as that in HepG2 cells. IFN gene expression in the M21



**Figure 3** Dot blot of total RNA from human tumor cells modified with HuIFN- $\beta$  gene, and fully selected with G418, probed with <sup>32</sup>P labeled HuIFN- $\beta$ -cDNA. Lane 1: Hep3B cells infected with MNAIFNB retroviruses; Lane 2: Hep3B cells infected with MNSIFNB retroviruses; Lane 3: HepG2 cells infected with MNAIFNB retroviruses; Lane 4: HepG2 cells infected with MNSIFNB retroviruses; Lane 5: M21 cells infected with MNSIFNB retroviruses.

and RZ94602 cell lines, which did not produce AFP, was inhibited after infection with MNAIFNB, MNAIFNA and AMNSIFNA retroviruses.

## DISCUSSION

*In vivo* gene therapy for cancer is more practicable than the *ex vivo* protocol in clinical trials. As for the *in vivo* protocol, the major problem is to induce the guaranteed specific expression of the gene of interest in tumor cells. *In vivo* gene therapy can be guaranteed if the transcription regulatory sequences (TRS) of "house-keeping gene" of the tumor characteristic proteins are employed to regulate gene expression. First, high expression levels of the gene of interest can be achieved if the gene is controlled by the TRS. The

concentration of interferon or expression of IFN gene at the tumor location is correlated with the antitumor effect<sup>[1]</sup>. Second, possible microenvironmental disorders caused by the expression of Class I IFN gene in non-tumoral tissues can be prevented. This is of great significance in cancer gene therapy.

Human IFN  $\alpha$  and IFN  $\beta$  bind to the same receptors and are called class I interferon. Class I IFN plays an important role *in vivo* in tumor immunosurveillance and tumor biotherapy. In the present study, human IFN  $\alpha$  and IFN  $\beta$  genes were individually introduced into the established hepatoma, renal cell carcinoma, and melanoma cell lines by means of the retrovirus. Expression of IFN genes, under the transcription control of AFP enhancer, was in the hepatoma-cell-specific manner, and correlated with the AFP production levels in the hepatoma cells. Some transcription regulatory proteins existing in the nuclei of the hepatoma cells are believed to be related to the hepatoma-specific gene expression. In the AFP-producing hepatoma cells, the transcription regulatory sequence of AFP "house-keeping gene" is controlled by the tissue-specific transcription regulatory proteins. After the transcription regulatory sequence carried by the retroviruses is integrated into the genome of the hepatoma cells, the regulatory proteins could also activate AFP enhancer in the same manner. Since enhancer is not dependent on origin and orientation, AFP enhancer may transactivate the promoter of viral origin and increase the transcription rate of the promoter in certain cell types.

Promoter interference between heterologous promoter and retroviral promoter has been documented. Retroviral LTR promoter and enhancer may silence some internal heterologous promoters<sup>[7]</sup>. In this study, construction of pAMNS-IFNA was carried out by the insertion of AFP enhancer core sequence into retroviral LTR region in order to silence LTR enhancer activity and increase the hepatoma cell-specific expression of human IFN- $\alpha$  gene. However, the hepatoma cells-specific expression was not significantly increased after the treatment. It was demonstrated that enhancer elements in retroviral LTR region did not significantly interfere with the function of AFP "house-keeping" gene enhancer.

Tumor-tissue-specific gene therapy is carried out in the thymidine kinase prodrug transformation gene therapy, but not in cytokine gene therapy and other immune gene therapies. It has been recently demonstrated that the transcription regulatory sequences

of AFP, carcinoembryonic antigen, and tyrosinase "house-keeping gene" regulated the thymidine kinase gene specifically expressed in the hepatocellular carcinoma, gastric cancer and melanoma cells<sup>[8-10]</sup>. Tumor tissue specific gene therapy is considered to be an important direction of gene therapy against cancer in the near future. Probability of helper virus production, which is generated by homologous recombination, is expected to be decreased on the condition of the transcription regulatory sequences existing in the recombinant retrovirus. The future of gene therapy is subsequently guaranteed.

## REFERENCES

- 1 **Cao GW**, Du P. Modern cancer biotherapy. Beijing: People Military Medical Publisher, 1995: 179-288
- 2 **Colombo MP**, Forni G. Cytokine gene transfer in tumor inhibition and tumor therapy: where are we now? *Immunol Today* 1994; **15**: 48-51 [PMID: 8155261 DOI: 10.1016/0167-5699(94)90131-7]
- 3 **Mizuno M**, Yoshida J, Sugita K, Hayashi Y, Yagi K. [Basic research for interferon gene therapy against malignant glioma]. *No Shinkei Geka* 1992; **20**: 547-551 [PMID: 1598130]
- 4 **Hwu P**, Yannelli J, Kriegler M, Anderson WF, Perez C, Chiang Y, Schwarz S, Cowherd R, Delgado C, Mulé J. Functional and molecular characterization of tumor-infiltrating lymphocytes transduced with tumor necrosis factor- $\alpha$  cDNA for the gene therapy of cancer in humans. *J Immunol* 1993; **150**: 4104-4115 [PMID: 8473752]
- 5 **Sambrook J**, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989: 1-500
- 6 **Miller AD**, Miller DG, Garcia JV, Lynch CM. Use of retroviral vectors for gene transfer and expression. *Methods Enzymol* 1993; **217**: 581-599 [PMID: 8386297 DOI: 10.1016/0076-6879(93)17090-R]
- 7 **Gunzburg WH**, Salmons B. Retroviral vectors. In: Lemoine NR, Cooper DN, eds. Gene therapy. London: Bios Scientific Publisher, 1996: 33-53
- 8 **Huber BE**, Richards CA, Krenitsky TA. Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. *Proc Natl Acad Sci USA* 1991; **88**: 8039-8043 [PMID: 1654555 DOI: 10.1073/pnas.88.18.8039]
- 9 **Tanaka T**, Kanai F, Okabe S, Yoshida Y, Wakimoto H, Hamada H, Shiratori Y, Lan K, Ishitobi M, Omata M. Adenovirus-mediated prodrug gene therapy for carcinoembryonic antigen-producing human gastric carcinoma cells *in vitro*. *Cancer Res* 1996; **56**: 1341-1345 [PMID: 8640823]
- 10 **Vile RG**, Hart IR. Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA. *Cancer Res* 1993; **53**: 3860-3864 [PMID: 8395331]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Hepatitis G virus infection in patients with chronic non-A–E hepatitis

Jin-Hong Chang, Lai Wei, Shao-Cai Du, Hao Wang, Yan Sun, Qi-Min Tao

Jin-Hong Chang, Lai Wei, Shao-Cai Du, Hao Wang, Yan Sun, Qi-Min Tao, Institute of Hepatology, People's Hospital, Beijing Medical University, Beijing 100044, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Qi Min Tao. Institute of Hepatology, People's Hospital, Beijing Medical University, Beijing 100044, China  
Telephone: +86-10-68314422-5726

Received: November 18, 1996

Revised: January 24, 1997

Accepted: February 15, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To elucidate the role of hepatitis G virus (HGV) infection in chronic non-A–E hepatitis and sequence the partial NS5 genome of HGV isolated from the serum of a Chinese patient with chronic non-A–E hepatitis

**METHODS:** Serum samples of patients with chronic non-A–E hepatitis were collected and total nucleic acids were extracted and subjected to reverse transcriptase-nested-polymerase chain reaction (RT-nested-PCR) using primers from the putative NS5 region of HGV genome. Then, 994bp cDNA was prepared from the positive serum, purified with electrophoresis of polyacrylamide gels, and directly sequenced using the dideoxy-mediated chain-termination method.

**RESULTS:** HGV-RNA was detected in 1 of the 35 patients with chronic non-A–E hepatitis. Compared with the 2 HGV isolates (PNF2161 and R10291) obtained from American patients, the HGV NS5 gene of this Beijing isolate (HG-G) showed homology of 88.0% and 89.2% respectively. On the other hand, in comparison with the West African isolate (GBV-C), the Beijing isolate showed homology of 93.5%. The patient showed persistent increase of alanine transaminase, but normal levels were achieved after interferon therapy with persistent positive HGV RNA.

**CONCLUSION:** HGV is one of the causes of chronic non-A–E hepatitis, but it may not be a very important cause. The nucleotide sequence of partial NS5 gene of HG-G was found to be highly homologous to the West Africa isolate.

**Key words:** Hepatitis G; Non-A–E hepatitis; Genes, viral; RNA, viral; DNA, viral; Polymerase chain reaction; Genome, viral

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chang JH, Wei L, Du SC, Wang H, Sun Y, Tao QM. Hepatitis G virus infection in patients with chronic non-A–E hepatitis. *World J Gastroenterol* 1997; 3(3): 143-146 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/143.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.143>

### INTRODUCTION

Since reliable detection of hepatitis C virus (HCV) and E virus (HEV) infections has now become possible, most of the cases of non-A, non-B hepatitis have been found to be caused by HCV and HEV. However, the etiology of some non-A, non-B hepatitis still remains unknown<sup>[1]</sup>, which highlights the possibility of additional causes of human non-A, non-B, non-C, non-D, non-E (non-A–E) hepatitis. Recently, a new RNA virus genome associated with human hepatitis, termed HGV (or GBV-C), was identified simultaneously by two American laboratories<sup>[2,3]</sup>; this virus is considered to be one of the causes of human cryptogenic (non-A–E) hepatitis.

Thus far, the role of HGV infection in chronic non-A–E hepatitis and the primary structure of HGV in Chinese patients have been seldom reported. We detected HGV-RNA in the sera of 35 patients with chronic non-A–E hepatitis in Beijing by using the RT-nested PCR method and sequenced the partial NS5 gene from one isolate positive for HGV RNA.

### MATERIALS AND METHODS

#### Subjects

Of 35 patients with chronic non-A–E hepatitis, 20 were male and 15 were female and aged at an average of 41.2 years. Six patients had history of transfusion, and the remaining 29 were sporadic cases. All were patients treated at the Institute of Hepatology, People's Hospital, BMU. The serum samples were stored at -20 °C. They all tested negative for anti-HAV IgM, HBsAg, anti-HBc, HCV-RNA, anti-HCV, and anti-HEV.

#### Reagents

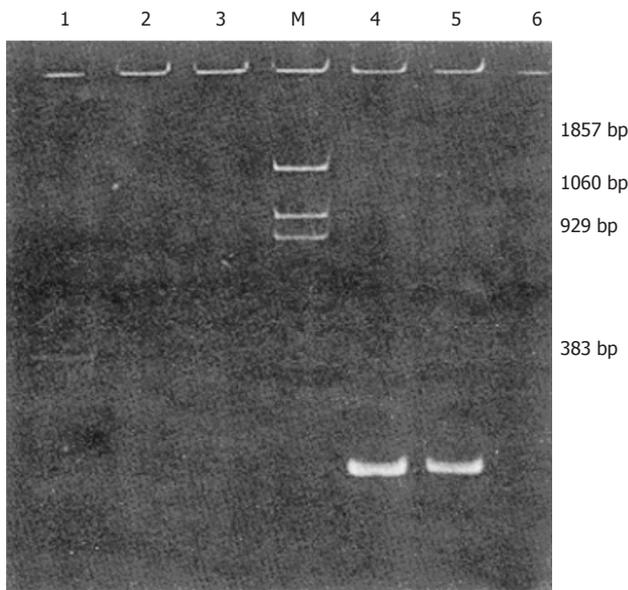
AMV-RT,dNTP, Taq DNA polymerase and DNA sequence kit used in this study are products of Promega.

#### Primers for HGV RNA detection

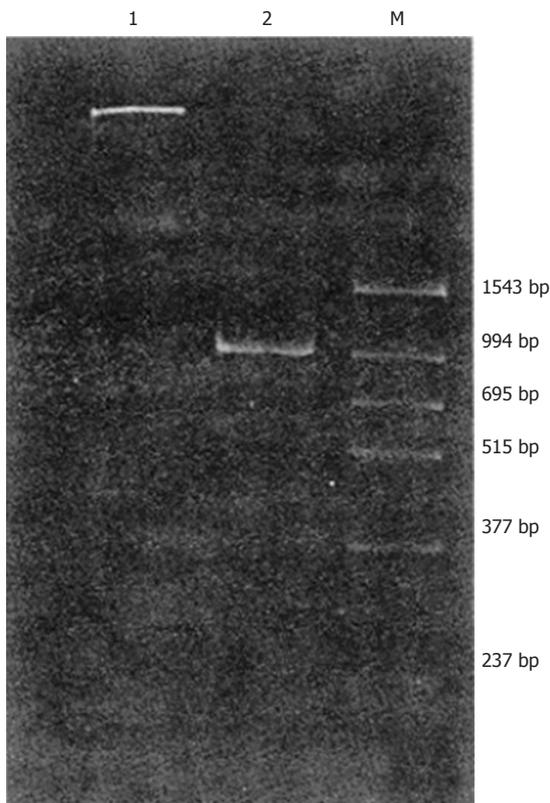
Oligonucleotide primers were designed as described previously<sup>[3]</sup> and were derived from the putative NS5 region of the HGV genome (Table 1). 51# and 52# (outer primers) as well as 53#, 54#(inner primers) were used for clinical detection, with the first PCR product being 400-bp in length and the second PCR product being 210-bp in length. Further, 52# and 55#(outer primers) as well as 56# and 57#(inner primers) were used for the amplification of a longer fragment as a sequence template (994 bp).

#### HGV RNA extraction, cDNA synthesis, amplification and sequence analysis

Total nucleic acid was extracted as described before<sup>[4]</sup>. The



**Figure 1** Electrophoretic pattern of RT-nested PCR for hepatitis G virus (HGV) RNA detection. 1, 4 were products of PCR from original sera, 2 and 5 products from 10<sup>-6</sup> diluted sera, 3 and 6 were negative controls. 1, 2 and 3 were the first PCR products, 4, 5 and 6 were the second PCR products. M was DNA marker, PBR 322/BstN I.

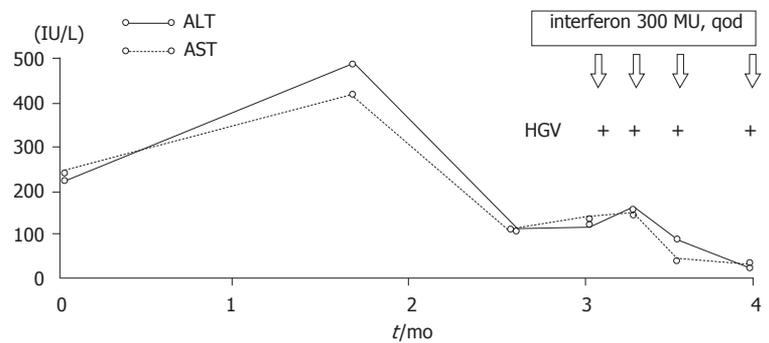


**Figure 2** RT-nested PCR for amplification of long fragment cDNA of hepatitis G virus (HGV) NS5 region. 1 was negative control, 2 was positive PCR product, M was DNA and PCR marker.

extracted HGV RNA was resuspended in diethylpyrocarbonate-treated water and subjected to denaturation at 70 °C for 1 min, followed by a quickly chilling it on ice. Thereafter, the reverse reaction mixture (10 µL reaction-containing AMV-RT buffer, 50 pmol of primer 52#, dNTPs 200 µmol/L each, 2 units of AMV-RT) was added to synthesize cDNA at 43 °C for one hour. The first PCR was performed in a volume of 50 µL, containing 50 pmol primer 51# or 55#, 1.5 units of Taq P, dNTPs 200 µmol/L each. For the second PCR reaction, a volume of 5 µL was removed from the first reaction and added to a tube containing the inner primers 53# and 54# or 56# and 57#, along with dNTPs, Taq polymerase and Taq buffer, as was the case in the first reaction. The first and second PCRs were carried out for 30 cycles, consisting of 94 °C for 60 s, 55 °C for 90 s and 72 °C for 120 s, with a 600-s extension at 72 °C at the end. The second amplified product was analyzed by electrophoresis in 6% polyacrylamide gels. The positive products were purified and directly sequenced using primers 56# and 57#, as per our

**Table 1** Primers for the detection of hepatitis G virus (HGV) RNA with RT-nested-PCR

Primer	GBV-C position	Sequence (5'-3')
51#	7614~7632	GTTACACTTATGAGGARGC
52#	7994~8013	GCRTCCACACAGATGGCGCA
53#	7739~7758	GAGATACTGAAGGGACTCC
54#	7930~7948	CTGGTTGGGGGTACTGG
55#	6885~6909	CTCTTTGGGTAGTAGCCGAGAGAT
56#	7925~7952	CYCGCTCRITTTGGGGTACTGGAAGGC
57#	6959~6981	TCGGTTACTGAGTGCAGCTCAGATGAG



**Figure 3** Clinical manifestation of the patient with chronic non-A-E hepatitis

previously described method<sup>[5]</sup>.

## RESULTS

### RT-nested-PCR for HGV RNA detection

Of 35 patients with chronic non-A-E hepatitis, 1 tested positive for HGV RNA (Figures 1 and 2).

### Clinical data of the HGV RNA positive patient with chronic non-A-E hepatitis

The patient who tested positive for HGV RNA was a 64-year-old woman who had received transfusion of 400 mL blood in 1979. She had not developed any symptoms until February 1996. She was found to have elevated serum ALT level at a physical check-up but tested negative for anti-HAV IgM, HBsAg, anti-HBc, HCV RNA, anti-HCV, and anti-HEV (Figure 3).

### The primary structure of Beijing isolate (HG-G) at nucleic acid level and amino acid level in HGV NS5 region

The nucleotide sequences of HG-G in HGV NS5 as well as those of West African isolate (GBV-C) and American isolates (PNF 2161, R10291) for comparison are shown in Figure 4. The amino acid sequences of the 4 isolates are shown in Figure 5. At a nucleotide level, HG-G homology was 87.95% and 89.23% when compared with two American isolates, and all isolates showed homology of 96.49% at the amino acid level. When compared with the West African isolate, HG-G showed homology of 93.50% and 97.77% at the nucleotide level and amino acid level, respectively. In the region sequenced, 16 proline, 8 cysteine and 3 glycosylated residues were detected.

## DISCUSSION

As reported before, about 20% patients with chronic hepatitis tested negative for serum markers of HBV and HCV, thereby indicating the existence of an unknown etiology in human hepatitis<sup>[1]</sup>. In 1995, HGV was found to be responsible for 9.49% of the cases of non-B non-C hepatitis and 8.33% of the cases of chronic non-A-E hepatitis<sup>[3]</sup>, which indicates that HGV is one of the causes of non-A-E hepatitis. In our research, 1 (2.9%) of 35 patients with chronic non-A-E hepatitis tested positively for HGV RNA. This relatively low detection rate may be related to the insufficient exclusion of other non-virus elements and racial/ethnic and geographic differences. Consistent with other reports<sup>[3]</sup>, many patients with chronic non-A-E hepatitis in our series tested negative for serum HGV RNA; this may be attributed to the lack of sufficient knowledge about the newly discovered virus in its affective factors of molecular biologic

CBV-C	tcggttacgg	agagcagctc	agatgagAAG	ACCCTGTCCG	TGACCTCCTC	GCAGGAGGAC	ACCCCGTCCT	CAGACTCATT	TGAAGTCATC	CAAGAGTGTG	
R10291			...	...C....	...T.....	.....T	.....	...T.....	C.....	.....C...	7158
PNF2161			...	...C....	...T.....	.....T	.....	...T.....	C...G....	....CC..	
HG-G			...	.....	...T...T..	.....T	.....	...G.....	.....	.....C...	
CBV-C	ATACTGCTGA	ATCAGAGGAA	AGCGTCTTCA	ACGTGGCTCT	TTCCGTACTA	AAAGCCTTAT	TTCCACAGAG	CGATGCCACA	CGAAAGCTAA	CGGTTAAGAT	
R10291	...G...A....	...GG.....	...T.....	.....	.....	G....G..	.....	T.....T	A.....T...	...C...C...G...	
PNF2161	...G...A...C..	...GGG.....	...T.....	.....	.....	.....	.....	...C...G...C..	A...G....T...	...C...C.....	
HG-G	.....	GAGT....T	.....	.....	...C....	.....	.....	...C.....	...C.....T...	.....G...	7258
CBV-C	GTCTTGCTGT	GTTGAGAAGA	GCCTAACACG	CTTCTTTTCT	TTAGGGTTGA	CCGTGGCTGA	CGTGGCTAGC	CTGTGTGAGA	TGGAGATCCA	GAACCATACA	
R10291	...AA.....	.....	...C...G...	...G....C...	...G.....	...G.....	T....C...T	.....	.....	.....	
PNF2161	...G.....C	...A...	...T...C...A	...G.....	...G.....	...G.....	...T...T.....	.....	...A.....	.....	
HG-G	...A.....	...G.....	...C...G...	.....C	...G...AC..	...T.....	.....	.....	...A.....	.....	7358
CBV-C	GCCTATTGTG	ACAAGGTGCG	CACTCCGCTA	GAATTGCAAG	TTGGGTGCTT	GGTGGGCAAT	GAACTTACCT	TTGAATGTGA	CAAGTGTGAG	GCACGCCAAG	
R10291	.....	.....	.....	.....	.....	.....	.....	.....	T.....	...TA...G....	
PNF2161	.....	...C....	.....T	.....G..	.....	.....	.....	.....	T.....	...TA...G....	
HG-G	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7458
CBV-C	AGACCCTTGC	CTCCTTCTCC	TACATATGGT	CCGGGGTCCC	ACTTACTCGG	GCCACTCCGG	CCAAACCACC	AGTGGTGAGG	CCGGTGGGGT	CCTTGTGTGT	
R10291	...TT...G...	.....	...T...T.....	...T.....G...	...T...G....A..	.....A...	...T.....	T.....	.....	.....	
PNF2161	...A...TG...	.....T	...T...T.....	...T...A...G...	G..G....A.....	.....G...	...G...T...	C.....	...T...C...	...T.....A...	
HG-G	...T...G...	T.....T	...C....	...T.....	.....A...	.....A...	.....	.....	.....	.....	7558
CBV-C	GGCAGACACC	ACCAAGGTCT	ACGTGACCAA	TCCGGACAAT	CTTGGGAGGA	GGGTGACAA	GGTGACTTTC	TGGCGGCTC	CTCGGTACA	CGACAAGTTC	
R10291	...T.....	...G...A...G...	...T...C...A...	C.....	.....A...	...A...G.....	.....C...	.....C...	...CA...C...	T....A...AT	
PNF2161	...C...T...	...T.....G...	...T...T.....	...A.....	...G...AC....	.....G.....	.....C...	...T.....	...A...T...	T...T...A...	
HG-G	.....	.....G...	.....	.....	.....	...A.....	.....C...	...T.....	...C.....	...A.....	7658
CBV-C	CTCGTGGACT	CGATCGAGGG	CGCTCGGAGA	GCTGCTCAAG	GCTGCCTAAG	CATGGGTTAC	ACTTATGAGG	AGGCAATAAG	GACTGTTAGG	CCGCATGCTG	
R10291	.....	...C.....	T...CA...G	...G.....	C....A....	.....	.....	...A.....	.....	...A.....	
PNF2161	.....	...T...T.....	...AA...G	...C.....	C.....	.....	.....	...A.....	...A...	...A.....	
HG-G	...T...	...A...	...G	.....	C.....	.....	.....	.....	.....	.....	7758
R10291	CCATGGGCTG	GGGACTAAG	GTGTCGGTCA	AGGACTTGGC	CACCCCTGCG	GGGAAGATGG	CTGTTATGAG	CCGGCTTCAG	GAGATACTTG	AAGGGACTCC	
PNF2161	.....	.....	...T...	...A...	...C....	.....	...C...C...	...A.....	.....	...G.....	
HG-G	.....	.....	...T...	.....	.....	.....	...C...C...	...A...A...C...A	.....	.....	7858
CBV-C	GGTCCCTTTT	ACCCTGACTG	TCAAAAAGGA	GGTGTCTTTC	AAAGATCGTA	AGGAGGAGAA	GGCCCCCGC	CTCATTGTGT	TCCCCCCCCT	GGACTTCCGG	
R10291		TT	G	.....	C	.....	.....	.....	.....	.....	
PNF2161	C	TT	G	.....	CG	.....	.....	.....	.....	.....	
HG-G	C	T	.....	.....	CC	.....	.....	.....	T	T	7924
CBV-C	ATAGCTGAAA	AGCTCATTCA	GGGAGACCCG	GGGCGGGTTG	CAAAGGCCGG	TGTTGGGGGG	GCTTACgcct	tccagtacac	ccccaaccg	cggg	
R10291	...G...	...T...CT	.....	...G...	...C...G...T	GT...G....	.....	.....	.....	.....	
PNF2161	.....	...CTT	...A	...C...A...	...C...G...T	GT...G....	...C....	.....	.....	.....	
HG-G	.....	...A...TT	.....	.....	.....	.....	...C....	.....	.....	.....	

Figure 4 Nucleotide sequence of partial hepatitis G virus (HGV) NS5 gene in the serum of a patient with chronic non-A-E hepatitis and its comparison with West African and American isolates. GBV-C is West African isolate; R10291 and PNF2161 are American isolates; HG-G is a patient with chronic non-A-E hepatitis. —denotes the same as GBV-C sequence. Capital letter is nucleotide detected, small letter is primer sequence.

CBV-C	KTLSVTSSQEDTPSSDSFEVIQECDTAESEESVFNVALSVLKALFPQSDATRKLTVMKSCCEKSVTRFFSLGLTVADVASLCMEIQNHTAYCDKVRTP
R10291	...P...S.....SE...G.....E.....R...N.....
PNF2161	...P...S.....SE...G.....Q.....
HG-G	...S.....D.....R.....
CBV-C	LELVQVGLVGNELTFECDKCEARQETLASFSYIWSGVPLTRATPAKPPVVRVPGSLLVADTTKVYVTPNDNVGRRVDKVTFWRAPRVHDKFLVDSIERAR
R10291	.....T.....
PNF2161	.....T.....K.....
HG-G	.....
CBV-C	RAAQGCLSMGYTYEEAIRTYEEAIRTVRPHAAMGWGSKVSVKDLATPAGKMAVHDLRQEIILEGTPVPFTLVKKEVFFKDRKEEKAPRLIVFPPLDFIAEKLIQGD
R10291	...A...Q.....L...
PNF2161	...A.....L...
HG-G	...A.....F.....L...
CBV-C	PGRVAKAGVGGAY
R10291	...VL...
PNF2161	...VL...
HG-G	.....

Figure 5 Amino acid sequence of hepatitis G virus (HGV) NS5 region in the serum of the patient with chronic non-A-E hepatitis and its comparison with West African and American isolates.

detection. In addition, it must be recognized that there may be other causes of human hepatitis besides the known factors, that is to say, HGV may not be a very important cause of chronic non-A-E hepatitis.

The patient, who tested positive for HGV RNA in our research had a history of receiving blood transfusion 17 years before, and she did not show any symptoms and an elevated serum ALT level until February 1996. She did not respond very well to that treatment, but her serum ALT level was normalized after interferon

treatment although HGV RNA continued to persist in her serum. Since we cannot find evidence of other hepatitis virus infection, we believe that HGV must be responsible for the chronic hepatitis in this case, although the clinical features are similar to those of hepatitis C.

The primary structure in HGV NS5 region in the Beijing isolate (HG-G) was also analyzed. In the region sequenced, 16 conserved proline residues and 8 conserved cysteine residues were detected; these residues may play an important role in the formation of the

spatial configuration, indicating that the product encoded by this region may be important in maintaining HGV function. With respect to the nucleic acid sequence and amino acid sequence, HG-G showed marked homology to the West African isolate. Since only 3 isolates of HGV sequences have been reported thus far, it is difficult to decide whether HG-G was of the same genotype as the West African isolate. Future studies on sequence analyses are needed for further research.

## REFERENCES

- 1 **Tao QM**, Wang Y, Du SC, Guo JP. Epidemiology of hepatitis B and C in China. In: Nishioka K, Shzaki H, Mishiro S, Oda T, eds, *Viral hepatitis and liver disease*. Tokyo: Springs, 1994: 412-415 [DOI: 10.1007/978-4-431-68255-4\_105]
- 2 **Leary TP**, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK. Sequence and genomic organization of GBV-C: a novel member of the flaviviridae associated with human non-A-E hepatitis. *J Med Virol* 1996; **48**: 60-67 [PMID: 8825712 DOI: 10.1002/(SICI)1096-9071(199601)48:1<60::AID-JMV10>3.0.CO;2-A]
- 3 **Linnen J**, Wages J, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Fong SK, Thomas H, Bradley D, Margolis H, Kim JP. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996; **271**: 505-508 [PMID: 8560265 DOI: 10.1126/science.271.5248.505]
- 4 **Chomczynski P**, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159 [PMID: 2440339 DOI: 10.1016/0003-2697(87)90021-2]
- 5 **Wei L**, Wang Y, Chen HS, Tao QM. Sequencing of hepatitis C virus cDNA with polymerase chain reaction directed sequencing. *Chinese Journal of Hepatology* 1996; **4**(2): 103-105

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Source of blood supply and embolization treatment in cavernous hemangioma and sclerosis of the liver

Gou-Wei Li, Zhong-Rong Zhao, Bao-Sheng Li, Xiao-Gong Liu, Zhi-Liang Wang, Qing-Feng Liu

Gou-Wei Li, Bao-Sheng Li, Xiao-Gong Liu, Zhi-Liang Wang, Qing-Feng Liu, Department of General Surgery, Second Teaching Hospital Xi'an Medical University, Xi'an 710004, Shaanxi Province, China

Zhong-Rong Zhao, Department of Radiology, Xi'an 710004, Shaanxi Province, China

Author contributions: All authors contributed equally to the work.

Supported by Science Foundation of Shaanxi Province, No.39330, and received the 3<sup>rd</sup> class Award for Scientific and Technical Progress by the Chinese Ministry of Health.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Guo-Wei Li, Supervisor of Doctoral postgraduate, having 40 papers published. Department of General Surgery, Second Teaching Hospital Xi'an Medical University, Xi'an 710004, Shaanxi Province, China  
Telephone: +86-29-7276936-29278

Received: April 16, 1996

Revised: June 13, 1996

Accepted: July 20, 1996

Published online: September 15, 1997

### Abstract

**AIM:** To investigate the source of the blood supply in cavernous hemangioma of liver (CHL), and provide a feasible treatment for CHL *via* the hepatic artery.

**METHODS:** (1) Portovenography, hepatic arteriography and portal vein staining were performed in 5 patients to determine the origin of the blood supply. Two casts of hepatic blood vessels from resected specimens were observed. (2) Clinical data from 75 patients (30 males, 45 females, aged 25-57 years, mean of 37.4) were obtained. Of these, 56 were of solitary type (44 on the right lobe, 12 on the left, with 4 having intraparenchyma), and 19 were of multiple type (9 on the right, 2 the left, 8 whole liver). Twenty-two patients were treated with sclerosis, 50 by embolization *via* hepatic artery, and 3 were excised.

**RESULTS:** In the 5 cases where portography was used, the contrast medium did not enter the tumor, and the tumor appeared as low density area, with the intrahepatic branches of the portal vein pushed aside. In the 5 cases with where portal vein staining was used, the normal liver parenchyma stained a deep blue; however, the tumor was not stained. The tumor area appeared as a round vacant cavity in the 2 specimen casts. For the 72 patients treated with sclerosis or embolization *via* hepatic artery or through interventional method, the tumors diminished by 10%-30% in diameter, and no tumors grew larger.

**CONCLUSION:** The blood supply of CHL originates from the hepatic

artery. Tumors treated with sclerosis and embolization decreased in size or got fibrotic.

**Key words:** Liver neoplasms/blood supply; Liver neoplasms/therapy; Hemangioma, cavernous/therapy; Embolization, therapeutic; Sclerotherapy

Li GW, Zhao ZR, Li BS, Liu XG, Wang ZL, Liu QF. Source of blood supply and embolization treatment in cavernous hemangioma and sclerosis of the liver. *World J Gastroenterol* 1997; 3(3): 147-149 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/147.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.147>

### INTRODUCTION

Cavernous hemangioma of the liver (CHL) is a common, benign, hepatic tumor. With the development and wide application of modern imaging technics, many more cases of CHL have been diagnosed in recent years. Currently, the conventional treatment for CHL is excision of the tumor. In this report, we studied the blood supply of CHL from December 1985 to November 1992, and we found that CHL is a malformation of hepatic arterial vasculature. In this study, 22 of 72 CHL patients were treated using sclerosis, while the other 50 were treated with gelfoam microspheres with lipiodol administered either during surgery or using hepatic arterial intubation *via* the femoral artery. In all cases, the results were satisfactory.

### MATERIALS AND METHODS

#### Portography and hepatic arteriography during operation

The portovenography and hepatic arteriography were performed in five patients as previously reported<sup>[1]</sup>.

#### Portal vein staining during the operation

In these five cases, the portal veins were intubated *via* the right gastroepiploic vein in the right gastric vein, or *via* direct puncture of the portal vein, and 60 mg of methylene blue diluted to a final volume of 20 mL was quickly injected. The staining of the liver parenchyma and the tumor body were then observed.

#### Pathology and the casts of resected specimens

The two resected CHL specimens from left lobes, 10 cm and 10.5 cm in diameter, were fixed with formalin and the sections were sent for serial pathological observations. Another resected specimen, 6 cm in diameter, was made into model cast by filling the hepatic vein (yellow) and portal venous branch (blue) with methyl methacrylate after vascular lavation.

### RESULTS

#### Radiographic observations

In the 5 cases with in which portography was used, the contrast



Figure 1 The tumor appeared as a filling defect.



Figure 3 Methylene blue staining of the liver except the tumor area.

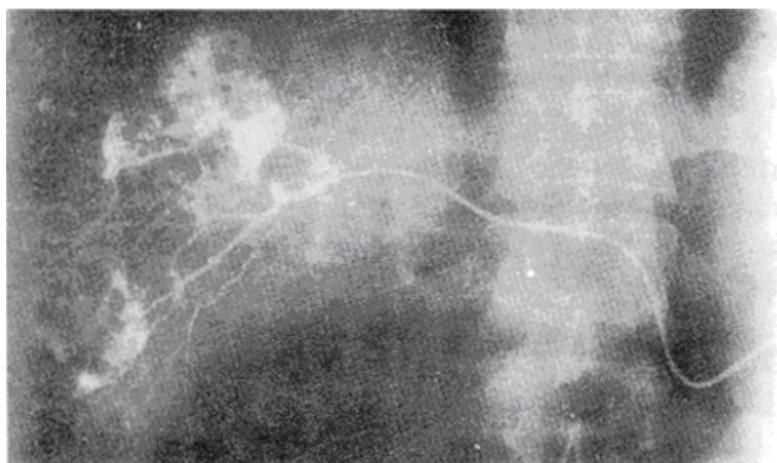


Figure 2 Radiograph by immediate visualization of tumor body after injection intubation of hepatic artery branch.



Figure 4 Specimen cast filled with methyl methacrylate via portal vein and hepatic vein, the tumor area appeared as a round vacant cavity.

medium could not enter the tumor, and the intrahepatic branches of portal vein were pushed aside by the tumor. During the liver parenchymal phase-contrast, the density of the normal tissue was obviously increased, while the tumor appeared to be of lower density, with sharp and clear boundaries. In 22 patients that underwent sclerosing therapy, hepatic arteriography revealed that the contrast medium immediately entered the tumor, and had a "cotton" or "popcorn" appearance. In these patients, an arteriovenous fistula was not observed (Figures 1 and 2).

#### **Methylene blue staining of the liver**

After the injection of methylene blue through portal vein, the normal liver parenchyma was stained homogeneously in a deep blue; however, the tumor was not appear stained, and the boundary between the normal liver tissue and the tumor was sharp (Figure 3).

#### **Observations on pathology and cast specimens**

In the serial sections of nine biopsies and resected specimens, no normal blood vessels or bile ducts were observed under microscopy. Although CHL did not have a capsule, it did have a clear boundary with the neighboring normal liver tissues.

The casting specimens showed that the eroded tumor left behind a round vacant area in the specimens. The stained branches of the portal vein did not extend into the tumor (Figure 4).

#### **Clinical data**

Among the 75 patients included in this study, 30 were males and 45 females, with an age ranged between 25-57 years, with a mean age of 37.4 years. All patients were diagnosed by ultrasound, with 66 confirmed by CT, 34 by selective coeliac arteriography, and 20 by ECT. The diameter of the CHL ranged 6-30 cm, with only 4 of them greater than 20 cm. Among these 75 patients, 56 cases were of solitary type, with 44 were on the right lobe, 12 on the left and 4 were intraparenchymatous. Nineteen of the 75 patients

had multiple type, with 9 on the right, 2 on the left and 8 in the whole liver. Of the original 75 cases, three cases were excised; the left hepatic artery was not shown on arteriography for 2 of them, however, but were confirmed during surgery. In the other 72 cases, 22 received sclerosing therapy, and 50 were treated with embolization (10 of these were interventional embolizations). Among the 75 cases, 2 were HBsAg positive, 1 HBsAb positive and none of them had cirrhosis. Twenty-one were proved to be CHL pathologically.

#### **Surgical methods**

Under direct view, the involved hepatic arterial branches were dissected and confirmed by methylene blue injection. The proximal end was ligated and intubated through the distal end, and the tube was then fixed with rubber pieces. The opening of the tube was filled with normal saline and fire sealed, before being pulled out through the abdominal wall puncture, and fixing an adhesive plaster on the skin. Through this tube, sclerosing treatment and repeated radiographic examinations were carried out<sup>[1]</sup>.

For the sclerosing therapy group, the patients were divided into three subgroups. Subgroup 1 ( $n = 8$ ) had 10-20 mL of 40% carbamide injected through the intubated arterial branch of the liver once daily for 20-30 d. Subgroup 2 ( $n = 6$ ) had 10 mL of 49.9% ethanol injected by the same route, once every three days, for a total of 5-7 injections. Subgroup 3 ( $n = 8$ ) had 2 carbamide injections and one ethanol injection administered alternatively, with usually 9 injections required.

For the embolization therapy group, 100 mg of gelfoam particles mixed with 8-16 mL of lipiodol emulsion was injected. Ten patients were given interventional embolization. Generally, one single embolization was adequate. It was only when the tumor diameter was greater than 10 cm that a second embolization was needed. In cases with multiple tumors less than 4 cm in diameter on the other lobe of the liver, anhydrous ethanol was injected into the tumor at multiple points. The injection was stopped when the tumor

appeared blanched or collapsed. After withdrawal of the needle, finger pressing was used to prevent bleeding and extravasation of ethanol.

### Cessation of therapy

In the sclerosing therapy group, periodical radiography was performed. The tube was pulled out until the tumor body could not be visualized. The embolization treatment was discontinued when the tumor had shrunk by 10% based on X-ray evaluation or after two-weeks or one month. When the diameter was beyond 10 cm, a second embolization was given. Among the 28 cases, a total of 36 embolizations were performed.

### Clinical criteria for CHL obliteration

(1) On intensified CT scanning, the "ring sign" and "half ring sign" reduced in size or disappeared totally after 3-6 mo. (2) The dynamic isotopic hepatic blood pool scanning showed a radioactive defect. And (3) Shrinkage of tumor on ultrasonography.

### Advantages and disadvantages of sclerosing therapy and embolization therapy

The carbamide in sclerosing therapy was not irritative, and usually it took 25 d to obliterate the CHL. Ethanol injection, however, elicited intolerable pain, which lasted for about 2 h, and generally required seven injections. Interventional embolization produced lasting pain, which often required pethidine; however, only 1-2 times were needed, and the pain attenuated on the second treatment.

### Follow up study

The 22 cases with sclerosing therapy were followed for 1-6 years, and CT and ultrasound examinations indicated the diameter of the tumor had shrunk by 20%-60%. In one patient who received a second operation, after one year, white and shining fibrous tissues were evident, which were confirmed pathologically. The other two female patients with tumors beyond 20 cm and who had previously had repeated skin petechiae, gained more than 5 kg of weight, and the petechiae disappeared after the treatment. The embolization group were followed for over six months, and the tumors for this group all diminished by 10%-30% in diameter, and none of the tumors grew any larger.

## DISCUSSION

Since the beginning of this century, CHL has been regarded as a portal venous malformation by many researchers, but ligation of branches of portal vein was unsatisfactory as a treatment<sup>[2-5]</sup>. Yamamoto was the first to determine that the morphology of sinusoids of CHL specimens were different from that of portal and hepatic vein, but similar to that of hepatic artery using electron microscopy<sup>[6]</sup>. At present, a shunt for cavernous hemangioma has not been identified. Based on the portovenographic findings, intubational imaging of hepatic arterial branch, liver staining, and observations of the cast vessels in the resected specimens, this study has demonstrated that CHL is a malformation of the hepatic artery, and its blood supply arises completely from the hepatic artery without an arteriovenous shunt. This provides the theoretical basis for treating CHL *via* the hepatic artery. This study found that both sclerosing and embolization therapy can shrink the tumor and replace it with fibrotic tissues.

Hepatic cavernous hemangioma can grow continuously. Previously, Niemann reported 55 cases that led to diffuse hemangioma in the liver, which easily ruptured, causing a mortality rate as high as 70%<sup>[4,7]</sup>. However, excision is difficult when the tumor is of the multiple type, especially when it is near the hilum, very large in size, occupying the whole lobe of the liver, or some other contraindication exists. Our experience has found that therapy *via* intubation of hepatic artery is safe, reliable, and causes less injury, and does not require a blood transfusion.

Sclerosing agents, such as carbamide and ethanol, both require a long duration of therapy, especially the carbamide. Ethanol often produces pain for a brief period. However, Lipiodol can selectively stay for a long time in both benign and malignant hepatic tumors with rich blood supply. In this study, all 28 patients that received embolization therapy demonstrated such characteristics. Lipiodol and gelfoam microspheres are both long acting embolization agents. Gelfoam microspheres can occlude the vessels by secondary inflammation and growth of granulation tissues. The microspheres still could be identified in a few areas under microscopy after 28 d, and the hemangioma was completely filled with granulation tissues and fibrous tissues<sup>[7]</sup>.

CHL is composed of cavernous blood sinusoids, without blood vessels, bile ducts or hepatocytes. Its blood supply primarily came from hepatic artery. The contrast agent and radioactive isotopes could quickly enter the periphery of the tumor, but diffused slowly and drained out after a long period, which accounts for the variegated feature of CHL. For example, the early visualization and late disappearance in hepatic angiography; the ring and half-ring signs on CT scanning, and the intensified imaging of blood pool radioactive scanning, is due to the fact that there are no hepatocytes within the tumor, making it appear that there is a radioactive defect in the static scanning. The above characteristics can also be the basis to assess the efficacy of occlusion treatment.

The variation of the hepatic artery is as high as 45%, particularly in the left lobe. This suggests that selective hepatic arteriography should be performed before intubation in order to raise the success rate of embolization therapy. Furthermore, interventional embolization therapy is only possible for certain patients. Since the hepatic artery tapers gradually, non-selective hepatic arteriography cannot show a complete picture of large and multiple hemangiomas, but rather requires superselective hepatic arteriography for the diagnosis and treatment of CHL.

## REFERENCES

- 1 Li GW, Liu XG, Li BSH, Wang ZL, Lei XY, Wang Y. Study on sclerosing therapy of hemangioma of the liver. *Chinese Journal of Experimental Surgery* 1992; **9**: 1-3
- 2 Kato M, Sugawara I, Okada A, Kuwata K, Satani M. Hemangioma of the liver. Diagnosis with combined use of laparoscopy and hepatic arteriography. *Am J Surg* 1975; **129**: 698-703 [PMID: 124140 DOI: 10.1016/0002-9610(75)90350-5]
- 3 Chao RS, Liu YF, Tian YS, He SG, Sheng K. Excision of cavernous hemangioma of the liver with decollement extra capsule. *J Practical Surg* 1989; **9**: 268-269
- 4 Li GC, Gu JZ. Child hemangioma. 1st ed. Shaanxi: Shaanxi Science and Technology Publication House, 1991: 83-87
- 5 Wakabayashi Y. Ligation of branches of portal vein against massive hemangioma of the right hepatic lobe. *Jpn J Gastroenterol* 1966; **63**: 245-249
- 6 Yamamoto K, Itoshima T, Ito T, Ukida M, Ogawa H, Kitada M, Hattori S, Mizutani S, Nagashima H. Scanning electron microscopy of a liver cavernous hemangioma. *Gastroenterol Jpn* 1983; **18**: 15-20 [PMID: 6832546]
- 7 Chen XL, Wang DQ, Zhong DC, Gua YS, Yang CH, Li XW. Study of embolizing hepatic artery with gelfoam microsphere. *Chinese Journal of Experimental Surgery* 1991; **8**: 166-167

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Survival and malignant phenotype changes of human hepatoma SMMC-7721 cell line induced by cryopreservation at -50 °C

Shi-Ming Jiang, Zhao-Hui Xu, Yan Zhang, Xian-Min Shi

Shi-Ming Jiang, Zhao-Hui Xu, Biology Department, Shandong Normal University, Jinan 250014, Shandong Province, China

Yan Zhang, Shanghai Institute of Cell Biology, Academia Sinica

Xian-Min Shi, Biology Department, Xiamen University

Author contributions: All authors contributed equally to the work.

Shi-Ming Jiang, Male, born on 1961-11-26. Master of science, graduated from Department of Biology, Shandong Normal University in 1985. Associate professor, having 28 papers and 1 book published.

Supported by a grant from Shandong Provincial Science and Technology Committee, No. K91236517.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Shi-Ming Jiang, Biology Department, Shandong Normal University, Jinan 250014, Shandong Province, China

Received: October 26, 1996  
Revised: December 19, 1996  
Accepted: January 22, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate the effect of cryopreservation at -50 °C on the human hepatoma SMMC-7721 cell line.

**METHODS:** With 15% DMSO as a cryoprotectant, the SMMC-7721 cells were cryopreserved at -50 °C, then thawed and recultured. The survival rate, mitotic index and LDH isoenzymes were compared between pre- and post-cryopreservation.

**RESULTS:** Thirteen hours after the thaw, the mitotic index of cryopreserved SMMC-7721 cells decreased by 1.09%. The mode scope of chromosome number (46-53) after cryopreservation tended to transfer to that of normal human cells, and the percentage of metaphases containing 46 chromosomes changed from 0% to 16%. LDH isoenzymes changed from H-like model (LDH3(29.3%) > LDH4 (26.8%) > LDH2 (25.3%) > LDH5 (14.9%) > LDH1 (3.6%) to M-like model (LDH4 (48.3%) > LDH5 (28.3%) > LDH3 (18.9%) > LDH2 (4.4%) > LDH1 (0%)). This suggests that the survival rate could reach over 95%.

**CONCLUSION:** Cryopreservation at -50 °C can be a convenient method for the cryopreservation of cell lines. However, cryopreservation at -50 °C is likely involved in the changes of the malignant phenotypes of the human hepatoma SMMC-7721 cell line, and may induce the differentiation of malignant cells.

**Key words:** Liver neoplasms; Carcinoma, hepatocellular; Cryopreservation; Tumor cells, cultured; Phenotype

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Jiang SM, Xu ZH, Zhang Y, Shi XM. Survival and malignant phenotype changes of human hepatoma SMMC-7721 cell line induced by cryopreservation at -50 °C. *World J Gastroenterol* 1997; 3(3): 150-152 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/150.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.150>

### INTRODUCTION

Cryopreservation and cryotherapy play important roles in the research and therapy of cancer. It has been reported that -50 °C is in the great ice crystal forming temperature region (-40 °C to -60 °C), in which cells are greatly damaged, even if they incubated at this temperature for a very short period of time (10 min)<sup>[1]</sup>. This is particularly damaging to the cellular membranes and the chromatin. Therefore, how to pass this temperature region successfully is a key step in the cryopreservation of not only for maintaining cell suspensions, but also whole organs, such as heart, kidney, etc. Many studies have shown that some chemical reagents, such as sodium butyrate, dimethyl sulfate (DMSO), cAMP Vitamin A, and Vitamin D3 can induce the differentiation of malignant cells *in vitro*<sup>[2-4]</sup>. Nothing is known about the differentiation of cancer cells induced by the cryopreservation, especially at -50 °C. In this report, we provide some evidence that cryopreservation at -50 °C may be involved in the changes of malignant phenotypes of human hepatoma SMMC-7721 cell line.

### MATERIALS AND METHODS

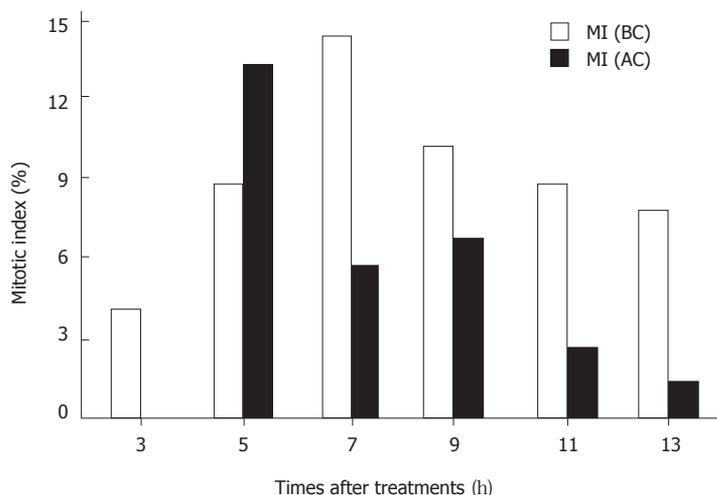
#### Cell culture and cryopreservation

The human hepatoma SMMC-7721 cell line was obtained from the Shanghai Cell Bank of Academia Sinica and maintained in our laboratory. The cells were cultured in RPMI 1640 medium (Gibco Inc) supplemented with 10%-20% fetal calf serum (FCS) and incubated at 37 °C, with 5% CO<sub>2</sub>/95% air. The cells attached and spread well on either untreated plastic plates or untreated glass flasks. In the cryopreservation study, the cells were firstly passaged in glass flasks. When the cells grew to exponent stages, the culture medium was removed, and the cells were released from the flask using 0.25% trypsin (in D-Hank's solution, pH 7.2) for 1 min. The cells were then re-suspended in RPMI 1640 medium. The cells were counted using a Bueker's chamber. Cell viability was detected by mixing the cell suspension with 0.5% aqueous solution of trypan blue (1:1). The cell suspension was adjusted to the concentration of 1-5 × 10<sup>6</sup>/mL with 85% RPMI-1640 medium and 15% ice cold DMSO before being

**Table 1** The effects of dimethyl sulfate concentration on the survival and the rate of attachment of SMMC-7721 cells after cryopreservation at -50 °C

5 0	5 10	10 0	10 10	15 0	15 10	20 0	20 10	30 0	30 10	40 0	40 10	DMSO (%)	FCS (%)
90 ± 2	90 ± 3	95 ± 2	98 ± 2	98 ± 1	99 ± 1	93 ± 4	95 ± 2	87 ± 4	98 ± 2	80 ± 4	83 ± 2		SR (%)
60 ± 3	65 ± 2	85 ± 4	90 ± 3	85 ± 3	90 ± 2	55 ± 2	60 ± 4	20 ± 4	25 ± 5	1 ± 1	2 ± 1		AR (%)

SR: Survival rate; AR: Attaching rate.



**Figure 1** The comparison of mitotic index of human hepatoma SMMC-7721 cells between pre and post cryopreservation.

placed at -50 °C in an ultracold freezer (Sanyo, Japan).

**The viability and reculture of cells after cryopreservation**

After being cryopreserved at -50 °C for 6 mo, the cells were taken out from the freezer and thawed in a 40 °C water bath, swirling until the ice was dissolved. The cell suspension was transferred into the centrifugation tube, centrifuged at 500 rpm for 3 min, the preservers were removed and washed with RPMI-1640 medium for 3 times by centrifugation. The cell viability was determined as described above. The cells were recultured in RPMI-1640 medium. The rate of attachment was determined after 12 h in culture by counting the unattached cells.

**The determination of mitotic index before and after cryopreservation**

After being cryopreserved and treated as described above, the cells were adjusted to the concentration of 2 × 10<sup>5</sup>/mL, plated in 24-well Costar tissue culture plastic dishes, and incubated at 37 °C, with 5% CO<sub>2</sub>/95% air for 3 h. After that the cells on cover slips in 4 parallel wells were taken out at an interval of 2 h, fixed in cooled methanol: acetic acid (3:1) and stained with Feulgen stain, and hematoxylin and eosin. The mitotic index of 1000 cells from each well were counted. The control group was treated in the same way as the experimental group but the cryopreservation step was omitted.

**The analysis of the mode scope of chromosomal number**

After being cultured for three days, the cryopreserved cells were given colchicine at a final concentration of 0.01 µg/mL and cultured for 6 h. The cells were then released from the flask using 0.25% trypsin and resuspended in RPMI-1640. After centrifuging the cell suspension to remove the trypsin, 0.075 mol/L KCl was added to the cells, and the cells were then incubated in a 37 °C water bath for 10 min. Following this, the cells were fixed with cooled methanol: acetic acid (4 °C) for three times. The chromosomes were spread onto slides and the routine method for Giemsa stain was used to examine the chromosome sample. The mode scope of chromosomal number of 100 metaphase figures was examined under a microscope.

**LDH isoenzyme analysis**

After being cultured to the exponent stage, the cryopreserved cells were collected and homogenized. The supernatant of the cells was collected and LDH isoenzyme analysis was done using PAGE electrophoresis. After staining, the LDH bands were scanned with a

gel scanning detector, and the percentage content of each band of the LDH was recorded.

**RESULTS**

**The survival and growth of cells after cryopreservation at -50 °C**

After being cryopreserved in different DMSO concentrations with RPMI-1640 medium, or different DMSO concentrations with 10% FCS and RPMI 1640 medium at -50 °C for 6 mo, the cells were rapidly thawed in 40 °C water bath and washed with RPMI-1640 medium 3 times. The survival and the rate of attachment after 12 h in culture are listed in Table 1.

In all cells frozen with DMSO, the viabilities detected by trypan blue resistant staining were all greater than 80%, which suggested that DMSO could protect cells from damage during cryopreservation at -50 °C. However, the rates of attachment were significantly different between DMSO concentrations, with a sharp decrease in attachment when treated with 30% DMSO, and very few cells attaching on the flasks in the group treated with 40% DMSO. Rather, the cells treated with 40% DMSO tended to gather in large cell clumps, remaining suspended in the medium, with many of the cells in a bubble like form. The addition of FCS to the cryoprotectant DMSO increased both the survival and the attaching rates at all concentrations. Based on the results of our study, we recommended using 10% and 15% DMSO with 85% RPMI 1640 medium as the in the freezing medium when cryopreserving a the cell line. The results clearly indicated that cryopreservation at -50 °C could be used as a practical and convenient method for cryopreserving SMMC-7721 cells.

**The effects of -50 °C cryopreservation on the mitotic index**

The comparison of mitotic index between SMMC-7721 cells that were not cryopreserved with those that were cultured for 13 h after 6 mo of cryopreservation is shown in Figure 1.

The MI of the cryopreserved cells was lower than that in controls at all time points examined, except for that in the 5<sup>th</sup> hour, when the MI was higher in the cryopreserved cells. The highest MI in the cryopreserved cells (13.31%) was 1.09% lower than that in the normal cultured cells. The results suggest that the cryopreservation at -50 °C could decrease the MI of SMMC-7721 cells.

**The effects of cryopreservation on the mode scope of chromosomal numbers**

After being cryopreserved more than ten times, the mode scope of chromosomal numbers ranged from 24 to 109, the main scope being 46-53 (58%) (Figure 2), and the percentage of cells with 46 chromosomes was 16%, which was significantly different from the SMMC-7721 cells in the time of establishment<sup>[5]</sup>.

**The effect of cryopreservation at -50 °C on the patterns of LDH isoenzymes**

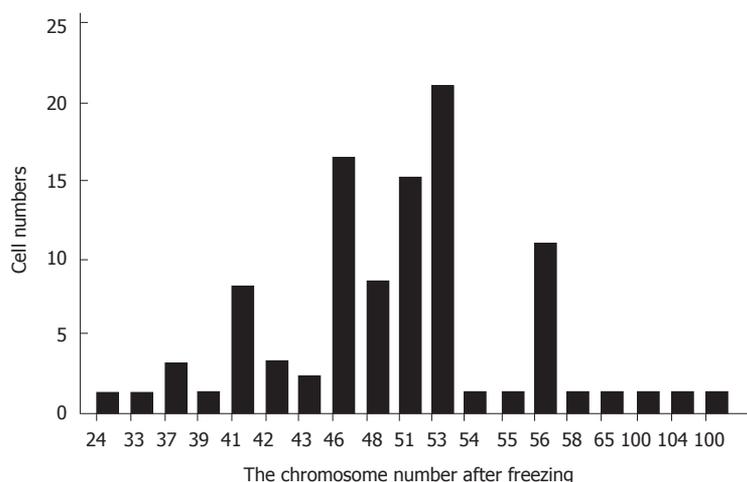
The changes of LDH patterns after cryopreservation at -50 °C are shown in Table 2.

After being cryopreserved at -50 °C, LDH2 and LDH3 contents decreased and the LDH4 and LDH5 contents increased as compared with the LDH patterns in the time of establishment of the SMMC-7721 cell line. These same differences were also evident in the cryopreserved cells when compared to the human hepatoma BEL7404 cell line. After cryopreservation, the content of LDH4 was the highest (48.3%), LDH5 the second (28.3%), with the sequence of the LDH activity in these cells being LDH4 > LDH5 > LDH3 > LDH2 > LDH1.

**Table 2** The effects of -50 °C cryopreservation on the activity of lactate dehydrogenase (%) of human hepatoma SMMC-7721 cell line

	LDH5	LDH4	LDH3	LDH2	LDH1
SC (AC)	28.3	48.3	18.9	4.4	0
SC (E)	14.9	26.8	29.3	25.3	3.6
BEL7404 cell	1.7	22.95	37.76	27.21	10.38

SC (AC): SMMC-7721 cell after cryopreservation; SC (E): SMMC-7721 cell in the establishment; BEL7404 cell: Another human hepatoma cell line.



**Figure 2** The effects of -50 °C cryopreservation on the chromosome numbers of human hepatoma SMMC-7721 cell line. Abbreviations: SC (AC): SMMC-7721 cell after cryopreservation; SC (E): SMMC-7721 cell in the establishment; and BEL7404 cell: another human hepatoma cell line

## DISCUSSION

Many chemical reagents, such as sodium butyrate, DMSO, dBcAMP, vitamin A, and vitamin D3 can induce the differentiation of cultured malignant cells, which suggested that these agents might be useful in the treatment of cancer. To our knowledge, the differentiation induced by cryopreservation at -50 °C has not been reported. In this report, we have found that the survival rate of human hepatoma cells SMMC-7721 at -50 °C is 95%, as indicated by the trypan blue resistant staining. These cells also had an 85% attachment rate, spreading on the surface of culture dishes when treated with 10%-15% DMSO as a cryoprotectant. DMSO could penetrate rapidly into the cells and decrease the great ice crystal formation, suggesting that DMSO concentration of 5%-40% could protect the cells from damage of great ice crystal at -50 °C, and the cell survival rates were all more than 80% with trypan blue resistant staining. However; high concentration of DMSO was toxic to cells, with a lower survival rate than lower concentrations of DMSO, but more importantly, the rates of attachment on the flasks after cryopreservation was much lower at 40% DMSO. These low rates of attachment might be induced by the high osmotic pressure in cells treated with high concentration of DMSO, which caused the cells to swell up and may have destroyed the cells in the process of revitrification. It is suggested that the cryopreservation at -50 °C with 10%-15% DMSO as a cryoprotectant is a practical and convenient method.

It is important to remember that -50 °C is in the great-ice-crystal forming temperature region in which cells can suffer great damage, and the cell membrane system might be changed greatly. This damage may induce many changes in physiology and biochemistry, maybe even in chromosomal and gene level. The success of cryopreservation at -50 °C and the high survival rate provided may not only be a convenient method for cryopreservation, but may also offer a new means for the study of gene expression and control in response to lower temperature.

We observed a decrease in the mitotic index of cryopreserved SMMC-7721 cells during the 13 hour culture at all time points

examined except for that in the 5<sup>th</sup> hour, in which the MI was higher than that in control group. This time point discrepancy might be due to the synchronism of some cells after cryopreservation, as many cells might be going to the M phase together. These results suggested that after the culture period, the growth rate in the cryopreserved group might be lower than that in the control group.

After being cryopreserved at -50 °C more than 10 times, the chromosomal number, the main scope of chromosomal number and LDH isoenzymes of SMMC-7721 cells were compared between cells both before and after cryopreservation. The cell line in the establishment was a superdiploid cell line, its scope of chromosomal number was 44-107, the main scope 54-58, and the percentage of cells with 46 chromosomes was 0%. After being cryopreserved, chromosomal number changed to a range of 24-109, the main scope to 46-53, the percentage of cells with 46 chromosomes to 16%. These suggested that the cells with superdiploid chromosomes had a lower tolerance to -50 °C than those with a normal chromosome complement, and after being cryopreserved at -50 °C several times, both the main scope and the scope of chromosomal number returned to a normal level. The LDH isoenzymes of SMMC-7721 cells in the establishment tended towards H pattern (LDH3 (29.3%) > LDH4 (26.8%) > LDH2 (25.3%) > LDH5 (14.9%) > LDH1 (3.6%)) (H pattern > M pattern). After being cryopreserved at -50 °C for several times, the LDH isoenzyme contents were changed to LDH4 (48.3%) > LDH5 (28.3%) > LDH3 (18.9%) > LDH2 (4.4%) > LDH1 (0%), but the normal LDH isoenzymes of human hepatocyte were of M pattern, that was LDH5 > LDH4 > LDH3 > LDH2 > LDH1. The comparison showed that the LDH pattern of SMMC-7721 cells was also returned to the normal pattern of hepatocyte cells after being cryopreserved with the distinct decreasing of LDH2 content and increasing of LDH4 and LDH5. This LDH pattern, which is different from that of human hepatoma cells, has showed that genes controlling LDH M pattern are located in the chromosome 11, and the M pattern genes are located on chromosome 12. Both the LDH pattern and the change of main scope of chromosome suggested the changes of LDH isoenzyme were induced by the change of chromosomes.

From our results, we may conclude that the cryopreservation at -50 °C with 10%-15% DMSO as a cryoprotectant is not only a practical and convenient method for storage, but also might induce the differentiation of human hepatocarcinoma SMMC-7721 cells, thus opening a new way for researching into the gene expression and control in response to lower temperature.

## REFERENCES

- 1 Sakai A, Kobayashi S, Oiyama I. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Rep* 1990; **9**: 30-33 [PMID: 24226373 DOI: 10.1007/BF00232130]
- 2 Yang SM. [DMSO induced differentiation of human gastric adenocarcinoma cell line MGC 80-3]. *Shiyan Shengwu Xuebao* 1994; **27**: 281-287 [PMID: 7801721]
- 3 Rizzo AM, Gornati R, Rossi F, Bernardini G, Berra B. Retinoic acid induces changes in *Xenopus* embryo glycolipid pattern. *Cell Biol Int* 1995; **19**: 895-901 [PMID: 8574216 DOI: 10.1006/cbir.1995.1027]
- 4 Okazai T, Bell RM, Hannun YA. Sphngomyelin turnover induced by vitamin D3 in HL-60 cells. Role in cell differentiation. *J Biol Chem* 1989; **264**: 19076-19080 [PMID: 2808413]
- 5 Dong RC, Zhou RH, Lu FD. The establishment and biological study of human hepatoma SMMC-7721 cells. *Academic Journal of Second Military Medical University* 1980; **1**: 5-9

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Effects of Linomide on growth and metastasis of implanted human gastric cancer in nude mice

Hou-Quan Tao, Yan-Zhen Lin, Hao-Ran Yin, Qin-Long Gu, Zheng-Gang Zhu, Ming Yao

Hou-Quan Tao, Yan-Zhen Lin, Hao-Ran Yin, Qin-Long Gu, Zheng-Gang Zhu, Ming Yao, Department of Surgery, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China

Hou-Quan Tao, having 9 papers published, Department of Surgery, Ruijin Hospital, Shanghai 200025, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Hou-Quan Tao, Professor, Department of Surgery, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China  
Telephone: +86-21-64370045

Received: December 24, 1996

Revised: February 19, 1997

Accepted: March 10, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To elucidate the effect of angiogenesis inhibitor, Linomide, on tumor growth and metastasis in nude mice implanted with human gastric cancer.

**AIM:** A metastatic model of gastric cancer was established using orthotopic implantation of histologically intact tumor tissues into the gastric wall of nude mice. Linomide (0, 80, 160 mg·kg<sup>-1</sup>) was given p.o. every day after the implantation, and the mice were sacrificed after 10 wk to detect tumor size and metastasis. The microvessel counts were measured by immunohistochemical staining using a monoclonal antibody against Human Factor VIII related antigen.

**RESULTS:** Linomide treatment significantly decreased the size of the implanted tumors (control group: 1.36 ± 0.81 cm<sup>3</sup> vs Linomide treated group: 0.84 ± 0.51 cm<sup>3</sup> and 0.62 ± 0.35 cm<sup>3</sup>, *P* < 0.05 and 0.01, respectively). Additionally, an antimetastatic effect of Linomide was clearly demonstrated in a dose dependent manner: mice given 80 mg·kg<sup>-1</sup> Linomide developed liver metastasis in 4 of 10 cases, mice given 160 mg/kg developed metastasis in only 1 of 10 mice, while it developed in 19 of 28 mice of the control group (*P* < 0.05 and 0.01, respectively). The number of metastatic foci was also significantly less in the treated group. Furthermore, the microvessel counts in tumors of treated mice was reduced by 33%-42% as compared with the control tumors (*P* < 0.01).

**CONCLUSION:** Linomide has a strong inhibitory activity against *in vivo* tumor growth and metastasis of gastric cancer, effectively suppressing the growth of the primary tumor, preventing liver metastasis, and attenuating the rate of neovascularization.

**Key words:** Linomide; Stomach neoplasms; Neoplasm metastasis; Liver neoplasms/secondary; Neovascularization, pathologic

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Tao HQ, Lin YZ, Yin HR, Gu QL, Zhu ZG, Yao M. Effects of Linomide on growth and metastasis of implanted human gastric cancer in nude mice. *World J Gastroenterol* 1997; 3(3): 153-155 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/153.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.153>

### INTRODUCTION

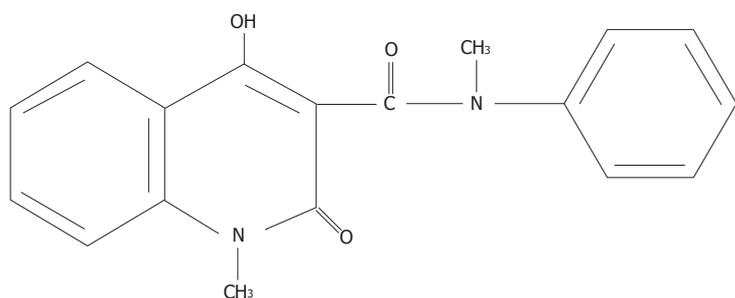
Although most gastric cancer is surgically resectable, tumor relapse and metastasis does develop in some patients. Therefore, many studies have attempted to address the control of relapse and metastasis in patients with these tumors. Neovascularization is not only the basis of tumor growth, but also the part of the complicated process of tumor metastasis, and is crucial to the development of hepatic metastasis. Since a small focus of tumor cells cannot grow at a secondary site without the induction of angiogenesis, it is expected that inhibition of angiogenesis should provide a potent form of therapy to prevent tumor recurrence and metastasis of gastric cancer.

Angiogenesis inhibitors have been reported to have inhibitory activity against both tumor growth and metastasis, and some have achieved good results in animal experiments. Specifically, Linomide, a quinoline-3-carboxamide (Figure 1), has been demonstrated to have inhibitory effects on the growth and metastasis of several rodent tumor models, and its antitumor mechanism may involve antiangiogenic effects<sup>[1-3]</sup>.

Implanting human tumor cells orthotopically into the corresponding organs of nude mice results in local tumor growth, and a much higher rate of metastasis. Human gastric cancer cells injected into the stomach wall of nude mice produced tumors that eventually metastasize to the liver, demonstrating that orthotopic implantation can enhance the metastatic potential of these tumor cells<sup>[4]</sup>. Recently, a new model of human gastric cancer was developed that avoids the disruption of tumor integrity, by using the orthotopic implantation of intact tumors<sup>[5]</sup>. Such a model should better reflect the original properties of human cancer, and could be of great value in the development of new drugs and new therapy strategies. In the present study, the inhibitory effect of Linomide on the local tumor growth and hepatic metastasis and the changing of microvessel density of tumors was examined in a nude mouse model of metastatic human stomach cancer using the orthotopic implantation of histologically intact tissues.

**Table 1** Inhibitory effect of Linomide in gastric cancer growth in nude mice

	<i>n</i>	Local tumor growth	Volume of local tumor (cm <sup>3</sup> )
Control	28	28/28 (100%)	1.36 ± 0.81
Linomide			
80 mg/kg	10	10/10 (100%)	0.84 ± 0.51 <sup>a</sup>
160 mg/kg	10	10/10 (100%)	0.62 ± 0.35 <sup>b</sup>

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, vs control group.**Figure 1** Structure of Linomide.

## MATERIALS AND METHODS

### Materials

The Linomide was a kind gift of Kabi Pharmacia Therapeutics (Helsingborg, Sweden). It was dissolved in an isotonic solution, and was administered to the mice in their drinking water. These concentrations did not affect total daily water intake of the mice. Control animals were given ordinary drinking water only.

### Animals

Male BALB/c nu/nu mice were obtained from the Shanghai Cancer Institute. In this study, only animals which were 6 to 8 wk old and weighed 20 to 22 g were used.

### Human gastric cancer xenografts

Human gastric adenocarcinoma cell line, SGC-7901, was kindly provided by the Shanghai Cancer Institute. Mice were inoculated s.c. in the flank with  $1 \times 10^6$  SGC-7901 cells, and the xenograft was maintained by serial transplantation in nude mice. Small pieces of tissue were resected aseptically during the exponential growth phase from these tumors and then implanted into nude mice. Mice were anesthetized, and a small midline incision was made to carefully expose the stomach wall. A part of the serosal membrane, about 3 mm in diameter, in the middle of the greater curvature of the glandular stomach was removed at the site where the tumor pieces were to be implanted. A tumor piece of 5 mm in diameter was then fixed on each injured site of the serosal surface with a 7.0 Dexon transmural suture. The stomach was then returned to the peritoneal cavity, and the abdominal wall and skin were closed with 5.0 Dexon sutures. The animals were kept in a specific-pathogen-free environment.

### Assay of tumor growth and hepatic metastasis

Animals were given Linomide at a dose of 80 or 160 mg/kg daily via the drinking water and the same volume of normal saline was given to other mice as control. Mice were sacrificed 10 wk after implantation, or earlier if they developed signs of distress. Autopsy was performed immediately, and the tumors growing on the gastric wall were removed, and its width (a) and length (b) were measured. Tumor volumes in cm<sup>3</sup> were calculated by using a standard formula:  $ab^2 \times 0.5236$ . The ratio of tumor inhibition were calculated as:

$$\frac{[(\text{Volume of control} - \text{Volume of experiment}) / \text{Volume of control}] \times 100\%}{}$$

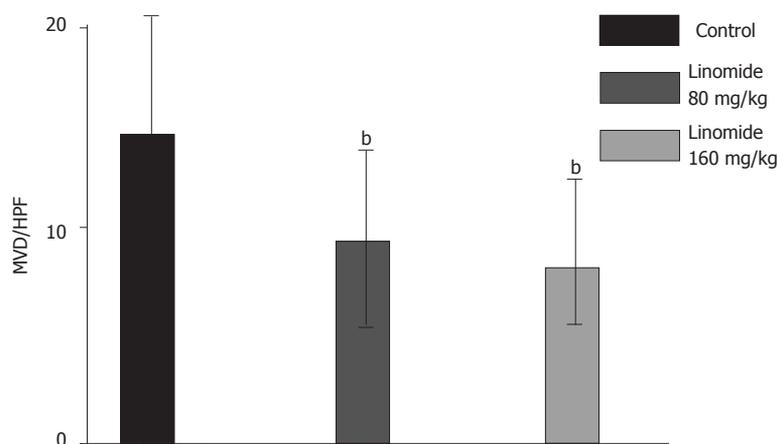
The liver was examined by routine histology to detect metastases.

### Microvessel staining and counting

Intratumoral microvessels were identified by immunostaining using anti-human FVIII related antigen monoclonal antibody, and microvessel density (MVD) was assessed as the count of endothelial

**Table 2** Inhibitory effect of Linomide in hepatic metastasis

	<i>n</i>	Ratio of metastasis	Metastatic foci
Control	28	19/28 (67.9%)	4.2 ± 1.4
Linomide			
80 mg/kg	10	4/10 (40.0%) <sup>a</sup>	2.5 ± 0.8
160 mg/kg	10	1/10 (10.0%) <sup>b</sup>	2

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, vs control group.**Figure 2** Blood vessel density (microvessel density; MVD) per high power field (HPF) in histological sections of gastric tumors from animals treated with different doses of Linomide. <sup>b</sup>*P* < 0.01 vs control.

deposits/mm<sup>2</sup> in the areas that were considered to be most active for neovascularization, as described previously<sup>[6]</sup>.

### Statistical analysis

Numerical values are expressed as the  $\bar{x} \pm s$ . Student's *t* test and the  $\chi^2$  test were used for statistical analysis. Differences were considered significant if *P* < 0.05.

## RESULTS

### Tumor growth

All tumor pieces implanted in the stomach showed local orthotopic growth. Treatment of Linomide significantly decreased the tumor volume as compared to the control group, and the inhibitory effect on tumor growth was related to the dose of Linomide. The ratio of inhibition of 80 and 160 mg/kg Linomide were 38.2% and 54.4% respectively. This result indicates that the growth of gastric carcinoma can be inhibited by Linomide (Table 1).

### Hepatic metastasis

Similar to human patients, the nude mouse model of metastatic human gastric cancer using orthotopic implantation of histologically intact tissue also saw extensive metastases in regional lymph nodes, peritoneum, liver, spleen and other tissues. To appraise the inhibitory effect of Linomide in gastric cancer metastasis, we examined the incidence of hepatic metastasis and the number of metastatic foci in our mouse model. We found that Linomide inhibited hepatic metastasis in a dose dependent manner. The number of metastatic foci in the liver was  $2.5 \pm 0.8$  in the 80 mg·kg<sup>-1</sup> group and 2 in the 160 mg·kg<sup>-1</sup> group. In contrast,  $4.2 \pm 1.4$  metastatic foci were found in the control group (Table 2).

### Effect of Linomide on tumor angiogenesis

The capillary density in tumor of Linomide (80 and 160 mg/kg) treated mice was reduced by 33%-42% as compared with the control group. These data suggest that Linomide attenuated the rate of neovascularization, but did not completely block the initial activation of angiogenesis, nor the capability of each capillary to grow (Figure 2).

## DISCUSSION

Recently, the mechanism of tumor angiogenic and angiogenic

inhibitors have become 'hot spots' in the field of tumor research. Since tumor growth is reported to generally depend on angiogenesis, angiogenic inhibitors should have an inhibitory effect on *in vivo* tumor growth. The angiogenic inhibitor, Linomide, has been reported to have an inhibitory effect on both tumor growth and metastasis in rodent prostatic cancer and melanoma. However, its effect has not been examined in human tumors. In this study, we investigated its inhibitory effect on the local growth and hepatic metastasis of human gastric cancer in a nude mouse model, constructed using the orthotopic implantation of histologically intact tissues. Although it was previously reported that Linomide had an inhibitory effect of tumor growth, because the tumors were inoculated s.c., the model may not reflect the natural environment of tumor growth. The host organ microenvironment can profoundly influence the growth of tumor cells. The model in the present study was more appropriate than heterotopic implantation models. In this study, we found that Linomide can inhibit gastric tumor growth, the ratio of inhibition of 80 and 160 mg·kg<sup>-1</sup> were 38.2% and 54.4%, respectively. The result is consistent with the hypothesis that the rapidly proliferating tumor is more angiogenesis dependent.

Kalland<sup>[3]</sup> had reported that continuous treatment with Linomide in mice reduced the rate of pulmonary metastasis by 85%. In the present study, we found that it inhibited hepatic metastasis in a dose-dependent manner. Hepatic metastasis was observed in only 1 or 10 (10%) of the mice treated with 160 mg/kg, and was decreased significantly in mice treated with either 80 or 160 mg/kg of Linomide as compared with the control group. This result demonstrated that Linomide has an inhibitory effect on the metastasis of human tumors.

Tumor cell metastasis occurs through a complicated process that involves five main steps: (1) angiogenesis, (2) adhesion to endothelial cell basement membrane, (3) local proteolytic destruction of the basement membrane, (4) migration into secondary site, and (5) proliferation at the secondary sites. Tumor growth and metastasis require the development of new vessels. Mature capillaries have a thickened basement membrane, but growing capillaries have fragmented basement membrane. Because growing capillaries are leaky, these new vessels also increase the opportunity for tumor cells to enter the circulation. This study found that the microvessel density of Linomide treatment group was decreased by 33%-42% as compared with control group, indicating that efficacy for hepatic metastasis by Linomide may depend on

the inhibition of angiogenesis at both the first and the final step of the metastatic process. Tumor cells rarely enter circulation because of the inhibition of capillary growth by Linomide. At the same time, tumor cells, when arriving at the target organs, cannot grow without the induction of angiogenesis. The tumor cells that arrived at the liver could not grow to a detectable mass since angiogenesis was inhibited by Linomide.

Vukanovic *et al*<sup>[1]</sup> thought that Linomide could inhibit tumor associated macrophage/monocyte number and their ability to secrete TNF- $\alpha$ . However, the precise mechanism of Linomide on human tumor neovascularization should be investigated further. Maeda *et al*<sup>[7]</sup> have reported that tumor recurrence and metastasis are closely correlated with microvessel count in gastric carcinoma. Our study indicated that hepatic metastasis of human gastric cancer was prevented by inhibiting tumor angiogenesis. Thus, hepatic metastasis may be prevented by angiogenesis inhibitors such as Linomide through reducing the opportunities for tumor cells to enter the circulation and inhibiting the growth of tumor cells arriving in the liver.

In summary, the angiogenesis inhibitor Linomide seems to be a potent tumor growth and metastatic inhibition agent, and might serve as a clinical treatment with further studies.

## REFERENCES

- 1 Vukanovic J, Isaacs JT. Linomide inhibits angiogenesis, growth, metastasis, and macrophage infiltration within rat prostatic cancers. *Cancer Res* 1995; **55**: 1499-1504 [PMID: 7533663]
- 2 Ichikawa T, Lamb JC, Christensson PI, Hartley-Asp B, Isaacs JT. The antitumor effects of the quinoline-3-carboxamide linomide on Dunning R-3327 rat prostatic cancers. *Cancer Res* 1992; **52**: 3022-3028 [PMID: 1591718]
- 3 Kalland T. Effects of the immunomodulator LS 2616 on growth and metastasis of the murine B16-F10 melanoma. *Cancer Res* 1986; **46**: 3018-3022 [PMID: 3486041]
- 4 Yamashita T. Manifestation of metastatic potential in human gastric cancer implanted into the stomach wall of nude mice. *Jpn J Cancer Res* 1988; **79**: 945-951 [PMID: 3141329 DOI: 10.1111/j.1349-7006.1988.tb00059.x]
- 5 Furukawa T, Fu X, Kubota T, Watanabe M, Kitajima M, Hoffman RM. Nude mouse metastatic models of human stomach cancer constructed using orthotopic implantation of histologically intact tissue. *Cancer Res* 1993; **53**: 1204-1208 [PMID: 8439965]
- 6 Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis - correlation in invasive breast carcinoma. *N Engl J Med* 1991; **324**: 1-8 [PMID: 1701519 DOI: 10.1056/NEJM199101033240101]
- 7 Maeda K, Chung YS, Takatsuka S, Ogawa Y, Onoda N, Sawada T, Kato Y, Nitta A, Arimoto Y, Kondo Y. Tumour angiogenesis and tumour cell proliferation as prognostic indicators in gastric carcinoma. *Br J Cancer* 1995; **72**: 319-323 [PMID: 7543771 DOI: 10.1038/bjc.1995.331]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Loss of heterozygosity and mRNA expression at deleted in colorectal cancer gene locus in gastric cancer

Dong-Xu Wang, Dian-Chun Fang, Yuan-Hui Luo, Wei-Wen Liu

Dong-Xu Wang, Dian-Chun Fang, Yuan-Hui Luo, Wei-Wen Liu, Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 630038, China

Dong Xu Wang, Physician in charge, Doctor of Medicine, having 9 papers published.

Author contributions: All authors contributed equally to the work.

Supported by the National Natural Science Foundation of China, No. 39470332.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Dong-Xu Wang, Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 630038, China  
Telephone: +86-811-5318301

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 15, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To assess the effects of the deleted in colorectal cancer (*DCC*) gene changes on the development and progression of gastric cancer.

**METHODS:** The loss of heterozygosity (LOH) and mRNA expression *DCC* gastric cancer using a PCR-based detection method.

**RESULTS:** LOH was found in 35.3% (18/51) of the specimens, and the LOH was more frequently detected in tumors from patients with stage III or IV cancer (50.5%) than those in stages I or II (14.3%) ( $P < 0.05$ ). The occurrence of LOH was not found to correlate with the histological type, tumor size, invasion depth and lymph node metastasis of gastric cancer. The mRNA expression of the *DCC* gene was studied in 26 of the 51 cases, of which LOE was found in 30.8% (8/26). LOE was not significantly correlated to LOH or other clinicopathological parameters.

**CONCLUSION:** LOH and LOE of *DCC* gene are frequently encountered in gastric cancer, and the LOH of *DCC* gene is a late event associated with progression of gastric cancer.

**Key words:** Stomach neoplasms; *DCC* gene; Gene expression; mRNA; Heterozygosity loss

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang DX, Fang DC, Luo YH, Liu WW. Loss of heterozygosity and mRNA expression at deleted in colorectal cancer gene locus in gastric cancer. *World J Gastroenterol* 1997; 3(3): 156-159 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/156.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.156>

### INTRODUCTION

Tumor suppressor genes play an important role in regulating normal cellular proliferation<sup>[1,2]</sup>. Conversely, inactivation of tumor suppressor genes at both alleles may allow a cell to lose normal growth controls and acquire a malignant phenotype. This inactivation may occur through a variety of mechanisms including deletion, rearrangement, point mutation, gene conversion, and binding of suppressor gene products with viral or cellular inactivating proteins<sup>[1,3]</sup>. The deleted in colorectal cancer (*DCC*) gene was first cloned based on frequent deletions affecting the 18q21 region in colon cancer<sup>[4]</sup>. Subsequently, loss of heterozygosity (LOH) or loss of expression (LOE) of *DCC* has been reported in several other tumor types, including breast<sup>[5,6]</sup>, pancreatic<sup>[7]</sup>, prostate<sup>[8]</sup> and testicular<sup>[9]</sup> carcinomas, glioblastomas<sup>[10]</sup> and hematological malignancies<sup>[11]</sup>.

In a study of human gastric cancer, chromosome 18q was frequently affected by the loss of heterozygosity detectable in more than 60% of cases<sup>[12]</sup>. However, there have been no studies reported on LOE of *DCC* gene in gastric cancer. In order to investigate the effects of the *DCC* gene abnormality on the development and progression of gastric cancer, LOH and LOE of *DCC* gene were examined using a PCR based detection method.

### MATERIALS AND METHODS

#### Tissue specimens

Tumor and corresponding noncancerous tissues were obtained from 51 patients who underwent surgical resection for gastric carcinoma between January 1993 and October 1996 at the Southwest Hospital. None of the patients had received any radiotherapy or chemotherapy preoperatively. Each pair of tumor and corresponding non-tumor tissues was stored at -80 °C immediately after the resection for experimental use. A 6 µm section was cut from each tissue and stained with hematoxylin/eosin for pathological diagnosis. After diagnostic confirmation, a visual assessment was made of the approximate proportion of tumor cells vs normal cells in the tumor. Only the specimens in which tumor cells represented ≥ 60% of the tumor tissue were accepted for LOH and LOE analysis.

#### Total RNA isolation and DNA extraction

Total RNA was prepared from tumor and noncancerous tissues using the acid guanidinium thiocyanate method<sup>[13]</sup> and high molecular weight DNA was extracted using proteinase K digestion and phenol chloroform isoamyl alcohol extraction as previously described<sup>[14]</sup>.

#### RT-PCR assay of *DCC* gene expression

RT-PCR was performed as described previously with some modifications<sup>[15]</sup>. *DCC* complementary DNA was amplified at 94 °C for 40 s; 49 °C for 40 s, and 72 °C for 1 min in a Perkin Elmer Thermocycler 2400 for 35 cycles. *DCC* primers were located on exons O and P, amplifying a 233 base pair fragment

**Table 1 Relationship of loss of heterozygosity (LOH) and mRNA expression (LOE) of deleted in colorectal cancer (*DCC*) with clinicopathological parameters**

Clinicopathologic parameters	LOH/informative (%)	LOE/No. examined (%)
Differentiation		
Well/moderate	4/12 (33.3)	1/6 (16.7)
Poor	12/28 (42.9)	5/14 (35.7)
Mucinous carcinoma	2/11 (18.2)	2/6 (33.3)
Tumor size		
< 5 cm	4/20 (20.0)	4/12 (33.3)
> 5 cm	14/31 (45.2)	4/14 (28.6)
Serosal invasion		
Absent	4/18 (22.2)	3/12 (25.0)
Present	12/33 (36.4)	5/14 (35.7)
Lymph node metastasis		
Absent	5/24 (20.8)	2/13 (15.4)
Present	13/27 (48.1)	6/13 (46.2)
Clinical staging		
Stages I - II	3/21 (14.3)	2/11 (18.2)
Stages III-IV	15/30 (50.0) <sup>a</sup>	6/15 (40.0)

<sup>a</sup>*P* < 0.05 as compared with that of stage I and II.

**Table 2 Relationship between loss of heterozygosity (LOH) and mRNA expression (LOE) of deleted in colorectal cancer (*DCC*) gene**

Groups	LOH	LOE	No. of cases (%)
1	+	+	4 (15.4)
2	-	-	14 (53.8)
3	-	+	4 (15.4)
4	+	-	4 (15.4)

+: Positive; and -: Negative.

from the human mRNA<sup>[16]</sup>. A fragment of this size cannot be amplified from genomic DNA, for the primers were designed to frame sequences that cross an intron on the *DCC* gene. RT-PCR without RNA or without reverse transcriptase were included in each experiment as negative controls. Primers used were 5' TTCCGCCATGGTTTTAAATCA 3' (*DCC* sense), and 5' AGCCTCATTTTCAGCCACACA 3' (*DCC* antisense).

### PCR-LOH analysis

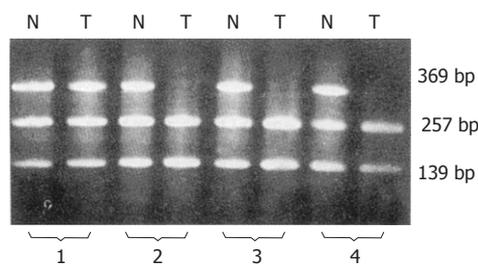
Fifty to 500 ng of genomic DNA were placed at 95 °C for 5 min in 20 μL buffer containing 10 mmol/L Tris (pH 8.3), 5 mmol/L KCl, 2.5 mmol/L MgCl<sub>2</sub>, 0.1 μg/μL bovine serum albumin, sense and antisense primers at 1 μM concentration. Then 2.5 units of Ampli Taq DNA polymerase was added and PCR was run at 94 °C for 40s; 56 °C for 40 s, 72 °C for 1 min, for 35 cycles. For M2 and M3 polymorphism<sup>[17]</sup>, PCR products were digested with MspI and analyzed on 25% agarose gels. For VNTR polymorphism<sup>[18,19]</sup>, PCR products were directly separated on 2.5 gels. The gel was then stained with ethidium bromide and photographed under UV light. The primers were: 5'TGCACCATGCTGAAGATTGT 3' (M2 sense), 5'AGTACAACACAAGGTATGTG 3' (M2 antisense); 5' CGACTCGATCCTACAAAATC 3' (M3 sense), 5' TCTACCCAGGTCTCAGAG 3' (M3 antisense); 5' GATGACATTTTCCCTCTAG 3' (VNTR sense), and 5' GTGGTTATTGCCTTGAAAAG 3' (VNTR antisense). Negative controls without genomic DNA were performed for each set of PCR reaction.

### Data analysis

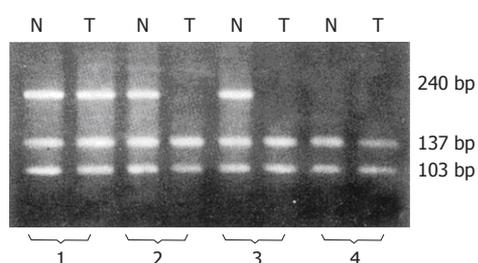
Photographs of thidium-stained gels were read by two observers independently. LOH and LOE was defined by a visible change in that allele: allele ratio in tumor compared to the matching normal tissues. A reduction of allelic intensity over 50% in tumor compared to the matching tissues was taken to be indicative of LOH or LOE, (Figures 1-3).

### Statistical analyses

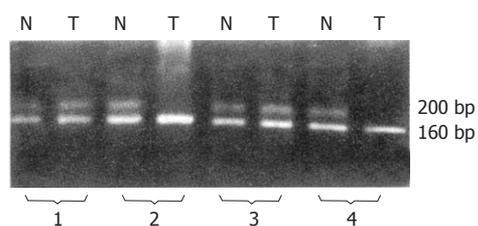
Associations between variables were made with the Chi square test, a *p* value of less than 0.05 was considered to be statistically significant.



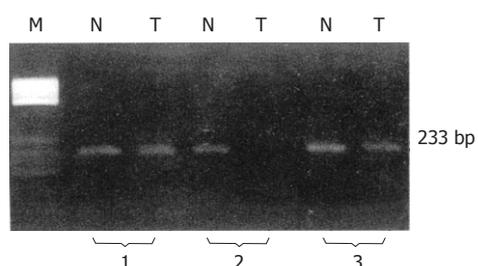
**Figure 1 Heterozygosity loss of deleted in colorectal cancer (*DCC*) gene (M2) of gastric cancer.** N: Normal tissue DNA; T: Tumor tissue DNA; 1: Heterozygote; 2-4: loss of heterozygosity (LOH).



**Figure 2 Heterozygosity loss of deleted in colorectal cancer (*DCC*) gene (M3) of gastric cancer.** N: Normal tissue DNA; T: Tumor tissue DNA; 1: Heterozygote; 4: Homozygote; 2 and 3: loss of heterozygosity (LOH).



**Figure 3 Heterozygosity loss of deleted in colorectal cancer (*DCC*) gene (VNTR) of gastric cancer.** N: Normal tissue; T: Tumor tissue DNA; 1 and 3: Heterozygote; 2 and 4: loss of heterozygosity (LOH).



**Figure 4 Loss of deleted in colorectal cancer (*DCC*) gene expression of gastric cancer.** N: Normal tissue; T: Tumor tissue; M: Marker of molecular weight, PBR 322/*Hae* III; 1 and 3: Normal expression; 2: loss of heterozygosity (LOH).

## RESULTS

LOH of the *DCC* gene was determined by PCR-LOH in 51 specimens of gastric cancer. In order to raise the assay sensitivity, three different sites, *i.e.*, M2, M3 and VNTR were used in this study. LOH of *DCC* was observed in 9 of 47 (19.0%) at M2, 7 of 50 (14.0%) at M3 and 3 of 26 (11.5%) at VNTR sites, respectively. If a positive allelic deletion of *DCC* was judged by LOH at one or any combination of these three sites, the incidence of LOH at the *DCC* locus was 35.3% (18/51). LOH was detected in 37.5% (6/16) of intestinal type of gastric cancer and 36.4% (12/33) of gastric type. Of the 51 cases of gastric cancer, 26 underwent the examination of expression of *DCC* mRNA, and LOE was observed in 30.8% (8/26) (Figure 4). The incidence of LOE was 44.4% (4/9) in the intestinal type and 23.5% (4/17) in gastric type.  $\chi^2$  test revealed no significant difference of the LOH and LOE between these two types of cancer (*p* > 0.05).

Correlation between LOH and LOE of *DCC* and clinicopathological data of gastric cancer are illustrated in Table 1.  $\chi^2$  test demonstrated that LOH of *DCC* was significantly higher in stages III and IV gastric cancer than that in stage I or II (*P* < 0.05). Correlation between

LOH and LOE is shown in Table 2. The paired data were analyzed with  $\chi^2$  test and no significant correlation was found between LOH and LOE ( $p > 0.05$ ).

## DISCUSSION

The *DCC* gene is located on the human chromosome 18q<sup>21.3</sup>. It was reported that the inactivation of this gene is closely related to the pathogenesis of colorectal, esophageal and pancreatic carcinoma, and this gene is considered a susceptible gene in gastrointestinal carcinomas. Uchino *et al.*<sup>[12]</sup> and Ranzani *et al.*<sup>[19]</sup> reported that the rate of LOH of *DCC* in gastric cancer was 61% and 42.9%, respectively; in our study, this rate was 35.5%. Together, these results suggest that *DCC* gene takes part in the pathogenesis of gastric cancer through its LOH.

To our knowledge, this is the first report on the expression of mRNA of *DCC* gene in gastric cancer, which confirmed the LOE of *DCC* mRNA in the gastric cancers with RT-PCR. This suggests that the inactivation of *DCC* in gastric cancer occurs in various patterns, and further confirms that the *DCC* gene is the susceptible gene of gastric cancer, playing an important role in the pathogenesis of gastric cancer.

The histological progression associated with the intestinal type of gastric cancer has been well documented, with apparent evolution through a sequence of superficial gastritis, intestinal metaplasia and dysplasia<sup>[20,21]</sup>. Lesions indicating an adenoma carcinoma sequence similar to that in the colorectum were also observed in the stomach<sup>[22]</sup>. In the present study we have found that the rate of LOE of *DCC* was as high as 44.4% in intestinal type of gastric cancer, which approached that of the rate seen in colorectal carcinoma<sup>[23]</sup>. However, the rate of LOE was rather low in gastric type of gastric cancer. Though there was no statistical difference between the two types of gastric cancer, similarities in genetic alterations between colorectal carcinoma and intestinal type of carcinoma may reflect a carcinogenic pathway common to colorectal carcinoma and gastric carcinoma. It was also found in our study that the rate of LOH of intestinal type of *DCC* was similar between the two types of gastric cancer. Whether the effects of LOH and LOE of *DCC* gene are different between the two types of gastric cancer needs further studies.

The *DCC* gene encodes a molecule which shares high homology with the neural cell adhesion molecule<sup>[16]</sup>. Cell adhesion molecules are cell surface receptors that play critical roles in a number of different processes, including embryogenesis, thrombosis, wound healing, cell homing, and immunoreactivity, as well as tumor progression and metastasis. The inactivation of *DCC* gene may result in malignant degeneration of the cells and aid in the invasion and metastasis of a tumor. Kato *et al.*<sup>[24]</sup> found that the incidence of LOH at *DCC* locus in colorectal carcinoma was significantly greater for patients with liver metastasis than for patients with no liver metastasis. Iino *et al.*<sup>[23]</sup> and Yanoshita *et al.*<sup>[13]</sup> observed that the expression of *DCC* gene in mRNA was greatly reduced or not detectable in invasive colorectal carcinoma in comparison with carcinoma in adenoma and intramucosal carcinoma. They indicated that the inactivation of the *DCC* gene was associated with the progression of early stage carcinoma to advanced stage. Itoh *et al.*<sup>[25]</sup> revealed that the expression level of *DCC* mRNA was lower in liver metastasis than in primary carcinoma. These findings imply that the inactivation of the *DCC* gene occurs in the late stage of colorectal carcinoma, and was of prognostic significance. There were similar conclusions in the study of esophageal and pancreatic carcinoma<sup>[7,26]</sup>. In our study, the rate of LOH of *DCC* rose along with the increase of tumor size and the depth of invasion and the metastasis to lymph nodes, and LOE of *DCC* frequently occurred in gastric cancer of stages III and IV with lymph node metastasis. Though these data did not find a statistical significance, they may suggest that *DCC* gene plays a definite role in the proliferation, invasion and metastasis of gastric cancer. The LOH rate of *DCC* in our study was significantly higher in the stages III and IV than that in stages I or II, which indicates that LOH of *DCC* occurs in the late stage and is related to the advances of the malignancy. Ranzani *et al.*<sup>[19]</sup> had similar findings as ours. Thus, it is expected that LOH

and LOE of *DCC* may be potentially used as a prognostic factor for gastric cancer. However, to accurately reveal the correlation of LOH and LOE of *DCC* with clinicopathologic factors and prognosis, a larger number of cases should be examined.

The interrelation of LOH and LOE of *DCC* gene was preliminarily studied and it was found that LOH does not seem to be necessary for LOE of *DCC* mRNA, which was similar to the finding of others<sup>[14,27]</sup>. There might be some other causes, such as alterations in sequences controlling transcriptional regulation, point mutation or insertions within the *DCC* gene, or alterations in other gene controlling *DCC* gene expression. Further studies are required to examine these possibilities.

## REFERENCES

- Weinberg RA. Tumor suppressor genes. *Science* 1991; **254**: 1138-1146 [PMID: 1659741 DOI: 10.1126/science.1659741]
- Malkin D, Friend SH. The role of tumour suppressor genes in familial cancer. *Semin Cancer Biol* 1992; **3**: 121-130 [PMID: 1511155]
- Levine AJ, Momand J, Finlay CA. The *p53* tumour suppressor gene. *Nature* 1991; **351**: 453-456 [PMID: 2046748 DOI: 10.1038/351453a0]
- Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; **319**: 525-532 [PMID: 2841597 DOI: 10.1056/NEJM198809013190901]
- Cropp CS, Lidereau R, Campbell G, Champene MH, Callahan R. Loss of heterozygosity on chromosomes 17 and 18 in breast carcinoma: two additional regions identified. *Proc Natl Acad Sci USA* 1990; **87**: 7737-7741 [PMID: 1977164 DOI: 10.1073/pnas.87.19.7737]
- Devilee P, van Vliet M, Kuipers-Dijkshoorn N, Pearson PL, Cornelisse CJ. Somatic genetic changes on chromosome 18 in breast carcinomas: is the *DCC* gene involved? *Oncogene* 1991; **6**: 311-315 [PMID: 2000224]
- Höhne MW, Halatsch ME, Kahl GF, Weinel RJ. Frequent loss of expression of the potential tumor suppressor gene *DCC* in ductal pancreatic adenocarcinoma. *Cancer Res* 1992; **52**: 2616-2619 [PMID: 1314700]
- Gao X, Honn KV, Grignon D, Sakr W, Chen YQ. Frequent loss of expression and loss of heterozygosity of the putative tumor suppressor gene *DCC* in prostatic carcinomas. *Cancer Res* 1993; **53**: 2723-2727 [PMID: 8504411]
- Peng HQ, Bailey D, Bronson D, Goss PE, Hogg D. Loss of heterozygosity of tumor suppressor genes in testis cancer. *Cancer Res* 1995; **55**: 2871-2875 [PMID: 7796415]
- Scheck AC, Coons SW. Expression of the tumor suppressor gene *DCC* in human gliomas. *Cancer Res* 1993; **53**: 5605-5609 [PMID: 8242611]
- Porfiri E, Secker-Walker LM, Hoffbrand AV, Hancock JF. *DCC* tumor suppressor gene is inactivated in hematologic malignancies showing monosomy 18. *Blood* 1993; **81**: 2696-2701 [PMID: 8490178]
- Uchino S, Tsuda H, Noguchi M, Yokota J, Terada M, Saito T, Kobayashi M, Sugimura T, Hirohashi S. Frequent loss of heterozygosity at the *DCC* locus in gastric cancer. *Cancer Res* 1992; **52**: 3099-3102 [PMID: 1591722]
- Kikuchi-Yanoshita R, Konishi M, Fukunari H, Tanaka K, Miyaki M. Loss of expression of the *DCC* gene during progression of colorectal carcinomas in familial adenomatous polyposis and non-familial adenomatous polyposis patients. *Cancer Res* 1992; **52**: 3801-3803 [PMID: 1319833]
- Fang DC, Luo YH, Lu R, Liu WW, Liu FX and Liang ZY. Loss of heterozygosity at APC, MCC and *DCC* genetic loci in colorectal cancers. *China National Journal of New Gastroenterology* 1995; **1**: 21-24
- Simon B, Weinel R, Höhne M, Watz J, Schmidt J, Körtner G, Arnold R. Frequent alterations of the tumor suppressor genes *p53* and *DCC* in human pancreatic carcinoma. *Gastroenterology* 1994; **106**: 1645-1651 [PMID: 8194712]
- Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 1990; **247**: 49-56 [PMID: 2294591 DOI: 10.1126/science.2294591]
- Parry PJ, Markie D, Fearon ER, Nigro JM, Vogelstein B, Bodmer WF. PCR-based detection of two MspI polymorphic sites at D18S8. *Nucleic Acids Res* 1991; **19**: 6983 [PMID: 1722309 DOI: 10.1093/nar/19.24.6983-a]
- Huang Y, Boynton RF, Blount PL, Silverstein RJ, Yin J, Tong Y, McDaniel TK, Newkirk C, Resau JH, Sridhara R, Reid BJ, Meltzer SJ. Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. *Cancer Res* 1992; **52**: 6525-6530 [PMID: 1423299]
- Ranzani GN, Renault B, Pellegata NS, Fattorini P, Magni E, Bacci F, Amadori D. Loss of heterozygosity and K-ras gene mutations in gastric cancer. *Hum Genet* 1993; **92**: 244-249 [PMID: 8406432 DOI: 10.1007/BF00244466]
- Jass JR. Role of intestinal metaplasia in the histogenesis of gastric carcinoma. *J Clin Pathol* 1980; **33**: 801-810 [PMID: 7430392 DOI: 10.1136/jcp.33.9.801]
- Correa P, Shiao YH. Phenotypic and genotypic events in gastric carcinogenesis. *Cancer Res* 1994; **54**: 1941s-1943s [PMID: 8137316]
- Tatsuta M, Iishi H, Baba M, Nakaizumi A, Uehara H, Taniguchi H. Expression of c-myc mRNA as an aid in histologic differentiation of adenoma from well differentiated adenocarcinoma in the stomach. *Cancer* 1994; **73**: 1795-1799 [PMID: 8137202 DOI: 10.1002/1097-0142(19940401)73:7<1795::AID-CNCR2820730704>3.0.CO;2-X]
- Iino H, Fukayama M, Maeda Y, Koike M, Mori T, Takahashi T, Kikuchi-Yanoshita R, Miyaki M, Mizuno S, Watanabe S. Molecular genetics for clinical management of colorectal carcinoma. 17p, 18q, and 22q loss of heterozygosity and decreased

- DCC* expression are correlated with the metastatic potential. *Cancer* 1994; **73**: 1324-1331 [PMID: 7906606 DOI: 10.1002/1097-0142(19940301)73:5<1324::AID-CNCR2820730503>3.0.CO;2-W]
- 24 **Kato M**, Ito Y, Kobayashi S, Isono K. Detection of *DCC* and Ki-ras gene alterations in colorectal carcinoma tissue as prognostic markers for liver metastatic recurrence. *Cancer* 1996; **77**: 1729-1735 [PMID: 8608570 DOI: 10.1002/(SICI)1097-0142(19960415)77:8<1729::AID-CNCR47>3.0.CO;2-Z]
- 25 **Itoh F**, Hinoda Y, Ohe M, Ohe Y, Ban T, Endo T, Imai K, Yachi A. Decreased expression of *DCC* mRNA in human colorectal cancers. *Int J Cancer* 1993; **53**: 260-263 [PMID: 7678832 DOI: 10.1002/ijc.2910530215]
- 26 **Miyake S**, Nagai K, Yoshino K, Oto M, Endo M, Yuasa Y. Point mutations and allelic deletion of tumor suppressor gene *DCC* in human esophageal squamous cell carcinomas and their relation to metastasis. *Cancer Res* 1994; **54**: 3007-3010 [PMID: 8187090]
- 27 **Enomoto T**, Fujita M, Cheng C, Nakashima R, Ozaki M, Inoue M, Nomura T. Loss of expression and loss of heterozygosity in the *DCC* gene in neoplasms of the human female reproductive tract. *Br J Cancer* 1995; **71**: 462-467 [PMID: 7880725 DOI: 10.1038/bjc.1995.94]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesion

Ji-Feng Wu, Yu-Lin Song, Guang-Lin Yang, Yu-Ming Dong, Dao-Bing Wang, Min-Pei Liu

Ji-Feng Wu, Yu-Lin Song, Guang-Lin Yang, Yu-Ming Dong, Dao-Bing Wang, Min-Pei Liu, Department of Pathology, Anhui Medical University, Hefei 230032, Anhui Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Ji-Feng Wu, Professor, Department of Pathology, Anhui Medical University, Hefei 230032, Anhui Province, China

Received: October 31, 1996

Revised: December 22, 1996

Accepted: January 30, 1996

Published online: September 15, 1997

### Abstract

**AIM:** To study the significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesions.

**METHODS:** Immunohistochemical staining and mucin histochemical staining were performed on paraffin-embedded sections from gastric benign and malignant lesions from 101 patients.

**RESULTS:** SC3A positive rate was 80.3% (57/71) in lesions of gastric carcinoma. The expression of SC3A was not related to the classification, differentiation, metastasis and or survival rates. The positive rate of SC3A in cancers secreting acid mucin (90.2%) or sulphomucin (91.3%) was higher than that in cancers without acid mucin (20.0%) or sulphomucin (60.0%) ( $P < 0.01$ ). The positive rate of sulphomucin was higher in cases of intestinal metaplasia with cancer (88.9%) than that of cases of intestinal metaplasia with a benign lesion (35.3%) ( $P < 0.01$ ). Additionally, the positive rate of SC3A with sulphomucin in intestinal metaplasia (60.9%) was higher than that without sulphomucin (31.3%) ( $P < 0.05$ ).

**CONCLUSION:** SC3A monoclonal antibody might be helpful in the diagnosis of gastric cancer and the discernment of histogenesis.

**Key words:** antibodies, monoclonal/metabolism; stomach neoplasms/immunology

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wu JF, Song YL, Yang GL, Dong YM, Wang DB, Liu MP. Significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesion. *World J Gastroenterol* 1997; 3(3): 159 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/159.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.159>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers

Guo-Jun Wu, Xiang-Nian Shan, Ming-Fa Li, Shao-Lin Shi, Qi-Ping Zheng, Long Yu, Shou-Yuan Zhao

Guo-Jun Wu, Shao-Lin Shi, Qi-Ping Zheng, Long Yu, Shou-Yuan Zhao, Institute of Genetics, Fudan University, Shanghai 200433, China

Xiang-Nian Shan, Ming-Fa Li, Biological Department of Nanjing Railway Medical College, Nanjing 210009, China

Guo Jun Wu, male, was born on Nov. 2, 1967 in Yuyao County, Zhejiang Province. He graduated from Nanjing Railway Medical College, and is a PhD candidate in genetics at the Institute of Genetics at Fudan University. He has published seven papers.

Author contributions: All authors contributed equally to the work.

Presented at the Fifth Congress of Chinese Genetics Society.

Supported by the National Natural Science Foundation of China (No. 39480018), Natural Science Foundation of Jiangsu Province, No. BK93154315, and Outstanding Youth Teachers Foundation of Jiangsu Province, No. QZ91014.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Guo Jun Wu, Institute of Genetics, Fudan University, Shanghai 200433, China  
Telephone: +86-21-65492222-4107

Received: November 2, 1996  
Revised: January 25, 1997  
Accepted: February 22, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To evaluate the role of *p53* in the development and progression of colorectal cancer and gastric carcinoma by analyzing the loss of heterozygosity (LOH) at 17p13.1 and 17p13.3.

**METHODS:** LOH at the *p53* gene locus and 17p13.3 were examined in 22 cases of gastric carcinoma and 14 cases of colorectal cancer by Southern blot analysis.

**RESULTS:** Of the 22 gastrosarcoma cases, 12 (54%) were heterozygous and LOH was detected in 6 (50%) of the 12 informative cases. In the 14 colorectal cancer cases, 10 (71%) were heterozygous, and LOH was detected in 6 (60%) of the 10 informative cases.

**CONCLUSION:** LOH at the *p53* gene locus is a frequent event in multiple step carcinogenesis progression. The high frequency of LOH at 17p13.3 suggests that there may be another tumor suppresser gene in that chromosome region.

**Key words:** Stomach neoplasms; Colorectal neoplasms; *p53* gene; Heterozygosity loss; Genes, suppressor, tumor

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wu GJ, Shan XN, Li MF, Shi SL, Zheng QP, Yu L, Zhao SY. Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers. *World J Gastroenterol* 1997; 3(3): 160-162 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/160.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.160>

### INTRODUCTION

Recent molecular biology studies have revealed that carcinogenesis is a multiple-step process involving many factors. The activation of some oncogenes and inactivation of some tumor suppresser genes play key roles in this process<sup>[1]</sup>. *p53* is a widely related tumor suppresser gene, but its function has not been clearly defined<sup>[2,3]</sup>. In this research, LOH of the *p53* gene in two common cancers, gastric cancer and colorectal cancer, was examined by Southern blot hybridization and RFLP analysis.

### MATERIALS AND METHODS

#### Samples

Twenty-two cases of gastric cancer and 14 cases of colorectal cancer were acquired from the affiliated hospital of Nanjing Railway Medical College. Tumor tissues and their corresponding normal tissues were obtained during surgery. The classification of tumor and normal tissues was performed by the Department of Pathology of Nanjing Railway Medical College.

#### Probes

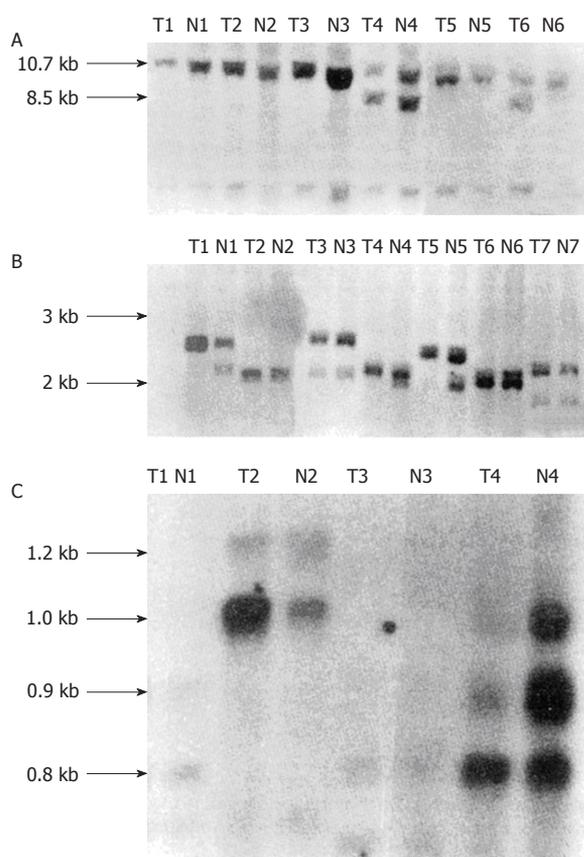
php53B is a *p53* cDNA probe located at 17p13.1. pYNZ22 is a VNTR (variable number of tandem repeats) probe located at 17p13.3. Both probes were obtained from ATCC (American Type Culture Collection).

#### Southern blot

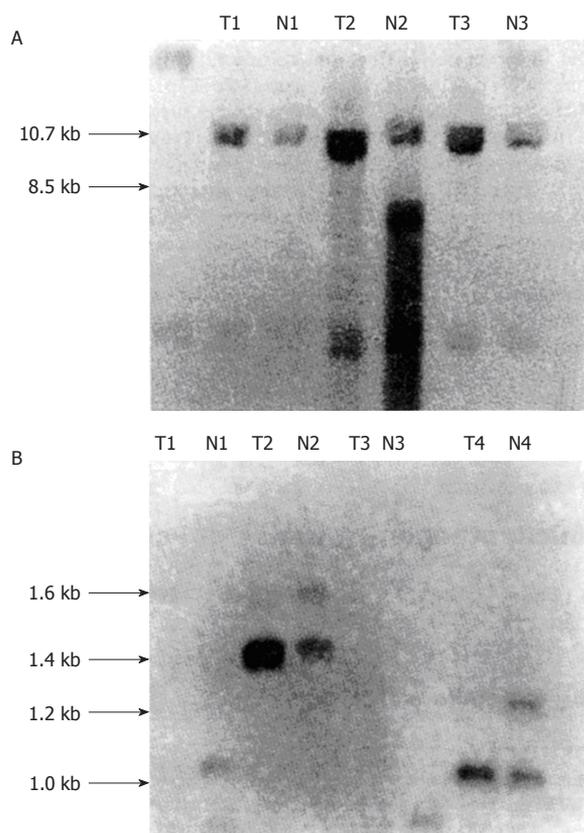
Genomic DNA was isolated from tumor and normal tissues according to standard methods<sup>[4]</sup>. It was then completely digested with specific restriction endonucleases, electrophoresed on agarose gels, denatured, and transferred to nylon filters. The filters were prehybridized for 8-12 h, hybridized for 24-36 h in 50% formamide at 42°C, washed and autoradiographed for 1-4 d at -70°C. Probes were radiolabeled using the random primer method.

#### LOH analysis

The hybridization results of each tumor tissue were compared to that of its normal tissue. If the normal tissue had two heterozygosity bands and its corresponding tumor tissue had only one



**Figure 1** Loss of heterozygosity (LOH) at 17p13 in gastric carcinoma. N: Normal DNA; T: Tumor DNA. (A) LOH at the *p53* locus. Genomic DNA was digested with *Sca*I. Sample 4 showed heterozygosity, and Sample 6 showed LOH. (B) LOH at YNZ22. Genomic DNA was digested with *Taq*I. Samples 3 and 7 showed heterozygosity. Samples 1 and 5 showed LOH. (C) LOH at YNZ22. Genomic DNA was digested with *Msp*I, LOH was detected in Samples 2 and 4.



**Figure 2** Loss of heterozygosity (LOH) at 17p13 in colorectal cancer. N: Normal DNA; T: Tumor DNA. (A) LOH at *p53*. Genomic DNA was digested with *Sca*I. LOH was detected in Sample 2. (B) LOH at YNZ22. Genomic DNA was digested with *Msp*I. LOH was detected in Samples 2 and 4.

homozygosity band, the patient was classified as having *p53* LOH.

## RESULTS

### *php53B* and *pYNZ22* RFLP fragments

Using the *Sca*I restriction enzyme, we identified 8.5 kb and 10.7 kb allelic fragments with the *php53B* probe. After use of the *Msp*I

**Table 1** Loss of heterozygosity (LOH) detected by the *php53B* and *pYNZ22* probes in gastric cancer

Locus	Restriction enzyme	Number of sample	Heterozygosity and LOH rate (%)	LOH and rate (%)
<i>p53</i>	<i>Sca</i> I	22	3 (14)*	2 (66)*
YNZ22	<i>Taq</i> I	8	4 (50)	2 (50)
	<i>Msp</i> I	14	6 (43)*	3 (50)*
17p13		22	12 (54)	6 (50)

\*One sample showed heterozygosity and LOH detected with both probes.

**Table 2** Loss of heterozygosity (LOH) detected by the *php53B* and *pYNZ22* probes in colorectal cancer

Locus	Restriction enzyme	Number of sample	Heterozygosity and LOH rate (%)	LOH and rate (%)
<i>p53</i>	<i>Sca</i> I	7	2 (20)	1 (50)
YNZ22	<i>Msp</i> I	14	8 (57)	5 (62)
17p13		14	10 (71)	6 (60)

The number in bracket shows the rate of heterozygosity or LOH.

restriction enzyme, we identified a group of alleles 0.5 kb to 1.3 kb with the *pYNZ22* probe. While using *Taq*I, we identified allele fragments ranging from 2 kb to 3 kb with the *pYNZ22* probe (Figures 1 and 2).

### LOH of 17p13 in gastric and colorectal cancer

We identified heterozygosity in all of the normal tissues from the 12 gastric cancer cases and ten colorectal cancer cases. They all showed hybridization bands of different lengths. We determined that six gastric cancer cases and six colorectal cancer cases exhibited LOH. The rate of detection was 50% and 60%, respectively. These details are shown in Tables 1 and 2.

## DISCUSSION

In his "two hit" theory, Knudson showed that the loss of function of tumor suppressor genes generally involves at least two genetic mutation events<sup>[5]</sup>. These mutations can be detected by Southern blot hybridization and analysis of the LOH occurrence rate. The closely linked polymorphic gene probes located nearby or inside the possible tumor suppressor gene are used to examine the tumor tissue and its normal adjacent tissue. Detection of LOH suggests that there is a tumor suppressor gene located in the region covered by the gene probe, and two mutation events may have occurred in the tumor suppressor gene.

In this study, we detected LOH in two gastric cancer cases and one colorectal cancer case using the *php53B* probe. As *php53B* is a *p53* cDNA probe, our data suggest that two mutation events occurred in the *p53* gene of some gastric cancer and colorectal cancer patients, and that the normal function of the wild type *p53* gene was lost. We also detected LOH in five gastric and colorectal cancer cases using the *pYNZ22* probe, which is located at 17p13.3 and is tightly linked with *p53*. These results further suggest that the inactivation of *p53* at 17p13.1 is involved in gastric and colorectal carcinogenesis.

To date, *p53* is the only tumor suppresser gene that has been assigned to chromosome 17p13. There are conflicting reports on whether the LOH detected by the *pYNZ22* probe only reflects *p53* inactivation. Studies in breast cancer have shown that LOH detected by *pYNZ22* primarily represents *p53* inactivation<sup>[6]</sup>. However, Coles *et al.*<sup>[7,8]</sup> suggested that there may be certain regulatory genes located between YNZ22 and *p53* that control *p53* gene expression. Damage to this gene, together with *p53* inactivation supposedly contribute to breast cancer carcinogenesis. In our research, only one case of gastric cancer showed LOH in both *p53* and the YNZ22 region, while the other cases did not demonstrate LOH in both *p53* and the YNZ22 locus. Therefore, further studies are necessary, including collecting more cases and using more restriction endonucleases, to determine whether LOH detected within *pYNZ22* in gastric and colorectal cancers represents *p53* inactivation or whether there is an additional regulatory gene near the YNZ22 locus that is mutated in

gastric and colorectal cancers.

---

## REFERENCES

- 1 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-I]
- 2 **Lane DP**. Cancer. p53, guardian of the genome. *Nature* 1992; **358**: 15-16 [PMID: 1614522 DOI: 10.1038/358015a0]
- 3 **Hollstein M**, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991; **253**: 49-53 [PMID: 1905840 DOI: 10.1126/science.1905840]
- 4 **Sambrook J**, Fritsch EF, Maniatis T. Molecular cloning Ed 2. New York: Cold Spring Harbor Laboratory Press, 1989: 914-923
- 5 **Knudson AG**. Hereditary cancer, oncogene and antioncogene. *Cancer Res* 1985; **24**(6): 1437-1443
- 6 **Singh S**, Simon M, Meybohm I, Jantke I, Jonat W, Maass H, Goedde HW. Human breast cancer: frequent p53 allele loss and protein overexpression. *Hum Genet* 1993; **90**: 635-640 [PMID: 8444469]
- 7 **Coles C**, Thompson AM, Elder PA, Cohen BB, Mackenzie IM, Cranston G, Chetty U, Mackay J, Macdonald M, Nakamura Y. Evidence implicating at least two genes on chromosome 17p in breast carcinogenesis. *Lancet* 1990; **336**: 761-763 [PMID: 1976143 DOI: 10.1016/0140-6736(90)93236-I]
- 8 **Kim CJ**, Kim WH, Kim CW, Lee JB, Lee CK, Kim YL. Detection of 17p loss in gastric carcinoma using polymerase chain reaction. *Lab Invest* 1995; **72**: 232-236 [PMID: 7853854]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Clinicopathogenic studies of acute diarrhea in children

Li-Min Cai, Chang Zhang, He Chen, Wei-Ping Jiang, Wen-Xiang Mao

Li-Min Cai, Chang Zhang, He Chen, Wei-Ping Jiang, Wen-Xiang Mao, Department of Infectious Diseases, The First People's Municipal Hospital of Wenling, Wenling 317500, Zhejiang Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Li-Min Cai, Department of Infectious Diseases, The First People's Municipal Hospital of Wenling, Wenling 317500, Zhejiang Province, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To identify etiologic pathogens of acute diarrhea in children and to determine the diagnostic value of stool pH.

**METHODS:** From May 1988 to April 1992, 368 children with acute diarrhea were studied. Fresh stools were routinely examined, and stool pH was tested with pH paper. Samples were placed in Cary-

Blair culture medium and were sent to the lab for bacterial isolation and identification. Rotavirus was identified in the supernatant by ELISA.

**RESULTS:** Thirty-one pathogens and 385 bacterial strains were found in the 368 samples, with a detection rate of 67.7%, including 37.8% of mixed infections. Among the bacteria families, vibrionaceae was the most common (39.7%), and among bacteria genera, aeromonas was the most common (26.8%). In bacterial diarrhea, stool pH tended to be basic, while in viral diarrhea it tended to be acidic.

**CONCLUSION:** There are 31 pathogens for children's acute diarrhea in this area. It is quite difficult to make an etiologic diagnosis only by clinical signs. However, stool pH is of some value for early disease diagnosis.

**Key words:** Diarrhea/etiology; Acute diseases; Diarrhea/diagnosis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Cai LM, Zhang C, Chen H, Jiang WP, Mao WX. Clinicopathogenic studies of acute diarrhea in children. *World J Gastroenterol* 1997; 3(3): 162 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/162.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.162>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## P21 and CEA expression and AgNOR counts in dimethylhydrazine-induced colon carcinoma in rats

Zhi-Gang Zhang, Jing-Ying Wu, Xiang-Dong Fu, Da-Kun Gu, Fang Fang

Zhi-Gang Zhang, Da-Kun Gu, Fang-Fang, Department of Pathology, Shanghai Medical College for Health Staff

Jing-Ying Wu, Xiang-Dong Fu, Ganquan Hospital, Shanghai Tiedao University

Zhi-Gang Zhang, works in the Department of Pathology, Shanghai Medical University, and has published ten papers and one book.

Author contributions: All authors contributed equally to the work.

\*This project was funded by the Youth Research Fund of Shanghai Municipal Health Bureau (131914Y5).

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Zhi-Gang Zhang, Associate Professor, Department of Pathology, Shanghai Medical College for Health Staff  
Telephone: +86-21-64041900/2009

Received: August 8, 1996  
Revised: October 13, 1996  
Accepted: November 19, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To study P21 and carcinoembryonic antigen (CEA) expression and to measure argyrophilic nucleolar organizer region (AgNOR) counts in various lesions of colonic mucosa and the mechanism of carcinogenesis.

**METHODS:** Thirty-eight male Wistar rats were injected with dimethylhydrazine (DMH) once a week for 25 wk. P21 and CEA expression was detected by immunohistochemical methods, and AgNOR was counted by silver staining paraffin sections from various colonic lesions.

**RESULTS:** The incidence of colonic carcinoma in DMH-treated rats was 71.05%(27/38), and lymph node metastasis occurred in six rats. Immunohistochemical studies showed that P21 was primarily expressed in dysplasia and carcinomas, while CEA was expressed in carcinomas and metastatic tumors. AgNOR counts were higher in dysplasia and carcinomas. There were significant differences in P21 and CEA expression between benign and malignant lesions ( $P < 0.05$ ). The difference in AgNOR counts was also significant between normal and dysplastic tissues, and between dysplasia and malignant lesions ( $P < 0.05$ ).

**CONCLUSION:** Dysplasia is a premalignant change of colonic carcinoma. The detection of P21 *via* immunohistochemistry and AgNOR counting may be an important clinical screening technique for colon carcinoma and premalignant lesions.

**Key words:** Colonic neoplasms; Carcinoembryonic antigen; Ras oncogene; Nucleolus organizer region; P21 oncogene

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhang ZG, Wu JY, Fu XD, Gu DK, Fang F. P21 and CEA expression and AgNOR counts in dimethylhydrazine-induced colon carcinoma in rats. *World J Gastroenterol* 1997; 3(3): 163-165 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/163.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.163>

### INTRODUCTION

The transformation-inducing genes most frequently detected in solid human tumors are members of the Ras family of oncogenes. It has been suggested that P21 overexpression in the early neoplasm stage may play an important role in carcinogenesis<sup>[1,2]</sup>. In this study, we examined P21 and CEA expression and quantified nuclear silver stain of the granules of nucleolar organizer region (AgNOR) in 1.2 dimethylhydrazine (DMH)-induced colonic carcinoma in rats to study the carcinogenesis mechanism.

### MATERIALS AND METHODS

#### Animals

Fifty male Wistar rats weighing approximately 170 g were randomly divided into a test group (T group,  $n = 40$ ) and control group (C group,  $n = 10$ ). Rats in the T group were subcutaneously injected with 20 mg/kg body weight DMH once a week. Rats in the C group rats were given 0.9% NaCl solution at the same time. DMH administration lasted 20 wk. At the 10<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup> week, one rat was sacrificed in each group, and 10 were sacrificed at the 20<sup>th</sup> week. The rest were continually fed with standard diet until the 25<sup>th</sup> week, when they were all sacrificed. Intestinal tissues were fixed in 10% neutral formalin.

#### Pathological and immunohistochemical examination

Tissue specimens were collected from the tumor, adjacent tissues and normal colonic mucosa in T group rats, and normal colon mucosa were collected from the C group. Serial paraffin sections were cut at 4  $\mu$ m thickness and were stained with hematoxylin and eosin (HE). The ABC immunohistochemical method was used for P21 staining, and the PAP method was used for CAE staining. The working dilution of the anti-P21 monoclonal antibody (Huamei Company) was 1:40, and 1:100 for the anti-CEA antibody (DAKO Company). PBS was used as a negative control.

#### AgNOR staining technique

A one-step silver staining method was used for AgNOR detection. The observation method and statistical standard were performed as

**Table 1 DMH (dimethylhydrazine)-induced carcinogenesis in rats**

Lesions	<i>n</i>	Incidence (%)	Number of mass
Well-differentiated adenocarcinoma	17	44.74	20
Undifferentiated adenocarcinoma	3	7.89	5
Mucinous adenocarcinoma	7	18.42	9
No tumor	11		
Total	38	71.05	34

**Table 2 Histological changes in colons of DMH (dimethylhydrazine)-treated rats**

Histological types	T group	C group
Normal epithelial cell	0	6*
Inflammation	10	4
Dysplasia	10	0
Adenoma	3	0
Carcinoma	34	0

\*With normal mucosa for all intestines.

**Table 3 The distribution of positive P21 and Carcino Embryonic Antigen (CEA) immunohistochemical staining in rat colonic lesions**

Histological types	<i>n</i>	p21 (%)	CEA (%)
Benign			
Normal epithelial cell	10	0 (0.0)	0 (0.0)
Adenoma	3	1 (33.3)	0 (0.0)
Dysplasia	10	6 (60.0)	3 (30.0)
Malignant			
Well-differentiated adenocarcinoma	20	14 (70.0)	16 (80.0)
Undifferentiated adenocarcinoma	5	1 (20.0)	4 (80.0)
Mucinous adenocarcinoma	9	7 (77.8)	6 (66.7)

*P* < 0.05 for p21 and CEA between benign and malignant lesions.

described previously<sup>[3]</sup>.

## RESULTS

### Animal experiments

Of the 38 rats in the T group (two rats unexpectedly died during the experiment and were excluded), 27 developed colonic carcinoma with a total of 34 masses. The incidence of carcinogenesis was 71.05% (27/38). Various colonic mucosal lesions are summarized in Table 1. The tumors were 1-13 mm in diameter and more frequently occurred in the distal colon. Most tumors appeared nodular in shape. Six rats developed mesenteric lymph node metastasis, and two developed gastric and pulmonary metastases.

### Histopathological changes

T group rats exhibited inflammation and atypical dysplasia of the colonic mucosa at the 10<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup> week (Figures 1 and 2). We observed adenomas and adenocarcinomas after 20 wk with metastasis (Table 2). We never observed dysplasia or tumor development in the control group.

### Immunohistochemical staining

P21 and CEA staining of colonic lesions is summarized in Table 3. Normal and inflammatory mucosa were negative for expression. P21 was weakly expressed in adenomas and primarily expressed in dysplasia and carcinoma (Figure 3), while CEA was expressed in dysplasia, adenocarcinoma and metastasis (Figure 4). The difference in P21 and CEA expression between benign and malignant colonic mucosal lesions in DMH-treated rats was statistically significant (*P* < 0.05).

### AgNOR count

The AgNOR counts in various colonic mucosa lesions were as follows: adenoma 2.7 ± 0.18, dysplasia 3.2 ± 0.21, and adenocarcinoma 4.6 ± 0.26, and normal epithelial cell 1.45 ± 0.08. In colonic adenocarcinoma, the AgNOR granules not only increased in number, but also became larger and irregular. The AgNOR counts were statistically significantly different between normal mucosa and dysplasia, as well as dysplasia and malignant tissue (*P* < 0.05).

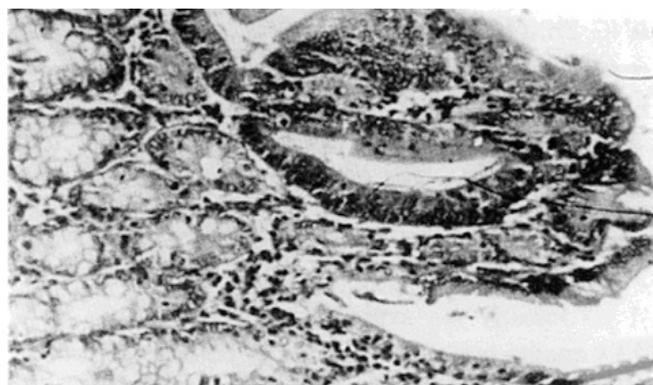


Figure 1 Dimethylhydrazine (DMH)-treated rats, dysplasia localized to the upper 1/3 of the colonic mucosa. HE × 100

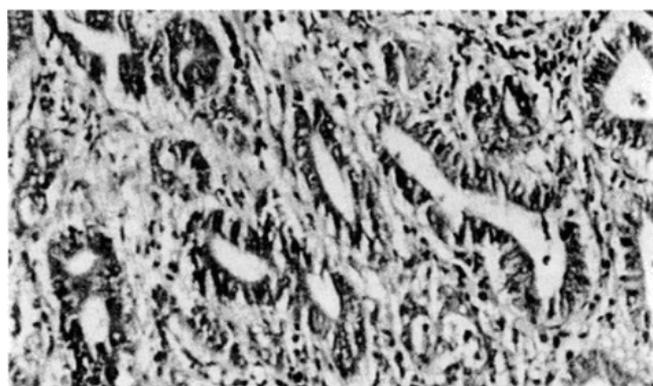


Figure 2 Dimethylhydrazine (DMH)-treated rat, colonic adenocarcinoma. HE × 100

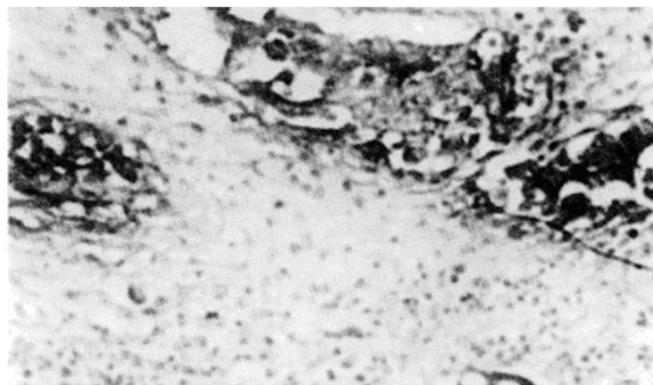


Figure 3 Colonic adenocarcinoma, positive P21 expression in the invasive carcinoma nest. ABC method × 200

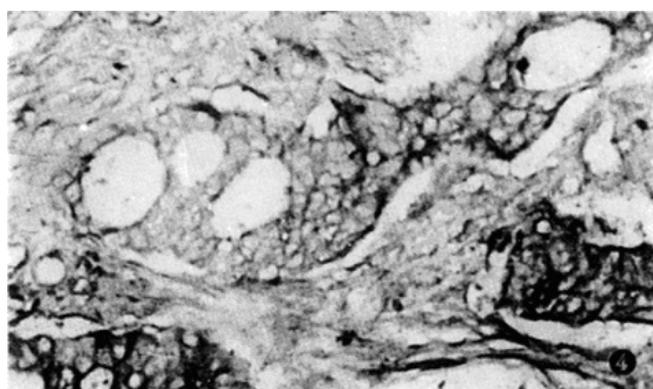


Figure 4 Colonic adenocarcinoma, positive CEA expression in the invasive carcinoma nest. PAP method × 200

However, among the various colonic carcinomas, the differences were not statistically significant (*P* > 0.05).

## DISCUSSION

We treated rats with DMH to induce colonic carcinoma and observed the sequential changes in the colonic mucosa. The marked dysplasia

in the epithelial cells initially involved one or two glands and was localized to the upper 1/3 of the mucosal layer (Figure 1). As the experiment progressed, the number of atypical glands increased, and mucosal noduli formed. Cell heterogeneity also increased, and ultimately led to colonic adenocarcinoma with invasion and metastasis, strongly suggesting that dysplasia is a premalignant lesion for colonic carcinoma.

The activation of oncogenes and P21 overexpression might be critical for malignant cell transformation<sup>[4]</sup>. P21 can inhibit the intercellular signal transmission, leading to loss of controlled cell mitosis. Therefore, P21 overexpression is an important sign of carcinogenesis<sup>[5]</sup>. In our study, we found that P21 protein was expressed in both dysplasia and adenocarcinoma. These data suggest that Ras may exert its effects on cell growth as early as the dysplasia stage to further promote cell transformation. The results support the hypothesis that dysplasia is a premalignant lesion of colonic carcinoma; therefore, ras P21 expression could be an early diagnostic indicator for premalignant lesions. CEA expression is another major marker in colonic carcinoma diagnosis. In DMH-induced colon carcinoma, dysplasia presented with P21 overexpression and increased AgNOR counts, while CEA was only expressed in carcinoma and metastasis. In metastasis, the AgNOR

granules increased and clustered, which is a typical indicator of the malignant colonic carcinoma phenotype. We suggest that P21 and CEA are two proteins expressed in colon carcinoma at different stages of differentiation. In conclusion, P21 immunohistochemical detection and AgNOR quantification important for clinical screening of colon carcinoma and premalignant lesions.

## REFERENCES

- 1 **Carneiro F**, David L, Sunkel C, Lopes C, Sobrinho-Simões M. Immunohistochemical analysis of ras oncogene p21 product in human gastric carcinomas and their adjacent mucosas. *Pathol Res Pract* 1992; **188**: 263-272 [PMID: 1625989 DOI: 10.1016/S0344-0338(11)81203-3]
- 2 **Viola MV**, Fromowitz F, Oravez S, Deb S, Schlom J. ras Oncogene p21 expression is increased in premalignant lesions and high grade bladder carcinoma. *J Exp Med* 1985; **161**: 1213-1218 [PMID: 3886828 DOI: 10.1084/jem.161.5.1213]
- 3 **Zhang ZG**, Zhai WR, Zhang JM, Zhong TL. Study on CEA with immunohistochemistry and AgNOR count in breast carcinoma (in Chinese with English abstract). *Chinese Journal of Histochemistry Cytochemistry* 1994; **3**: 374-378
- 4 **He KL**. Oncogene and growth factor (in Chinese). In: Tang ZY, eds *Modern oncology*, Shanghai: Shanghai Medical University Publisher 1993: 71-75
- 5 **Spandidos DA**, Kerr IB. Elevated expression of the human ras oncogene family in premalignant and malignant tumours of the colorectum. *Br J Cancer* 1984; **49**: 681-688 [PMID: 6733017 DOI: 10.1038/bjc.1984.108]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Double-bullet radioimmunotargeting therapy in 31 primary liver cancer patients

Ying-De Wu, De-Nan Zhou, You-Quan Gang, Xiao-Hua Hu, Zhi-Ge Li, Xiang-Qun Song, Hai-Ping He, Ke-Zheng Yang, Bing-Yan Huang

Ying-De Wu, Xiao-Hua Hu, Zhi-Ge Li, Xiang-Qun Song, Ke-Zheng Yang, Bing-Yan Huang, Department of Chemotherapy, Affiliated Cancer Hospital, Guangxi Medical University, Nanning 530021, Guangxi Province, China

De-Nan Zhou, You-Quan Gang, Hai-Ping He, Guangxi Cancer Institute, Nanning 530021, Guangxi Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Ying-De Wu, Department of Chemotherapy, Affiliated Cancer Hospital, Guangxi Medical University, Nanning 530021, Guangxi Province, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To observe the effect of double bullet immunotargeting therapy with chemotherapy and internal radiotherapy on primary liver cancer.

**METHODS:** The polyclonal horse antibody against human AFP (anti-AFPAb) and the monoclonal murine antibody against human AFP (anti-AFPMcAb) were used as carriers, and  $^{131}\text{I}$  and mitomycin C (MMC) were used as warheads to form double bullet, *i.e.*  $^{131}\text{I}$  anti-AFPMcAb-MMC (double bullet 1) and  $^{131}\text{I}$  anti-AFPAb-MMC (double bullet 2) prepared using the modified chloramine T method. Double

bullet targeting therapy was administered by intravenous drip once a month in 31 patients (treatment group) with unresectable primary liver cancer. Among them, 4, 17 and 10 patients were administered 1, 2 and 3 times, and the median radiation dose (MBq/case) was  $193.5 \pm 37.74$ ;  $651.9 \pm 232.4$ , and  $992.0 \pm 230.5$  respectively.

**METHODS:** Tumor shrinkage, decrease in AFP, and 1 and 2 -year survival rates were significantly higher than the control groups who received transarterial infusion (TAI) or transarterial chemoembolization (TACE) at the same time (50.0%, 15/30 vs 30.0%, 9/30,  $P < 0.05$ ; 66.7%, 18/27 vs 28.0%, 7/25,  $P < 0.01$  and 50.0%, 34.0% vs 33.0%, 3.3%,  $P < 0.01$ , respectively). Furthermore, the tumor progression rate (10%) in the treatment group was significantly lower than that of the control group (40.0%,  $P < 0.01$ ).

**CONCLUSION:** Double bullet target therapy is more effective than traditional therapies due to the synergistic effects of the antibody, radioisotope, and anticancer agents, which together, enhance tumor killing.

**Key words:** Liver neoplasms/therapy; Immunotherapy; Alpha fetoproteins; Antibodies, monoclonal

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wu YD, Zhou DN, Gang YQ, Hu XH, Li ZG, Song XQ, He HP, Yang KZ, Huang BY. Double-bullet radioimmunotargeting therapy in 31 primary liver cancer patients. *World J Gastroenterol* 1997; 3(3): 165 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/165.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.165>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Diagnostic value of occult fecal blood testing for colorectal cancer screening

Kun Chen, Deng-Ao Jiao, Shu Zheng, Lun Zhou, Hai Yu, Ya-Chang Yuan, Kai-Yan Yao, Xing-Yuan Ma, Yang Zhang

Kun Chen, Deng-Ao Jiao, Yang Zhang, Department of Epidemiology, Zhejiang Medical University, Hangzhou 310031, Zhejiang Province, China

Shu Zheng, Lun Zhou, Hai Yu, Institute of Cancer Research, Zhejiang Medical University, Hangzhou, Zhejiang Province, China

Ya-Chang Yuan, Kai-Yan Yao, Xing-Yuan Ma, Institute of Cancer Prevention & Control, Jianshan, Zhejiang Province, China

Kun Chen, male, was born on August 23, 1960, and graduated from the School of Public Health, Zhejiang Medical University, with a Master's degree in public health. He is an associate professor, and Deputy Director of the school and Director of the Epidemiology department, where he studies tumor epidemiology. He has published 35 papers.

Author contributions: All authors contributed equally to the work.

Supported by the National "8<sup>th</sup> 5-year" Science and Technology Development Projects (No.85-914-01).

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Kun Chen, Department of Epidemiology, Zhejiang Medical University, Hangzhou 310031, Zhejiang Province, China  
Telephone: +86-571-7022700-570

Received: October 26, 1996  
Revised: December 21, 1996  
Accepted: January 19, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To evaluate the diagnostic value of occult fecal blood testing in mass colorectal cancer screening.

**METHODS:** A reverse passive hemagglutination reaction fecal occult blood test (RPHA-FOBT) and colorectal cancer risk factor quantitative method were used as preliminary screening for colorectal cancer. A 60-cm fiber optic colonoscopy was used to validate the preliminary screen and was used to detect colorectal cancer in a community of 75813 subjects.

**RESULTS:** Compared to the 60-cm fiber optic colonoscopy as a standard reference, FOBT has a sensitivity of 41.9%, specificity of 95.8%, Youden's index of 0.38, and positive predictive value of 0.68%. These results increased with subject age from the first detection. A 3-year follow up in the target mass showed that all new cases had initially been FOBT-negative.

**CONCLUSION:** The value of FOBT as an indicator of colorectal cancer in mass screening is limited.

**Key words:** Colorectal neoplasms/diagnosis; Occult blood; Mass

screening; Risk factors; Colonoscopy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen K, Jiao DA, Zheng S, Zhou L, Yu H, Yuan YC, Yao KY, Ma XY, Zhang Y. Diagnostic value of occult fecal blood testing for colorectal cancer screening. *World J Gastroenterol* 1997; 3(3): 166-168 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/166.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.166>

### INTRODUCTION

Fecal occult blood testing (FOBT) was first reported by Greigor in 1967 as a useful index in mass colorectal cancer screening<sup>[1]</sup>. Immunochemical FOBT is currently used worldwide<sup>[2-5]</sup> to detect early colorectal cancer, but the sensitivity, specificity, and positive predictive value of this method varies greatly with the differences in the selected masses. Reverse passive hemagglutination reaction fecal occult blood testing (RPHA-FOBT) was established by Zhou *et al*<sup>[4]</sup> in 1987. Since then, this method has been used among patients and colorectal cancer high risk populations with histories of rectal polyps or ulcers. The sensitivity for the two groups was 89% and 64%, respectively, and the positive predictive values were 100% and 1.5%, respectively. These results were statistically significantly different<sup>[6]</sup>. The value of RPHA-FOBT as a mass screening indicator and its relationship with a 3-year cumulative incidence rate (CIR) of colorectal cancer in a population aged  $\geq 30$  years is reported in this study.

### MATERIALS AND METHODS

RPHA-FOBT and colorectal cancer risk factor quantitative methods<sup>[7]</sup> were implemented as a preliminary screening procedure, and a 60-cm fiber optic colonoscopy was performed as an accurate screening from May 1989 to May 1990 in Jianshan County, an area of the highest colorectal cancer incidence rate in China<sup>[8]</sup>. In this study, 75813 subjects were randomly selected from ten towns in Jianshan County. Of the 62611 subjects tested (82.6%), 43 colorectal cancer cases were identified, with a total detection rate of 68.7/10.5. A total of 70% of the 43 cases were classified as either Dukes A or B, the early stages of colorectal cancer.

The entire population studied was surveyed for colorectal cancer incidence. Fifty-three new cases were identified from May 1990 to May 1992, totaling 96 cases within 3 years from May 1989 to May 1992, making the CIR 153.3 per 10<sup>5</sup> people.

RPHA-FOBT kits were purchased from the Basic Medical Sciences Institute of Zhejiang Medical University<sup>[3,4]</sup>. Fecal samples were sent to the local hospital by the examiners, smeared on slides, and transferred to the Lab of Cancer Research Institute of Zhejiang

**Table 1** Diagnostic value of RPHA-FOBT (passive hemagglutination reaction fecal occult blood testing) by age group

Sex	n	Sensitivity (%)	Specificity (%)	J	PV (+)
Men	30177	43.5	95.9	0.39	0.81
Women	32434	40	95.7	0.36	0.56
Total	62611	41.9	95.8	0.38	0.68

**Table 2** Diagnostic value of RPHA-FOBT (reversed passive hemagglutination reaction fecal occult blood testing) by age group

Age (yrs)	Sex	Case/mass	Sensitivity (%)	Specificity (%)	PV(+) (%)
30-39	Men	23/30177	43.5	95.9	0.81
	Women	20/32434	40	95.7	0.56
	Total	43/62611	41.9	95.8	0.68
40-49	Men	22/19026	40.9	95.9	1.15
	Women	18/20517	38.9	95.6	0.77
	Total	40/39543	40	95.8	0.94
50-59	Men	19/10891	47.4	95.9	1.96
	Women	15/12497	46.7	95.3	1.18
	Total	34/23388	47.1	95.6	1.52
≥ 60	Men	8/4856	50	95.2	1.68
	Women	9/6219	55.6	94.8	1.53
	Total	17/11075	52.9	95	1.6

**Table 3** CIR1 and CIR3 (One-year cumulative incidence rate and three-year cumulative incidence rate) by sex and RPHA-FOBT (reversed passive hemagglutination reaction fecal occult blood testing) Results

Sex	FOBT (+)				FOBT (-)			
	n	CIR1	CIR3	<i>u</i> <sup>1</sup>	n	CIR1	CIR3	<i>u</i> <sup>1</sup>
Men	1236	809.1	809.1	0	28941	44.9	148.6	4.01 <sup>b</sup>
Women	1417	564.6	564.6	0	31017	38.7	112.3	3.35 <sup>b</sup>
Total	2653	678.5	678.5	0	59958	41.7	130.1	5.55 <sup>b</sup>

<sup>b</sup>*P* < 0.01; <sup>1</sup>*u* = (|*x*<sub>1</sub> - *x*<sub>2</sub>|) / √(*x*<sub>1</sub> + *x*<sub>2</sub>).

Medical University.

## RESULTS

### Diagnostic value of RPHA-FOBT in mass colorectal cancer screening

FOBT sensitivities in this natural community were 43.5% in males and 40.0% in females, but the specificity was over 95%. There was no statistical significance in Youden's index (J) between males and females (0.39 vs 0.36, *u* = 0.199, *P* > 0.05, Table 1).

The total positive predictive value (PV) of RPHA-FOBT was 0.68% (Table 1). This indicated that endoscopic screening is too large of a scale for epidemiologists, as only approximately seven subjects among 1000 that screened positive with RPHA-FOBT. There was no statistical significance in the positive predictive value (0.81% vs 0.56%,  $\chi^2 = 0.583$ , *P* > 0.05) between men and women.

### Relationship between age and RPHA-FOBT

Colorectal cancer prevalence rates vary with age, and the mass screening results correlated well with the prevalence rate. As the American Association of Cancer has suggested, people over the age of 40 should take the FOBT annually<sup>[9]</sup>. As shown in Table 2, the diagnostic index increased with the initial age of screening; *i.e.*, the sensitivity rose from 43.5% to 50.0% in men, and from 40.0% to 55.6% in women, and the positive predictive value (PV+) increased by 1%.

### Follow-up of the target population

We surveyed 62611 subjects for three years to observe the long-term values of the RPHA-FOBT results for colorectal cancer. We compared the one-year CIR (CIR1) and three-year CIR (CIR3) in FOBT-positive and -negative subjects in females and males. Because FOBT-positive subjects underwent endoscopic screening, there was no change between CIR1 and CIR3 of the FOBT-positive subjects (*u* = 0), while there were statistically significant differences between CIR1 and CIR3 of FOBT-negative females and males. In FOBT-negative males and females, the CIR3 was 2.3 and 1.9 times higher than CIR1, respectively. These results demonstrate that

many colorectal cancer patients were misdiagnosed because of their negative FOBT results (Table 3), and the false negative rate was very high.

## DISCUSSION

We used RPHA-FOBT and the colorectal cancer risk factor quantitative method as preliminary screening procedures, and a 60-cm fiber optic colonoscopy, which could reach the splenic curve, as the accuracy screening procedure in FOBT-positive subjects and the high risk population indicated by the quantitative method. We determined the FOBT diagnostic values by comparing the results from FOBT to the results of the 60-cm fiberoptic colonoscopy. It was previously reported<sup>[10]</sup> that 82% of 3147 Chinese colorectal cancer patients had their cancer initiate in the colon below the splenic curve, suggesting that approximately 20% of cases cannot be identified by this method. However, because of its ease of use and simple preparation, it is still considered a relatively reliable and practical method for reference standard. We examined more than 3000 subjects by 60-cm fiberoptic colonoscopy in our study, and no new cases were identified during a 3-year follow up.

There have not been any similar reports in China about the application of RPHA-FOBT and its value in mass screening. In some studies abroad<sup>[2,5,6,11]</sup>, the sensitivity of immunochemical FOBT ranged from 33% to 50%, which was similar to our results (Table 1). However, our results differed from the previous studies among a special population using the same materials and methods<sup>[3,4]</sup>, suggesting that more than 50% of cases in a normal population cannot be detected using FOBT. The specificity of RPHA-FOBT is over 95%, but when estimated together with other diagnostic indexes, such as the Youden's index (0.39 in males, 0.36 in females in this study), its practical value for screening colorectal cancer was limited.

PV(+), the percentage of patients among FOBT-positive subjects, is another useful index to estimate the diagnostic value of FOBT. Hardcastle *et al.*<sup>[12]</sup> identified 618 FOBT-positive subjects in 27651 individuals, among which 65 subjects were colorectal cancer patients, giving a PV(+) of 10.5%, which is higher than results (PV(+) 4.8%) reported by Kewenter *et al.*<sup>[13]</sup>. The PV(+) reported by Gregorio *et al.*<sup>[6]</sup> was 7.5%, while the PV(+) in our study (0.68%), which was significantly lower than previous reports.

As Gregorio *et al.*<sup>[6]</sup> reported, the PV(+)'s of FOBT were 3% and 9%, respectively, in population less than or more than 60 years old. The difference was significant, but the difference in PV(+) between sexes was not significant (male 7%, female 10%), similar to our results (Table 2). Results in our study suggested that the older the initial age of screening, the higher the rate of PV(+). The PV(+) in our study was lower than in previous other reports. The PV(+) would increase if we defined older initial ages of the screened population to reduce the workload in the screening.

The long-term value of FOBT as a colorectal cancer diagnostic index is not perfect. The CIR1 and CIR3 values were similar in FOBT-positive male and female subjects, but compared to CIR1, the CIR3 in FOBT-negative males and females was approximately 3.3 and 2.9 times higher (*P* < 0.01). The results in Table 3 show that these FOBT-negative subjects should also be monitored to quickly identify and treat new cases.

In summary, when used alone, FOBT is not a satisfactory indicator in mass colorectal cancer screening. Therefore, it is necessary to search for and develop new testing methods, or "concentrate" the target mass. We recommend that the initial age of screening should serve as an important factor in mass colorectal cancer screening.

## REFERENCES

- 1 Greegor DH. Occult blood testing for detection of asymptomatic colon cancer. *Cancer* 1971; **28**: 131-134 [PMID: 5110619 DOI: 10.1002/1097-0142(197107)28:1<131::AID-CNCR2820280125>3.0.CO;2-I]
- 2 Hardcastle JD. The prospects for mass population screening in colorectal cancer. *Cancer Surv* 1989; **8**: 123-138 [PMID: 2680067]
- 3 Yu H. [Evaluation of RPHA fecal occult blood test in screening for colorectal cancer]. *Zhonghua Zhongliu Zazhi* 1990; **12**: 108-110 [PMID: 2401172]

- 4 **Zhou PH**, Zhang WZ. [Experimental study on reverse passive hemagglutination for the detection of human fecal occult blood]. *Zhonghua Yixue Zazhi* 1987; **67**: 671-672 [PMID: 3130160]
- 5 **Khubchandani IT**, Karamchandani MC, Kleckner FS, Sheets JA, Stasik JJ, Rosen L, Riether RD. Mass screening for colorectal cancer. *Dis Colon Rectum* 1989; **32**: 754-758 [PMID: 2503341 DOI: 10.1007/BF02562123]
- 6 **Gregorio DI**, Lolachi P, Hansen H. Detecting colorectal cancer with a large scale fecal occult blood testing program. *Public Health Rep* 1992; **107**: 331-335 [PMID: 1594743]
- 7 **Chen K**, Jiao DA, Yu H, Zhou L, Zheng S, Ma XY, et al. A study of quantitative method in mass screening colorectal cancer. *Chinese Journal of Oncology* 1993; **15**: 37-40
- 8 **Cancer prevention & control office for the Minister of Health**. Investigation and analysis of malignant tumors death in China. Beijing: The Press of People-s Health, 1979: 198
- 9 **The U.S. Preventive Services Task Force**. Guide to clinical preventive services. Maryland: William & Wilkins, 1989: 4
- 10 **National Cooperative Group on Pathology and Prognosis of Colorectal Cancer**. [Study of Dukes' staging of colorectal carcinoma by the prognosis. National Cooperative Group on Pathology and Prognosis of Colorectal Cancer]. *Zhonghua Zhongliu Zazhi* 1986; **8**: 144-145 [PMID: 3021417]
- 11 **Simon JB**. Occult blood screening for colorectal carcinoma: a critical review. *Gastroenterology* 1985; **88**: 820-837 [PMID: 3917961]
- 12 **Hardcastle JD**, Thomas WM, Chamberlain J, Pye G, Sheffield J, James PD, Balfour TW, Amar SS, Armitage NC, Moss SM. Randomised, controlled trial of faecal occult blood screening for colorectal cancer. Results for first 107,349 subjects. *Lancet* 1989; **1**: 1160-1164 [PMID: 2566735 DOI: 10.1016/S0140-6736(89)92750-5]
- 13 **Kewenter J**, Björk S, Haglind E, Smith L, Svanvik J, Ahrén C. Screening and rescreening for colorectal cancer. A controlled trial of fecal occult blood testing in 27,700 subjects. *Cancer* 1988; **62**: 645-651 [PMID: 3292038 DOI: 10.1002/1097-0142(19880801)62:3<645::AID-CNCR2820620333>3.0.CO;2-#]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Difference between periportal and pericentral Kupffer cells in lipopolysaccharide uptake in rats

Xian-Ming Chen, Jin-Chun Liu, Rei-Ling Xu, Xue-Hui Ma, Yuan-Chang Zhao, De-Wu Han

Xian-Ming Chen, Rei-Ling Xu, Xue-Hui Ma, Yuan-Chang Zhao, De-Wu Han, Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Jin-Chun Liu, First Teaching Hospital of Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Xian-Ming Chen, Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To reveal the difference in the ability of Kupffer cells in the periportal and pericentral regions of the liver to uptake lipopolysaccharides (LPS) injected into the portal vein.

**METHODS:** Male Wistar rats were divided into two groups: normal control group ( $n = 6$ ) and GdCl<sub>3</sub>-treated group ( $n = 8$ ). Sixteen hours before the experiment, rats in the GdCl<sub>3</sub>-treated group were injected with GdCl<sub>3</sub> *via* the tail vein to eliminate Kupffer cell function specifically in the periportal region. LPS at a dose of 20 µg/100 g body weight was injected into rats of both groups *via* the portal vein. Zero, 2, 5, 10, 30, and 60 min after LPS injection, liver samples were

obtained and the distribution of LPS in Kupffer cells was observed by immunofluorescence imaging of monoclonal antibody-specific LPS staining using a confocal laser scanning microscope.

**RESULTS:** In the normal control group, positive reactions to LPS were found in Kupffer cells in the periportal region with the peak at two minutes after LPS injection. Kupffer cells in the pericentral region showed the peak at five minutes after LPS injection, but its fluorescent intensity to LPS at the peak time in the cytoplasm was significantly lower than that of Kupffer cells in the pericentral region. In the GdCl<sub>3</sub>-treated group, Kupffer cells in the pericentral region showed the peak at two minutes following LPS injection, and the LPS fluorescent intensity showed no significant difference from that of the normal control rats at the peak point. No significant changes of LPS fluorescent intensities were found in Kupffer cells in the periportal region at various time points following LPS injection in GdCl<sub>3</sub>-treated rats.

**CONCLUSION:** Kupffer cells in the periportal and pericentral regions showed differences in LPS uptake *via* the portal vein.

**Key words:** Liver/metabolism; Kupffer cells; Lipopolysaccharides/metabolism; Portal vein; Endotoxins; *Escherichia coli*

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen XM, Liu JC, Xu RL, Ma XH, Zhao YC, Han DW. Difference between periportal and pericentral Kupffer cells in lipopolysaccharide uptake in rats. *World J Gastroenterol* 1997; 3(3): 168 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/168.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.168>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Effects of metoclopramide on gastrointestinal myoelectric activity in rats

Xiao-Min Qin, Hong-Fang Li, Long-De Wang

Xiao-Min Qin, Hong-Fang Li, Department of Physiology, Lanzhou Medical College, Lanzhou 730000, Gansu Province, China

Long-De Wang, Hospital of Gansu Traditional Chinese Medical College, Lanzhou 730020, Gansu Province, China

Xiao-Min Qin, Associate professor of physiology, Director of Basic Medical Department, with 30 published papers and seven books.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Xiao Min Qin, Associate professor, Department of Physiology, Lanzhou Medical College, Lanzhou 730000, Gansu Province, China

Received: December 20, 1996

Revised: February 25, 1997

Accepted: March 19, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To investigate the effects of metoclopramide (MCP) action on myoelectric activity in the antrum and small intestine.

**METHODS:** Ten healthy male Wistar rats, weighing 250-350 g, were anesthetized with ketamine hydrochloride (100 mg/kg, intramuscularly). Four pairs of bipolar stainless steel electrodes 3 mm apart were implanted on the serosal surface of the antrum at one, 10 and 20 cm distal to the pylorus. Five to ten days following the operation, the gastrointestinal myoelectric activity of fasted rats after intramuscular injection of 2.5, six and 12 mg/kg MCP was recorded using a 8-channel EEG machine, and these values were quantitatively compared with the myoelectric activity after saline injection.

**RESULTS:** In fasted rats, 2.5 mg/kg MCP increased the amplitude of spike activity ( $402.0 \pm 138.4 \mu\text{V}$ , vs  $345 \pm 163.4 \mu\text{V}$ ,  $P < 0.05$ ) and the percentage of the slow wave-containing spike bursts ( $60.4\% \pm 22.0\%$  vs  $47.4\% \pm 22.5\%$ ,  $P < 0.01$ ) of small intestine (1 cm distal to the pylorus), but did not affect the myoelectric activity of the antrum. Six and 12 mg/kg MCP increased the amplitude of both the slow wave ( $332.8 \pm 200.1 \mu\text{V}$  vs  $191.2 \pm 143.9 \mu\text{V}$ ,  $P < 0.01$ ;  $330.0 \pm 197.1 \mu\text{V}$  vs  $191.2 \pm 143.9 \mu\text{V}$ ,  $P < 0.05$ ) and the spike activity of the antrum ( $180.5 \pm 69.7 \mu\text{V}$  vs  $121.8 \pm 63.3 \mu\text{V}$ ,  $P < 0.05$ ;  $174.5 \pm 71.7 \mu\text{V}$  vs  $123.8 \pm 63.3 \mu\text{V}$ ,  $P < 0.05$ ), while in small intestine (1 cm distal to the pylorus) only the amplitude of spike activity ( $407.3 \pm 179.0 \mu\text{V}$  vs  $345.0 \pm 163.4 \mu\text{V}$ ,  $P < 0.05$ ;  $456.0 \pm 145.4 \mu\text{V}$  vs  $345.0 \pm 163.4 \mu\text{V}$ ,  $P < 0.05$ ) and the percentage of the slow wave containing spike bursts ( $61.7\% \pm 26.5\%$  vs  $47.4\% \pm 22.5\%$ ,  $P < 0.01$ ;  $59.1\% \pm 17.3\%$  vs  $47.4\% \pm 22.5\%$ ,  $P < 0.01$ ) was increased and the latent period significantly prolonged ( $2.5 \pm 0.35 \text{ min}$  vs  $0.77 \pm 0.18 \text{ min}$ ,  $P < 0.01$ ).

**CONCLUSION:** Different mechanisms may be involved in enhancing the myoelectric activity of the antrum and small intestine following MCP administration.

**Key words:** Metoclopramide; Stomach; Intestine; Myoelectric activity

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Qin XM, Li HF, Wang LD. Effects of metoclopramide on gastrointestinal myoelectric activity in rats. *World J Gastroenterol* 1997; 3(3): 169-170 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/169.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.169>

### INTRODUCTION

Metoclopramide (MCP) improves gastroduodenal coordination<sup>[1]</sup>, relieves postsurgical and diabetic gastroparesis<sup>[2,3]</sup>, and increases the spike activity during migrating motor complexes (MMC) of small intestine without disrupting the fasting pattern in dogs<sup>[4]</sup>. While these studies suggest that MCP complicates pharmacological action, few have studied the effects of MCP on gastrointestinal myoelectric activity. It is important to compare how different doses impact MCP's effect on the myoelectric activity of the antrum and small intestine. Such studies might be useful both for investigating the side effects of MCP and for further understanding the mechanisms controlling gastrointestinal motility.

### MATERIALS AND METHODS

Ten healthy male Wistar rats, weighing 250-350 g, were anesthetized with ketamine hydrochloride (100 mg/kg, intramuscularly). Four pairs of bipolar stainless steel electrodes (3 mm apart) were implanted onto the serosal surface of the gastrointestinal tract. The first pair was placed on the antrum 0.5 cm from the pylorus and the others on the small intestine at one, 10 and 20 cm distal to the pylorus. The free ends of the electrodes were moved subcutaneously to the back of the neck. Recordings began five to ten days after operation. Electric activity was registered with a 8-channel EEG machine (ND-82B, Shanghai) with a time constant of 0.1 s and a high cutoff frequency of 30 Hz.

In the first, second and third series of experiments, MCP (The Seventh Pharmaceutical Factory of Wuxi) doses were 2.5, six and 12 mg/kg. Each experiment lasted one week and the myoelectric activities were recorded every other day.

The frequency and amplitude of the slow wave and spike activity from the antrum and small intestine were recorded and analyzed 30min following MCP administration, and the number of slow wave-containing spike bursts was also quantified. The results were represented as mean  $\pm$  SD and analyzed statistically by Student's *t*

**Table 1** Effects of metoclopramide on myoelectric activity in the antrum of rats ( $\bar{x} \pm s$ )

	Slow wave		Spike activity
	Frequency (num/min)	Amplitude (V)	Amplitude (V)
Control	4.6 ± 0.3	191.2 ± 143.9	121.8 ± 63.3
2.3 mg/kg	4.5 ± 0.3	192.4 ± 140.1	128.8 ± 54.9
6 mg/kg	4.6 ± 0.3	332.8 ± 200.1 <sup>b</sup>	180.5 ± 69.7 <sup>a</sup>
12 mg/kg	4.5 ± 0.2	330.0 ± 197.1 <sup>b</sup>	174.5 ± 71.7 <sup>a</sup>

Values represent mean ± SD (*n* = 8); <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 vs control.

**Table 2** Effects of metoclopramide on myoelectric activity in the small intestine of rats ( $\bar{x} \pm s$ )

Sit	Groups	Slow wave		Spike activity	
		Frequency (num/min)	Amplitude (V)	Amplitude (V)	Spike bursts (%)
1 cm	Control	38.2 ± 1.4	172.0 ± 101.9	345.0 ± 163.4	47.4 ± 22.5
	2.5 mg/kg	28.2 ± 1.6	175.0 ± 81.9	402.0 ± 138.4 <sup>a</sup>	60.4 ± 22.0 <sup>b</sup>
	6 mg/kg	38.0 ± 1.3	173.7 ± 110.1	407.3 ± 179.0 <sup>a</sup>	61.7 ± 26.5 <sup>b</sup>
	12 mg/kg	37.6 ± 1.1	179.0 ± 116.4	456.0 ± 145.4 <sup>b</sup>	59.1 ± 17.3 <sup>b</sup>
10 cm	Control	37.3 ± 1.4	201.2 ± 110.1	335.8 ± 138.6	38.1 ± 12.9
	2.5 mg/kg	36.9 ± 1.6	201.7 ± 113.3	383.6 ± 150.5	55.4 ± 16.1 <sup>b</sup>
	6 mg/kg	37.0 ± 1.2	210.7 ± 105.2	412.5 ± 158.8 <sup>b</sup>	54.3 ± 25.1 <sup>b</sup>
	12 mg/kg	37.2 ± 1.3	202.5 ± 103.8	452.6 ± 144.3 <sup>b</sup>	58.8 ± 15.6 <sup>b</sup>
20 cm	Control	35.9 ± 1.1	153.2 ± 94.7	322.0 ± 93.1	37.9 ± 14.5
	2.5 mg/kg	36.6 ± 1.2	149.1 ± 69.1	338.2 ± 162.7	46.3 ± 12.1 <sup>a</sup>
	6 mg/kg	36.2 ± 1.2	150.2 ± 50.2	356.4 ± 117.9	60.8 ± 22.6 <sup>c</sup>
	12 mg/kg	35.6 ± 1.4	152.9 ± 73.4	347.3 ± 159.6	59.7 ± 24.2 <sup>b</sup>

Values represent mean ± SD (*n* = 28); <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, <sup>c</sup>*P* < 0.001 vs control.

test, with *P* < 0.05 considered significant.

## RESULTS

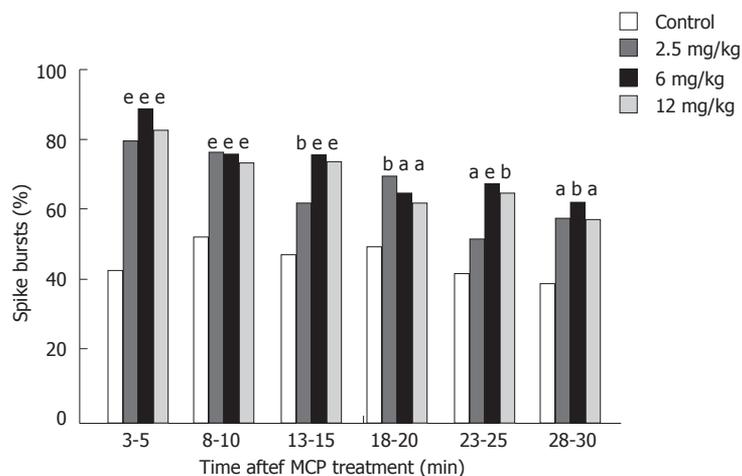
After control recordings following saline injection were obtained at 40 min and 60 min, rats that were fasted for 16-18 h were injected intramuscularly with MCP. The lowest dose of 2.5 mg/kg MCP significantly increased the amplitude of spike activity and the percentage of slow wave-containing spike bursts in the small intestine, however did not affect the myoelectric activity in the gastric antrum (Tables 1 and 2). Nevertheless, the intermediate dose of 6 mg/kg and the highest dose of 12 mg/kg increased the amplitude of spike activity and the percentage of slow wave-containing spike bursts in the small intestine, while also raising the amplitude of both the slow wave and spike activities in the antrum. MCP did not affect the amplitude of the slow wave at any of the four electrode sites (Tables 1 and 2). MCP effects on the electric activity in the small intestine were dose-independent (Table 2, Figure 1).

The latency times of MCP's effect on the gastric antrum vs the small intestine were significantly different, with 0.7 ± 0.18 min in the antrum and 2.5 ± 0.35 min in the small intestine at 1 cm distal to the pylorus.

## DISCUSSION

Gastrointestinal myoelectric activities are divided into slow wave and spike activity (fast wave). Slow wave pace and direct gastric contraction. Thus, the alteration of slow wave would influence the mechanical contraction<sup>[4]</sup>. The spike activity can improve gastrointestinal motility<sup>[5]</sup>. The gastrointestinal myoelectric activity is a sensitivity index of gastrointestinal motility.

In this study, we find that MCP enhances gastrointestinal myoelectric activity, which is consistent with results reported by previous investigations<sup>[6]</sup>. Our results, however, also show significant differences in drug responses between the antrum and



**Figure 1** Dose-dependent effects of MCP (metoclopramide) on the percentage of slow wave-containing spike bursts at 1 cm distal to the pylorus of the small intestine. Note the significant increase in spike bursts induced by MCP. <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, <sup>e</sup>*P* < 0.001 vs control.

small intestine. Firstly, the drug only increased the spike activity in the small intestine, while both the slow wave and the spike activity in the antrum increased. Secondly, MCP's effect on the myoelectric activity of the small intestine was not dose-dependent, as reported by 6 Wingate, *et al*<sup>[6]</sup> and 7 Achem-Karam, *et al*<sup>[7]</sup> in dogs. Nevertheless, the minimal effective doses (or threshold dose) of this drug varied between the antrum and small intestine. For example, a dosage as small as 2.5 mg/kg increased myoelectric activity in only the small intestine, but did not have any effect on the antrum. Thirdly, the latency periods of this drug between the antrum and small intestine were markedly different (*i.e.* 0.70 ± 0.18 min vs 2.50 ± 0.35 min, respectively). These results suggest that the biochemical control of motor activity is not consistent along the gastrointestinal tract. MCP, an analogue of procainamide<sup>[8,9]</sup>, could directly act on the smooth muscle cholinergic M receptor, while also causing acetylcholine release from the postganglionic cholinergic nerve endings<sup>[10]</sup>, as well as increased myoelectric activity in the antrum. However, this increased spiking activity in the small intestine may not be the result of direct action, as other mechanisms are also likely to be involved.

## REFERENCES

- 1 Johnson AG. The action of metoclopramide on human gastroduodenal motility. *Gut* 1971; **12**: 421-426 [PMID: 4996971 DOI: 10.1136/gut.12.6.421]
- 2 Reynolds JC, Putnam PE. Prokinetic agents. *Gastroenterol Clin North Am* 1992; **21**: 567-596 [PMID: 1516959]
- 3 Patterson DJ. Prokinetic agents in postgastrectomy patients. *Gastroenterol Clin North Am* 1994; **23**: 313-325 [PMID: 7915253]
- 4 You CH, Chey WY. Study of electromechanical activity of the stomach in humans and in dogs with particular attention to tachygastria. *Gastroenterology* 1984; **86**: 1460-1468 [PMID: 6143703]
- 5 Chow E, Huizinga JD. Myogenic electrical control activity in longitudinal muscle of human and dog colon. *J Physiol* 1987; **392**: 21-34 [PMID: 3446780 DOI: 10.1111/jphysiol.1987.sp016767]
- 6 Wingate D, Pearce E, Hutton M, Ling A. Effect of metoclopramide on interdigestive myoelectric activity in the conscious dog. *Dig Dis Sci* 1980; **25**: 15-21 [PMID: 6243532 DOI: 10.1007/BF01312727]
- 7 Achem-Karam SR, Funakoshi A, Vinik AI, Owyang C. Plasma motilin concentration and interdigestive migrating motor complex in diabetic gastroparesis: effect of metoclopramide. *Gastroenterology* 1985; **88**: 492-499 [PMID: 3965339]
- 8 McCallum RW. Review of the current status of prokinetic agents in gastroenterology. *Am J Gastroenterol* 1985; **80**: 1008-1016 [PMID: 4072997]
- 9 Ramirez B, Richter JE. Review article: promotility drugs in the treatment of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 1993; **7**: 5-20 [PMID: 8094981 DOI: 10.1111/j.1365-2036.1993.tb00064.x]
- 10 Hay AM, Man WK. Effect of metoclopramide on guinea pig stomach: critical dependence on intrinsic stores of acetylcholine. *Gastroenterology* 1979; **76**: 492-496 [PMID: 428704]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Characteristics of upper digestive tract diseases in Bohai Bay fishermen

Yuan-Ben Wang, Yuan-Ping Wang, Jing Zou, Bao-Jie Bai, Guo-Chun Ren, Ben-Qing Cai

Yuan-Ben Wang, Yuan-Ping Wang, Jing Zou, Department of Gastroenterology, 271 Hospital of PLA, Tianjin 300191, China

Bao-Jie Bai, Guo-Chun Ren, Ben-Qing Cai, Qikou Town Hospital, Huanghua City, Hebei Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Yuan-Ben Wang, Department of Gastroenterology, 271 Hospital of PLA, Tianjin 300191, China

Received: January 28, 1997

Revised: March 22, 1997

Accepted: April 17, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To study the characteristics of upper digestive tract diseases (UDTDs) in fishermen who live in Bohai Bay.

**METHODS:** An investigation was carried out in 1488 fishermen with symptoms of UDTDs (aside from liver, biliary and pancreatic diseases) during the time period between December 1991 and February 1995. This investigation included medical history evaluations, physical, gastroscopic and pathological examinations, tests for *Helicobacter pylori* (*H. pylori*) infection, and analysis of the nitrate content in their drinking water.

**RESULTS:** Among the 1488 subjects investigated, 1467 suffered from one or more of the 14 UDTD diseases, most of which were chronic atrophic gastritis (CAG, 1103 cases), peptic ulcers (268 cases), and cancer of the upper digestive tract (25 cases).

**CONCLUSION:** The incidence rate of UDTDs tends to be high among fishermen due to their particular living habits, the high nitrate content of their drinking water, etc. In addition, the clinical manifestations of UDTDs in fishermen are significantly different from those of the inland residents.

**Key words:** Digestive tract disease; Gastroscopy; nitrate; *Helicobacter pylori*; Gastritis, atrophic; Peptic ulcer; Digestive system neoplasms

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang YB, Wang YP, Zou J, Bai BJ, Ren GC, Cai BQ. Characteristics of upper digestive tract diseases in Bohai Bay fishermen. *World J Gastroenterol* 1997; 3(3): 171-173 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/171.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.171>

### INTRODUCTION

Owing to their special living environment and habits, the incidence and clinical manifestations of the UDTDs found in fishermen may be different from those of inland residents. To gain a clear understanding of the characteristics that are specific to the UDTDs in fishermen, an investigation was carried out on fishermen and their family members from a fishing village near Bohai Bay, Qikou town, Hebei Province, from December 1991 to February 1995. These studies will provide important insight into developing improved methods for disease prevention and treatment.

### MATERIALS AND METHODS

#### Subjects

1488 subjects who required medication for the symptoms of upper digestive tract diseases were recruited for the investigation, which accounted for 6.62% of the 22479 individuals in the town (the annual mean population), and 2.08% of the 71479 individuals who had visits to the doctor due to upper digestive tract symptoms over the three years. There were 830 men and 658 women ranging in age from 15 to 78 years who were involved in this study, with 879 patients aged 21-40 years, accounting for 59.07% of the total number of cases. The ratio of men to women was 1.26 to 1. Patients with liver, biliary or pancreatic diseases were excluded from the study. 1430 cases had a detailed record of disease course, which ranged from one month to 30 years, however the majority of cases (1108, 77.48%) covered less than five years. The primary signs and symptoms included slight epigastric pain that was either regular or irregular in 1039 (72.65%) cases, heartburn or acid regurgitation in 801 (56.01%), anorexia in 727 (50.83%), epigastric distension after meals in 563 (39.37%), epigastric tenderness in 883 (61.74%) and other rare symptoms such as belching, nausea and/or vomiting, hematemesis and melena.

#### Methods

A physical examination as well as a detailed inquiry into each patient's medical history was carried out. All patients who were suspected of having liver, biliary or pancreatic disease received liver function tests and B-mode ultrasonographies. 1488 patients without any liver, biliary or pancreatic diseases were enrolled into the study and underwent complete examinations of the esophagus and stomach using GIF-XQ 20 gastroscopy. The gastroscope reached to the duodenal papilla and beyond in 1463 patients, however failed to pass through the obstructed site in 25 patients with cancer. Photographs of recurring phenotypes were taken for 131 cases. Gastritis was diagnosed based on the Criteria and Schemes of Chronic Gastritis by Fibroscopy constructed by the National Seminar on Fibroscopy in 1978. Peptic ulcer, on the other hand, was diagnosed based on the diagnostic and staging criteria proposed by Sakia T, and other diseases were diagnosed based on the corresponding criteria in China. Biopsy specimens were obtained from 503 patients at two to four random sites and

subjected to pathological examination. Subsequent diagnoses were made according to the Pathologic Diagnostic Criteria for Abnormal Gastric Mucosa proposed by the National Conference on Pathology in Zhengzhou in 1978. Investigations into these patient's life habits included smoking history, alcohol consumption, eating habits (consuming very sweet or salty foods), ingesting non-steroid anti-inflammatory agents and eating schedules. The nitrate content of the resident's drinking water was measured by the Tianjin Detection Center for Environment Protection, and the shrimp pastes were cultured for fungus. In the late stage cancers, biopsy specimens were acquired from 171 patients to test for *H. pylori* using the rapid urease test.

## RESULTS

### Gastroscopy

Of the 1488 patients undergoing gastroscopy, 21 showed no obvious signs of abnormalities. The remaining 1467 cases were diagnosed as simple atrophic gastritis (692, 47.17%), simple atrophic gastritis (163, 11.11%), simple bulbar duodenitis (232, 15.82%), peptic ulcer (268, 18.26%), esophagus cardia gastric fundus peptic ulcer cancer (15, 1.02%), gastric cancer (10, 0.68%), or other diseases (87, 5.93%). These non-cancer diagnoses included reflux esophagitis, esophagus and stomach polyps, diverticulum of the esophagus, stomach or duodenum, inflammation of the stomach's cardiac region, achalasia, hiatus hernia, gastrolithiasis, acute gastric mucosal disease, and gastroduodenal ascariasis. Fourteen different diseases types were detected by gastroscopy. There were 1103 (75.18%) cases of CAG found in this study, including 411 cases of atrophic gastritis that accompanied by other diseases, 268 cases of peptic ulcers (including 91 gastric ulcers), 31 pyloric ulcers, 117 duodenal bulbar ulcers and 29 postbulbar ulcers. Among them, 60 patients had complex ulcers, including multiple active gastric ulcers and duodenal ulcers. Kissing ulcers were also seen frequently. In this series, 625 (42.60%) were identified as having two to five different kinds of disease by gastroscopy.

### Pathological examination

Biopsy specimens from 503 cases were pathologically examined; 121 were diagnosed as superficial gastritis, peptic ulcers or cancers of the upper digestive tract, and 382 were identified as atrophic gastritis. The latter included 152 mild (39.79%), 216 moderate (56.54%), and 14 severe (3.66%) cases, with the moderate and severe CAG (230) making up 60.20% of the total cases of atrophic gastritis. Four patients were under 20 years old, of whom one had mild and three had moderate atrophic gastritis.

### Living habits

The medical histories of the 1414 patients revealed that 836 (59.12%) used to smoke 20 cigarettes per day for over one year, 476 (33.66%) drank 150 g or more of spirits per day for over one year, 746 (52.75%) consumed pungent and irritant foods regularly, and 404 (28.57%) drank sugar-water on a frequent basis. All patients ate dried salt fish and shrimp paste almost daily. More than half of the patients had extremely irregular dining habits owing to their sea-based work. Only a small minority of patients ingested non-steroid anti-inflammatory agents. All patients had more than three types of the life-habits specified above. The nitrate content in the drinking water was 400 g/L (the permissible content is less than 250 g/L). Additionally, rugose colony of candida was observed in the shrimp paste cultures.

### Detection of *H. pylori*

One hundred and seventy-one patients tested positive for *H. pylori* using the rapid urease test, of which 112 (65.48%) were positive and 59 negative (34.50%).

## DISCUSSION

UDTDs are common diseases with complicated etiologies, however their incidence rates have not been reported to date. The incidence of UDTs in the population under investigation was as high as

98.59%, however this does not represent the true rates of UDTD detection and incidence in fishermen because they make up only 6.62% of the local population. Nevertheless, our results may suggest a potential high USTD incidence rate among the fishermen in Bohai Bay. The main categories of UDTDs detected in this population included CAG, peptic ulcers, cancer of the esophagus-cardia-gastric fundus and gastric cancer. The manifestations of these three diseases were not in agreement with those previously reported in the Chinese literature. According to the data reported by Li SN, the detection rate of atrophic gastritis was 6.58% in a 15-25 year old group studied and 34.62% in a separate group ages 65 years and older in regions with high incidences of gastric cancer, while 0% and 6.90% rates, respectively, were found in low incidence regions. In comparison, the reported detection rate in China ranged from 3.20% to 34.62%. In this study, the detection rate of CAG was 74.12% using gastroscopy and 75.94% *via* pathological examination, which are markedly higher values than those mentioned above. This suggests that the incidence of CAG was significantly higher in Chinese fishermen than in the inland residents ( $\chi^2 > 6.63$ ,  $P < 0.01$ ). Up until now, there have been no reports on the degree of atrophy in UDTD. Our results show that the mild, moderate and severe atrophies of the gastric mucosa were 39.79%, 56.54% and 3.66%, respectively, in the 382 cases diagnosed by pathological examination. The moderate and severe atrophies, however were increased up to 60.20%. Furthermore, there were three moderate atrophic cases in the four CAG patients below 20 years of age. It is clear that CAG tends to occur in younger individuals and is significantly more severe in fishermen than in inland residents. In addition, rough gastric mucosa with obvious protrusions, pitting, bleeding, erosion, cobblestone-like morphologies and pseudohypertrophy were often seen in fisherman patients with moderate to severe, but rarely found in inland patients. The incidence of peptic ulcers was reported to be 10%<sup>[2]</sup>. Huang XQ and Qian AC<sup>[3]</sup> concluded that approximately 10% of the Chinese population suffered from peptic ulcers, with the ratio of male to female ranging from 2:1 to 7.4:1<sup>[2,4]</sup>. Among peptic ulcers, 70%-80% were duodenal ulcers<sup>[5]</sup>, 17.5%-20% were gastric ulcers, 5% were complex ulcers, and among the duodenal ulcers 5% were postbulbar ulcers<sup>[5]</sup>. In this series of patients, the ratio of men to women was 1.26:1, and there were 43.65% duodenal bulbar ulcers, 19.86% postbulbar ulcers, 33.95% gastric ulcers and 22.38% complex ulcers. In comparison with values reported in the literature, there were relatively higher detection rates of gastric, postbulbar, and complex ulcers and lower detection rates of duodenal bulbar ulcers ( $\chi^2 > 6.63$ ,  $P < 0.01$ ). In addition, pyloric ulcers and multiple ulcers were also more common in fishermen vs inland residents. Notably, both esophageal and gastric carcinomas are common in China. For example, there are 19 counties and towns in China where the standardized mortality rates from esophageal cancer are over 100/100000; the mortality rates of gastric cancer is 20.95/100000 for males and 10.16/100000 for females<sup>[5]</sup>. The fishermen in this study accounted for 1.02% and 0.68% of the male and female population, respectively, therefore suggesting that Qikou Town has a high incidence rate of esophageal and gastric cancers.

The characteristics of UDTDs found in Bohai Bay fishermen could be related to the several factors. Firstly, coastal residents often consume sodium-rich food such as salt fish and shrimp paste. Long-term high sodium intake can lead to increased osmotic pressure in both the stomach and duodenum and stimulate osmotic pressure receptors on the duodenal wall. As a result, gastric emptying time is prolonged leading to the build up of noxious substances from consumed food in the stomach. This increased stimulation to gastric mucosa causes severe injury, ultimately resulting in chronic gastritis. In addition, nitrates in food are often converted to nitrites by the activity of bacterium reductases, which increase gastric cancer risk. Additionally, excessive long-term smoking can stimulate gastric acid secretion, causing incompetence of the pyloric sphincter, reflux of both bile and duodenal juice into the stomach, elevation of pepsinogen levels in serum, decrease of bicarbonate levels in pancreatic juice and a decrease of prostaglandin E2 levels in serum. The increase in aggressive factors and decrease in defensive

factors could lead to injury of the gastric and duodenal mucosa, resulting in inflammation and the generation of peptic ulcers in both the stomach and duodenum. Another possible reason why UDTD incidence is higher in fishermen than in inland residents is the nitrate content of their drinking water, which was almost twice the level set by the state. Long-term ingestion of nitrate-rich water could lead to accumulation of nitrate in the body. Nitrate is a main source of nitrosamine and nitrosylamine, which are both carcinogenic substances. High nitrate content in food may therefore be an important cause of esophageal and gastric cancers. Furthermore, consuming food that is either too hot or too sweet, in addition to drinking spirits, not only enhances aggressive factors that cause injury to gastric mucosa, but also weakens the defensive factors in the stomach, ultimately resulting in an imbalance of the two factors and the onset of gastritis and gastric ulcers.

Most experts believe that *Hp* infection is related to the occurrence and progress of both gastritis and peptic ulcers, however its relationship to gastric cancer requires further study.

The candida in shrimp paste is an opportunistic pathogenetic fungus, but it does not cause damage to the gastric mucosa under normal conditions. However if the gastric internal environment

changes due to the previously mentioned four factors, candida may act as a pathogen by contributing to UDTDs, which were more prevalent in fishermen compared with inland residents.

It has been suggested that the successful prevention and treatment of UDTDs in fishermen depends upon improving both their social environment and living habits, in addition to pharmacotherapy - lowering nitrate content of their drinking water, minimizing salt fish and shrimp paste consumption, giving up smoking and alcohol, and eating foods with minimal irritants.

---

## REFERENCES

- 1 **Zhang YC.** Mucosal pathology and biopsy of chronic gastritis. Gastric pathology and biopsy of gastric mucosa. eds, Shenyang: Liaoning Publishing House, 1988:32-33
- 2 **Dai ZY,** Chen HZ, Din XJ. Peptic ulcer. Practical internal Medicine. 8th ed. Beijing: Peoples Health Publishing House, 1986:1286-1289
- 3 **Huang XQ,** Qian AC. Peptic ulcer. Ed 1. Tianjin: Tianjin Technical Translation Press, 1993:293-295
- 4 **Zhou L.** Chronic peptic ulcer. Practical Digestive Disease in Elderly. Ed 1. Beijing: People's Military Medical Publisher, 1988:38-48
- 5 **Zhen ZT,** Huang CT, Wang ZT. Gastric and esophageal cancer. Gastroenterology. Ed 2. Beijing People's Health Publishing House, 1993:138-357

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Relation between bile acids and myocardial damage in obstructive jaundice

Yi-Ping Mu, Shu-You Peng

Yi-Ping Mu, Shu-You Peng, Department of Surgery, Second Affiliated Hospital, Zhejiang Medical University, Hangzhou 310009, Zhejiang Province, China

Yi Ping Mou, male, born on May 15, 1965 in Huangyan County of Zhejiang Province, graduated from Wenzhou Medical College and obtained a Bachelors degree in 1986. From 1990 to 1995, under the instruction of Professor Peng Shu You, he was engaged in the diagnosis and surgical treatment of hepatobiliary and pancreatic diseases, the study on the pathophysiology of obstructive jaundice and on the clinical nutriology, and obtained an MD. He is now working in the Department of Surgery, Second Affiliated Hospital, Zhejiang Medical University, and has 15 published papers.

Author contributions: All authors contributed equally to the work.

Supported by the Natural Science Foundation of Zhejiang Province, No. 394177.

A preliminary report was presented at the 95<sup>th</sup> China International Meeting on Haptic, Biliary and Pancreatic Surgery.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Yi-Ping Mu, Department of Surgery, Second Affiliated Hospital, Zhejiang Medical University, Hangzhou 310009, Zhejiang Province, China

Received: September 26, 1996  
Revised: November 16, 1996  
Accepted: December 23, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate the morphologic changes of the myocardium and its relationship to serum bile acids in obstructive jaundice.

**METHODS:** Part 1: 35 rats were randomly assigned to three groups: Group I (BDL1,  $n = 11$ ), the common bile duct (CBD) was ligated and severed and mice were then sacrificed after one week. Group II (BDL2,  $n = 11$ ), the CBD was ligated and severed and mice were then killed after two weeks. Group III (SO,  $n = 13$ ), the CBD was isolated. Hearts were collected for morphologic studies and blood was taken to determine the total serum bile acids (TBA). Part 2: 13 rats received gastric intubation of 10% 4 mL/kg sodium cholate. Their serum TBA and the heart's morphologic changes were then examined.

**RESULTS:** One to two weeks after the CBD was ligated and severed, damage was evident in the mitochondria within the myocardium and the serum TBA was significantly increased. When rats were administered sodium cholate to make their peak blood concentration mimic the average blood concentration in BDL2, a similar degree of myocardial damage was observed.

**CONCLUSION:** An increase in endogenous bile acids is one causative factor of myocardial damage in obstructive jaundice.

**Key words:** Cholestasis; Bile acids and salts; Jaundice; Myocardium/pathology; Mitochondria, heart

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Mu YP, Peng SY. Relation between bile acids and myocardial damage in obstructive jaundice. *World J Gastroenterol* 1997; 3(3): 174-176 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/174.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.174>

### INTRODUCTION

Patients who suffer from jaundice due to extrahepatic biliary obstruction experience high rates of both postoperative complications and death. According to a recent research review, surgery to treat obstructive jaundice has a 10%-25% mortality rate as well as a 56% rate of major complications<sup>[1]</sup>. It is generally accepted that both cardiovascular instability and predisposition to hypotension and shock are main some of the primary causes for the development of postoperative life-threatening complications such as renal failure. Nevertheless, the mechanism of these postoperative complications remains unclear.

Previous studies on isolated cholemia demonstrated that cholemia might lead to cardiac depression. This result suggested that myocardial dysfunction causes circulatory failure in jaundice patients<sup>[2,3]</sup>. The aim of this study is to further investigate whether there is morphologic myocardial damage. Since serum bile acids have detergent properties and are elevated in obstructive jaundice, this study also investigates the relationship between bile acids and myocardial damage.

### MATERIALS AND METHODS

Part I : 35 male Sprague-Dawley (SD) rats weighing 200-250 g were randomly divided into three groups: group I (BDL1,  $n = 11$ ), the common bile duct (CBD) was ligated and severed, and rats were sacrificed after one week: group II (BDL2,  $n = 11$ ), the CBD was also ligated and severed, however the rats were sacrificed after two weeks: group III (SO,  $n = 13$ ), the CBD was isolated and the rats were sacrificed after one week. When the rats were sacrificed, heart specimens were collected for both light and electron microscopic examination, and blood was taken to determine the total bile acid (TBA) and to perform the biochemical liver function test.

Part II : 13 male SD rats weighing 200-250 g received gastric intubation of 10% 4 mL/kg sodium cholate (Sigma, USA). The changes in both their serum TBA and myocardial morphologies were

**Table 1** Changes in serum total bile acid (TBA) and biochemical liver function test

	SO group	BDL1 group	BDL2 group
TBA( $\mu\text{mol/L}$ )	60.83 $\pm$ 14.8	243.12 $\pm$ 129.72 <sup>a</sup>	355.30 $\pm$ 238.38 <sup>a,c</sup>
TB( $\mu\text{mol/L}$ )	5.37 $\pm$ 3.16	108.24 $\pm$ 38.33 <sup>a</sup>	80.03 $\pm$ 37.62 <sup>a</sup>
DB( $\mu\text{mol/L}$ )	1.71 $\pm$ 1.20	76.19 $\pm$ 26.43 <sup>a</sup>	57.57 $\pm$ 33.45 <sup>a</sup>
AKP(u/L)	29.27 $\pm$ 8.96	51.49 $\pm$ 15.05 <sup>a</sup>	55.21 $\pm$ 18.94 <sup>a</sup>
$\gamma$ -GT(u/L)	5.77 $\pm$ 4.32	31.43 $\pm$ 20.15 <sup>a</sup>	31.00 $\pm$ 23.87 <sup>a</sup>
ALT(u/L)	49.38 $\pm$ 24.53	235.57 $\pm$ 235.36	153.22 $\pm$ 148.39 <sup>a</sup>
AST(u/L)	209.46 $\pm$ 104.47	1112.86 $\pm$ 1138.88 <sup>a</sup>	622.33 $\pm$ 536.56 <sup>a</sup>

<sup>a</sup> $P < 0.05$ , significant difference from sham value; <sup>c</sup> $P < 0.05$ , significant difference from BDL1 group. TB: Total bilirubin; DB: Direct bilirubin; AKP: Alkaline phosphatase;  $\gamma$ -GT:  $\gamma$ -glutamyl transpeptidase; ALT: Alanine transaminase; AST: Aspartate transaminase.

examined one, two, three, four, and 24 h later.

Specimens for light microscopic studies were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E). Specimens for electron microscopic studies were double fixed in osmic acid and glutaraldehyde, stained with uranyl acetate, embedded in EPON-812 epoxy resin, and observed under a Philip EM 412 electron microscope.

Total bile acid (TBA) was determined enzymatically using 3- $\alpha$  hydroxysteroid dehydrogenase.

All results represent the mean  $\pm$  SD. Means were compared using the Student's *t* test.  $P < 0.05$  was considered statistically significant.

## RESULTS

Part I : Rats subjected to BDL ligation and severing (BDL groups 1 and 2) had significantly higher serum TB and TAB than the sham operated (SO) group (Table 1).

Morphological studies using light microscopy showed that vascular dilation was only evident within the myocardial interstitium for both BDL groups 1 and 2. Using electron microscopy, however, the myocardial mitochondria appeared swollen, decreased and distorted both both groups, with cristae and outer membranes that either looked obscured or had completely disappeared. Nevertheless, the changes seen in the BDL2 group were more obvious than those in the BDL1 group (Figures 1-3).

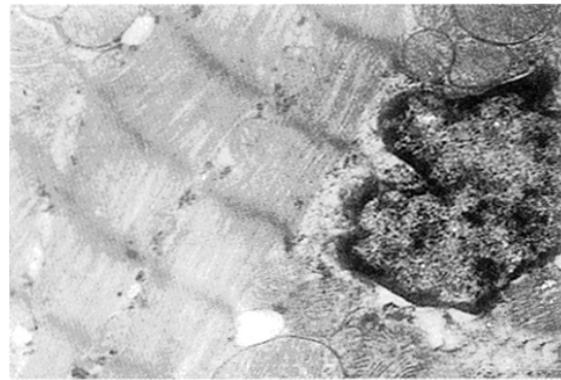
Part II : The serum TBA one, two, three, four, or 24 h following sodium cholate ingestion were 47.7, 132.8, 332.7, 115.0 and 64.3  $\mu\text{mol/L}$ , respectively. The mitochondria in the myocardium appeared swollen and decreased, with cristae and outer membranes that either looked indistinct or had disappeared (Figure 4).

## DISCUSSION

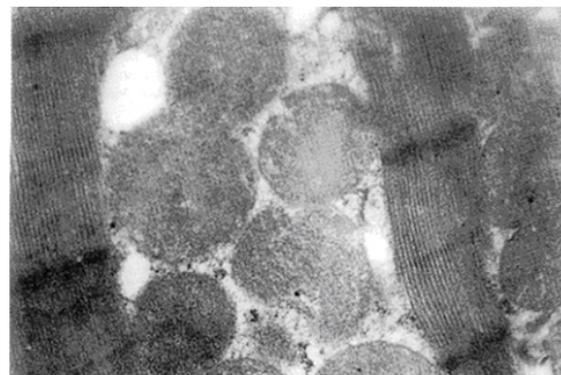
Cardiovascular predisposition and instability to both shock and hypotension have been reported in obstructive jaundice patients. Central and peripheral mechanisms seem to be responsible for increased susceptibility to shock and decreased vascular responsiveness. Recent studies on animal models of isolated cholemia that were induced using choledochocaval anastomosis demonstrated that the mean left ventricular time (LVET) decreased, whereas the mean preejection period (PEP) and mean PEP/LVET were increased. These results suggested that the performance of the left ventricle in isolated cholemia was impaired<sup>[2,3]</sup>. However, the component of bile that was responsible for this impaired performance remained unclear. It also was unclear whether bile caused any morphological changes within the myocardium.

Bile acids are the chief constituents of bile. Due to its mild detergent properties, it may prove cytotoxic at certain concentrations<sup>[4,5]</sup>. Since serum bile acids are elevated in obstructive jaundice, it is important to investigate the relationship between bile acids and the myocardial damage characteristic of obstructive jaundice.

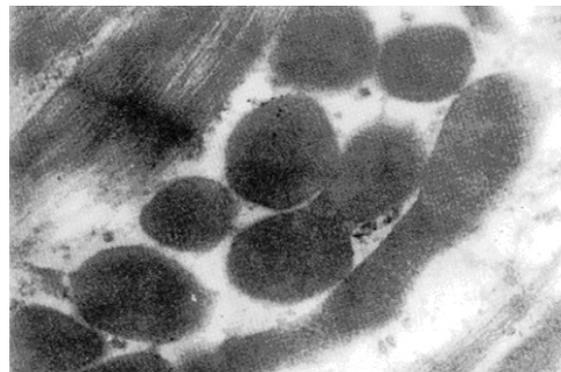
This study demonstrates that 1-2 wk after the CBD was ligated and severed, the serum TBA raises significantly and the myocardial mitochondria decrease in number and swell, exhibiting cristae that appear distorted, curled or entirely dissolved. When rats ingested sodium cholate until their peak blood concentration



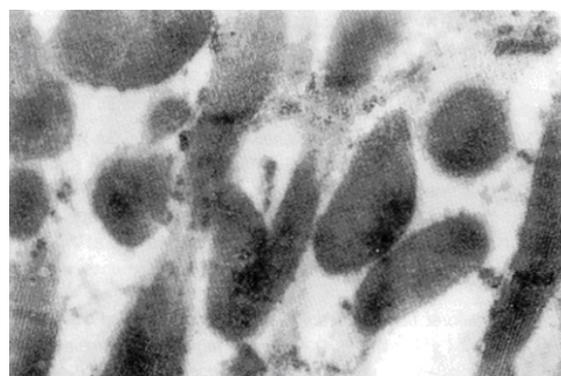
**Figure 1** Electron micrograph of rat myocardium from the sham operated (SO) group ( $\times 14000$ ).



**Figure 2** Electron micrograph of rat myocardium from the BDL1 group. The mitochondria appear swollen, with indistinct outer membranes and obscured cristae ( $\times 21000$ ).



**Figure 3** Electron micrograph of rat myocardium from the BDL2 group. Mitochondria appear distorted and curdled, with indistinct outer membranes and cristae ( $\times 21000$ ).



**Figure 4** Electron micrograph of myocardium from sodium cholate-fed rats. The number of mitochondria are decreased and appear distorted, with indistinct outer membranes and cristae ( $\times 21000$ ).

mimicked the average blood concentration in obstructive jaundice, their myocardium was damaged to a similar degree. These results suggest that the myocardial mitochondria was damaged, thus indicating that endogenous bile acids have a significant deleterious effect that contributes to obstructive jaundice.

It remains unclear how the myocardium can protect itself from the damage caused by bile acids. Previous studies in rats demonstrated that ursodeoxycholic acid could limit histologic

liver alterations and portal hypertension typically induced by bile duct ligation<sup>[6]</sup>. Using the hemolysis of erythrocytes as a model of cytotoxicity, Sagawa *et al.*<sup>[7]</sup> demonstrated that both liposomes and hydrophilic bile acids (such as ursodeoxycholate acid) may protect against hydrophobic bile salt-induced cell membrane damage. We anticipate that the operative mortality and morbidity rates would be decreased if perioperative measures against bile acids toxicity were taken to improve cardiac function<sup>[8]</sup>.

## REFERENCES

- 1 **Scott-Conner CE**, Grogan JB. The pathophysiology of biliary obstruction and its effect on phagocytic and immune function. *J Surg Res* 1994; **57**: 316-336 [PMID: 8028341 DOI: 10.1006/jsre.1994.1151]
- 2 **Green J**, Beyar R, Sideman S, Mordechovitz D, Better OS. The "jaundiced heart": a possible explanation for postoperative shock in obstructive jaundice. *Surgery* 1986; **100**: 14-20 [PMID: 3726756]
- 3 **Zhiwei Q**, Qingpei L, Zhewei F. Experimental study on the influence of obstructive jaundice on cardiac function. *Acta Universitatis Medicinalis Secundae Shanghai* 1993; **13**: 88-90
- 4 **Mu YP**, Peng SY. The acute and chronic toxicity of bile acids. *Foreign Medical Sciences. Surgery* 1995; **22**: 76-78
- 5 **Mu YP**, Peng SY. Advance in the studies on the cytotoxicity of bile acids. *Foreign Med Sci: Physiol Pathophysiol* 1995; **15**: 163-165
- 6 **Poo JL**, Feldmann G, Erlinger S, Braillon A, Gaudin C, Dumont M, Lebec D. Ursodeoxycholic acid limits liver histologic alterations and portal hypertension induced by bile duct ligation in the rat. *Gastroenterology* 1992; **102**: 1752-1759 [PMID: 1568585]
- 7 **Sagawa H**, Tazuma S, Kajiyama G. Protection against hydrophobic bile salt-induced cell membrane damage by liposomes and hydrophilic bile salts. *Am J Physiol* 1993; **264**: G835-G839 [PMID: 8498510]
- 8 **Mu YP**, Peng SY. Toxicity of bile acids in obstructive jaundice. *Chinese Journal of Practical Surgery* 1996; **16**: 16-17

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Immunohistochemical study on endocrine-like tumor cells in colorectal carcinomas

Dao-Cun Wang, Li-Dong Wang, Yun-Ying Jia, Yi-Qing Liu, Chang-Wei Feng, Fu-Ai Tang, Qi-Zhou, Zhen-Feng Li, Guang-Lin Cui

Dao-Cun Wang, Li-Dong Wang, Yun-Ying Jia, Yi-Qing Liu, Chang-Wei Feng, Fu-Ai Tang, Qi-Zhou, Zhen-Feng Li, Guang-Lin Cui, Department of Gastroenterology, the Second Affiliated Hospital, Henan Medical University, Zhengzhou 450003, Henan Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Dao-Cun Wang, Department of Gastroenterology, the Second Affiliated Hospital, Henan Medical University, Zhengzhou 450003, Henan Province, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 24, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To evaluate the clinical significance of endocrine-like tumor cells in human colorectal carcinomas.

**METHODS:** The immunohistochemistry method (ABC) using a rabbit polyclonal antibody against human chromogranin A (CGA) was employed to determine changes in endocrine-like tumor cells from the surgically resected colorectal carcinoma tissues of patients (35 males and 27 females, aged from 19 to 78 years, with a mean age of 50.3 years). Of the 62 specimens, 44 were from rectal carcinomas, 18 from colonic carcinomas, 14 from lymph nodes and 48 from non-involvement. Dukes classification revealed 19 of the cases were in

stage A, 29 cases were in stage B and 14 cases were in stage C. All of the specimens were fixed with 10% formalin, embedded with paraffin and cut into 5  $\mu$ m sections. Additionally, the correlations among CGA-positive tumor cells, as well as the clinicopathologic data, age and sex of the patients, were also investigated.

**RESULTS:** CGA-positive tumor cells were found in 35.5% of the patients with colorectal cancers, representing 20.0% (5 of 25) and 45.9% (17 of 37) of the aged and non-aged, respectively. These differences were significant ( $\chi^2$  test,  $P < 0.05$ ). Nevertheless, no significant correlations were found between the CGA-positive tumor cells and the sex, Dukes stages, tumor location, degree of histological differentiation or presence of lymph node metastasis.

**CONCLUSION:** The low incidence of endocrine-like tumor cells found in the aged patients may be a new pathological feature for colorectal carcinomas, which could explain why the aged patients usually had a better prognosis. The exact significance of these findings requires further characterization.

**Key words:** Colorectal neoplasms/pathology; Chromogranin A; Immunohistochemistry

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang DC, Wang LD, Jia YY, Liu YQ, Feng CW, Tang FA, Zhou Q, Li ZF, Cui GL. Immunohistochemical study on endocrine-like tumor cells in colorectal carcinomas. *World J Gastroenterol* 1997; 3(3): 176 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/176.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.176>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Analysis of fibronectin, fibronectin receptor and interleukin-1 in patients with cirrhosis treated by the Yanggan Jieyu decoction

Hong Wu, Jie-Sheng Gao, Jian-Zhen Fan, Jing Huang, Jan-Wei Deng

Hong Wu, Jie-Sheng Gao, Jian-Zhen Fan, Jing Huang, Jan-Wei Deng, The Second Affiliated Hospital of Hunan Medical University, Changsha 410011, Hunan Province, China

Hong Wu, professor of clinical immunology and integrated traditional Chinese and western medicine, graduated from Hunan Medical College, in 1961, studied in the U.S.A. from 1988-1990.

Author contributions: All authors contributed equally to the work.

Supported by the Hu National Natural Sciences Foundation of China, No. 86030617; and by the Hunan Province, No.92110.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Jie-Sheng Gao, The Second Affiliated Hospital of Hunan Medical University, Changsha 410011, Hunan Province, China  
Tel: +86-486-731-5550495

Received: June 13, 1996  
Revised: October 9, 1996  
Accepted: November 16, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate the effects of the Yanggan Jieyu (YGJY, nourishing the liver and alleviating mental depression) decoction on the plasma concentrations of fibronectin (FN), fibronectin receptor (FNR), tumor necrosis factor alpha (TNF- $\alpha$ ), and the activity of interleukin-1 (IL-1) in patients with cirrhosis.

**METHODS:** Thirty-four cases of decompensated cirrhosis were divided into the YGJY decoction treatment group and the control group (patients received standard treatment). FN, FNR and TNF- $\alpha$  were measured by ELISA and expressed as mg/L (FN, FNR) and ng/L (TNF- $\alpha$ ). IL-1 was measured by mice thymocyte proliferation using a  $\beta$  scintillation counter and was expressed as cpm.

**RESULTS:** In the YGJY decoction treatment group, FN and TNF- $\alpha$  levels increased significantly ( $P < 0.01$  and  $P < 0.05$ , respectively), and FNR and IL-1 levels decreased significantly ( $P < 0.05$  and  $P < 0.05$ , respectively). In the control group, FN, FNR, TNF- $\alpha$ , and IL-1 levels did not significantly change.

**CONCLUSION:** YGJY decoction could prevent hepatic fibrosis by adjusting the plasma levels of FN, FNR, TNF- $\alpha$  and IL-1, which could mediate cirrhosis formation. This data is of clinical significance.

**Key words:** Liver cirrhosis; Yanggan Jieyu decoction; Fibronectin; Fibronectin receptors; Tumor necrosis factor; Interleukin-1; Traditional Chinese Medicine

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All

rights reserved.

Wu H, Gao JS, Fan JZ, Huang J, Deng JW. Analysis of fibronectin, fibronectin receptor and interleukin-1 in patients with cirrhosis treated by the Yanggan Jieyu decoction. *World J Gastroenterol* 1997; 3(3): 177-179 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/177.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.177>

### INTRODUCTION

Recent reports have shown that serum levels of some extracellular matrix proteins and cytokines, such as fibronectin and interleukin-1, may be biomarkers of hepatic fibrosis, as well as important molecules for initiating hepatic fibrosis<sup>[1-3]</sup>. Using the autoimmune hepatitis and cirrhotic model of C<sub>57</sub> BL/6 mice, it was suggested that the Yanggan Jieyu (YGJY, nourishing the liver and alleviating mental depression) decoction could prevent liver tissue damage<sup>[4]</sup>. Clinical studies have confirmed that many cirrhotic patients were in the state of Gan Yu Xue Xu (stagnancy of the Qi and deficiency of blood in the liver). In this study, we evaluated the curative effect of the YGJY decoction in cirrhosis.

### MATERIALS AND METHODS

#### Patients

Thirty-four patients (30 men and 4 women ranging in ages from 25 years to 68 years) diagnosed with chronic cirrhotic Hepatitis B virus (HBV) were included in this study. Their diagnoses were based on clinical and laboratory evaluations<sup>[5]</sup>. All of the cases were decompensated cirrhosis and 80% of the cases also had cirrhotic ascites).

#### Treatment

The patients were randomly divided into two groups. Twenty patients were treated with the YGJY decoction (35 g per day for 8 wk). The decoction is made of Bupleuri (10 g), Lycium barbarum (10 g), Rapid Angelicae Sinensis (10 g), and Radix Glycyrrhizae (5 g). The remaining 14 patients were treated by the standard methods. Twenty healthy volunteers served as controls.

#### Plasma FN and FNR analysis

The FN and FNR levels were assayed by ELISA. FN was detected by using a specific anti-FN rabbit serum (rabbit immunoglobulin to human fibronectin provided by Dakopatts, Denmark)<sup>[1,2]</sup>. FNR was detected by using an anti-FNR mouse antibody (Takara Biolaboratory, Japan). The results were expressed as mg/L.

#### IL-1 production and analysis

IL-1 was produced from human mononuclear cells (macrophages) after stimulation with 40  $\mu$ g of LPS (Sigma) and 3  $\mu$ g of indomethacin (Sigma). Thymocyte proliferation was analyzed using the thymocytes from 4 to 8 wk old BALB/C mice. The samples were

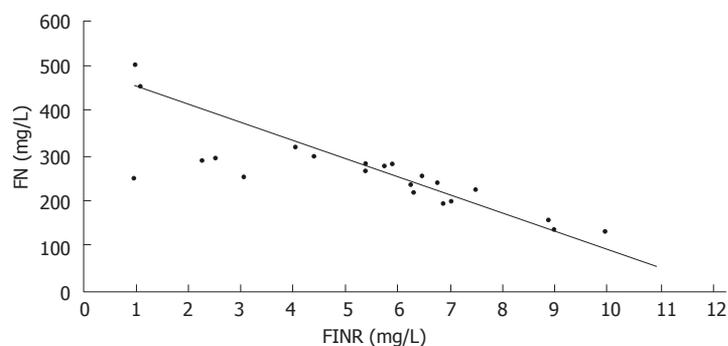


Figure 1 Serum FNR level in relation with plasma FN in 34 patients with chronic liver diseases (liver cirrhosis).

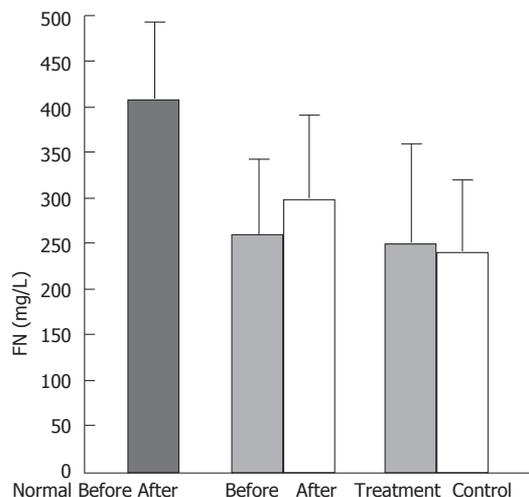


Figure 2 Serum levels of fibronectin in the hepatic fibrosis patients treated with the YGJY (Yanggan Jieyu) decoction.

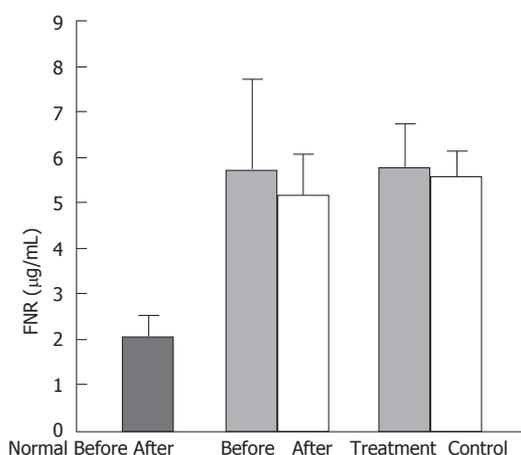


Figure 3 Serum levels of the fibronectin receptor in the hepatic fibrosis patients treated with YGJY (Yanggan Jieyu) decoction.

incubated in the presence or absence of 0.3 mg/L conA (Sigma). Sample dilutions were assayed for IL-1 activity by the incorporation of tritiated thymidine into the cells and counted with a  $\beta$  scintillation counter. The results were expressed as cpm<sup>[6]</sup>.

### Serum TNF- $\alpha$ analysis

The serum TNF- $\alpha$  concentrations were determined by the ELISA (Genzyme corporation, Cambridge, MA, United States).

### Statistical analysis

The mean values in the control and cirrhotic patients before and after treatment were compared by the Student's *t* test. The correlation between FN and other parameters of hepatic fibrosis were evaluated by a linear regression analysis. Data were expressed as average  $\pm$  standard deviation.

## RESULTS

In the healthy control group, mean plasma FN levels were 413.0

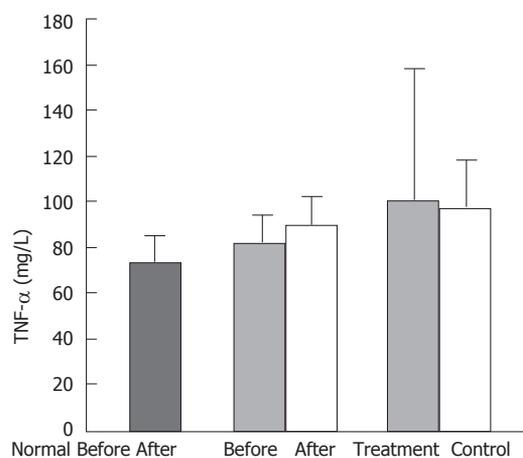


Figure 4 Tumor necrosis factor released in hepatic fibrosis patients treated with the YGJY (Yanggan Jieyu) decoction.

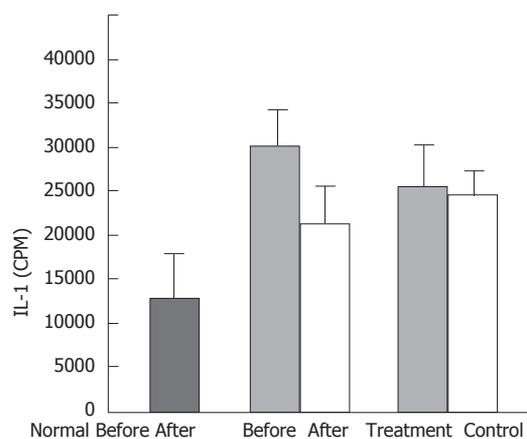


Figure 5 Interleukin-1 release in hepatic fibrosis patients treated with the YGJY (Yanggan Jieyu) decoction.

$\pm$  72.5 mg/L, FNR levels were  $2.3 \pm 0.4$  mg/L, TNF- $\alpha$  levels were  $72.3 \pm 8.6$  ng/L, and IL-1 activity was  $1320.6 \pm 419.2$  cpm. In the experimental group, cirrhotic patients had significantly decreased FN levels ( $248 \pm 97.0$  mg/L,  $P < 0.01$ ), and significantly increased FNR levels ( $5.5 \pm 2.3$  mg/L,  $P < 0.01$ ), TNF- $\alpha$  levels ( $97.4 \pm 29.4$  ng/L), and IL-1 activity, ( $2760.8$  cpm,  $P < 0.05$ ), compared with the healthy control group. A negative correlation was observed between the serum concentrations of FN and FNR ( $P < 0.01$ ,  $r = -0.6534$ ) (Figure 1).

After treatment with the YGJY decoction, the FN levels significantly increased ( $247.9 \pm 97.2$  mg/L to  $298.3 \pm 93.2$  mg/L,  $P < 0.01$ ) (Figure 2). The FNR levels significantly decreased after YGJY treatment ( $5.6 \pm 2.7$  mg/L to  $4.3 \pm 2.3$  mg/L,  $P < 0.01$ ) (Figure 3). The TNF- $\alpha$  levels significantly increased after YGJY treatment ( $83.9 \pm 7.1$  ng/L to  $93.6 \pm 12.0$  ng/L,  $P < 0.05$ ) (Figure 4). The activity of IL-1 after the YGJY treatment decreased significantly ( $2760.8 \pm 813.6$  cpm to  $1922.3 \pm 847.0$  cpm,  $P < 0.01$ ) (Figure 5). In the standard treatment group, the FN levels, FNR levels, TNF- $\alpha$  levels, and the activity of IL-1 were not significantly different ( $P > 0.05$ , Figures 2-5).

### Clinical profiles

In 63.5% of patients with cirrhotic HBV, the serum globulin level was down-regulated to normal levels after treatment with YGJY decoction. However, the serum globulin level returned to normal in only 23.4% of patients treated by standard methods.

## DISCUSSION

Recent studies on cirrhosis have focused on the interactions between cytokines and the extracellular matrix (ECM)<sup>[1,8,9]</sup>. Several reports have shown that serum levels of some ECM molecules, such as type III procollagen peptide, types I and IV collagen, and fibronectin, were biomarkers of hepatic fibrosis, and the accumulation of ECM molecules played a major role in liver function impairment. The FN and FNR are the main components of the

extracellular matrix. We found that in patients with decompensated liver cirrhosis, the plasma FN was significantly lower and FNR was significantly higher, with a strong negative correlation ( $r = -0.6534$ ). The TNF- $\alpha$  levels and IL-1 activity were also increased as compared with the normal subjects. Hagiwata *et al.*<sup>[10]</sup> observed that recombinant human IL-1 could increase FN in the liver of rats, and also could directly increase the transcription of type I, III and IV collagen. IL-1 may act synergistically with TNF- $\alpha$  to induce hepatitis<sup>[9]</sup>.

These data show that serum FNR, IL-1 and TNF- $\alpha$  could up-regulate and FN could down-regulate the liver fibrosis process. We found that IL-1 activity and FNR levels, which could indicate increased fibrosis of the liver, were strongly down-regulated by treatment with YGJY decoction. While FN, which could indicate decreased liver fibrosis, was strongly up-regulated by YGJY decoction treatment. These changes showed that YGJY decoction could prevent liver damage and inhibit hepatic fibrosis.

TNF- $\alpha$  is a multifunctional cytokine, which is hypothesized to regulate inflammatory and pathological processes and orchestrate necrosis and regeneration. We detected plasma levels of TNF- $\alpha$  and nitrate in cirrhotic rats by reproduction with CCl<sub>4</sub>. The TNF- $\alpha$  levels were significantly higher after YGJY treatment than before treatment. It has been shown that TNF- $\alpha$  can positively regulate liver cell regeneration and hepatocyte proliferation in rats induced by the nitrate<sup>[11]</sup>. Further studies are necessary to clarify the effect and mechanism of YGJY decoction in hepatocyte proliferation and regeneration.

## REFERENCES

- 1 **Yamauchi M**, Nakajima H, Ohata M, Hirakawa J, Mizuhara Y, Nakahara M, Kimura K, Fujisawa K, Kameda H. Detection of fibronectin receptor in sera: its clinical significance as a parameter of hepatic fibrosis. *Hepatology* 1991; **14**: 244-250 [PMID: 1830562 DOI: 10.1002/hep.1840140207]
- 2 **Gabrielli GB**, Casaril M, Bonazzi L, Baracchino F, Bellisola G, Corrocher R. Plasma fibronectin in liver cirrhosis and its diagnostic value. *Clin Chim Acta* 1986; **160**: 289-296 [PMID: 3539410 DOI: 10.1016/0009-8981(86)90196-8]
- 3 **Matsuoka M**, Pham NT, Tsukamoto H. Differential effects of interleukin-1 alpha, tumor necrosis factor alpha, and transforming growth factor beta 1 on cell proliferation and collagen formation by cultured fat-storing cells. *Liver* 1989; **9**: 71-78 [PMID: 2785237]
- 4 **Wu H**, Wang ZM, Fan JT, Deng JW, Fen TJ. Effect of six kinds of decoctions on experimental autoimmune liver diseases. *Bulletin of Hunan Medical University* 1991; **16**: 263-267
- 5 **Chen JZ**, Li ZM. Internal medicine. Ed 3. Beijing: The People-s Medical Publishing House 1989: 389-396
- 6 **Habicht GS**, Beck G, Benach JL, Coleman JL, Leichtling KD. Lyme disease spirochetes induce human and murine interleukin 1 production. *J Immunol* 1985; **134**: 3147-3154 [PMID: 2984284]
- 7 **Pizzaro TT**, Malinowskak K, Kovacs EJ, Chancy J, Bobinson JA, Piclinini LA. Induction of TNFB and FNF (Lymphotoxin) gene expression during rate Cardige gllogr of regection (Abstract). *FASEB J* 1991; **5**(A): 1708
- 8 **Dinaretto CA**. Interleukin-1 and Interleukin? *antagonism blood*. 1991; **77**: 1627-1862
- 9 **Gantner F**, Leist M, Lohes AW, Germaann RG, Tes G. Concaavalin A induced T cell-mediated hepatic injury in mice: the role of tumor necrosis factor. *Hepatology* 1995; **2**: 190-198
- 10 **Hagiwata T**, Suzuid H, Kano C, Kashiwag I, Ykyama Y, Onoiaki K. Regulation of fibronectin synthesis by interleukin-1 and interleukin-6 in rat hepatocytes. *Am J Pathol* 1990; **136**: 39-47
- 11 **Kubo Y**, Yasunaga M, Masuhara M, Terai S, Nakamura T, Okita K. Hepatocyte proliferation induced in rats by lead nitrate is suppressed by several tumor necrosis factor alpha inhibitors. *Hepatology* 1996; **23**: 104-114 [PMID: 8550029]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Effects of electro-acupuncture on 5-HT, NOS and the gastric mucosa of stress rats

Shun-Li Zhu, Guan-Sun Xu, Zhen-Jiu Wang, Quan-Zhu Chen, Jie Jiao

Shun-Li Zhu, Guan-Sun Xu, Zhen-Jiu Wang, Quan-Zhu Chen, Jie Jiao, Institute of Acupuncture and Meridians, Anhui College of Traditional Chinese Medicine, Hefei 230038, Anhui Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Shun-Li Zhu, Institute of Acupuncture and Meridians, Anhui College of Traditional Chinese Medicine, Hefei 230038, Anhui Province, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To study the effects of electro-acupuncture (EA) on 5-hydroxytryptophan (5-HT) levels, nitrous oxide (NOS) levels, nitric oxide (NO) levels, and the gastric mucosa in stress rats.

**METHODS:** The changes of 5-HT and NOS were measured in the gastric mucosa, and NO and 5-HT were measured in the serum by biochemical methods. The gastric mucosa was examined pathohistologically in the stress rats with gastric mucosa damage

after EA. The changes before and after stress by EA were compared.

**RESULTS:** EA decreased the gastric mucosa damage index in the stress rats ( $2.71 \pm 0.40$  to  $1.86 \pm 0.69$ ,  $P < 0.01$ ). EA normalized NOS level in gastric mucosa to the control group. The changes before stress by EA was more obvious than that after stress. EA lowered the 5-HT levels in the gastric mucosa ( $\mu\text{g/g}$  wet weight,  $6.91 \pm 3.08$  to  $4.51 \pm 1.62$ ,  $P < 0.01$ ). EA recovered the NO level in serum of the stress rats ( $\mu\text{mol/L}$ ,  $5.78 \pm 1.49$  to  $7.91 \pm 1.11$ ,  $P < 0.05$ ), and increased the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in serum continuously.

**CONCLUSION:** EA stimulation normalizes the NOS and NO levels in the gastric mucosa of stress rats. EA also lowers the high 5-HT levels and induces NO release.

**Key words:** Electroacupuncture; Serotonin/acu-moxibustion effects; Aminoacid oxidoreductases/acu-moxibustion effects; Gastric mucosa/Acu-moxibustion effects

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhu SL, Xu GS, Wang ZJ, Chen QZ, Jiao J. Effects of electro-acupuncture on 5-HT, NOS and the gastric mucosa of stress rats. *World J Gastroenterol* 1997; 3(3): 179 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/179.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.179>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Alterations of erythrocyte ATPase activity and oxygen consumption in patients with liver-blood deficiency syndrome

Lin-Jie Shi, Jun-Fan Liu, Zi-Qiang Zhang, Yi-Qin Lu, Yi-Gang Shu, Guo-Lin Chen, Zhi-Hua Xin, Jin-Yao Xu

Lin-Jie Shi, Zi-Qiang Zhang, Guo-Lin Chen, Zhi-Hua Xin, Institute of Integrated TCM and Western Medicine, Hunan Medical University, Changsha 410008, Henan Province, China

Jun-Fan Liu, Yi-Qin Lu, Jin-Yao Xu, Department of Biochemistry and Institute of Blood Biochemistry

Yi-Gang Shu, Institute of Hematology, Xiang Ya Hospital

Lin Jie Shi, male, born on 1942-12-26 in Linxiang, Hunan Province, graduated from Hunan College of TCM in 1968. He is an Associate Professor of Integrated TCM and Western Medicine, focuses on liver diseases and TCM, and has 22 papers published.

Author contributions: All authors contributed equally to the work.

Supported by the National Natural Science Foundation of China, No.39170881.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Lin-Jie Shi, Associate Professor, Institute of Integrated TCM and Western Medicine, Hunan Medical University, Changsha 410008, Henan Province, China  
Telephone: +86-731-4440388-3805  
Fax: +86-731-4440312

Received: December 13, 1996  
Revised: February 6, 1997  
Accepted: March 13, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate the pathophysiology of erythrocyte energy metabolic changes of patients with the traditional Chinese Medicine (TCM) liver-blood deficiency syndrome (LBDS).

**METHODS:** Erythrocyte membrane ATPase activity and oxygen consumption rate (OCR) were determined in 66 patients with LBDS, including 35 patients with iron deficiency anemia and 31 patients with chronic aplastic anemia. Thirty healthy adults served as controls.

**RESULTS:** ATPase activity and OCR were decreased in patients with LBDS.

**CONCLUSION:** The decreased erythrocyte ATPase activity and OCR might cause the energy hypometabolism in LBDS patients.

**Key words:** Erythrocytes; Cell membrane; Oxygen consumption; Adenosine triphosphatase; Liver-blood deficiency syndrome; Iron-deficiency anemia; Aplastic anemia

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Shi LJ, Liu JF, Zhang ZQ, Lu YQ, Shu YG, Chen GL, Xin ZH, Xu JY. Alterations of erythrocyte ATPase activity and oxygen consumption in patients with liver-blood deficiency syndrome. *World J Gastroenterol* 1997; 3(3): 180-181 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/180.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.180>

### INTRODUCTION

Erythrocytes, the most common blood cell, exhibit metabolic characteristics. We have conducted hemorrheologic studies in patients with liver-blood deficiency syndrome (LBDS)<sup>[1]</sup>, and observed that their hematocrit (Hct) was significantly decreased. This indicated a reduced erythrocyte count. There are few reports on erythrocyte metabolic alterations in patients with LBDS. In this study, the erythrocyte membrane ATPase activity and the erythrocyte oxygen consumption rate (OCR) were determined in patients with anemia, including iron deficiency anemia and chronic aplastic anemia.

### MATERIALS AND METHODS

#### Diagnostic criteria

General clinical data are listed in Table 1. The patients enrolled in this study all had traditional Chinese Medicine (TCM) differentiated LBDS syndrome diagnosed by two clinicians according to our certified standard<sup>[2]</sup>. The symptoms included dizziness, decreased visual acuity and/or blurred vision, numbness of the extremities, face, lips, and nails appeared pale and malnourished, tongue appeared pale, and pulse taut and/or thready. Patients presenting with decreased visual acuity and/or blurred vision and numbness of the extremities along with an additional two symptoms (excluding those with YinXu, YangXu and Qixu) were diagnosed with LBDS. Iron deficiency anemia (IDA) was diagnosed according to the "Diagnostic Criteria and Curative Improvement Standard of Clinical Diseases"<sup>[3]</sup>. Chronic aplastic anemia (CAA) was diagnosed according to the June 1987 Baoji Conference revised standard<sup>[4]</sup>. Healthy adult blood donors served as controls.

#### Instruments

Equipment used included a portable automatic balanced recorder (XWT-104, Shanghai Dahua Instrument Factory), a Clark electrode and SP-2 dissolved oxygen assay controller (China Academy Vegetal Physiology Institute), a 2219-II thermostat circulation water bath (LKB), and a 751 spectrometer (Shanghai 3<sup>rd</sup> Analytic Instrument Factory).

#### Preparation of the erythrocyte membrane

Five milliliters of heparin anticoagulated fasting venous blood was centrifuged at 3000 rpm for 10 min. The buffy coat was discarded. The remainder was washed with isotonic Tris-HCl (310 mOsm, pH

**Table 1** General clinical data

	<i>n</i>	Male/Female	Age (yr)	Diseases
Control group	30	15/15	34.2 ± 10.4 (20-46)	
Erythrocyte membrane ATPase	31	11/20	36.6 ± 13.6 (19-56)	IDA 16, CAA 15
Erythrocyte OCR	35	13/22	35.8 ± 15.2 (21-48)	IDA 19, CAA 16

**Table 2** Comparison of ATPase activities

Groups	<i>n</i>	Mg <sup>2+</sup> -ATPase	Na <sup>+</sup> -K <sup>+</sup> -ATPase		Ca <sup>2+</sup> -ATPase
			(μmol Pi·mg protein·h)		
Control	30	0.300 ± 0.160	0.250 ± 0.120		0.620 ± 0.170
LBDS	31	0.130 ± 0.072 <sup>b</sup>	0.154 ± 0.081 <sup>b</sup>		0.530 ± 0.159 <sup>a</sup>
CAA	15	0.132 ± 0.044 <sup>b</sup>	0.156 ± 0.067 <sup>b</sup>		0.562 ± 0.130
IDA	16	0.132 ± 0.091 <sup>b</sup>	0.152 ± 0.093 <sup>b</sup>		0.468 ± 0.215 <sup>a</sup>

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 vs control.

**Table 3** Comparison of erythrocyte OCR (oxygen consumption rate)

Groups	<i>n</i>	Oxygen consumption rate (μL 100-h·mL compressed RBC)
Control	30	107.26 ± 18.46
LBDS	35	82.25 ± 36.39 <sup>b</sup>
CAA	16	68.83 ± 24.83 <sup>b</sup>
IDA	19	100.17 ± 13.86 <sup>a</sup>

<sup>a</sup>*P* < 0.05 vs CAA, <sup>b</sup>*P* < 0.01 vs control.

7.4) twice at a ratio of 1:30 with cool hypotonic Tris-HCl (20 mOsm, 2 mmol/L EDTA, pH 7.4). After centrifugation at 12000 rpm for 35 min, a pink fluid precipitant could be seen adhering to the wall of the tube. This was washed with hypotonic solution twice, and a white cell membrane preparation was obtained. The whole procedure was performed at 0 °C to 4 °C, and the membrane product was stored at -20 °C. Twenty minutes before the ATPase activity assay, the membrane was dissolved with 2 g/100 L saponin. The membrane protein content was determined by the improved Lowry method<sup>[5]</sup>.

#### Determination of erythrocyte membrane ATPase activity

The Mg<sup>2+</sup>-ATPase, Na<sup>+</sup>-K<sup>+</sup>-ATPase, and Ca<sup>2+</sup> ATPase activities were assessed according to Reinila *et al*<sup>[6]</sup>.

#### Determination of erythrocyte oxygen consumption rate

The erythrocyte oxygen consumption rate (OCR) was determined using the film oxygen electrode Clark technique and formula<sup>[7]</sup>.

#### Statistics

Results were expressed as mean ± standard deviation. A *t*-test and ANOVA were used for statistical analysis.

## RESULTS

The Mg<sup>2+</sup>-ATPase, Na<sup>+</sup>-K<sup>+</sup>-ATPase, and Ca<sup>2+</sup> ATPase activities in patients with LBDS were significantly decreased as compared with the healthy controls, (*P* < 0.01, *P* < 0.01, and *P* < 0.05, respectively) (Table 2). The patients diagnosed with CAA did not have a significant difference in the Ca<sup>2+</sup>-ATPase activity compared to normal controls.

The erythrocyte OCR was generally decreased in the LBDS patients compared with the healthy controls. The erythrocyte OCR

of patients diagnosed with IDA was not significantly significant from the healthy controls (*P* > 0.05). The erythrocyte OCR of patients diagnosed with CAA was significantly decreased from the healthy controls (*P* < 0.01). The difference between the IDA and CAA patients was significant (*P* < 0.01) (Table 3).

## DISCUSSION

Na<sup>+</sup>-K<sup>+</sup>-ATPase is responsible for the active transport of sodium and potassium across the membrane, which maintains a high intracellular concentration of potassium and a low intracellular concentration of sodium. ATP is required for the active transport of these molecules<sup>[8]</sup>. If there is a decrease of Na<sup>+</sup>-K<sup>+</sup>-ATPase on the erythrocyte membrane, then there will be an increase of intracellular sodium concentrations, which could lead to a hypoenergetic status of the erythrocytes. Furthermore, if phosphorylation of membrane proteins is impaired in the ATPase deficient erythrocyte, then the formation of membrane protein polymers will be hindered. This affects cytoskeleton stability<sup>[9]</sup>, resulting in abnormalities of the erythrocyte structure. Therefore, Na<sup>+</sup>-K<sup>+</sup>-ATPase is essential for the maintenance of the normal morphology, structure and function of the erythrocyte<sup>[10]</sup>. In mature erythrocytes, glucose catabolism is very active in order to provide the sodium pump with energy and to maintain the normal functioning of the erythrocytes (90% of the energy from glycolysis and 10% from the pentose phosphate pathway)<sup>[7]</sup>.

We observed that the activities of the ATPases, including the Mg<sup>2+</sup>-ATPase, the Na<sup>+</sup>-K<sup>+</sup>-ATPase and the Ca<sup>2+</sup> ATPase, were significantly decreased compared to the normal controls. However, no differences were observed between the patients diagnosed with IDA and CAA. In addition, the oxygen consumption rate of the LBDS patients was decreased compared to the controls, especially the patients with CAA. Taken together, the results suggest that the erythrocyte ATPase activity and OCR are decreased in LBDS patients, which could lead to pathophysiological changes of decreased energy metabolism.

## REFERENCES

- 1 Shi LJ, Cheng CH, Shu YG, Lou TL, Chen GL, Zhao JF. Examination of hemorrhheology and erythrocyte deformability of patients with LBDS. *Bulletin of Hunan Medical University* 1996; **21**: 131-133
- 2 Cheng GT, Xue SQ. Current diagnostic criteria of diseases. Beijing: Xueyuan Publishing House 1991: 710-712
- 3 Health Ministry of PLA (ed). Clinical diagnostic criteria and curative improvement standard. Beijing: People's Army Surgeon Publisher House 1989: 140-141
- 4 Diagnostic criteria of aplastic anemia. *Chinese Journal of Hematology* 1987; **8**: C4
- 5 Li H, Lu YQ, Peng XH, Liu JF. Effect of Ginseng total saponin on the benzene induced rat aplastic anemia erythrocyte utilization of four hexoses. *Bulletin of Hunan Medical University* 1994; **19**: 381-384
- 6 Reinila M, MacDonald E, Salem N, Linnoila M, Trams EG. Standardized method for the determination of human erythrocyte membrane adenosine triphosphatases. *Anal Biochem* 1982; **124**: 19-26 [PMID: 6214964 DOI: 10.1016/0003-2697(82)90214-7]
- 7 Arnott RD, White R, Jerums G. Effect of thyroid status on ouabain binding to the human lymphocyte. *J Clin Endocrinol Metab* 1982; **54**: 1150-1156 [PMID: 6281292 DOI: 10.1210/jcem-54-6-1150]
- 8 de Wardener HE, MacGregor GA. Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. *Kidney Int* 1980; **18**: 1-9 [PMID: 7218655 DOI: 10.1038/ki.1980.104]
- 9 Gratzer WB. The red cell membrane and its cytoskeleton. *Biochem J* 1981; **198**: 1-8 [PMID: 7034726 DOI: 10.1042/bj1980001]
- 10 Lin JC. Blood biochemistry. Beijing: People's Health Publishing House 1988: 230-232

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## The effects of *Astragalus membranaceus* on oxygen consumption in the intestine

Shao-Zhi Li, Xiao-Hong Tan

Shao-Zhi Li, Research Laboratory of Diagnosis, Hunan College of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China

Xiao-Hong Tan, The First Affiliated Hospital of Hunan College of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China

Li Shao Zhi, male, was born on 1953-10-11 in Changsha City, Hunan Province, and graduated from the Hunan College of Traditional Chinese Medicine as a postgraduate in 1984. He is an Associate Professor of Diagnosis, Director of Research Laboratory of Diagnosis, Hunan College of Traditional Chinese Medicine, and has 34 papers and two books published.

Author contributions: All authors contributed equally to the work.

Supported by U.S. NIH research grant HL-15231.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Shao-Zhi Li, Associate Professor, Research Laboratory of Diagnosis, Hunan College of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China  
Telephone: +86-731-5556660-794 (H)

Received: November 1, 1996  
Revised: January 11, 1997  
Accepted: February 18, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate the effects of *Astragalus membranaceus* (AM) on intestinal oxygen consumption both *in vivo* and *in vitro*.

**METHODS:** The oxygen consumption of the intestine was measured using an arteriovenous (A-V) O<sub>2</sub> difference analyzer after treatment with AM in the intestinal lumen of ten healthy, anesthetized mongrel dogs. The effects of AM on the oxygen consumption of the intestinal mucosa *in vitro* were observed using constant volume manometers.

**RESULTS:** The oxygen consumption of the intestine *in vivo* increased significantly ( $P < 0.05$  or  $P < 0.01$ ) after treatment with AM compared to the saline control. The oxygen consumption significantly increased after treatment with the 30% AM dilution and the 50% AM dilution compared to that of the 10% AM dilution ( $P < 0.05$ ). There was no significant difference between the 30% AM dilution and the 50% AM dilution ( $P > 0.05$ ). The effects of AM on oxygen consumption of the intestinal mucosa *in vitro* were similar to those *in vivo*. After treatment with the 5% AM dilution and the 1% AM dilution, the intestinal oxygen consumption increased compared to the control (Krebs Ringer phosphate buffer (KRPB)) ( $P < 0.05$  and  $P < 0.01$ , respectively). There was no significant difference between treatment with the 10% AM dilution and the KRPB control ( $P > 0.05$ ).

**CONCLUSION:** AM improved the function of intestinal oxidative metabolism.

**Key words:** *Astragalus membranaceus*; Oxygen consumption; Small intestine metabolism

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Li SZ, Tan XH. The effects of *Astragalus membranaceus* on oxygen consumption in the intestine. *World J Gastroenterol* 1997; 3(3): 182-184 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/182.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.182>

### INTRODUCTION

It was reported that *Astragalus membranaceus* (AM) could stabilize the configuration of deoxyhemoglobin, decrease hemoglobin's affinity for oxygen, shift the oxygen hemoglobin dissociation curve to the right, increase hemoglobin's capacity for transporting oxygen, and decrease the from an oxygen supply deficiency<sup>[1,2]</sup>. AM can also decrease the oxygen consumption of cardiac muscle and maintain the balance of oxygen supply in the blood<sup>[3]</sup>. However, its effects on oxygen consumption in the intestine has not been investigated. This study observed the effects of AM on oxygen consumption in the intestine both *in vivo* and *in vitro*.

### MATERIALS AND METHODS

#### Animal model

Ten healthy mongrel dogs (15-20 kg) of either sex were fasted for 24 h and anesthetized with pentobarbital sodium (30 mg/kg). All animals were ventilated with a positive pressure respirator (Harvard Apparatus, United States) that was adjusted to achieve normal blood pH, O<sub>2</sub> tension, and CO<sub>2</sub> tension before each experiment. A midline abdominal incision was made and a segment of jejunum (20-40 g) about 30 cm aboard to the ligament of Treitz was exteriorized. A rubber tube was placed in the lumen of the segment for introduction and withdrawal of the AM solution. After heparin sodium (500 U/kg) was administered intravenously, a vein draining in the jejunal segment and a femoral artery were cannulated for continuous measurement of intestinal oxygen consumption of the observed segment. The measured blood was directed to a reservoir, which was pumped back to the animal *via* a femoral vein at a rate equal to the total outflow in order to maintain the blood equilibrium. Both ends of the segment were ligated to the adjacent jejunum to exclude collateral flow after the rubber tube was placed into the lumen of the segment (Figure 1). The segment was covered with a plastic sheet and kept at 37 °C with a heat lamp and a

**Table 1** Effects of AM (*Astragalus membranaceus*) on the intestinal oxygen consumption *in vivo* (average  $\pm$  standard deviation, mL $\cdot$ min $\cdot$ 100 g)

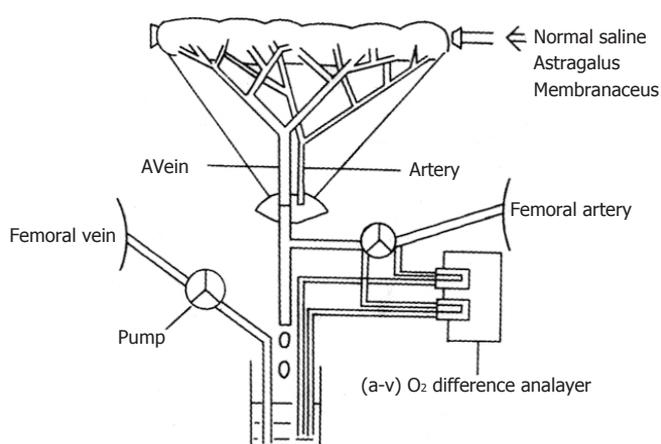
Groups	n	0-3 min	4-7 min	8-11 min	12-15 min
A. Normal saline	10	2.62 $\pm$ 0.51	2.71 $\pm$ 0.54	2.68 $\pm$ 0.60	2.58 $\pm$ 0.58
B. 10% concentration	10	2.96 $\pm$ 0.64	3.48 $\pm$ 0.66 <sup>a</sup>	3.87 $\pm$ 0.69 <sup>b</sup>	4.01 $\pm$ 0.72 <sup>b</sup>
C. 20% concentration	10	3.18 $\pm$ 0.70	4.16 $\pm$ 0.78 <sup>bc</sup>	4.82 $\pm$ 0.82 <sup>bc</sup>	4.77 $\pm$ 0.83 <sup>b</sup>
D. 30% concentration	10	3.23 $\pm$ 0.76	4.35 $\pm$ 0.79 <sup>bc</sup>	4.74 $\pm$ 0.86 <sup>bc</sup>	4.76 $\pm$ 0.84 <sup>b</sup>

<sup>a</sup>P < 0.05, B,C,D vs A; <sup>b</sup>P < 0.01, B,C,D vs A; <sup>c</sup>P < 0.05, C,D vs B.

**Table 2** Effects of AM (*Astragalus membranaceus*) on the oxygen consumption of the intestinal mucosa *in vitro* (average  $\pm$  standard deviation, mL $\cdot$ min $\cdot$ 100 g)

Groups	n	0-3 min	4-7 min	8-11 min	12-15 min
A. Normal saline	10	2.62 $\pm$ 0.51	2.71 $\pm$ 0.54	2.68 $\pm$ 0.60	2.58 $\pm$ 0.58
B. 10% concentration	10	2.96 $\pm$ 0.64	3.48 $\pm$ 0.66 <sup>a</sup>	3.87 $\pm$ 0.69 <sup>b</sup>	4.01 $\pm$ 0.72 <sup>b</sup>
C. 20% concentration	10	3.18 $\pm$ 0.70	4.16 $\pm$ 0.78 <sup>bc</sup>	4.82 $\pm$ 0.82 <sup>bc</sup>	4.77 $\pm$ 0.83 <sup>b</sup>
D. 30% concentration	10	3.23 $\pm$ 0.76	4.35 $\pm$ 0.79 <sup>bc</sup>	4.74 $\pm$ 0.86 <sup>bc</sup>	4.76 $\pm$ 0.84 <sup>b</sup>

<sup>a</sup>P < 0.05, B,C,D vs A; <sup>b</sup>P < 0.01, B,C,D vs A; <sup>c</sup>P < 0.05, C,D vs B; <sup>d</sup>P < 0.01, C,D vs B.



**Figure 1** Representation of the animal model used to measure the oxygen consumption in the intestine after treatment with AM (*Astragalus membranaceus*).

thermoregulator (Yellow Spring Instrument, United States)<sup>[4,5]</sup>.

### Methods of measurement

Intestinal oxygen consumption *in vivo* was measured by an arteriovenous (A-V) O<sub>2</sub> difference analyzer (A-Vox. Systems, United States). After the operation was performed, normal saline was used to wash the lumen three times. Ten milliliters of normal saline was placed into the lumen while the intestinal blood flow, motility, and blood pressure were continuously measured for 15 min. This procedure was repeated every 15 min until the blood flow, motility, and blood pressure reached a steady state. Then the effects of different concentrations of AM on oxygen consumption were measured. Each dog was given each AM concentration three times. The intestinal oxygen consumption was recorded from 0-3 min, 4-7 min, 8-11 min, and 12-15 min.

The oxygen consumption of the intestinal mucosa *in vitro* was measured using constant volume manometers (Warburg Apparatus, United States). Segments of jejunum were harvested from anesthetized mongrel dogs. The tissue was immediately placed in fresh oxygenated ice-cold Krebs Ringer phosphate buffer (KRPB) solution. The container was surrounded by ice in order to keep the temperature low. Blood was removed from the tissue by perfusing KRPB *via* the local artery after the lumen was washed. The tissue was cut open along the mesenteric border, and intact mucosal sheets were obtained by gently separating the mucosa from the underlying gut wall using a microscope slide. Small pieces of mucosa, 1 mm x 2 mm, were obtained by cutting. These small pieces were transferred to the fresh, oxygenated, cold KRPB. Numerous small pieces were blotted on filter paper, weighed to the nearest 0.15 g, and placed in Warburg flasks. Each flask contained a total volume of 3 mL of incubation media that included oxygenated KRPB with 0.5 mL of one of the concentrations of AM in the experimental groups or without AM in the control group. To measure the absorption of carbon

dioxide, the center well of the flask contained 0.2 mL of 10% KOH and 0.1 g of filter paper. The flasks were attached to manometers, placed in a water bath (37 °C), and shaken at 90 cycles per minute. After a 10-15 min equilibration, manometer pressure readings were obtained every 10 min for 1 h. Additional mucosal tissue samples of 5-6 g were weighed, oven dried, and re-weighed to obtain the percentage dry weight, which was used to calculate the dry weight of the experimental samples.

### Preparation of experimental solutions

AM was purchased from the Hebei Company of Medicinal Materials. Two hundred milliliters of distilled water was added to a flask containing 50 g of AM. The flask was put on an electric furnace to be decocted until the solution became 100 mL. This was labeled as the 50% concentration of AM. This solution was further diluted to 30%, 10%, 5%, 1% and 0.5% with distilled water. Fresh AM dilutions were prepared on each experimental day.

KRPB solution was prepared with NaCl (7.106 g/L), KCl (0.365 g/L), CaCl<sub>2</sub> (0.138 g/L), MgSO<sub>4</sub> (0.01 g/L), Na<sub>2</sub>HPO<sub>4</sub> (2.29 g/L), and glucose (0.9 g/L). First, NaCl, KCl, MgSO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub> were placed in distilled water and stirred. At the same time, the solution was gassed with a combination of 95% O<sub>2</sub> and 5% CO<sub>2</sub> for 10 min. CaCl<sub>2</sub> dissolved in 100 mL of distilled water was slowly added into the KRPB solution. A fresh solution was prepared on each experimental day.

### Statistical methods

Analysis of variance (ANOVA) was employed to detect the differences among the four groups. The Q-test (Newman-Keuls test) was used to compare the differences between two groups.

## RESULTS

Six different concentrations of AM (50%, 30%, 10%, 5%, 1% and 0.5%) were tested in both *in vivo* and *in vitro* experiments to determine the most effective concentration. This study reported the effects of the most effective concentration and the concentration above and below the most effective concentration.

The effects of AM on the intestinal oxygen consumption *in vivo* are reported in Table 1. After treatment with the AM solution, the intestine increased the oxygen consumption, especially after 4-7 min, compared to the saline control ( $P < 0.05$  or  $P < 0.01$ ). The intestinal oxygen consumption was different among the different AM dilutions. The oxygen consumption was significantly increased ( $P < 0.05$ ) after treatment with the 30% and 50% AM dilutions compared to the 10% dilution. There was no significant differences in oxygen consumption between the 30% and 50% AM dilutions ( $P > 0.05$ ).

The effects of AM on the oxygen consumption of the intestinal mucosa *in vitro* is reported in Table 2. There was no significant difference between the 10% AM dilution and the KRPB control ( $P > 0.05$ ). The intestinal oxygen consumption was increased after treatment with the 5% and 1% AM dilutions. The oxygen consumption significantly increased ( $P < 0.05$ ) at 50 min and 60 min after treatment with the 1% AM dilution compared to that of the KRPB control. The oxygen consumption significantly increased ( $P < 0.05$  or  $P < 0.01$ ) at 20 min, 30 min, 40 min, 50 min, and 60 min after treatment with the 5% AM dilution compared to the KRPB control, the 10% AM dilution, and the 1% AM dilution.

## DISCUSSION

AM has been shown to protect cultured cardiac muscle cells during a glucose and oxygen deficient period. The proposed mechanism was that AM could stabilize the function of cardiac muscle cells, protect the mitochondria and lysosomes, and enhance the resistant capacity of oxygen deficiency<sup>[6]</sup>. In addition, AM has been shown to lower the oxygen consumption of the heart and liver mitochondria in guinea rats, increase the formation of 2, 3-diphosphoglycerate in healthy human red blood cells (*in vitro*), and improve hemoglobin's capacity for transporting oxygen<sup>[1]</sup>. After a 25% concentrated solution (1 mL/kg) of AM was injected into the empty stomachs of healthy, awakened dogs, the cycle duration of the interdigestive myoelectric

complex was changed. Phase I was shortened and phase II were prolonged in the jejunum. The cycle duration was also extended and showed an increase of action potential in phase II. Taken together, this data indicated that AM could strengthen the movement and muscle tone of the intestine<sup>[7]</sup>.

Our previous study also showed that the intestinal blood flow and motility increased after treatment with the AM solution into the canine intestine *in vivo*. This revealed that AM could improve the physiological function of the intestine. This study also indicated that the pharmaceutical effects of AM on different organs were different.

Guo *et al*<sup>[8]</sup> reported that the injection of AM could directly cause peripheral vasodilatation. According to Kviety's and Granger's<sup>[9]</sup> analysis of 50 papers about the effects of vasoactive agents on oxygen uptake, vasodilators can increase oxygen uptake. Changes in oxidative metabolism, blood flow, and capillary density appeared to be the major mechanism by which vasoactive agents alter splanchnic oxygen uptake. Under *in vitro* conditions, any drug-induced changes in tissue oxygen uptake are assumed to reflect only changes in tissue oxidative metabolism. Accordingly, AM probably has the pharmaceutical action of increasing oxygen consumption.

In order to probe the effect of AM on oxygen consumption in this study, the canine intestine was treated with different dilutions of AM. Its effect on the intestinal oxygen consumption was observed. We observed that AM treatment could significantly increase the intestinal oxygen consumption *in vivo* compared to the saline control. This indicated that AM could improve the physiological function of the intestine. To eliminate the interference involved in *in vivo* experiments, the effects AM treatment on intestinal oxygen consumption *in vitro* were also measured. We observed that AM treatment could also significantly increase the intestinal oxygen consumption compared to the KRPB control.

The effects of AM treatment on the intestinal oxygen consumption varied at different dilutions. This might be explained by the use of different experimental methods. In the *in vivo* experiments, AM was placed into the intestine. This method limited the effect of the AM on the surface of the tissue. While in the *in vitro* experiments, AM was put in the flasks containing small pieces of intestinal mucosa. In this method, the surface of the tissues was in contact with the AM constantly. Therefore, different pharmaceutical

responses were observed among the AM dilutions between the *in vivo* and *in vitro* experiments.

We observed that the pharmaceutical function of AM was to increase the intestinal oxygen consumption. It was consistent with the report that AM could improve the intestinal function<sup>[7]</sup>. Other studies have shown different effects of AM on oxygen consumption in different organs (*i.e.* decreased oxygen consumption in cardiac muscle cells and liver mitochondria, and increased intestinal oxygen consumption *in vivo* and *in vitro*). The mechanism, by which AM affects oxygen consumption, awaits further studies.

## ACKNOWLEDGMENTS

The authors are grateful to Dr Adamu Alemayehu and to Wayne Assing, an English teacher from the United States, for their help with the manuscript.

## REFERENCES

- 1 Zhang XR, Liu CM, Chen WW. Effect of Qixue injection on heart oxygen consumption (in Chinese). *Chinese Journal of Integrative Medicine* 1987; 7: 606-607
- 2 Lei ZY, Wang SR. Effect of Astragalus Membranaceus on cardiovascular system. *Chinese Journal of Integrative Medicine* (in Chinese) 1993; 13: 443-446
- 3 Chen JC, Li SY, Miao LJ, Yan A. A study about effect of Astragalus Membranaceus on ultrastructure of cardiac-muscle cells cultured in medium lack of glucose and oxygen (in Chinese). *New J TCM* 1990; 22: 52-53
- 4 Chou CC, Alemayehu A, Mangino MJ. Prostanoids in regulation of postprandial jejunal hyperemia and oxygen uptake. *Am J Physiol* 1989; 257: G798-G808 [PMID: 2512816]
- 5 Alemayehu A, Lock KR, Coatney RW, Chou CC. L-NAME, nitric oxide and jejunal motility, blood flow and oxygen uptake in dogs. *Br J Pharmacol* 1994; 111: 205-212 [PMID: 8012697 DOI: 10.1111/j.1476-5381.1994.tb14045.x]
- 6 Li SY, Chen JC, Huang X, Yan A, Miao LJ. The study about the mechanism of Qi-boosting function of Astragalus Membranaceus (in Chinese). *New J TCM* 1987; 19: 51-52
- 7 Yang DZ, Bi QH, Din AL, Ying CZ. Effect of Astragalus Membranaceus on Myoelectric activity of small intestine (in Chinese). *Chinese Journal of Integrative Medicine* 1993; 13: 616-617
- 8 Guo ZG, Xu SW, Jia HJ, Zhou HH, Zhang FL, Shen L. The peripheral vasodilation effect of Hunan (*Astragalus Membranaceus* (fisch) Bunge) and a comparison with that of r-Aminobutyric acid (GABA). *J TCM* 1980; 21: 73-76
- 9 Kviety's PR, Granger DN. Vasoactive agents and splanchnic oxygen uptake. *Am J Physiol* 1982; 243: G1-G9 [PMID: 7046475]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Ultrastructural observation of the gastric mucosa in chronic gastritis patients treated by traditional Chinese medicine

Zi-Li Zhang, Ji-Kang Bu, Jian-Xiong Zhao

Zi-Li Zhang, Ji-Kang Bu, Department of TCM, the First Hospital of Lanzhou Medical College, Lanzhou 730000, Gansu Province, China

Jian-Xiong Zhao, The Research Section of Integrated Traditional Chinese and Western Medicine, Lanzhou Medical College, Lanzhou 730000, Gansu Province, China

Zi-Li Zhang, male, was born on November 16, 1963 in Jingtai County, Gansu Province, and graduated from the Department of Medicine, Lanzhou Medical College in 1986. He is a Deputy chief physician, and has 31 papers published.

Author contributions: All authors contributed equally to the work.

Supported by the Foundation of Gansu Educational Commission, No.948-25.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Zi-Li Zhang, Department of TCM, the First Hospital of Lanzhou Medical College, Lanzhou 730000, Gansu Province, China  
Telephone: +86-931-8414480-6261

Received: September 18, 1996  
Revised: December 22, 1996  
Accepted: December 30, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To demonstrate the relationship between the ultrastructural changes of the gastric mucosa and the syndrome differentiation in chronic gastritis.

**METHODS:** Sixteen chronic gastritis patients with Piweixuhan (PXG, the cold of insufficiency syndrome of the spleen and the stomach) and fifteen chronic gastritis patients with Ganweibuhe (GBG, incoordination syndrome of the liver and the stomach) were treated with Jianpiwenwei decoction (JWD, invigorating the spleen and warming the stomach) or Shuganhewei decoction (SHD, dispersing the stagnated Liver Qi and regulating the stomach), respectively for three months. Before and after treatment, a gastroscopy was performed and the gastric mucosa was collected from the lesser curvature of the antrum of each patient. The ultrasections were observed and photographed under the JEM-100C X electron microscope.

**RESULTS:** The common ultrastructural anomalies of the two types of chronic gastritis were the plasmacyte infiltration and the lesions of the mucosal epithelial cells, chief cells and antral mucous cells. There were obvious differences between the two types. In PXG, the predominant lesion of the chief cells was swelling of the mitochondria, while in GBG the rough endoplasmic reticulum was enlarged in the chief cells and the plasmacytes. After treatment, most cases of the ultrastructural lesions reverted to normal or improved.

**CONCLUSION:** There was a close relationship between the ultrastructural changes of gastric mucosa and the syndrome differentiation of chronic gastritis. JWD and SHD could significantly improve the ultrastructural lesions of the gastric mucosa.

**Key words:** Gastritis/TCM therapy; Gastric mucosa/ultrastructure; Traditional Chinese medicine

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhang ZL, Bu JK, Zhao JX. Ultrastructural research on chronic gastritis treated by traditional Chinese medicine. *World J Gastroenterol* 1997; 3(3): 185-188  
Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/185.htm>  
DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.185>

### INTRODUCTION

Chronic gastritis is a common disease that is difficult to cure. Diagnosis and treatment of chronic gastritis by traditional Chinese medicine (TCM) has had a good therapeutic effect. In this study, we investigated the relationship between the ultrastructural changes of the gastric mucosa based on the TCM differentiation of chronic gastritis. The therapeutic effects of the TCM differentiation treatment on the gastric mucosa were also tested.

### MATERIALS AND METHODS

#### Patients

Sixteen patients with Piweixuhan (PXG, the cold of insufficiency syndrome of the spleen and the stomach) and fifteen patients with Ganweibuhe (GBG, incoordination syndrome of the liver and the stomach) were selected from the inpatients at the First Affiliated Hospital of Lanzhou Medical College. They were diagnosed according to the TCM differentiation standard of chronic gastritis in "Guiding Principle of Clinical Research of New Drugs" by the Chinese Ministry of Public Health.

#### Methods

The patients with PXG were treated with a Jianpiwenwei decoction (JWD, invigorating the spleen and warming the stomach). This decoction contained 10 g of Dangshen (*Radix codonopsis pilosula* e), 20 g of Fuling (*Poria*), 15 g of Baizhu (*Rhizoma atractylodis macrocephalae*), 5 g of Muxiang (*Radix Aucklandiae*), 5 g of Shenggancao (*Radix glycyrrhizae*), 10 g of Zhibanxia (*Rhizoma pinelliae preparata*), 5 g of Sharen (*Fructus amomi*), 10 g of Gaoliangjiang (*Rhizoma alpiniae officinarum*), 10 g of Yanhusuo (*Rhizoma corydalis*), and 10 g of Chaomaiya (*Fructus hordei germinatus*).

The patients with GBG were treated with a Shuganhewei



Figure 1 The mitochondria were enlarged in the chief cells in chronic gastritis patients differentiated into the PXG category.



Figure 2 The rER in chief cells enlarged and some showed intracisternal sequestration in chronic gastritis patients differentiated into the GBG category.

decoction (SHD, dispersing the stagnated Liver Qi and regulating the stomach). This decoction contained 10 g of Chaihu (*Radix bupleuri*), 15 g of Baishao (*Radix paeoniae alba*), 10 g of Zhiqiao (*Fructus aurantii*), 5 g of Muxiang (*Radix aucklandiae*), 10 g of Shenggancao (*Radix glycyrrhizae*), 10 g of Yujin (*Radix curcumae*), 15 g of Yanhusuo (*Rhizoma corydalis*), 10 g of Baizhi (*Radix angelicae dahuricae*), 10 g of Danggui (*Radix angelicae sinensis*), and 10 g of Jineijin (*Endothelium corneum galli*).

Patients were given 200 mL of the respective decoctions orally for three months, twice a day. The crude content of the two prescriptions was 500 g/L. Other related medications were not given during the observation period.

#### Assessment of the therapeutic effects

Gastroscopy was performed before and after the treatment. The gastric mucosa was collected from the lesser curvature of the antrum and immediately immersed in a cold 3% glutaraldehyde solution. The specimens were rinsed with phosphate buffer solution, post-fixed with 1% osmium tetroxide, dehydrated in graded steps with increasing percentages of ethanol, embedded with Epon 812, sectioned with an ultratome, and double stained with uranium acetate and lead citrate. The ultrasections were observed and photographed under the JEM 100CX transmission electron microscopy.

The main ultrastructural lesions were divided into three grades: severe, moderate and mild. After treatment, the therapeutic effects were determined according to the following assessment standard: recovery (the ultrastructural lesions disappeared completely), marked effectiveness (the ultrastructural lesions lightened by two grades), effective (the ultrastructural lesions lightened by one grade), ineffective (the ultrastructural lesions had no obvious changes). All data were analyzed statistically by the  $\chi^2$  test, and significance was set at  $P < 0.05$ .

## RESULTS

We observed that the mitochondria in the chief cells were swelled, its cristae were reduced and shortened, and some even displayed vacuolization. Some displayed pyknosis (Figure 1). The mitochondria lesions were classified into three grades: severe (the injured mitochondria exceeded 2/3 of all the mitochondria in the cytoplasm), moderate (the number of injured mitochondria was between 1/3 and 2/3 of all the mitochondria in cytoplasm), and mild (the injured mitochondria were less than 1/3 of all the mitochondria in cytoplasm).

We observed that the rough endoplasmic reticulum (rER) in

the chief cells were expanded, showed vesicles and vacuoles, and some even had intracisternal sequestration<sup>[1]</sup> (Figure 2). The rER enlargement was also graded into three degrees: mild (the rER enlargement was less than 1/3 of the cytoplasm or two times less than the area of the normal rER), moderate (the rER enlargement accounted for 1/3 to 2/3 of the cytoplasm or exceeded four times of the area of the normal rER), and severe (the rER enlargement was more than 2/3 of the cytoplasm or six times more than the area of the normal rER, or formed intracisternal sequestration).

We observed that the intercellular space in the mucosal epithelial and antral mucous was widened. This change was also graded into the following three degrees: severe (the intercellular space was significantly enlarged and exceeded the width of the cells, the cell processes became slight and dispersed, and lymphocytes appeared in some spaces), moderate (the width of the intercellular space was between the severe and mild degrees), and mild (the intercellular space was small, and the cell processes became shorter and smaller). In addition, we observed that the surface microvilli were short, small and sparse, the organelles were reduced, the mucous granules decreased, the cells atrophied, and the electron density increased. In a few patients, the mucous epithelial cells became stratified epithelium.

We observed heterotypical and deformed nuclei appeared in the mucosal epithelial cells and the antral mucous cells. Many nuclei were markedly enlarged and the nucleoli increased in number and size. The nuclear membrane folded and sank, which thereby led to nucleus deformation (Figure 3). This deformation was divided into three grades: severe (the sinking depth exceeded the short radius of the nucleus), moderate (the sinking depth or scope was between the mild and the severe grades), and mild (the sinking depth was less than 1/3 of the short radius of nucleus, or the sinking scope was less than 1/3 of the nuclear circumference).

We observed that the lymphocytes and plasmacytes infiltrated into the gastric mucosa. The lymphocytes were normal. Some appeared in the enlarged mucous intercellular space or between the glandular epithelial cells. In the plasmacytes, the rER enlarged to various degrees. We utilized the same grading method as that for the chief cells (Figure 4).

In addition, we found that the capillary endothelial cells in some patients were swelled or disappeared after treatment. There were a small number of G cells scattered in the antral mucosa. The rER was enlarged in some of these cells. In our study, the parietal cells, the zymogen granules in the chief cells, the stomach body's mucous cells, and the mucigen granules were nearly normal.

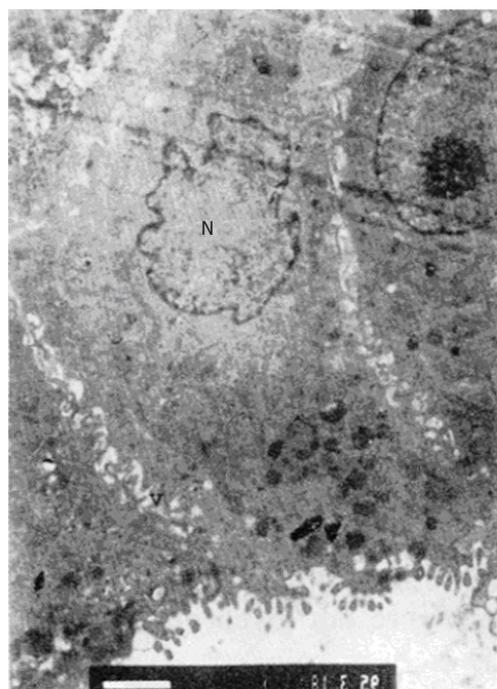
The therapeutic effects on the ultrastructural lesions of the gastric mucosa are presented in Tables 1 and 2.

**Table 1** The lesions and the therapeutic effects on the mitochondria and rough endoplasmic reticulum (rER) in chief cells

Differentiation	Total cases	Organelle lesions	Injured cases (%)	Markedly effective	Effective	Ineffective	Total effective rate (%)
PXG	16	Mitochondria <sup>b</sup>	13 (81.3)	7	4	2	84.6
		rER <sup>b</sup>	3 (18.8)	1	1	1	1.0
GBG	15	Mitochondria	3 (20.0)	0	2	1	66.7
		rER	12 (80.0)	8	2	2	83.3

<sup>b</sup>*P* < 0.01, compared with GBG.**Table 2** The therapeutic effect of the intercellular space, nuclear membrane and plasmacyte

Ultrastructural changes	Differentiation	Injured Cases (%)	Recovery	Markedly effective	Effective	Ineffective	Total effective Rate (%)
	GBG	12 (80.0)	2	5	3	2	83.3
The intercellular space of antral mucous cells widened	PXG	13 (81.3)	4	5	2	2	84.6
	GBG	13 (86.7)	3	6	3	1	92.3
The nuclear membrane of the mucosal epithelial cells and the antral mucous cells folded and sank in	PXG	10 (62.5)	3	3	2	2	80.0
	GBG	10 (66.7)	2	4	3	1	90.0
The rER in the plasmacyte enlarged	PXG	6 (37.5) <sup>a</sup>	1	3	0	2	66.7
	GBG	12 (80.0)	4	3	3	2	83.3

<sup>a</sup>*P* < 0.05, compared with GBG.**Figure 3** The intercellular space of antral mucosal epithelial cells was enlarged. N: Deformed nucleus.**Figure 4** The plasmacytes infiltrated and the intercellular space was enlarged. rER: Enlarged rER

## DISCUSSION

Optical microscopy is the primary means to diagnose the degree and the type of pathological changes in gastritis. However, it is difficult to detect the early stage lesions of organelles in the cells and to determine the nature of the lesions by optical microscopy because of its limited resolution. Moreover, the pathological grading under optical microscope is not very precise. In order to explore a more objective, reliable and precise method to determine therapeutic effects and pathological grading, we designed this study, which combined the TCM differentiation treatment and observation of the ultrastructure of the cells. Although there were some studies about the ultrastructural study of chronic gastritis<sup>[2-5]</sup>, there were few studies about the relationship between the ultrastructural changes of the gastric mucosa and the TCM differentiation of chronic gastritis.

This study showed that the ultrastructural lesions in the chief cells were significantly different between the two types of gastritis. PXG was characterized by swelled mitochondria, while GBG was characterized by expanded rER. This morphological difference revealed that the TCM differentiation of chronic gastritis has a modern ultramicropathological basis. Ultrastructural research on chronic gastritis could greatly contribute to its treatment and

differentiation.

This study also found that more than 80% of chronic gastritis patients had widened intercellular space of the mucosal epithelial and the antral mucous cells. This observation was consistent with the results of animal experiments reported in the literature<sup>[3,6]</sup>. On the other hand, more than 60% of the cases had deformed nuclei, the nuclear membrane was folded and sank in, and the nucleolus increased in number and size. This might be a compensated hyperfunction after the gastric mucosal barrier and the antral mucous cells were damaged. Liang *et al*<sup>[3]</sup> thought that the intercellular space enlargement was caused by mesenchyme edema. However, our results showed that the cell atrophy was a direct cause of intercellular space enlargement, which was consistent with our experimental results in a rat model<sup>[6]</sup>.

The lymphocyte and plasmacyte infiltration into the gastric mucosa of chronic gastritis was reported previously, which indicated that chronic gastritis was closely related to the patient's immunity. We observed plasmacyte infiltration in 58.1% of the patients with the rER enlarged to various degrees. Interestingly, the plasmacyte infiltration appeared in 80.0% of PXG patients, but only in 37.5% of GBG patients, which indicated that the GBG may be more closely related with immune functions. This has been confirmed by our experimental research<sup>[6]</sup> and helps to explain why the lesions of chief

cells were significantly different between the two types of gastritis. We hypothesize that the rER enlargement in chief cells may be a result of immune damage. In PXG patients, the main changes of the chief cells were mitochondrial lesions. It is well known that the cAMP content in cells of PXG patients is reduced (and thus is ATP reduced), the mitochondrial permeability is increased, which causes swelling and vacuolization. As a result, energy is decreased, cell function is lowered, and cells are atrophied, which led to gastric mucosal atrophy. In this study, parietal cells were normal, which was not consistent with the literature<sup>[2-5]</sup>. This difference needs to be examined further.

We observed that the ultrastructural lesions in PXG and GBG patients were significantly improved after treatment with JWD and SHD. We hypothesized that JWD would improve the mitochondrial lesions by increasing the cAMP content in cells, while SHD would improve the rER enlargement by stabilizing the plasmacytes and inhibiting the excessive immune reaction. Both prescriptions could strengthen the resistance against diseases, regulate the patients' immune function, and accelerate blood circulation of the gastric mucosa, thus promoting the recovery of the ultrastructural lesions of the gastric mucosa.

We also observed a few G cells in the antrum, in which some rER were enlarged. But we did not observe any other changes, such as secretory granules vacuolization and reduction reported by Ren *et al.*<sup>[2]</sup>. The rER enlargement of G cells mainly appeared in GBG patients, which probably was caused by the neuroendocrine disorder of G cells. SHD could make the enlarged rER in G cells recover by regulating its neuroendocrine function.

In addition, in the mesenchyme of the gastric mucosa in a few patients, the capillary endothelium swelled and the capillary cavity narrowed leading to dysfunction of the microcirculation. After treatment, these changes disappeared, suggesting that the two decoctions could significantly improve the microcirculation of the gastric mucosa. Therefore, both recovery of the mucosal epithelium and gland and the improvement of the microcirculation of gastric mucosa should be emphasized in treating chronic gastritis so as to increase the therapeutic effects.

## REFERENCES

- 1 **Song SX**, Be AH, Li JL, Bu JK, Wang YL, Sun SY. Electron microscopic basis for medicine. Beijing: Beijing Publishing House, 1992: 183-227
- 2 **Ren HY**, Chen GX, Nui LD, Lu GH, Hao WQ, et al. Observations of pathomorphologic changes on 150 chronic atrophic gastritis patients by treatment based on syndrome differentiation. *Chinese Journal of Integrated Traditional and Western Medicine* 1993; **13**: 144-146
- 3 **Liang P**, Chen LY, Yang SM, Chen BF, Lin JX, Pan XZ, et al. A study of the ultramicroscopic structure of chronic atrophicgastritis with data of gastric mucosa biopsy in 20 cases. *Chinese Journal of International Medicine* 1982; **21**: 220-223
- 4 **Dong YM**. [Scanning and transmission electron microscopy of chronic atrophic gastritis in 20 cases]. *Zhonghua Binglixue Zazhi* 1987; **16**: 50-53 [PMID: 2957082]
- 5 **Li QM**, Wu YF, Huang QH, Deng YF, Wu JL, et al. Ultrastructure of gastric mucosa and pathogenesis in spleen-energy deficiency. *Chinese Journal of New Gastroenterology* 1994; **2**: 156-157
- 6 **Bu JK**, Zhang ZL, Zhao JX. Stereological and ultrastructural research on the rats with chronic gastritis treated based on the differential diagnosis of traditional Chinese medicine (TCM). *China National Journal of New Gastroenterology* 1996; **2**: 76-78

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Characteristics of saliva secreted by patients with TCM-Piyinxu

Xue-Zhong Guan, Mu-Xin Wei, De-Zhen Chen, Yu-Chun Gu, Zhen-He Sun, Shu-Ying Bei

Xue-Zhong Guan, Mu-Xin Wei, De-Zhen Chen, Yu-Chun Gu, Zhen-He Sun, Shu-Ying Bei, Department of Traditional Chinese Medicine, Zhongshan Staff Sanatorium, Nanjing 210014, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Xue-Zhong Guan, Department of Traditional Chinese Medicine, Zhongshan Staff Sanatorium, Nanjing 210014, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To investigate various characteristics of saliva secreted by patients with TCM-Piyinxu (Spleen-yin deficiency).

**METHODS:** Twenty-five individuals with Piyinxu (15 males and 10 females; age range 26-70 years, mean age = 45 years) diagnosed based on criteria used in traditional Chinese medicine, were compared with 20 individuals with Shenyinxu (Kidney-yin deficiency) (11 males, 9 females; age range 35-75 years, mean age = 50) and 30 normal individuals (17 males, 13 females; age range 35-65 years, mean age = 49 years). After acid stimulation, the saliva flow in each group was measured, and the levels of amylase and protein in saliva

were determined using an automatic biochemical analyzer. The resultant data were analyzed using the Kruskal-Wallis test and one-way factorial ANOVA test.

**RESULTS:** The flow rates of saliva and amylase in Piyinxu patients ( $0.27 \pm 0.016$  mL/min and  $2134.13 \pm 343.51$  IU/min, respectively) were lower than those in normal subjects ( $0.46 \pm 0.027$  mL/min and  $3501.63 \pm 1099.63$  IU/min, respectively,  $P < 0.01$ ), but higher than those in the Shenyinxu group ( $0.13 \pm 0.051$  mL/min and  $951.62 \pm 383.17$  IU/min, respectively,  $P < 0.01$ ). The three groups showed no significant difference in their level of total salivary protein (Piyinxu group,  $3.07 \pm 0.60$  g/L; Shenyinxu group,  $3.01 \pm 0.90$  g/L, and control group,  $2.94 \pm 1.13$  g/L,  $P = 0.869$ ), amount of amylase per saliva volume, or their ratio of amylase to protein in secreted saliva ( $P = 0.173$  and  $P = 0.436$ , respectively).

**CONCLUSION:** Piyinxu patients showed altered rates of saliva and amylase secretion when compared with those parameters in patients with Shenyinxu and normal subjects.

**Key words:** Spleen asthenia/physiopathology; Yin deficiency/physiopathology; Salivation

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Guan XZ, Wei MX, Chen DZ, Gu YC, Sun ZH, Bei SY. Characteristics of saliva secreted by patients with TCM-Piyinxu. *World J Gastroenterol* 1997; 3(3): 188 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/188.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.188>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Treatment of postoperative gastric cancer with the Fuzheng Huoxue anticancer prescription

A-Gao Zhou, Da-Wei Huang, Yu-Xiong Ding, Hua Jiang, Ming-Lin Tang

A-Gao Zhou, Yu-Xiong Ding, Ming-Lin Tang, Department of Traditional Chinese Medicine, Shanghai Second Medical University, Shanghai 200025, China

Da-Wei Huang, Renji Hospital, Shanghai Second Medical University, Shanghai 200025, China

Hua Jiang, Shanghai Institute of Tumor, Shanghai 200025, China

A-Gao Zhou, male, born on 1948-09-08 in Shanghai. Graduated from the Department of Clinical Medicine, Shanghai Traditional Chinese Medical College in 1975. Associate professor, Master of medicine; mainly devoted to the treatment of gastric cancer with traditional Chinese medicines or an integrated traditional Chinese and Western medicine approach. Has published 23 papers.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. A-Gao Zhou, Associate professor, Department of Traditional Chinese Medicine, Shanghai Second Medical University, Shanghai 200025, China Telephone: +86-21-63846590-505

Received: December 15, 1996

Revised: January 26, 1997

Accepted: February 5, 1997

Published online: September 15, 1997

### Abstract

**AIM:** To study effects of the Fuzheng Huoxue anticancer prescription (Traditional Chinese Medicine) in treatment of gastric cancer.

**METHODS:** Sixty-nine patients with histologically confirmed mid- or late-stage gastric cancer were assigned to two groups. The treatment group included 35 cases (26 males and 9 females; 2 patients aged 33-40 years, 18 patients aged 41-60 years, and 15 patients aged 61-75 years; mean group age = 58.4 years). The control group included 34 cases (23 males and 11 females; 4 patients aged 33-40 years, 16 patients aged 41-60 years, and 14 patients aged 61-75 years; mean group age = 56.8 years). The two groups were not significantly different in sex, age, their clinical and pathological stages of disease or operation mode. The two groups of patients were given similar treatments; however, patients in the treatment group were given the Fuzheng Huoxue anticancer prescription. In animal studies, SGC-7901 gastric cancers cells were inoculated into the backs of 30 nude mice under sterile conditions. After inoculation, the nude mice were randomly allocated to a control group, a traditional Chinese medicine group, and a chemotherapy group ( $n = 10$  mice per group). The total weight of the 10 mice in each group was similar. Each nude mouse in the control group received 0.5 mL of saline solution each day. Mice in the traditional Chinese medicine group received 0.5 mL of the Fuzheng Huoxue anticancer prescription (containing 1.5 g crude drug) each day, while mice in the chemotherapy group were

intraperitoneally injected with 1 mg of 5-Fu once a week for 8 wk.

**RESULTS:** Prior to treatment, the mean OKT8 percentage among gastric patients in the treatment group was  $45.94\% \pm 8.45\%$ , the mean OKT4/OKT8 ratio was  $0.89 \pm 0.19$ , the mean AT-III concentration was  $29.9 \pm 7.9$  mg/dL, the mean Fa value was  $50.4\% \pm 24.4\%$ , and the mean  $\beta$ -TG concentration was  $91.0 \pm 25.9$  ng/dL. Prior to treatment, the mean percentage of OKT8 cells among patients in the control group was  $49.21\% \pm 6.60\%$ , the OKT4/OKT8 ratio was  $0.94 \pm 0.20$ , the AT-III concentration was  $32.3 \pm 7.2$  mg/dL, the mean Fa value was  $57.3\% \pm 24.6\%$ , and the mean  $\beta$ -TG concentration was  $87.5 \pm 34.2$  ng/dL. After treatment, the mean OKT8 percentage among patients in the treatment group was  $33.52\% \pm 7.80\%$ , the mean OKT4/OKT8 ratio was  $1.47 \pm 0.51$ , the mean AT-III concentration was  $38.8 \pm 5.5$  mg/dL, the mean Fa value was  $102.6\% \pm 31.6\%$ , and the mean  $\beta$ -TG concentration was  $62.3 \pm 15.1$  ng/dL. After treatment, the mean OKT8 percentage among patients in the control group was  $42.22\% \pm 7.07\%$ , the mean OKT4/OKT8 ratio was  $1.12 \pm 0.24$ , the mean AT-III concentration was  $30.9 \pm 8.0$  mg/dL, the mean Fa value was  $64.6\% \pm 26.9\%$ , and the mean  $\beta$ -TG concentration was  $67.0 \pm 42.1$  ng/dL. These data indicate that after treatment, the immunologic function of the T lymphocytes of gastric cancer patients in the treatment group was significantly improved ( $P < 0.01$ ). Additionally, the hypercoagulability in the treatment group was also improved ( $P < 0.001$ ), and the mean OKT4/OKT8 ratio, antithrombin III (AT-III) concentration, and fibrinolytic activity, etc. had all become normalized. The one-year (86%), 3-year (69%), and 5-year (40%) survival rates in the treatment group were all higher than those in the control group ( $P < 0.05$ ). The mean tumor weights in the control, traditional medicine, and chemotherapy groups were  $0.895 \pm 0.289$  g,  $0.433 \pm 0.177$  g, and  $0.357 \pm 0.142$  g, respectively. The tumor-inhibition rates in the traditional Chinese medicine group and chemotherapeutic group (51.6% and 60.1%, respectively) were significantly better than that in the control group ( $P < 0.001$ ). The mean tumor weight in the traditional Chinese medicine group ( $24.68 \pm 1.93$  g) was significantly higher than that in both the treatment group ( $22.96 \pm 1.87$  g) and control group ( $22.47 \pm 2.18$  g).

**CONCLUSION:** The Fuzheng Huoxue anticancer prescription can not only replenish vital functions (Zhengqi), correct a hypercoagulatory state, improve immunologic function, and extend patient survival times, but may also directly inhibit gastric tumor growth without producing toxic side effects.

**Key words:** Stomach neoplasms/TCM therapy; Fuzheng Huoxue; survival rate; T lymphocyte subsets; Medicine Chinese traditional

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhou AG, Huang DW, Ding YX, Jiang H, Tang ML. Treatment of postoperative gastric cancer with the Fuzheng Huoxue anticancer prescription. *World J Gastroenterol* 1997; 3(3): 189-191 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/189.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.189>

**Table 1 Comparison of T lymphocyte subgroup levels before and after treatment (mean ± SD)**

Groups		<i>n</i>	OKT3 (%)	OKT4 (%)	OKT8 (%)	OKT4/OKT8
Treatment	Before	15	56.10 ± 8.54	41.81 ± 6.18 <sup>a</sup>	45.94 ± 8.45 <sup>b</sup>	0.89 ± 0.19 <sup>b</sup>
	After	20	58.68 ± 9.98	41.89 ± 8.70 <sup>a</sup>	33.52 ± 7.80 <sup>d</sup>	1.47 ± 0.51 <sup>d</sup>
Control	Before	13	61.87 ± 5.01	46.61 ± 6.79	49.21 ± 6.60 <sup>b</sup>	0.94 ± 0.20 <sup>b</sup>
	After	12	56.11 ± 8.50	46.24 ± 6.10	42.22 ± 7.07 <sup>bd</sup>	1.12 ± 0.24
Healthy person		15	61.66 ± 6.42	48.11 ± 8.51	31.02 ± 4.96	1.57 ± 0.32

<sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.001 as compared with the healthy subjects group; <sup>c</sup>*P* < 0.05; <sup>d</sup>*P* < 0.001 as compared with pretreatment.

**Table 2 Changes in hypercoagulatory parameters before and after treatment in the two groups (mean ± SD)**

Groups		<i>n</i>	AT-III (mg/dl)	FA (%)	β-TG (ng/dl)
Treatment	Before	35	29.9 ± 7.9 <sup>b</sup>	50.4 ± 24.4 <sup>b</sup>	91.0 ± 25.9 <sup>b</sup>
	After	35	38.8 ± 5.5 <sup>d</sup>	102.6 ± 31.6 <sup>d</sup>	62.3 ± 15.1 <sup>bd</sup>
Control	Before	34	32.3 ± 7.2 <sup>a</sup>	57.3 ± 24.6 <sup>b</sup>	87.5 ± 34.2 <sup>b</sup>
	After	34	30.9 ± 8.0 <sup>b</sup>	64.6 ± 26.9 <sup>b</sup>	67.0 ± 42.1 <sup>bc</sup>
Healthy persons			36.4 ± 8.3 (59)	90.3 ± 24.3 (30)	25.0 ± 8.2 (103)

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 as compared with healthy subjects; <sup>c</sup>*P* < 0.05, <sup>d</sup>*P* < 0.001 as compared with pretreatment. The number of individuals is shown in parentheses.

## INTRODUCTION

From July 1986 to July 1995, we used the Fuzheng Huoxue (strengthening the body resistance and promoting blood circulation) anticancer prescription to treat postoperative patients with mid- or late-stage gastric cancer, as well as nude mice with transplanted human gastric cancers. The treatment results are reported below.

## CLINICAL INVESTIGATION

### Clinical data

This study enrolled 69 patients with histologically confirmed mid- or late-stage gastric cancer, and who were being treated at Renji Hospital, affiliated with the Shanghai Second Medical University. The patients were assigned to two study groups. The treatment group included 26 males and 9 females; (two patients aged 33-40 years, 18 patients aged 41-60 years, and 15 patients aged 61-75 years; mean group age = 58.4 years). The control group consisted of 23 males and 11 females, (4 patients aged 33-40 years, 16 patients aged 41-60 years, and 14 patients aged 61-75 years; mean group age = 56.8 years). The pathological stage of each patient was determined according to the TNM classification system, as modified by the Chinese Cooperative Group in Gastric Cancer<sup>[1]</sup>. In the treatment group, 8 cases were stage II, 15 were stage III, and 12 were stage IV. Twenty-seven cases underwent a palliative operation and 11 cases underwent a radical operation. In the control group, 11 cases were stage II, 11 cases were stage III, and 12 cases were stage IV. Twenty-three cases received a palliative operation, and 11 cases received a radical operation. The two groups showed no significant difference in sex, age, clinicopathologic stage or operation mode. The healthy individuals were recruited from staff at the Shanghai Second Medical University and its affiliated hospitals.

### Methods and parameters of observation

The Fuzheng Huoxue anticancer prescription contained Codonopsis pilosula (Franch.) N annf (15 g), Astragalus membranaceus (Fisch.) Bge (15 g), Atractylodes macrocephala Koidz (12 g), Poria cocos (Schw.) Wolf (12 g), Rehmannia glutinosa (Gaertn.) Lib osch (12 g), Adenophoratetraphylla (Thunb.) Fisch (15 g), Salvia miltiorrhiza Bge (15 g), and Angelica sinensis (Oliv.) Diels (12 g). One dose of this mixture was given to patients once a day. The control prescription contained Citrus tangerina Hort. et Tanaka (12 g), Magnolia officinalis Rehd. et Wils (12 g), Amomum villosum Lour (6 g), Oryza sativa L (15 g), and Hordeum vulgare L (15 g), and was also given as a single dose, once a day.

### Treatment methods

Starting at one month after an operation, decoctions of the Fuzheng

Huoxue anticancer prescription, the control prescription, and FT-207 manufactured by the Shanghai No.12 Pharmaceutical Factory, (100-200 mg, three times daily) were given to the designated groups for five days.

### Measurements of patient survival and assay methods

Patient survival rates were calculated by the direct method. T-lymphocyte subgroups, antithrombin III levels, fibrinolytic activity (Fa), and β-thromboglobulin (β-TG) were measured by a fluorescent immunoassay method<sup>[2]</sup>, rocket electrophoresis<sup>[3]</sup>, fibrolytic area<sup>[4]</sup>, and radioimmunoassays, respectively.

## EXPERIMENTAL INVESTIGATION

### Materials and methods

**Materials** An oral formulation of the Fuzheng Huoxue anticancer prescription with a crude medicine concentration of 3.0 g/mL was autoclaved and then stored at low temperature. Human gastric cancer cell line SGC-7901 was purchased from the Shanghai Institute of Pharmaceutics. Female nude mice aged 6-8 wk and 5-Fu (bat. No. 930712) were purchased from the Shanghai Tumor Institute, and the Shanghai Haipu Pharmaceutic Factory, respectively.

**Methods** SGC-7901 cells were inoculated into the backs of nude mice under sterile conditions. The mice were weighed on the next day, and then randomly allocated to a control group, a traditional Chinese medicine group, and a chemotherapy group (*n* = 10 mice per group). There was no significant difference in the total body weight of the nude mice in each group. A 0.5 mL dose of saline solution was instilled into the stomach of each mouse in the control group each day, while 0.5 mL of the Fuzheng Huoxue anticancer decoction was instilled into the stomach of each mouse in the traditional Chinese medicine group. Mice in the chemotherapy group were intraperitoneally injected with 1 mg of 5 FU once a week for 8 wk.

**Observation parameters** Each mouse was monitored for its body weight, tumor weight, and any tumor inhibitory effects.

### Experimental results

**Tumor weight and tumor inhibition** The mean tumor weights in the control, traditional Chinese medicine, and chemotherapy groups were 0.895 ± 0.289 g, 0.433 ± 0.177 g, and 0.357 ± 0.142 g, respectively. While compared with tumor growth in the control group, the tumor inhibition rates in the traditional Chinese medicine group and chemotherapeutic group were 51.6% and 60.1%, respectively, (*P* < 0.001).

**Body weight of the nude mice** The mean body weights of the nude mice in each group before and after treatment were 21.50 ± 1.51 g and 22.47 ± 2.18 g, respectively, in the control group, 21.50 ± 0.47 g and 24.68 ± 1.93 g, respectively, in the traditional Chinese medicine group, and 21.55 ± 0.64 g and 22.96 ± 1.87 g, respectively, in the chemotherapy group. None of these differences before and after treatment were statistically significant (*P* > 0.05). However, when compared with the mean pretreatment weight, the mean body weights in the traditional Chinese medicine and chemotherapy groups were significantly higher after treatment (*P* < 0.05). Furthermore, when compared with the mean body weight in the control group, the mean body weight in the traditional Chinese medicine group showed a significant increase (*P* < 0.05).

## RESULTS

### Comparison of survival rates between the treatment and control groups

Among the 35 patients in the treatment group, 31 survived > 0.5 years, 30 (86%) survived > 1 year, 24 (69%) survived > 3 years, and 14 (40%) survived > 5 years. Among the 34 patients in the control group, 28 (82%) survived > 0.5 years, 22 (65%) survived

> 1 year, 14 (41%) survived > 3 years, and 6 (18%)> 5 years. The 1, 3, and 5-year survival rates in the treatment group were all significantly higher than those in the control group ( $P < 0.05$ ).

The numbers of different T lymphocyte subgroups in the peripheral blood of subjects in the two groups before and after treatment are shown in Table 1. The immunologic function of subjects in both groups showed significant improvement after treatment.

Changes in the hypercoagulatory parameters of subjects in both groups before and after treatment are shown in Table 2. While both groups were in a hypercoagulatory state before treatment, the hypercoagulatory state of the treatment group showed significant improvement after treatment. The values for AT-III and Fa in the treatment group did not significantly differ from those in healthy individuals ( $P > 0.05$ ). The control subjects remained in a hypercoagulatory state.

## DISCUSSION

Patients with mid- or late-stage gastric cancer are usually deficient in Zheng qi, and have an impaired immunologic response<sup>[5]</sup>. The immunologic state of a human is closely correlated with tumor occurrence and development; furthermore, immune responses mediated by T lymphocytes play a major role in tumor immunity.

Our study showed that prior to treatment, the suppressor T lymphocytes of gastric cancer patients were hyperactive and the patients showed a poor immune response. Zhengxu (weakened body immunity) decreases a patient's anticancer response as well as their ability to tolerate chemotherapy. The Fuzheng method is widely used to treat tumors by practitioners of traditional Chinese medicine or combined traditional and Western medicine, and especially in Beijing, Shanghai, Tianjing, and Fujian<sup>[6,7]</sup>, China.

Gastric cancer is associated with blood stasis, and the extent of blood stasis is correlated with the severity and outcome of the disease<sup>[8]</sup>. AT-III is the most important anti-agglutination agent found in plasma. Fa directly reflects the activity of proplasminogen, and  $\beta$ -TG directly reflects the extent of blood stasis. The results of our study showed that prior to treatment, the patients with gastric cancer were in a state of hypercoagulation, and this was the basis for our clinical use of the Huoxue Huayu method. Zhengxu and

blood stasis are basic characteristics of patients with mid- or late-stage gastric cancer. In addition to the Fuzheng method, the Huoxue method can also be used to treat such patients. The Fuzheng Huoxue anticancer prescription produced better clinical effects in treating postoperative patients with mid- or late-stage gastric cancer as compared with the effects shown in control patients. The immunologic function and hypercoagulation status of the patients improved significantly. These results suggest that the Fuzheng Huoxue anticancer prescription can not only replenish Zheng qi, enhance the anticancer response of patients, and reduce the side effects of chemotherapy, but can also improve blood stasis and circulation, as well as immunologic function, and is thus beneficial for treating tumors and extending patient survival times. Our animal studies proved that the Fuzheng Huoxue anticancer prescription inhibited tumor growth without producing any significant toxic side effects or affecting the body weights of the nude mice. Based on these results, we feel the Fuzheng Huoxue anticancer prescription has a bright future for use in cancer management. While the Fuzheng Huoxue anticancer prescription may directly inhibit or kill gastric tumor cells, these activities require further confirmation.

## REFERENCES

- 1 **Xu GW**. Gastric cancer. 1st ed. Beijing: People Health Publishing House 1987: 85-89
- 2 **Zheng WF**. Medical immunomethod. Ed 1. Beijing: People-s Health Publishing House 1989: 128
- 3 **Zhou BZ**. Practical techniques in electrophoresis and immunoelectrophoresis. Wuhan: Hupei Science and Technology Publishing House 1988: 155-163
- 4 **Harmis BD**, Likewood D. Experimental methods in gel electrophoresis of protein. Beijing: Science Publishing House 1986: 161
- 5 **Wang GT**, Zhu JS, Xu JY. Activity detection of subgroup of T lymphocyte, NK cell and interferon in peripheral blood of patients with advanced gastric cancer. *Shanghai Medical Journal* 1995; **18**: 109-110
- 6 **Yu GQ**, Liang FY. Development of study in treating tumor with Fuzheng Peiben. *China Tumor Information* 1990; **3**: 10-13
- 7 **Li PW**. The prospect of the treatment of tumor with integrated traditional Chinese and western medicine through the changes in papers on tumor of recent 16 years. *Chinese Journal of Integrated Traditional and Western Medicin* 1993; **13**: 45-46
- 8 **Zhou AG**, Huang DW, Ding YX, Chen MF, Li DH, Jiang SJ. Investigation on blood stasis syndrome of patients with gastric malignant tumor before and after operation and before death. *Chinese Journal of Integrated Traditional and Western Medicine* 1990; **10**: 540-541

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Effects of tetrandrine on gastric mucosa and liver in portal hypertensive rats

Yi Mu, Yao-Zong Shen, Yi-Fang Chu

Yi Mu, Yao-Zong Shen, Yi-Fang Chu, Research Laboratory of Hepatobiliary Surgery, Xuzhou Medical College, and Department of Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221002, Jiangsu Province, China

Author contributions: All authors contributed equally to the work.

Supported by a grant from the Natural Science Foundation of the Educational Committee of Jiangsu Province, No.920098.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Yi Mu, Associate Professor, having 15 papers published. Research Laboratory of Hepatobiliary Surgery, Xuzhou Medical College, and Department of Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221002, Jiangsu Province, China  
Telephone: +86-516-5698950-2009

Received: August 8, 1996

Revised: October 2, 1996

Accepted: November 10, 1996

Published online: September 15, 1997

### Abstract

**AIM:** To study the effects of tetrandrine on portal hypertensive gastric mucosal lesions.

**METHODS:** Portal hypertensive models were induced in Wistar rats by 60% CCl<sub>4</sub> 3 mL/kg body weight through subcutaneous injection, once every 4 d for 56 d. The animals were randomly divided into portal hypertension, tetrandrine and propranolol groups and subsequently, treated by normal saline, tetrandrine and propranolol respectively for 15 d. Some healthy rats were used as control group. Portal venous pressure (PVP), gastric mucosal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) content, gastric mucosal blood flow (GMBF), gastric adherent mucus (GAM), ALT, ALP and serum total bilirubin (STB), were measured and liver tissues were observed histologically.

**RESULTS:** In tetrandrine group and propranolol group, PVP was significantly lower ( $1.43 \pm 0.13$ ,  $1.45 \pm 0.12$  vs  $1.89 \pm 0.18$  kPa;  $P < 0.01$ ) and gastric mucosal PGE<sub>2</sub> content ( $138.59 \pm 12.68$ ,  $129.98 \pm 14.31$  vs  $104.65 \pm 12.97$  pg/mg;  $P < 0.01$ ), GMBF ( $11.80 \pm 3.47$ ,  $10.54 \pm 3.63$  vs  $6.61 \pm 2.82$  mL·h·kg;  $P < 0.05$ ) and GAM ( $3.01 \pm 0.15$ ,  $2.98 \pm 0.21$  vs  $2.24 \pm 0.26$  mg;  $P < 0.01$ ) was significantly higher than that in portal hypertension control group. In tetrandrine group intrahepatic proliferative fibrous tissues were reduced and serum ALT ( $47.67 \pm 25.90$  vs  $189.33 \pm 41.21$  King U;  $P < 0.01$ ), ALP ( $0.22 \pm 0.04$  vs  $0.31 \pm 0.06$   $\mu\text{mol}\cdot\text{s}^{-1}/\text{L}$ ;  $P < 0.01$ ) and STB ( $4.75 \pm 0.76$  vs  $11.12 \pm 2.93$   $\mu\text{mol}/\text{L}$ ;  $P < 0.01$ ) were lowered as compared with those in portal hypertension control group. ALT ( $209.34 \pm 36.91$  vs  $189.33 \pm 41.21$  King U;  $P > 0.05$ ) and STB ( $11.63 \pm 3.01$  vs  $11.12 \pm 2.93$   $\mu\text{mol}/\text{L}$ ;  $P > 0.05$ ) in propranolol group were not different

from that in portal hypertension control group, but it showed more marked hepatocellular degeneration and necrosis and elevation of ALP ( $0.46 \pm 0.05$  vs  $0.31 \pm 0.06$   $\mu\text{mol}\cdot\text{s}^{-1}/\text{L}$ ;  $P < 0.01$ ).

**CONCLUSION:** Tetrandrine can improve the functions of gastric mucosa and liver, and facilitate the absorption of intrahepatic proliferative fibrous tissues. Propranolol can aggravate hepatitis though it may improve portal hypertensive gastric mucosal lesions.

**Key words:** Liver gastric mucosa; Hypertension, portal; Tetrandrine; Propranolol

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Mu Y, Shen YZ, Chu YF. Effects of tetrandrine on gastric mucosa and liver in portal hypertensive rats. *World J Gastroenterol* 1997; 3(3): 192-194 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/192.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.192>

### INTRODUCTION

Gastric mucosal lesions (GML) is one of common causes of upper gastrointestinal bleeding in portal hypertensive (PHT) patients with cirrhosis. So far, there have been no ideal therapy for this lesion. Although some reports have suggested that propranolol is effective for GML, attention has been paid to the side effects of this drug<sup>[1]</sup>. It has been demonstrated that tetrandrine can lower the portal venous pressure (PVP) with no significant effects on peripheral circulation<sup>[2]</sup>, and decrease the serum procollagen-III-peptide concentration in cirrhotic patients<sup>[3]</sup>. Therefore, in this study we observed the effects of tetrandrine on PVP, the functions of gastric mucosa and liver, and liver histology in PHT rats with cirrhosis, and tetrandrine was compared with propranolol, in an attempt to investigate effects of tetrandrine on PHT GML.

### MATERIALS AND METHODS

#### Animals and portal hypertension induction

A total of 148 male Wistar rats, weighing 200-300 g ( $254.5 \pm 31.4$ ) were used. Of them, 124 were treated by CCl<sub>4</sub>, 24 served as normal controls. The 124 rats received 60% CCl<sub>4</sub> (solution in rapeseed oil) 3 mL/kg by subcutaneous injection once every 4 d for 56 d. PHT model was formed in 72 rats, which was confirmed by the histology and the PVP measurement (more than 1.47 kPa).

#### Experimental groups

The 72 PHT rats were randomly divided into 3 groups, 24 in each: PHT control group, received normal saline 10 mL/kg tid through peritoneal injection, for 15 d; Tetrandrine group, received NS through same administration and forage containing tetrandrine

**Table 1 Portal venous pressure (PVP), gastric mucosal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) content, gastric mucosal blood flow (GMBF) and gastric adherent mucus (GAM) ( $\bar{x} \pm s$ )**

Groups	PVP (kPa)	PGE <sub>2</sub> (pg/mg)	GMBF (mL·h·kg)	GAM (mg)
PHT	1.89 ± 0.18 <sup>d</sup>	104.65 ± 12.97 <sup>d</sup>	6.61 ± 2.82 <sup>c</sup>	2.24 ± 0.26 <sup>d</sup>
Propranolol	1.45 ± 0.12 <sup>b</sup>	129.98 ± 14.31 <sup>b</sup>	10.54 ± 3.63 <sup>a</sup>	2.98 ± 0.21 <sup>b</sup>
Tetrandrine	1.43 ± 0.13 <sup>b</sup>	138.59 ± 12.68 <sup>b</sup>	11.80 ± 3.47 <sup>a</sup>	3.01 ± 0.15 <sup>b</sup>
Normal	1.24 ± 0.10 <sup>bc</sup>	162.03 ± 13.84 <sup>bd</sup>	18.86 ± 4.37 <sup>bd</sup>	3.25 ± 0.16 <sup>b</sup>

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, *vs* portal hypertensive (PHT) controls group; <sup>c</sup>*P* < 0.05, <sup>d</sup>*P* < 0.01, *vs* propranolol group.

**Table 2 Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum total bilirubin (STB) ( $\bar{x} \pm s$ )**

Groups	ALT (king U)	ALP (μmol·s <sup>-1</sup> /L)	STB (μmol/L)
PHT	189.33 ± 41.21	0.31 ± 0.06 <sup>d</sup>	11.12 ± 2.39
Propranolol	209.34 ± 36.91	0.46 ± 0.05 <sup>b</sup>	11.63 ± 3.01
Tetrandrine	47.67 ± 25.90 <sup>bd</sup>	0.22 ± 0.04 <sup>ad</sup>	4.75 ± 0.76 <sup>bd</sup>
Normal	36.42 ± 21.53 <sup>bd</sup>	0.21 ± 0.04 <sup>ad</sup>	4.96 ± 0.91 <sup>bd</sup>

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 *vs* portal hypertensive (PHT) controls group; <sup>d</sup>*P* < 0.01 *vs* propranolol group.

**Table 3 The liver fibrosis degree**

Groups	Grade 4+ + +	Grade 3+ +	Grade 2+ +	Grade 1- -
PHT	6	2	0	0
Propranolol	4	3	1	0
Tetrandrine <sup>ac</sup>	0	4	2	2
Normal <sup>bd</sup>	0	0	1	7

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, *vs* portal hypertensive (PHT) controls group; <sup>c</sup>*P* < 0.05, <sup>d</sup>*P* < 0.01, *vs* propranolol group.

(150 mg·kg<sup>-1</sup>·d) for 15 d; propranolol group, received propranolol 35 mg/kg tid peritoneal injection, for 15 d; normal group, through 24 healthy rats served as controls.

Each group was then randomly divided into 3 subgroups, 8 in each. In the 1<sup>st</sup> subgroup PVP and gastric mucosal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) content were measured and liver histology and liver function were examined. Gastric mucosal blood flow (GMBF) was measured in the 2<sup>nd</sup> subgroup and gastric adherent mucus (GAM) measured in the 3<sup>rd</sup> subgroup.

### Methods

**PVP measurement.** Laparotomy was performed under 60% urethane (5 mL/kg by peritoneal injection) anesthesia in rats fasted for 24 h. The portal vein was punctured with a needle and PVP was measured by a manometer.

**Gastric mucosal PGE<sub>2</sub> content measurement.** The stomach was excised, then put it on a piece of ice to scrape gastric mucosa. The gastric mucosal PGE<sub>2</sub> content was measured by radioimmunoassay<sup>[4]</sup>.

**Gastric mucosal blood flow measurement.** The rats fasted for 24 h, under the same anesthesia, GMBF was measured by neutral red clearance test<sup>[5]</sup>.

**Gastric adherent mucus measurement.** The rats fasted for 24 h, under the same anesthesia, the stomach was excised and opened along the lesser curvature, turned over the gastric wall. GAM was measured by alcian blue staining<sup>[6]</sup> and result was shown by adherent dye amount.

**Liver function test.** A blood sample 2.5 mL was taken for determining ALT, ALP and serum total bilirubin (STB). This was performed by the same staff from the laboratory department of our hospital.

**Liver histology.** The histological specimens were made by the pathology department of our hospital, stained with hematoxylin and eosin (HE) and observed under light microscope.

### Statistical analysis

Analyses of variance among groups for data measurement were made with F test, and difference between two groups was compared with *t* test. For ranked data, multiple samples were compared with

*H* test, and difference between two groups was compared with *U* test. Results were considered statistically significant at *P* < 0.05.

## RESULTS

There were no significant differences in PVP between tetrandrine and propranolol groups (*P* > 0.05), PVP in both the groups was significantly lower than that in PHT control group (*P* < 0.01), but higher than that in healthy group (*P* < 0.05), (Table 1).

Gastric mucosal PGE<sub>2</sub> content and GMBF showed no significant difference (*P* > 0.05) between tetrandrine and propranolol groups, and were significantly higher in both the groups than that in PHT control group (*P* < 0.01; *P* < 0.05), but lower than that in healthy group (*P* < 0.01), (Table 1).

There was no significant difference in GAM between tetrandrine and propranolol groups (*P* > 0.05). The values in both the groups were significantly higher than that in PHT control group (*P* < 0.01), but without significant difference as compared with the healthy group (*P* > 0.05), (Table 1).

In ALT and STB, difference between PHT and propranolol groups was not significant (*P* > 0.05). ALT and STB were significantly higher than in tetrandrine and healthy groups (*P* < 0.01), with no significant difference (*P* > 0.05) between the latter two groups, (Table 2).

ALP in propranolol group was significantly higher than that in PHT control group (*P* < 0.01), (Table 2). It was significantly higher than that in tetrandrine and healthy groups (*P* < 0.01; *P* < 0.05), with no significant difference (*P* > 0.05) between the latter two groups.

In PHT group, pseudolobules were found in most cases, but hepatocytic degeneration and necrosis were not obvious, few red cells were found in sinusoid of the liver. In propranolol group, pseudolobules or a large quantity of proliferative fibrous tissues were found, hepatocytic degeneration and necrosis were more marked with few red cells found in sinusoid of the liver. In tetrandrine group, intrahepatic proliferative fibrous tissue was less than that in PHT group and pseudolobules disappeared, and many red cells were found in the sinusoid of the liver, no hepatocytic degeneration and necrosis were found.

According to the degree of intrahepatic proliferative fibrous tissue, four grades were established. Grade 4, pseudolobules; Grade 3, a large quantity of fibrous tissue; Grade 2, a little fibrous tissue; Grade 1, no proliferative fibrous tissues were found. Results showed that there was no significant difference between propranolol and PHT groups (*P* > 0.05). In tetrandrine group, fibrous tissue was significantly decreased as compared with that in PHT control group (*P* < 0.05). There was significant difference (*P* < 0.01) between healthy and other groups. These data are shown in Table 3.

## DISCUSSION

The pathogenesis of PHT GML was the weakening of gastric mucosal protective mechanism resulting from the structural and functional impairment of mucosal microcirculation consequent upon PHT. Gastric acid and pepsin were not the main causes of this lesion<sup>[7]</sup>. Therefore, treating PHT GML by reducing PVP has been a main orientation of study in the world. Gastric mucosal PGE<sub>2</sub> content, GMBF and GAM are important factors of gastric mucosal protective mechanism so that the change of these factors will reflect the therapeutic effects on PHT GML.

It has been reported that propranolol may be effective for PHT GML. Our results showed that propranolol could significantly reduce PVP and increase the gastric mucosal PGE<sub>2</sub> content, GMBF and GAM. Our previous electron microscopic observation showed that propranolol could significantly improve the pathological state of gastric mucosal capillaries and increase the mucigen granules of gastric mucosal epithelial cells<sup>[8]</sup>. These demonstrated that propranolol is indeed useful in improving PHT GML. Propranolol is a β adrenoreceptor-blocking agent, and its effect depends on constricting splanchnic blood vessels, decreasing blood flow into the portal venous system, reducing PVP and improving the dilatation state of gastric mucosal microcirculation. Nevertheless, in this study, ALT and STB in propranolol group were not decreased, while ALP

was increased as compared with PHT group. These suggest that propranolol could not decrease hepatocytic damage, but increase the intrahepatic obstruction on the contrary. The liver histologic observation also proved this. Because propranolol reduces PVP through decreasing portal venous blood flow and constricting blood vessel, hepatic blood supply is further reduced, resulting in marked liver damage. Moreover, further reduction of the blood flow passing through the liver causes increased blood ammonia and incidence of cerebrosis.

Effects of tetrandrine on gastric mucosa in PHT rats were almost the same as propranolol, but their mechanisms of decreasing PVP were different. Tetrandrine, a calcium-channel blocking agents, inhibits calcium ion acrossing the calcium channel of vascular smooth muscle by blocking receptors on cell membrane, leading to a decrease in intracellular calcium ion concentration, and relieving excitation-constricting coupling. As a result, smooth muscle loosened, intra and extra-hepatic portal venous resistance decreased and PVP reduced<sup>[2]</sup>. Furthermore, it can inhibit the activity of adenylate cyclase on cell membrane, lower the intracellular cyclic adenosine monophosphate (cAMP) levels, thus disturbing the phosphorylation process of thymine, inhibiting the collagen synthesis of hepatocytes and Ito cells<sup>[9]</sup>. In this study, we also found that tetrandrine can reduce intrahepatic proliferative fibrous tissues. So PHT GML was improved because of the decreased PVP and portal venous backward blood flow. Besides, results showed that tetrandrine can significantly lower ALT, ALP and STB to the normal. From these results, it is supposed that tetrandrine alleviate hepatocytic necrosis and intrahepatic obstruction. Histologic observation also proved this. These are due to the dilatation of intra and extra-hepatic portal vein and the decrease in intrahepatic fibrous tissue and portal venous

resistance, resulting in increased hepatic blood supply and better hepatic nutritional stat us.

In conclusion, tetrandrine not only can improve the function of gastric mucosa and liver, but also facilitate the absorption of intrahepatic proliferative fibrous tissues. This study suggests that tetrandrine might be a more appropriate drug for PHT GML as compared with propranolol.

## REFERENCES

- 1 **Mu Y**, Shen SG, Shen YZ. Recent developments of study on portal hypertensive gastric mucosal lesions. *Chinese Journal of Genetical Surgery* 1993; **2**:114-117
- 2 **Li DG**, Lu HM, Li XH, Chen JW, Jiang ZM, Wang XL, et al. Use of calcium-channel blockers in cirrhotic patients with portal hypertension. *National Medical Journal of China* 1990; **70**: 370-374
- 3 **Li DG**, Xia WX, Lu HM, Li XH, Jiang ZM, Qiang QZ, et al. Significance of serum procollagen-III-peptide in reflecting the therapeutic effects of calcium-channel blockers on hepatic fibrosis. *Chinese Journal of International Medicine* 1990; **29**: 453-456
- 4 **Mu Y**, Shen YZ, Chu YF, Zheng JL, Zhang MZ. Radioimmunoassay of gastric mucosal prostaglandin E2 in portal hypertensive rats with cirrhosis. *Acta Academiae Medicinae Xuzhou* 1992; **12**: 299-301
- 5 **Zhan MC**, Zhang JF. Effect of intra-cerebroventricular injection of pentagastrin on gastric mucosal blood flow in rats. *Chinese Journal of Digestion* 1990; **10**: 335-337
- 6 **Li JY**, Zhang XJ, Lu QH. Effect of intra-cerebroventricular injection of bombesin on indomethacin induced gastric cancer in rats. *Acta Physiol Sinica* 1987; **39**: 495-530
- 7 **Mu Y**, Shen YZ, Liu ZF, Han B, Chu YF. Pathogenesis of gastric mucosal lesions in portal hypertension. *Chinese Journal of Pathophysiology* 1994; **10**: 210-214
- 8 **Mu Y**, Shen YZ, Han B, Chu YF. The Ultrastructure of gastric mucosa in portal hypertensive rats and the changes after tetrandrine, salavia miltiorrhizae and propranolol therapies. *Acta Academiae Medicinae Xuzhou* 1993; **13**: 109-112
- 9 **Fan LY**, Kong XQ, Gao CF. Effects of tetrandrine on DNA and collagen synthesis of rat Ito cells and hepatocytes. *Chinese Journal of Digestion* 1994; **14**: 281-283

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Cost-effectiveness study on treatment of duodenal ulcer

Shi-Yao Chen, Ji-Yao Wang, Jie-Chen, Xi-De Zhang, Shan-Shen Zhang

Shi-Yao Chen, Ji-Yao Wang, Jie-Chen, Xi-De Zhang, Shan-Shen Zhang, Department of Gastroenterology, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Shi-Yao Chen, Department of Gastroenterology, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To compare the efficiency of therapy with a 2-week regimen of amoxicillin plus metronidazole and six weeks of Tagamet (AMT group) vs the efficacy of therapy with 6 wk of omeprazole plus 2 wk of amoxicillin (OA group) for ulcer healing, *Helicobacter pylori* (Hp) eradication, and decreasing the recurrence of duodenal ulcers.

**METHODS:** This cost-effectiveness analysis was based on results shown in a randomized controlled trial conducted in 1995 in patients with a duodenal ulcer (OA group, 46 patients; AMT group, 43

patients) and treated at class grade III A hospitals in Shanghai, China.

**RESULTS:** The costs of treatment in the AMT group were less than those in the OA group for ulcer healing (¥546.25 vs ¥1296.76 per case,  $P < 0.01$ ), Hp eradication (¥702.32 vs ¥1742.53 per case,  $P < 0.01$ ), and decreasing ulcer recurrence (¥640.39 vs 1424.54 per case,  $P < 0.01$ ). Direct costs comprised the major cost involved in treatment of duodenal ulcers. The difference in the cost of treating ulcers in the two groups was primarily due to the costs of the different drugs. There was no significant difference between the two groups regarding their direct non-medical costs and indirect costs.

**CONCLUSION:** When based on therapeutic effectiveness and financial costs, AMT therapy was more cost-efficient than OA therapy. AMT therapy is recommended for its low cost, acceptable ulcer healing rates, ability to cure of an Hp infection, and especially when treating patients with an ulcer < 1 cm in diameter.

**Key words:** Duodenal ulcer; *Helicobacter pylori*; Cost-effectiveness analysis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen SY, Wang JY, Chen J, Zhang XD, Zhang SS. Cost-effectiveness study on treatment of duodenal ulcer. *World J Gastroenterol* 1997; 3(3): 194 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/194.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.194>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Endoscopic ligation for benign and malignant lesions of upper digestive tract

Yu-Long Chen, Yong-Zhong Chen, Jian-Xiang Zou, Xue-Li Li

Yu-Long Chen, Yong-Zhong Chen, Jian-Xiang Zou, Department of Gastroenterology, The First Affiliated Hospital of Henan Medical University, Zhengzhou, 450052, Henan Province, China

Xue-Li Li, Department of Pathology, People's Hospital of Henan Province, Zhengzhou 450003, Henan Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Yu-Long Chen, Professor, having 30 papers and 3 books published. Department of Gastroenterology, The First Affiliated Hospital of Henan Medical University, Zhengzhou, 450052, Henan Province, China  
Telephone: 0371-3921761

Received: August 8, 1996

Revised: October 2, 1996

Accepted: November 10, 1996

Published online: September 15, 1997

**Key words:** Esophageal and gastric varices; Polyp; Liver neoplasms

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen YL, Chen YZ, Zou JX, Li XL. Endoscopic ligation for benign and malignant lesions of the upper digestive tract. *World J Gastroenterol* 1997; 3(3): 195-196 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/195.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.195>

### INTRODUCTION

Endoscopic techniques have been used to treat variceal hemorrhage for > 50 years, and are now accepted as first line treatment for this type of esophageal bleeding. While injective sclerotherapy can control hemorrhage in 90% of cases, bleeding reoccurs and complications occur in up to 55%<sup>[1]</sup> and 40%<sup>[2]</sup> of cases, respectively. As a result of these problems, better methods for endoscopic treatment of variceal hemorrhage have been continuously studied. Banding ligation was first reported in humans in 1990<sup>[3]</sup>, and has since become an important method used in endoscopic therapy. Moreover, this method has shown reliable effects when used to manage esophageal varices. Based on such reports, we used banding ligation to resect polyps and early stage cancers of the upper digestive tract, and achieved satisfactory results.

### MATERIALS AND METHODS

#### Patients

A total of 174 patients underwent endoscopic ligation. Among these

patients, 78 were treated for a variceal hemorrhage (these included 4 patients with a primary hepatic cancer, 10 patients who experienced re-bleeding after spleen resection, and 2 patients who experienced re-bleeding after a TIPS operation). Prior to undergoing ligation, these patients had bleeding frequencies that ranged from 1 to 8 times/year (mean = 2.35 times/year). Twenty-one of the patients had a polyp in the upper digestive tract (14 cases in the gastric antrum, 4 in the gastric body, 2 in the lower esophagus, and 1 in the duodenum). The polyps could be morphologically characterized as one of three types: (1) long pedunculated, (2) sub-pedunculated, and (3) thick, and had diameters ranging from 0.3 cm to 0.9 cm. Five of the polyps proved to be early stage cancers (2 cases of early esophageal cancer, and 3 cases of gastric antral cancer *in situ*).

#### Methods

All ligations were performed using an Olympus XQ10 XQ20 endoscope (Olympus Corporation; Tokyo, Japan), and either a plastic or stainless steel ligation device. Other equipment included a silicon rubber band (used in ligation), trip wire, inner cylinder, and an outer cylinder. A ligation device fixed at the end of the endoscope was plunged into the digestive tract, and placed in close proximity to the lesion. The lesion was then sucked into the ligation device, and a prestressed rubber band was released over the entrapped lesion by pulling the trip wire; after which, the lesion was ligated. All patients underwent endoscopic re-examinations for > 1 year. The therapeutic effectiveness of the ligation procedure was evaluated by both endoscopic observations and histopathological examinations.

### RESULTS

While a majority of the 78 esophageal variceal patients who underwent ligation were cured, four patients died because the treatment did not stop the progress of their disease. The overall effectiveness of ligation was 94.8% (74/78 cases, Table 1), and the mean bleeding frequency decreased from 2.35 times/year before treatment to 0.15 times/year after treatment. While four patients reported slight dysphagia, no other complications occurred.

Table 2 shows the sloughing off times for the polyps and early stage cancers following treatment with endoscopic ligation; these times ranged from 4 to 10 d, and nearly 100% of the lesions disappeared. Furthermore, biopsy results showed no malignant cells in the resected specimens obtained from cancer patients treated with ligation. A small ulcer remained after lesion sloughing in 18 cases; however, these lesions usually healed 10 d later, and there was no subsequent bleeding or lesion recurrence. Histopathological examinations showed some infiltration of inflammatory cells at the site of the wound, but no necrosis was observed.

### DISCUSSION

Banding ligation was the most important development in endoscopic

**Table 1** Ligation compared with sclerotherapy for the variceal bleeding

<i>n</i>	Effective rate ( <i>n</i> )	Bleeding times (yr)		Rebleeding (%)	Complication ( <i>n</i> )	
		B-T	A-T		Dysphagia	Others
LT	78 94.8% (74/78)	2.35	0.15	3.80%	4	0
ST	32 90.6% (29/32)	2.4	1.1	30%	3	9

B-T: Before treatment; A-T: After treatment; LT: Ligation treatment; ST: Sclerotherapy

**Table 2** The sloughing off time for the ligated polyp and early cancers

Lesion type	<i>n</i>	Cases with different sloughing off time		
		4-5 (d)	6-8 (d)	9-10 (d)
Polyp				
TP	8	5	3	0
SP	7	4	3	0
LP	6	1	4	1
Early cancer <sup>1</sup>				
Gastric CA	3	1	2	0
Esophageal Ca	2	0	2	0

<sup>1</sup>No cancerous cells were found in the resected specimen of 5 early stage cancers. TP: Thick polyp; SP: Sub pedunculated polyp; LP: Long pedunculated polyp.

therapy. We have used endoscopic ligation (EL) to treat esophageal variceal bleeding since 1992, and achieved satisfactory results. We also conducted a study which compared banding ligation and sclerotherapy in the management of esophageal variceal bleeding. The results indicated that EL was highly effective. Moreover, the treated patients experienced a quick recovery and had a low rebleeding rate. The 78 patients who received ligation for esophageal variceal bleeding required significantly fewer treatments, as well as treatments of shorter duration, when compared to patients treated with conventional sclerotherapy. Additionally, the patients treated with EL experienced fewer complications, as only 4 patients reported slight dysphagia, and other complications associated with sclerotherapy, (*e.g.* fever, pleura infiltration, and esophageal stricture) were not observed.

Also, when compared with a portacaval shunt operation, EL produced no effect on liver blood flow. In cases where splenic hyperfunction is not sufficiently severe to necessitate performing a splenectomy, EL can serve as an alternative to portal-azygos disconnection. Moreover, EL might be the first choice for patients who experience re-bleeding after a portacaval shunt or portal azygos disconnection, as it has the advantages of safety, convenience, and causing little or no injury. We also used EL to treat 2 patients undergoing Tips operations, and achieved satisfactory results.

Endoscopic ligation has recently been used in treatment of gastroenteric polyps and in cases requiring early cancer resection. Ligation by itself usually blocks blood flow to the polyp or cancer; this induces lesion ischemia or tissue necrosis, which causes the ligated lesion to eventually fall off. We noticed that a very small ulcer

was present after a lesion fell off; however, the ligation procedure was a progressive process in which tissue damage and healing occurred almost simultaneously when the stretched silicon rubber band was recovered.

Similar to performing a microwave resection<sup>[4]</sup>, different methods should be used to ligate lesions of different morphological types. When ligating sub-pedunculated and thick polyps, as well as early stage cancers, the "O" type of rubber band should be used and released at the base of the polyp, while segmental ligation should be performed when treating long pedunculated polyps. When treating polyps with a longer pedicle, we used the "U" ligation method, which allowed the ligation device to approach the juncture of the polyp base and pedicle. Next, suction was applied through the endoscope, and the rubber band was released over the entrapped polyp ("U" shape).

The authors of this paper suggest that ligation management should be selected for the resection of early stage cancers of the upper digestive tract, and especially situ cancers; because using this method can spare the patient from the risks and pain associated with an operation. In cases where a histopathological examination reveals the presence of malignant cells, ligation should be performed within 24 h after biopsy so as to guarantee the correct site for ligation. If esophageal cancer is confirmed, the tissue can be treated with Lugol's solution, and then ligated at the lightly stained site<sup>[5]</sup>. Such patients should receive regular followup examinations with endoscopy and histopathology following their ligation treatment.

The scaling time of a lesion depends on its hardness and the elasticity of the "O" rubber band. The scaling time is shorter when treating soft lesions with an "O" rubber band of better elasticity.

The current commercially available inner cylinders have a diameter of 0.9 cm, and if the polyp is too large, it cannot be sucked into the device. Furthermore, the ligation method has limited usefulness for treating colon polyps, because no currently available colonoscope has a matched ligation device. Therefore, the methods and equipment used for ligation require further development.

## REFERENCES

- 1 MacDougall BR, Westaby D, Theodossi A, Dawson JL, Williams R. Increased long-term survival in variceal haemorrhage using injection sclerotherapy. Results of a controlled trial. *Lancet* 1982; **1**: 124-127 [PMID: 6119510 DOI: 10.1016/S0140-6736(82)90378-6]
- 2 Schuman BM, Beckman JW, Tedesco FJ, Griffin JW, Assad RT. Complications of endoscopic injection sclerotherapy: a review. *Am J Gastroenterol* 1987; **82**: 823-830 [PMID: 3307389]
- 3 Van Stiegmann G, Goff JS, Sun JH, Hruza D, Reveille RM. Endoscopic ligation of esophageal varices. *Am J Surg* 1990; **159**: 21-5; discussion 25-6 [PMID: 2294799 DOI: 10.1016/S0002-9610(05)80602-6]
- 4 Chen YL, Li JC and Tian DF. Studies on the endoscopic microwave method for gastroenteric-polyp resection. *Endoscope* 1990; **7**: 212-213
- 5 Yang CJ, Ren X, Tan CH, Zhu EQ, Zhu CN, Wang CJ. Evaluation of diagnosing esophageal lesions diagnosed with Lugol's solution staining. *Endoscope* 1995; **12**: 195-197

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Changes in mucosal permeability to lipopolysaccharide in the colon of chronic alcoholic rats

Xian-Ming Chen, Rui-Ling Xu, Xue-Hui Ma, Yuan-Chang Zhao, De-Wu Han

Xian-Ming Chen, Rui-Ling Xu, Xue-Hui Ma, Yuan-Chang Zhao, De-Wu Han, Department of Pathophysiology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Xian-Ming Chen, Department of Pathophysiology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1996  
Published online: September 15, 1997

### Abstract

**AIM:** To evaluate the effects of chronic alcohol abuse on mucosal permeability to lipopolysaccharide (LPS) in the colon of rats.

**METHODS:** *Escherichia coli* LPS (20 mg/L) was injected into the colon of chronic alcoholic rats ( $n = 10$ ) that had been supplied with Lieber diet every other day for six weeks. Before and 5, 10, 20, and 30 min after LPS injection, portal vein blood samples were obtained and the LPS levels in the blood were measured. The distribution of LPS in the colon tissues was observed with confocal laser scanning

microscopy by immunofluorescence technique using a monoclonal antibody specific to the lipid A region of LPS. Normal rats were used as the controls ( $n = 6$ ).

**RESULTS:** Before LPS injection, LPS levels in the portal vein blood of chronic alcoholic rats were significantly higher than that of the normal controls ( $3.56 \pm 0.67$  ng/L vs  $2.45 \pm 0.15$  ng/L,  $P < 0.01$ ). At 5, 10, 20, and 30 min after LPS injection, LPS levels were significantly higher than that before LPS injection ( $173.56 \pm 3.45$  ng/L,  $154.78 \pm 0.57$  ng/L,  $43.89 \pm 0.67$  ng/L,  $45.38 \pm 0.89$  ng/L vs  $3.56 \pm 0.67$  ng/L, respectively,  $P < 0.01$ ). Most mucosal cells in the chronic alcoholic rats showed strong positive reactions to LPS, but in the normal rats, there were no significant changes in portal vein blood LPS levels and in the fluorescence reactions to LPS in the mucosal cells after LPS injection.

**CONCLUSION:** Chronic alcohol abuse results in a significant increase in LPS permeability in the colon mucosa cells of rats.

**Key words:** Colon/metabolism; Lipopolysaccharide/metabolism; *Escherichia coli*; Alcohol; Endotoxins

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen XM, Xu RL, Ma XH, Zhao YC, Han DW. Changes in mucosal permeability to lipopolysaccharide in the colon of chronic alcoholic rats. *World J Gastroenterol* 1997; 3(3): 196 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/196.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.196>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Effects of Radix Rehmanniae on gastric acid secretion and gastric ulcer formation in rats

Zhu-Li Wang, Lin Li

Zhu-Li Wang, Department of Physiology, Sun Yat Sen University of Medical Sciences, Guangzhou 510089, Guangdong Province, China

Lin Li, Department of Traditional Chinese Medicine, Third Affiliated Hospital, Sun Yat Sen University of Medical Sciences, Guangzhou 510089, Guangdong Province, China

Zhu Li Wang, MD, male, born on February 6, 1963, in Changsha, Hunan Province, graduated from the Department of Medicine, Sun Yat Sen University of Medical Sciences in 1984. Associate Professor and Vice Director of the Department of Physiology, engaged in the study of digestive physiology, has published more than 10 academic papers.

Supported by the CMB Foundation, No. 2231061.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Author contributions: All authors contributed equally to the work.

Correspondence to: Dr. Zhu Li Wang, MD, Department of Physiology, Sun Yat Sen University of Medical Sciences, Guangzhou 510089, Guangdong Province, China  
 Telephone: +86-20-87778223-3274

Received: November 11, 1996

Revised: January 23, 1997

Accepted: February 25, 1997

Published online: September 15, 1997

**Key words:** Radix Rehmanniae; Stomach ulcer; Gastric acid/secretion; Gastric juice

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang ZL, Li L. Effects of Radix Rehmanniae on gastric acid secretion and gastric ulcer formation in rats. *World J Gastroenterol* 1997; 3(3): 197 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/197.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.197>

### INTRODUCTION

The traditional Chinese medicine Radix Rehmanniae (RR), or Chinese foxglove root, is the root of *Rehmannia glutinosa* Libosch, a Scrophulariaceae plant. It is divided into three types based on differences in its preparation and action: fresh (dRR), dry (dRR), or steamed (sRR). RR contains 23 glucosides (main component: iridoid glucoside), eight carbohydrates, over

20 amino acids, and inorganic ions and trace elements. Its pharmacological effects on many systems are known but not that on the gastrointestinal tract.

### MATERIALS AND METHODS

#### Animals

Sprague Dawley rats of both sexes, weighing 160–180 g, were supplied by our university's Centre of Experimental Animals.

#### Collection and measurement of gastric juice

According to Shay's method, solid food but not drinking water was withheld 48 h before the experiment for all rats. After ether anesthesia, the abdominal wall was incised and the pylorus was ligated. Two hours later, the rat was killed and its cardia was ligated. The stomach was removed and an incision was made along the greater curvature to collect and measure the gastric juice. To calculate the total acidity and total acid output, the gastric acid was titrated with 0.01 mol/L NaOH solution to pH 7 on a Type ZD<sub>2</sub> titrator.

#### Gastric ulcer model

The operative procedure was the same as above. The stomachs were removed, opened, washed with saline, and mucosal lesions were examined with a magnifying glass to count the number and incidence of ulcers 10–12 h after pylorus ligation. The ulcer inhibition rate was calculated as follows:

Ulcer inhibition rate = [(Average number of ulcers in treatment group – Average number of ulcers in control group)/Average number of ulcers in treatment group] × 100%

#### Preparation and administration of decoction

The dRR and sRR were provided by the Third Affiliated Hospital Pharmacy of Traditional Chinese Medicines. dRR or sRR (20 g) was placed in 200 mL deionized water (prepared by our university's Department of Chemistry), decocted for 30 min, filtered, and 150 mL deionized water was added to the filtrates, which were decocted for 20 min and filtered. The two filtrates were combined and concentrated to 20-mL volume, producing a 100% decoction of dRR or sRR that could be diluted to a 50% decoction with water. A 2.0-mL decoction or deionized water (control) was injected slowly into the duodenum after pylorus ligation in all the rats.

#### Statistical analysis

The data were analyzed statistically using computer software. Analysis of variance was used for comparing the difference among sample means; the *q* test (Newman-Keuls test) was used for multiple comparison. The data were logarithmically transformed when the homogeneity of variance was not met. If the variances were not equal after logarithmic transformation, we used the rank-sum test (*k*-ws test) and  $\chi^2$  test (for contingency table data) to analyze the difference in rates.

### RESULTS

#### Effects of dRR and sRR on gastric juice secretion and ulcer

Tables 1–3 show that dRR and sRR remarkably inhibited gastric acid secretion and ulcer formation in the pylorus-ligated rats.

#### Dose-effect relationship

The volume of gastric juice and total acid output in the 50% dRR group were higher than that in the 100% dRR group ( $P < 0.05$ ) (Table 1). The volume of gastric juice, total acidity, and total acid output in the 50% sRR group were also higher than that in the 100% sRR group ( $P < 0.05$ ) (Table 2). The results suggest that the inhibitory effects of dRR and sRR on gastric juice secretion may be dose-dependent.

#### Comparison between dRR and sRR

The volume, total acidity, and total acid output in the 100% dRR group were all higher than that in the 100% sRR group ( $P < 0.05$  or 0.01). Other differences were not statistically significant ( $P > 0.05$ ) (Tables 1–3).

### DISCUSSION

Some evidence has proven that the increased gastric juice secretion after pylorus ligation is due to excitation of the mucosal baroreceptors of the antrum and the activation of the vago-vagal reflex, and ulcer formation is related to the copious secretion of gastric juice. Therefore, the acid inhibitory effect of dRR and sRR may result from antagonization of the excitatory action of the vagus nerve. The anti-ulcer action of dRR and sRR may be in agreement with that of the acid inhibition; however, other mechanisms cannot be excluded.

The chemical components of dRR differ somewhat from that of sRR, as do the medical effects. The acid inhibitory effect of sRR is slightly stronger than that of dRR, but the relationship between the difference in activity and ingredients remains unclear.

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

Table 1 Effects of dry Radix Rehmanniae (dRR) on gastric juice secretion ( $\bar{x} \pm s$ )

Groups	n	Volume (mL)	Total acidity ( $\mu\text{mol/mL}$ )	Total acid output ( $\mu\text{mol}$ )
Control	10	2.9 ± 1.9	86.3 ± 16.1	241.3 ± 124.6
100% dRR	10	0.5 ± 0.6 <sup>a</sup>	41.9 ± 29.1 <sup>a</sup>	32.4 ± 56.6 <sup>a</sup>
50% dRR	9	1.4 ± 0.9 <sup>a</sup>	61.3 ± 33.6	104.3 ± 100.2 <sup>a</sup>
F value		15.319	6.772	11.226

The data were tested with analysis of variance, *q* test was used in multiple comparison. <sup>a</sup> $P < 0.05$ , as compared with control.

Table 2 Effects of steamed Radix Rehmannia (sRR) on gastric juice secretion ( $\bar{x} \pm s$ )

Groups	n	Incidence	Number of ulcer	Inhibitory rate
Control	10	90%	10.2 ± 12.3	—
100% dRR	12	33%	3.1 ± 7.6 <sup>a</sup>	69.60%
100% sRR	11	36%	1.1 ± 2.2 <sup>a</sup>	89.20%
		$\chi^2 = 8.27$	$F = 5.5112$	

The data were analyzed with *k*-ws test. "0" means no acid (pH > 7), <sup>a</sup> $P < 0.05$ , compared with control.

Table 3 Effects of 100% dRR and 100% sRR on gastric ulcer formation in pylorus ligated rats ( $\bar{x} \pm s$ )

Groups	n	Volume (mL)	Total acidity ( $\mu\text{mol/mL}$ )	Total acid output ( $\mu\text{mol}$ )
Control	10	2.9 ± 1.9	86.3 ± 16.1	241.3 ± 124.6
100% sRR	6	< 0.1 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
50% sRR	10	0.9 ± 0.6 <sup>a</sup>	73.3 ± 29.1	72.5 ± 53.4 <sup>a</sup>

The means were tested with analysis of variance, *q* test was used in multiple comparison. The rate was analysed with  $\chi^2$  test for contingency table data. <sup>a</sup> $P < 0.05$ , compared with control.

## Detection method for peripheral venous *AFP* mRNA in hepatocellular carcinoma

Cheng-Jin Hu, Dao-Li Yang

Cheng-Jin Hu, Dao-Li Yang, Department of Immunology, General Hospital of Jinan Military Area, PLA, Jinan 250031, Shandong Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Cheng-Jin Hu, Department of Immunology, General Hospital of Jinan Military Area, PLA, Jinan 250031, Shandong Province, China  
Telephone: +86-5315953171-65823

Received: November 11, 1996

Revised: January 6, 1997

Accepted: January 30, 1997

Published online: September 15, 1997

**Key words:** Liver neoplasms; Carcinoma, hepatocellular; Alpha-fetoproteins; MRNA; Polymerase chain reaction

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Hu CJ, Yang DL. Detection method for peripheral venous *AFP* mRNA in hepatocellular carcinoma. *World J Gastroenterol* 1997; 3(3): 198-199 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/198.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.198>

### INTRODUCTION

Hepatocellular carcinoma (HCC) is a major cause of death in patients with chronic liver disease. One of the difficulties in managing HCC is its complex character: intrahepatic metastasis, venous invasion, and distant metastasis can occur. In metastasis, tumor cells are scattered from the original site, spread hematogenously, and are arrested at the small vessels. Thus, detecting tumor cells in the circulation might predict tumor metastasis<sup>[1]</sup>. However, the number of cells in circulation could be too low to be detected morphologically.

Recently, tumor-associated genes in circulating tumor cells (predicting the presence of tumor cells in the circulation) could be detected by PCR in patients with prostate cancer with distant metastasis or in patients with neuroblastoma<sup>[2,3]</sup>. In HCC cells, the human alpha-fetoprotein (*AFP*) gene is transcribed prominently, but not in normal adult cells<sup>[4]</sup>. Thus, detecting HCC-associated gene transcription (*AFP* mRNA) in the circulation might be related to the hematogenous metastasis of HCC, even though overt metastasis might be obscure.

In the present study, a sensitive nested reverse transcription-PCR (RT-PCR) assay for detecting HCC cells in the blood was investigated.

### MATERIALS AND METHODS

#### Cell line

The BEL-7402 HCC cell line<sup>[5]</sup> (a gift from Dr. Liu from the Shandong Medical Institute cell bank) was maintained in RPMI 1640 medium containing 15% fetal bovine serum. It served as the positive control for *AFP* mRNA expression.

#### *AFP* cDNA clone plasmid

(70 g/L, made by our laboratory).

#### Specimen preparation

Peripheral blood from healthy volunteers was collected in a disposable syringe containing heparin. One thousand BEL-7402 cells were added to 5 mL whole blood, and serial dilutions of tumor cells were made. Each dilution contained 1000, 100, 10, and 1 BEL-7402 cell per 5 mL whole blood.

#### Preparation of nuclear cells from peripheral blood

After adding an equal volume of 3% gelatin solution to the tube containing 5 mL blood, the tube was incubated for 5 min at room temperature. The supernatant was collected and centrifuged at 500 × *g* for 5 min. Residual erythrocytes were lysed by adding distilled water, and isotonicity was restored after 30 sec by adding the same volume of 1.8% NaCl solution. After 5-min centrifugation at 500 × *g*, the cells were immediately frozen using liquid nitrogen and stored until used.

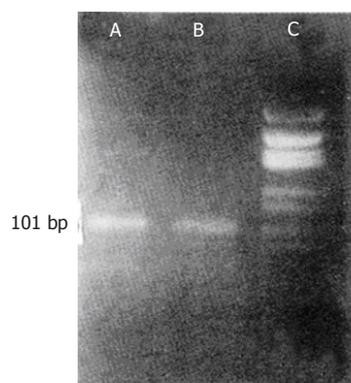
#### Total RNA extraction, complementary DNA (cDNA) synthesis, and RT-PCR

Using an RNA extraction system (prepared by our laboratory), total RNA was extracted from the nuclear cell component of the peripheral blood. About 5 μg RNA was extracted from 5 mL blood and 1 μg RNA was extracted from 10.5 BEL-7402 cells.

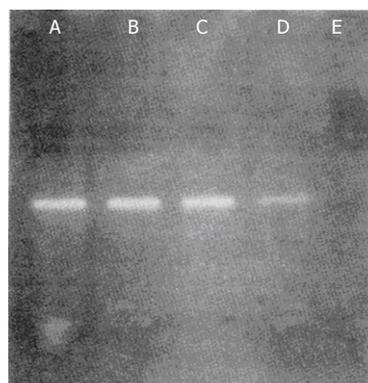
RNA (1 μg), which was heated at 95 °C for 10 min and rapidly cooled in ice water, was mixed with 3 μL 10 × buffer (pH 8.3, Tris-HCl), 0.6 μL 10 mmol/L dNTPs, 2.5 μL 25 mmol/L MgCl<sub>2</sub>, 0.5 μL each of primer #1 (upstream, 5'-ACTGAATCCAGAACAAGCTGCATAG-3') and primer #2 (downstream, 5'-TGCAGTCAATGCATCTTTACCA-3'), and 2 U reverse transcriptase, and the volume was adjusted to 30 μL by adding diethylpyrocarbonate-treated water and covered with liquid paraffin. The cDNA was synthesized by incubating the mixture at 37 °C for 30 min. After initial denaturation at 94 °C for 5 min, RT-PCR was performed according to the temperature profile (94 °C for 45 sec, 54 °C for 60 sec, 72 °C for 45 sec) for 35 cycles. The reaction was terminated by 5-min heating at 72 °C and was then cooled to 4 °C.

#### Nested PCR

The primers used in the nested PCR for *AFP* mRNA were #3



**Figure 1** Detection of *AFP* mRNA in BEL-7402 cells and in alpha-fetoprotein (AFP) cDNA clone plasmid. Lane A: AFP cDNA clone plasmid; B: BEL-7402 cell; C: Marker.



**Figure 2** Detection of tumor cells in 5 mL blood. Lanes A-D: 1000, 100, 10, and 1 BEL-7402 cell, respectively. Lane E: Blank control.

(upstream, 5'-TGGAATAGCTTCCATATTGGATTC-3') and #4 (downstream, 5'-AAGTGGCTTCTTGAACAACTGC-3').

The amplified RT-PCR product (2  $\mu$ L) was mixed with the second PCR buffer [3  $\mu$ L; 100 mmol/L Tris-HCl (pH 8.3), 10 mmol/L MgCl<sub>2</sub>], 0.35  $\mu$ L 42 mmol/L each primer (#3 and #4), 1.5 U *Taq* DNA polymerase, and the mixture was diluted to 30  $\mu$ L with distilled water and covered with liquid paraffin. The temperature profile of the nested PCR was the same as that of the RT-PCR described above.

### Gel electrophoresis

The amplified product was electrophoresed on 3.8% agarose gel and stained with ethidium bromide. The size of the amplified nested PCR product of *AFP* mRNA was 101 bp.

## RESULTS

### Absence of *AFP* mRNA in blood of healthy volunteers

The nested PCR did not detect *AFP* mRNA in the nuclear cell component of the peripheral blood from the healthy volunteers.

### Detection of *AFP* mRNA in BEL-7402 cells and *AFP* cDNA clone (Figure 1).

### Sensitivity of nested PCR for tumor cell detection

BEL-7402 cells ( $10^4$ ) were suspended in 5 mL whole blood from healthy volunteers and sequentially diluted with blood. *AFP* mRNA-specific PCR products (101 bp) could be observed in each lane,

which represented 1, 10, 100, and 1000 BEL-7402 cells in 5 mL normal blood, respectively (Figure 2).

## DISCUSSION

To detect tumor cells in blood, the demonstration of tumor-associated genes has been developed using PCR. In a recent study, albumin mRNA in blood was detected as a marker of circulating hepatocytes<sup>[6]</sup>. Some researchers have indicated that *AFP* production is increased in HCC cell lines such as BEL-7402, and *AFP* gene expression is increased during carcinogenesis<sup>[4]</sup>. Thus, we developed a nested PCR assay to detect the HCC-associated tumor gene transcript *AFP* mRNA in the nuclear cell component of blood, as the number of circulating tumor cells in patients with HCC accompanying metastasis to the distant organs might be too low. To explain the sensitivity of nested PCR for detecting HCC cells in the blood, BEL-7402 cells, established from hepatoblastoma and that produce adequate amounts of *AFP*, were mixed with blood<sup>[5]</sup>. The nested PCR could detect *AFP* mRNA when 1–10 tumor cells were present in 5 mL blood. Therefore, if BEL-7402 cells were circulating in a body (total volume of blood: around 5000 mL), it would be possible to detect *AFP* mRNA when more than 1000 tumor cells are present in the circulation. Although a moderate number of circulating tumor cells might be present, as assessed from the detection of the BEL-7402 cells, there might be several steps for the establishment of metastasis<sup>[1]</sup> such as tumor cell adhesion to the vascular endothelium, migration into the extracellular space, and proliferation at the metastatic foci. During this process, host organ immunological reactions take place, and some tumor cells might be damaged by immunocompetent cells, the mechanical force of the blood flow, or by platelet aggregation.

These results suggest that the highly sensitive nested PCR assay is useful for demonstrating the hematogenous spread of tumor cells. Further studies are being carried out in our laboratory, focusing particularly on predicting metastasis and post-therapeutic changes in patients with HCC by detecting circulating tumor cells.

## ACKNOWLEDGMENTS

We thank Mr. Li-Zhen Yong and Guo-Ying Song, Sino American Biotechnology Company, for their excellent technical assistance.

## REFERENCES

- Hart IR, Saini A. Biology of tumour metastasis. *Lancet* 1992; **339**: 1453-1457 [PMID: 1376386 DOI: 10.1016/0140-6736(92)92039-I]
- Moreno JG, Croce CM, Fischer R, Monne M, Vihko P, Mulholland SG, Gomella LG. Detection of hematogenous micrometastasis in patients with prostate cancer. *Cancer Res* 1992; **52**: 6110-6112 [PMID: 1382851]
- Mattano LA, Moss TJ, Emerson SG. Sensitive detection of rare circulating neuroblastoma cells by the reverse transcriptase-polymerase chain reaction. *Cancer Res* 1992; **52**: 4701-4705 [PMID: 1380888]
- Matsumura M, Niwa Y, Kato N, Komatsu Y, Shiina S, Kawabe T, Kawase T, Toyoshima H, Ihori M, Shiratori Y. Detection of alpha-fetoprotein mRNA, an indicator of hematogenous spreading hepatocellular carcinoma, in the circulation: a possible predictor of metastatic hepatocellular carcinoma. *Hepatology* 1994; **20**: 1418-1425 [PMID: 7527002 DOI: 10.1002/hep.1840200607]
- Zhu HD. Human hepatocellular carcinoma cell line BEL-7402. *Chinese Journal of Cell Biology* 1984; **6**: 91
- Hillaire S, Barbu V, Boucher E, Moukhtar M, Poupon R. Albumin messenger RNA as a marker of circulating hepatocytes in hepatocellular carcinoma. *Gastroenterology* 1994; **106**: 239-242 [PMID: 8276187]

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Clinicopathological risk factors and prognostic evaluation in hepatocellular carcinoma recurrence after surgery

Yi-Min Dai, Han Chen, Neng-Jin Wang, Can-Rong Ni, Wen-Ming Cong, Song-Ping Zhang

Yi-Min Dai, Han Chen, Neng-Jin Wang, Can-Rong Ni, Wen-Ming Cong, Song-Ping Zhang, Department of Pathology, Second Military Medical University, Shanghai 200433, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Dr. Yi-Min Dai, Department of Pathology, Second Military Medical University, Shanghai 200433, China

Received: October 31, 1996  
Revised: December 22, 1996  
Accepted: January 30, 1997  
Published online: September 15, 1997

### Abstract

**AIM:** To analyze the clinicopathological risk factors in hepatocellular carcinoma recurrence after surgery.

**METHODS:** We used significance testing ( $\chi^2$  and Student's *t*-test) of single and multiple factors, and Wilcoxon Cox tropic examination; a retrospective clinicopathological analysis was performed on 156 cases of hepatocellular carcinoma after hepatectomy.

**RESULTS:** Of the 156 cases, 68.4%, 57.3%, 46.7%, 31.5%, and

28.6% had one, two, three, four, and five postoperative tumor-free years, respectively; the total recurrence rate was 53.2% (83/156). In the 83 recurrent cases, 65 were intrahepatic subclinical, with a resection rate of 78.3% (65/83). The relevant factors involved in recurrence were: male gender, tumor number and size, capsule infiltration, and portal vein involvement. These factors were an obvious influence on the prognosis of the patients with postoperative hepatocellular carcinoma ( $P < 0.05$ ). In the recurrent liver carcinomas, 63.1% of tumor nodes (41/65) were at the ipsilateral segment of the primary tumor nodes.

**CONCLUSION:** Male gender, tumor number and size, capsule infiltration, and portal vein involvement are factors for postoperative hepatocellular carcinoma recurrence. Recurrence is mainly unicentral. The right front liver lobe is the segment with a high rate of recurrence.

**Key words:** Liver neoplasms/surgery; Carcinoma, hepatocellular/surgery; Neoplasm recurrence, local; Prognosis; Risk factors

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Dai YM, Chen H, Wang NJ, Ni CR, Cong WM, Zhang SP. Clinicopathological risk factors and prognostic evaluation in hepatocellular carcinoma recurrence after surgery. *World J Gastroenterol* 1997; 3(3): 199 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/199.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.199>

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S

## Endoscopic haemoclip ligation of pedunculated polyp before polypectomy

Yong-Guang Wang, KF Binmoeller, Zeng-Lie Li, N Soehendra

Yong-Guang Wang, Zeng-Lie Li, Digestive Endoscopic Research Centre of Shaanxi Province, Shaanxi Provincial Hospital, Xi'an 710068, Shaanxi Province, China

KF Binmoeller, N Soehendra, Department of Endoscopic Surgery, University Hospital Eppendorf, Hamburg, Germany

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-)

Correspondence to: Yong-Guang Wang, MD, PhD, Digestive Endoscopic Research Centre of Shaanxi Province, Shaanxi Provincial Hospital, Xi'an 710068, Shaanxi Province, China

Received: March 14, 1996

Revised: May 13, 1996

Accepted: June 11, 1997

Published online: September 15, 1997

Key words: Colonic polyps/surgery; Colonoscopy; Ligation, haemoclip

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang YG, Binmoeller KF, Li ZL, Soehendra N. Endoscopic haemoclip ligation of pedunculated polyp before polypectomy. *World J Gastroenterol* 1997; 3(3): 200 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i3/200.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i3.200>

### INTRODUCTION

Metallic haemoclips have been endoscopically placed in the gastrointestinal tract for treating bleeding lesions and closing perforations<sup>[1,2]</sup>. A further potential application is ligating pedunculated polyps prior to polypectomy as a prophylactic measure to prevent bleeding. We used metallic haemoclips to treat two patients with pedunculated colonic polyps and bloody diarrhoea successfully.

### CASE REPORT

#### Case 1

A 58-year-old white man with a colonic polyp was referred to our unit for polypectomy. He had a history of chronic renal failure secondary to chronic glomerulonephritis for 14 years and required regular dialysis with 1 × 5000 IU heparin. Colonic examination was performed with a colonoscope (CF-IT 200, Olympus Corp., Tokyo), and three pedunculated polyps were found. Two non-bleeding polyps, both 1.5 cm, were in the sigmoid flexure; the other was 0.8 cm and had a longer stalk, measured about 0.8 cm in length and 0.4 cm in diameter; and was in the descending colon. There was active oozing (Forrest I b) over the top of the polyp with the longer stalk. A standard polypectomy snare

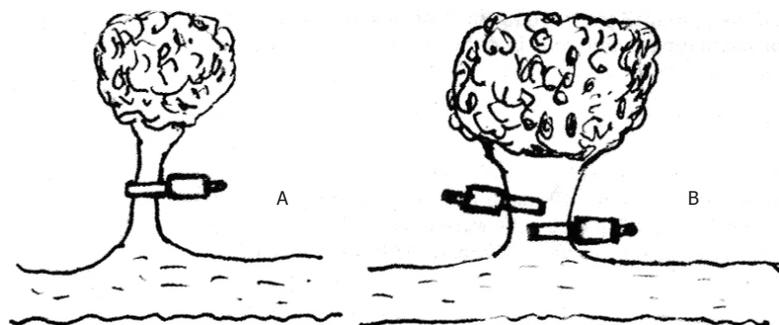


Figure 1 The principle of clip ligation for pedunculated polyps prior to polypectomy to stop bleeding or as a prophylactic measure to prevent bleeding. A: When the stalk of the polyp is thin (with a diameter > 0.4 cm), one clip is sufficient for complete ligation of the stalk. B: When the diameter of the stalk is > 0.4 cm, two clips are needed for complete ligation of the stalk.

was used to snare the polyps, and metallic clips with a delivery catheter (MD-850, HX-3L, Olympus Corp.) were used for clip ligation. Routine polypectomy was performed for the two non-bleeding polyps. In one, a small vessel fistula (Forrest II a) was seen and a clip was applied to ligate it. To prevent post-polypectomy bleeding, the stalk was ligated completely with one clip before the procedure was performed in the remaining polyp. There was no bleeding after the procedure. For the bleeding polyp, the stalk was ligated to stop the bleeding: first, the clip and delivery catheter were inserted through the endoscope and then the clip was opened, and the handle of the delivery catheter was rotated to change the direction of the clip prongs to grasp the stalk.

Thereafter, the delivery catheter was pushed and the clip was closed. Two clips were required to ligate the stalk completely. There was no massive bleeding after the polypectomy. No complications were observed by clipping and there was no recurrent bleeding during the 4-week follow-up.

#### Case 2

A 48-year-old Chinese woman with recurrent bloody diarrhoea for four years and fresh bloody diarrhoea again for four days was admitted to our hospital for colonoscopic examination. She had no history of fever, weight loss, or haemorrhoids. The total colon was examined with a colonoscope (CF-30I, Olympus Corp.), and a 2.0-cm polyp with a long stalk, about 1.0 cm in length and 0.3 cm in diameter, was found in the sigmoid colon. There was a fresh blood clot on the top of the polyp. We used the same technique as in case 1 and ligated the stalk completely with one clip.

No massive bleeding occurred after the polypectomy, but a blood vessel fistula without active bleeding was found. No complications or recurrence of bleeding were observed during the 8-week follow-up.

### DISCUSSION

Ligation using suture or metallic clips is a basic surgical technique for preventing postoperative bleeding. Generally, there are nourishing blood vessels in the stalk of the pedunculated polyp, and their diameter depends on the size of the polyp and the diameter of the stalk. It is essential to ligate the vessels completely or to prevent postoperative bleeding in pedunculated polyps with or without active bleeding. The principle of clip ligation for pedunculated polyps is shown in Figure 1. The number of clips required for complete ligation of the stalk depends on the diameter of the stalk.

The improved metallic haemoclip and clip delivery system have made clips easier to apply. Clip ligation of the bleeding vessel achieves immediate haemostasis comparable to surgical ligation. A previous study has shown that endoscopic haemoclip placement is a highly effective and safe method for treating non-variceal gastrointestinal bleeding<sup>[1]</sup>. We have used the clips and succeeded in closing a 0.5-cm perforation after snare excision of a 3.7 cm × 3.4 cm gastric leiomyoma<sup>[2]</sup>. As case 1 was on dialysis with heparin and had long-stalked polyps with and without bleeding, he was at high risk for polypectomy-induced bleeding and secondary massive bleeding. We used metallic clips to ligate the stalk, arresting the bleeding immediately and avoiding massive post-polypectomy bleeding. The stalk of the pedunculated polyp should be clipped completely.

Recently, the detachable snare was introduced as a new haemostatic device for preventing postoperative bleeding in pedunculated polypectomy, and obtained good results<sup>[3]</sup>. A comparative study of haemoclips and detachable snares for ligating pedunculated polyps before polypectomy is needed in the near future.

In conclusion, as a prophylactic measure, haemoclips can be used to ligate the stalk of a polyp as a new indication either with or without bleeding before polypectomy.

### REFERENCES

- 1 Binmoeller KF, Thonke F, Soehendra N. Endoscopic hemoclip treatment for gastrointestinal bleeding. *Endoscopy* 1993; 25: 167-170 [PMID: 8491134 DOI: 10.1055/s-2007-1010277]
- 2 Binmoeller KF, Grimm H, Soehendra N. Endoscopic closure of a perforation using metallic clips after snare excision of a gastric leiomyoma. *Gastrointest Endosc* 1993; 39: 172-174 [PMID: 8495838 DOI: 10.1016/S0016-5107(93)70060-7]
- 3 Lee MS, Park CW, Lee JS, Cho SW, Shim CS. Endoscopic polypectomy by use of the detachable snare for prevention of post polypectomy bleeding (Abstract). *Gastrointest Endosc* 1995; 41: 307

S- Editor: Filipodia L- Editor: Jennifer E- Editor: Hu S



Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



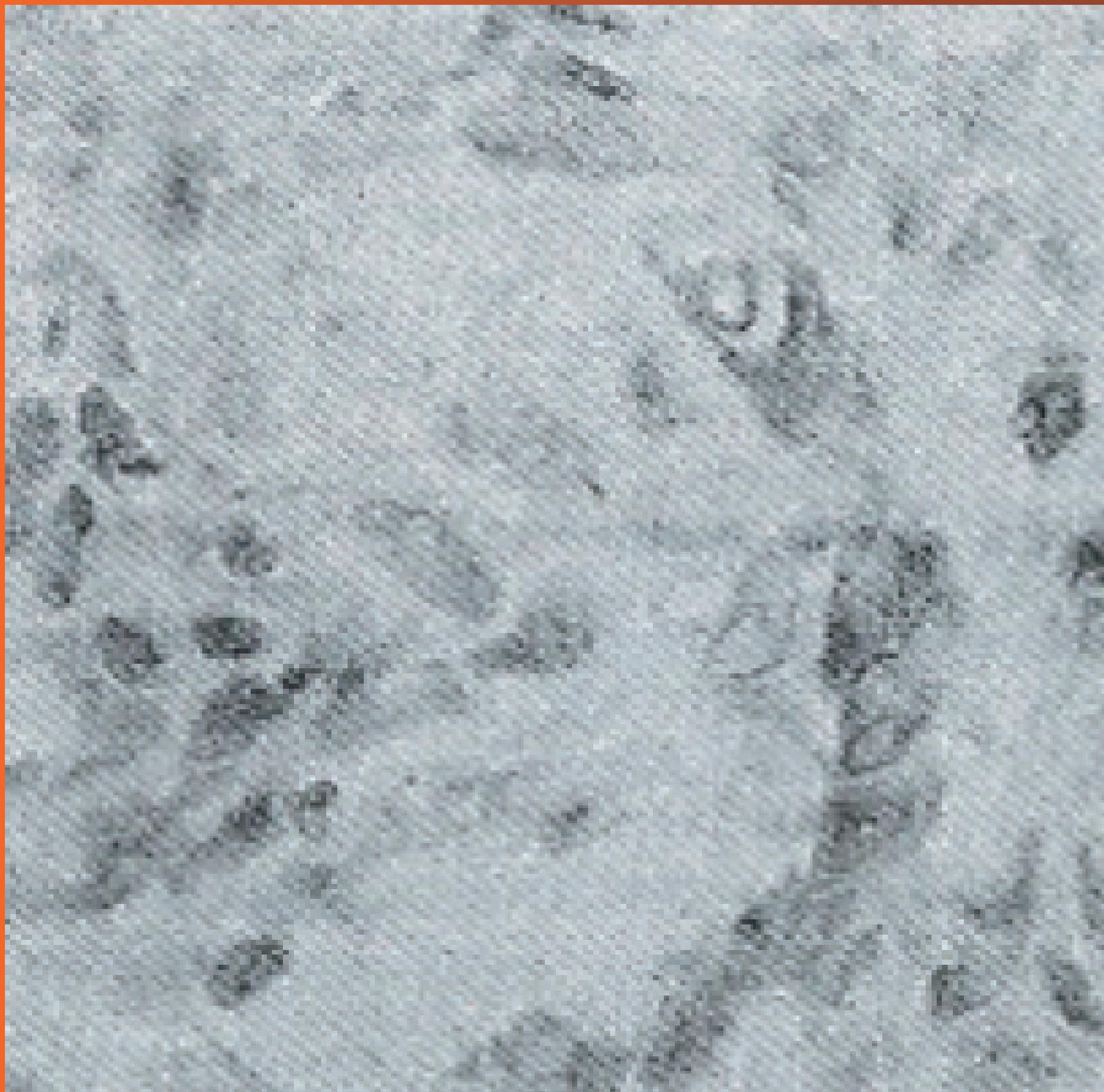
ISSN 1007 - 9327



9 771007 932045

# World Journal of *Gastroenterology*

*World J Gastroenterol* 1997 December 15; 3(4): 201-270



**COMMENTARY**

- 201 Somatostatin: likely the most widely effective gastrointestinal hormone in the human body  
*Huang XQ*
- 205 Advances and applications of enteroscopy for small bowel  
*Zhou DY, Jiang B, Yang XS*

**ORIGINAL RESEARCH**

- 208 SClinical significance of CD44v mRNA detection by PCR in peripheral blood of patients with hepatocellular carcinoma  
*Liu PF, Wu MC, Cheng H, Qian GX, Fu JL*
- 210 Analysis of two constitutive forms of microsomal heme oxygenase in different rat tissues  
*Xia ZW, Li YZ, Chen SN, Shen QX, Ben XM, Yu SC*
- 213 Effects of endotoxin on expression of *ras*, *p53* and *bcl-2* oncoprotein in hepatocarcinogenesis induced by thioacetamide in rats  
*Yang JM, Han DW, Liang QC, Zhao JL, Hao SY, Ma XH, Zhao YC*
- 218 Effects of somatostatin analog on splanchnic hemodynamics and plasma glucagon level in portal hypertensive rats  
*Wu ZY, Zhang XJ, Jiao Z, Chen ZP, Kuang YL*
- 221 Overproduction of nitric oxide inhibits vascular reactivity in portal hypertensive rats  
*Li XR, Wu JS, He ZS, Ma QJ, Gao DM*
- 225 Plasma D (-)-lactate as a new marker for diagnosis of acute intestinal injury following ischemia-reperfusion  
*Yao YM, Yu Y, Wu Y, Lu LR, Sheng ZY*
- 228 Establishment and application of an experimental model of human fetal hepatocytes for investigation of the protective effects of silybin and polyporus umbellatus polysaccharides  
*Wang MR, Le MZ, Xu JZ, He CL*
- 231 Comparative study of different interventional therapies for primary liver cancer  
*Liu Q, Jia YC, Tian JM, Wang ZT, Ye H, Yang JJ, Sun F*
- 234 Anti-human AFP variant monoclonal antibody in radioimmunoassay of primary hepatocellular carcinoma  
*Liu Y, Wu MC, Chen H, Zhang BH, Qian GX, Pan WZ, Qiang MY*
- 236 Comparative study on proliferation activity in small hepatocellular carcinoma related to hepatitis virus B and C  
*Yu SJ*
- 238 Multicenter randomized study on Me-CCNU, 5-FU and ADM vs ACNU, 5-FU and ADM for treatment of advanced gastric cancer  
*Xiao SD, Li DH, Zhang DZ, Shen MJ, Zhu XT, He GF, Zhao TP, Xi LP, Deng XC, Wang M, Wang XL, Chen Q, Zhang YP, Yao CL, Bao JG, Tong GW, Zhu LF, Jiang H, Minoru K*

- 242 Comparison of preoperative TN staging of gastric carcinoma by endoscopic ultrasonography with CT examination  
*Guo W, Zhang YL, Li GX, Zhou DY, Zhang WD*
- 246 Treatment of cancerous ascites and radical gastrectomy with intraperitoneal hyperthermic double-distilled water and cis-diaminodichloro-platinum perfusion  
*Chen ZX, Chen JP, Chen Z, Peng DS, Zhen JX, Tan JS*
- 249 Pharmacokinetics of four 5-FU preparations administered rectally to rats and rabbits  
*Zhang X, Wang CS, Wu GZ, Ling BD, Liang RH, Fang XS, Xin TY*
- 251 Determination of  $\beta$ -glucuronidase in human colorectal carcinoma cell lines  
*Feng S, Song JD*
- 253 Clinical significance of CA19-9 in diagnosis of digestive tract tumors  
*Zhao JZ, Wu BH*
- 255 Analysis of amino acid constituents of gallstones  
*Chen Y, Wang LL, Xiao YX, Ni JH, Yu Y*
- 257 A comparative study of changing patterns of concanavalin A-binding proteins in early stage of cholesterol gallstone formation  
*Chen YQ, Cai D, Zhang YL, Hua TF*
- 260 Comparative study of biliary trace elements and clinical phenotypes in Wilson's disease  
*Ren MS, Fan YX, Yang RM, Han YZ, Wu GJ, Xin YR, Yu L*

**INTEGRATED TRADITIONAL CHINESE AND MODERN MEDICINE**

- 263 Seropharmacological effects of Fuzheng Huayudecoction on rat Ito cell morphology and functions in culture  
*Liu CH, Liu C, Liu P, Xu LM*
- 266 Clinical and experimental studies on stomach carcinoma treated with Yangwei Kangliu granules  
*Lu WP, Sun GZ, Piao BK, Dong HT, Yang ZY, Lin HS*

**CASE REPORT**

- 248 Pancreatitis associated with herpes zoster: A case report  
*Wang HG, Yang GZ, Li HY*

**BRIEF REPORT**

- 237 Effect of garlic on micronuclei frequency in peripheral blood lymphocytes of rats with N-methyl-N'nitro-Nnitrosoguanidine-induced gastric carcinoma and precancerous lesions.  
*Su Q, Luo ZY, Ou YG, Li YQ, Zhou JG, Zhang D*
- 245 Radioimmunoassay-detected basal level of epidermal growth factor in gastric juice of 86 healthy Chinese volunteers  
*Zhang L, Zhang ML, Yan YQ, Liang DX*
- 250 Influence of diet intake on liver function test  
*Cheng SQ, Zhang JF, Zhang ZF, Qian MY, Guo XL, Shang WZ, Li DJ*

- 252 AgNOR and rasp21 expression in gastric mucosal lesions caused by *Helicobacter pylori* infection  
*Gao HJ, Lu XZ, Zhang XY, Zhao ZQ*
- 262 Altered expression of tumor suppressors p16 and Rb in gastric carcinogenesis  
*Zhou Q, Zou JX, Chen YL, Yu HZ, Wang LD, Li YX, Guo HQ, Gao SS, Qiu SL*
- 265 Influence of fever on biliary elements of guinea pigs  
*Lü HD, Tian MG, Zhang XP, Li HL*
- 269 Effect of esophageal cancer- and stomach cancerpreventing vinegar on N-nitrosoproline formation in the human body  
*Guo XK, Wang TJ, Gu JF*
- 270 Gastric emptying and plasma levels of gastrointestinal hormones in patients with peptic ulcer  
*Chen J, Li JM, Li XH, Hao HS, Fu SH*

**ABOUT COVER**

Yang JM, Han DW, Liang QC, Zhao JL, Hao SY, Ma XH, Zhao YC. Effects of endotoxin on expression of *ras*, *p53* and *bcl-2* oncoprotein in hepatocarcinogenesis induced by thioacetamide in rats. *World J Gastroenterol* 1997; 3(4): 213-217

**AIMS AND SCOPE**

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

**INDEXING/ABSTRACTING**

*World Journal of Gastroenterology* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central.

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Rui-Fang Li*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ze-Mao Gong*  
Proofing Editorial Office Director: *Jin-Lei Wang*

**NAME OF JOURNAL**  
*World Journal of Gastroenterology*

**ISSN**  
ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

**LAUNCH DATE**  
October 1, 1995

**FREQUENCY**  
Quarterly

**EDITORS-IN-CHIEF**  
**Bo-Yong Pan**, President of China Speciality Council of Gastrology and China Association of Huatuo Medicine, Room 12, Building 621, the Fourth Military Medical University, Xi'an 710033, Shaanxi Province, China

**Lian-Sheng Ma**, Member of the Speciality Committee of Digestive Diseases, Chinese Association of Combined Traditional Chinese and Western Medicine, Taiyuan Research & Treatment Centre for Digestive Diseases, Taiyuan 030001, Shanxi Province, China

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Gastroenterology*  
Room 903, Building D, Ocean International Center,  
No. 62 Dongsihuan Zhonglu, Chaoyang District,  
Beijing 100025, China  
Telephone: +86-10-59080039  
Fax: +86-10-85381893  
E-mail: editorialoffice@wjgnet.com  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
December 15, 1997

**COPYRIGHT**  
© 1997 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at [http://www.wjgnet.com/bpg/g\\_info\\_19970116143427.htm](http://www.wjgnet.com/bpg/g_info_19970116143427.htm)

**ONLINE SUBMISSION**  
<http://www.wjgnet.com/esps/>

## Somatostatin: Likely the most widely effective gastrointestinal hormone in the human body

Xiang-Qian Huang

Xiang-Qian Huang, Department of Internal Medicine, Affiliated General Hospital, Tianjin Medical University, Tianjin 300052, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Xiang-Qian Huang, Professor of Medicine, Chairman, Tianjin Society of Internal Medicine, Box 235, Tianjin Medical University, 22 Qixiangtai Road, Tianjin 300070, China

Received: January 3, 1997

Revised: March 14, 1997

Accepted: April 6, 1997

Published online: December 15, 1997

**Key words:** Somatostatin/Physiology; Somatostat in/analogs and derivatives; Receptors, somatostatin; Octreotide/Diagnostic use; Octreotide/Therapeutic use

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Huang XQ. Somatostatin: likely the most widely effective gastrointestinal hormone in the human body. *World J Gastroenterol* 1997; 3(4): 201-204 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/201.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.201>

Somatostatin (SS) was discovered from rat hypothalamus in 1968 and identified as a 14-amino acid peptide (SS-14) in 1973. Research achievements from studies of this fascinating hormone have drawn attention of scientists worldwide, yet it is not yet the time to make a complete conclusion on its broad distribution, physiologic functions, and roles in diagnosis and treatment of diseases. The results reported in the accumulated literature, however, do suggest its potential vast significant influence.

### SS DISTRIBUTION<sup>[1]</sup>

SS-producing cells (D-cells) exist at high densities throughout the central and peripheral nervous systems, in the endocrine pancreas and the gut, as well as in small numbers in the thyroid, adrenals, submandibular glands, kidneys, prostate and placenta (Table 1). In rats, gut-localized SS accounts for about 65% of the total body distribution, followed by brain localization at 25% and the pancreas and the remaining organs at 5% each. The approximate relative amounts of SS in the central nervous system are mainly in the cortex (49%) and spinal cord (30%). The D-cells in pancreatic islets are proximal to the other hormone-producing islet cells (*i.e.* A (glucagon-producing), B (insulin-producing), and PP (pancreatic polypeptide-producing) cells) and function to mediate function and inhibit hypersecretion of glucagon, insulin and pancreatic polypeptide

*via* paracrine signaling. The D-cells are also located in the mucosal glands located throughout the cardiac region of the stomach to the rectum, and their content of SS accounts for more than 90% of that in the entire gastrointestinal tract.

The SS in neurons populate both the submucosal and myenteric plexuses in all segments of the gastrointestinal tract, accounting for less than 10% of the total. Compared to SS concentration in plasma, that in cerebral spinal fluid is twice as much and in semen is 200-fold as high. Amniotic fluid is rich in SS. It is also present in some tumor tissues, including those of lung cancer, colon cancer and gastroenteropancreatic endocrine tumors. The extensive existence of SS in large amounts in the human body support the notion that it likely plays a significantly influential role in both physiologic and pathologic conditions.

### SS BIOLOGIC/PHYSIOLOGIC ACTIONS<sup>[1,2]</sup>

SS exerts inhibitory effects on various organs. The broad array of SS actions includes regulation of neurotransmission, glandular secretion, smooth muscle contractility and cell proliferation. SS has been shown to inhibit virtually every known exocrine and endocrine secreted factor, including growth hormone, prolactin, thyrotropin, corticotropin, insulin, glucagon, vasoactive intestinal polypeptide, pancreatic polypeptide, gastrin, cholecystokin, motilin, neurotensin, thyroxin (T<sub>4</sub>), triiodothyronine (T<sub>3</sub>), calcitonin, aldosterone, catecholamine and renin. It has also been shown to inhibit splanchnic blood flow and movement functions (*i.e.* smooth muscle contractility), such as contractility of stomach, intestine and the biliary system, as well as gastric emptying, contractility of sphincter of Oddi, prolongation of gastrointestinal transit time, secretion such as for gastric acid and bicarbonate from the exocrine pancreas, release of pepsin, trypsin and the hemopoietic intrinsic factor as well as bile flow, absorption of glucose and other carbohydrates, amino acids and triglycerides, activity of the nervous system such as acetylcholine release from the gut mural myenteric plexuses, proliferation of cells and tumor growth, platelet aggregation, and function of activated immune cells. SS is also capable of stimulating water and electrolyte absorption, activating opioid receptors, and appears to excite the central neurons. The above-mentioned extensive inhibitory functions of SS suggest that, the SS is a widespread and powerful inhibitor capable of influencing a number of organs and tissues, and therefore of mediating homeostasis.

### SOMATOSTATIN ANALOGUES<sup>[2-4]</sup>

To date, the clinical utility of SS has been limited due to its very short half-life (less than 3 min). Octreotide, the first synthetic SS analogue, first described by Bauer *et al* in 1982, possesses longer half life (up to 2 h) and could be injected intermittently, and even administered orally. This agent is also able to inhibit the release of growth hormone, glucagon, and insulin by 45-, 11-, and 1.3-times respectively, and more powerfully than SS-14 and without causing

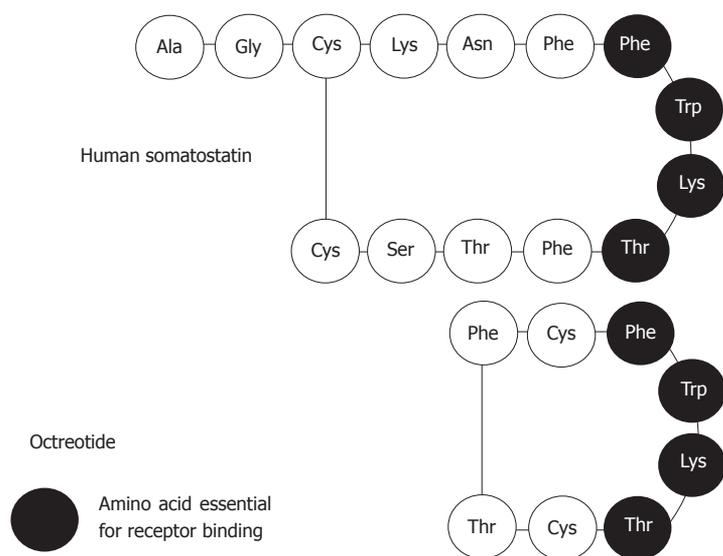
**Table 1 Localization of somatostatin**

Organs	Type of Cells	Location
Major sites		
Nervous system	Neurons	Hypothalamus Cerebral cortex Limbic system Basal ganglia Major sensory systems Spinal cord Dorsal root ganglia Autonomic ganglia
Pancreas	D-cells	Islets
Gut	D-cells Neurons	Mucosal glands Submucous and myenteric plexuses
Minor sites		
Adrenal		Scattered medullary cells
Placenta		Cytotrophoblasts in chorionic villi
Reproductive organs		Testis, epididymis, prostate
Submandibular Gland	D-cells	Scattered ductal cells
Thyroid	C-cells	Scattered parafollicular cells, coexisting with calcitonin
Urinary system		Scattered cells in renal glomerulus and collecting ducts
Miscellaneous		Thymus, heart, eyes, erythrocytes

**Table 2 The 5 subtypes of human SS receptors**

Feature	SS receptors				
	Subtype 1	Subtype 2	Subtype 3	Subtype 4	Subtype 5
Amino acid sequence	361	369 (A), 346 (B)	418	388	364
Chromosomal location	14	17	22 20	20 22	16
G protein coupling	Yes	Yes	Yes	Yes	Yes
Effector system					
Adenylate cyclase activity	Reduced	Reduced	Reduced	Reduced	Reduced
Tyrosine phosphatase activity	Increased	Increased	Not investigated	Not investigated	Not investigated
Possible binding affinity to receptors					
SS-14	++	++	++	++	++
SS-28	++	++	++	++	++
Octreotide	±	++	+	±	++
Vapreotide (RC-100)	±	++	+	+	+++
Lanreotide (BIM-23014)	±	++	+	+	+++
<sup>1</sup> Distribution in normal human tissue	Brain, lungs, stomach, jejunum, kidneys, liver, pancreas	Brain, kidneys	Brain, pancreas	Brain, lungs	Brain, heart, adrenal glands, placenta, pituitary, small intestine, skeletal muscle

<sup>1</sup>Not all human tissues were tested simultaneously. This table was modified from Steven *et al.*



**Figure 1 Amino acid composition of somatostatin and octreotide.**

rebound hypersecretion of hormones. Octreotide, an 8-amino acid, was modified from SS-14 (Figure 1), with the 7<sup>th</sup> to 10<sup>th</sup> amino acids being maintained in common since they are the 4 amino acids essential for receptor binding. Octreotide has similar pharmacological effects as native SS, and its widespread clinical use had illuminated its manifold physiological functions and values in clinical application. The mechanism underlying SS analogues' clinical utility in diagnosis and treatment is the SS receptors that are present on tissue membranes and which specifically bind to the SS analogues, resulting in cell effects.

There are 5 subtypes of SS receptors (Table 2), which are

identical in 42%-60% of their amino acid sequences and belong to a superfamily of receptors. The genes for these subtypes, located on different chromosomes, have suggested their different functions in different organs. As such, a variety of SS analogues might exert pharmacological actions by the specific binding with the various subtypes. The effects of treatment with SS analogues depend on both the ligands and the receptor subtypes.

### OCTREOTIDE USE FOR DIAGNOSIS<sup>[5,6]</sup>

Radiolabelled SS analogue, administered intravenously and then binding to the SS receptors on tumor tissues is the resulting formation of radiolabelled aggregation. Scintigraphy using a  $\gamma$  camera allow for visualization of such SS receptor-positive tumors. <sup>111</sup>Octreotide is the most commonly used SS analogue because of the high density of SS receptors on neuroendocrine tumors of the gastroenteropancreatic system, with the total positivity rate ranging from 50% to 100%, and in most of the groups investigated it is above 80%. Metastatic liver tumors show lower rates of positivity. Pituitary adenoma may usually be visualized by scintigraphy. Adenocarcinomas originating from the breast, kidney, colon or ovary also express SS receptors. In <sup>111</sup>octreotide scintigraphy imaging, the diagnostic reliability and rate of positivity are better than the other current diagnostic imaging techniques. The diagnostic efficiency depends upon the amount of SS receptors expressed on the tumor cells. Octreotide injection therapy does not affect the diagnostic efficiency, but can increase the therapeutic effect.

### OCTREOTIDE USE FOR TREATMENT<sup>[2,7,8]</sup>

Octreotide is the most widely used drug among the SS analogues. Its indications and clinical uses are as follows:

### **Pituitary adenomas and gastroenteropancreatic endocrine tumors**

These tumors have a large number of SS receptors and are treated with octreotide effectively, especially the growth hormone-secreting pituitary tumors (acromegaly). The agent is usually delivered as a subcutaneous injection of 100 µg three times a day, and may result in rapid control of symptoms and hormone secretion. In about half of the patients treated with octreotide, the size of the tumor is reduced. Some cases require larger doses, up to 300 µg or 600 µg daily, to achieve the maximal benefit, but a dose above 600 µg per day rarely has additional effect. Because of the high sensitivity observed in elderly patients with growth hormone-secreting tumors, octreotide can be recommended as a primary treatment. There have been no reports of loss of the suppressive effect of octreotide on growth hormone secretion in patients with acromegaly, even after 10 years or more of uninterrupted treatment.

Pancreatic islet-cell tumors express SS receptors in more than 80% of cases, and daily injection of 150 µg to 300 µg may improve the clinical outcome in 50% to 85% of cases. This outcome may be mediated by both a direct inhibitory effect on hormone production by the tumor and indirect effects, such as on the resorption of intestinal fluid, the production of gastric acid, or intestinal contractility. Octreotide treatment is often effective initially, but after weeks to months of treatment the symptoms worsen and tumor hormone secretion increases in virtually all the patients; while this effect can be reversed by increasing the dose of octreotide, eventually the drug becomes ineffective in all patients. This resistance phenomenon may be explained by the growth of clones of tumor cells that lack SS receptors. Octreotide is especially valuable in controlling the symptoms associated with vipoma or glucagonoma, and the remaining islet cell tumors often express subtypes of the SS receptor that do not bind octreotide, decreasing the beneficial effect. Reportedly, 70%-90% of metastatic carcinoid tumors respond well to octreotide, with effective control of the symptoms and of the overproduction of serotonin or tachykinin. It may also temporarily inhibit tumor growth, and in about 20% of patients the enlarged lymph nodes and liver metastases shrink. The most important benefits of octreotide are improvements to patient quality of life and patient survival.

### **Probable indications**

In accordance with its various functions, including suppression of endocrine and exocrine secretions of the gastroenteropancreatic system, reduction of splanchnic blood flow, prolongation of gastrointestinal transit time and stimulation of water and electrolytic absorption, octreotide can be used to treat the following diseases: variceal acute bleeding (by 48-h continuous intravenous administration, and which has a similar effect of emergency sclerotherapy); pancreatic fistula; post-elective pancreatic surgery complications when applied perioperatively; and some refractory secretory diarrheas (as in autoimmune disease syndrome, short bowel syndrome, radiation colitis, and chemotherapy conditions).

### **Potential role in oncologic treatment<sup>[2]</sup>**

Octreotide has not been used formally as a clinical oncologic treatment, but several studies have showed its promise in this realm. Octreotide has been shown to inhibit tumor growth in animals and of tumor cell lines *in vitro*, exerting antiproliferative effects on tumor cells *via* its effects on the SS receptors. The efficiency of octreotide on tumors depends on both the presence and amount of SS receptors on tumor cells. Tumors of the nervous system have SS receptors, and these tumors account for 50% of solid tumors in children and adolescents, including the neuroblastoma, meningioma, low grade astrocytoma and medulloblastoma.

### **Octreotide in combination with anti-cancer drugs<sup>[9-11]</sup>**

In Weckbecker's report, octreotide was applied in combination with the anti-cancer drugs mitomycin, doxorubicin, 5-fluorouracil, or Taxol to a pancreatic tumor cell line and to tumor-bearing nude mice. The results showed that the inhibitory effects of the anti-cancer drugs were enhanced markedly in a synergistic or additive manner, strongly suggesting the clinical utility of octreotide in combination with chemotherapeutic agents for tumor therapy.

Tamoxifen is an anti-oestrogenic agent used to treat breast cancer, but achievement of long-term effects in metastatic diseases is rare because developing resistance to the drug is common. Studies both *in vitro* and *in vivo* showed that the anti-proliferative effects of octreotide can enhance the anti-neoplastic effects of tamoxifen. Compared to tamoxifen mono-treatment, co-administration of octreotide plus tamoxifen yields better results with low toxicity and significantly lowered serum insulin-like growth factor I bioactivity.

### **Octreotide for SS-receptor-mediated radiotherapy**

A β-emitter-labelled octreotide (90Y-SDZ 413) binds to SS receptors on rat pituitary cells and inhibits growth hormone release. Administration of 90Y-SDZ 413 to a nude mouse model with SS receptor-positive tumors led to SS receptor-specific accumulation of the labelled conjugate (tumor/muscle ratio: 52/1), and a single treatment of 90Y-SDZ 413 led to a decrease of 25% in tumor mass. These results suggest that receptor-targeted radiotherapy by means of a 90Y-labelled octreotide represents a new strategy for the treatment of SS receptor-positive tumors.

The mechanism by which octreotide exerts its therapeutic effects on cancers has not been fully elucidated, but studies have indicated that the effects occur as a result of direct anti-proliferative activities on the tumor cells and direct inhibition of angiogenesis in the tumor mass, and as a result of indirect inhibition of the secretion of growth hormone, insulin, and insulin-like factor I. A variety of tumors express different subtypes of the SS receptor, and each of the different kinds of SS analogues may not have high affinity with all subtypes of the SS-receptor. High affinity binding between an SS analogue and its specific subtypes is key for its optimal efficacy on tumors.

### **Miscellaneous experiences in octreotide treatment<sup>[13-15]</sup>**

In patients with systemic sclerosis, octreotide can resolve the chaotic and non-propagative intestinal motility patterns so that they become well-coordinated-nearly to the extent as in healthy individuals-and can cause symptoms to subside. For patients with irritable bowel syndrome, octreotide can reduce the symptom of rectal pressure that underlies rectal urgency, thereby relieving a key component of the disease manifestation. In patients with dumping syndrome, octreotide can alleviate dumping by slowing gastric emptying, inhibiting insulin release, decreasing enteric peptide secretion, increasing intestinal absorption of water and sodium, slowing monosaccharide absorption, increasing gut transit time and preventing haemodynamic changes. For patients with psoriasis, a 12-wk course of octreotide treatment can ameliorate most symptoms. For patients with pancreatitis, octreotide can also correct orthostatic hypotension and suppress the inflammatory reaction, the mechanism underlying its therapeutic effect involves inhibiting pancreatic exocrine and endocrine secretions and rectifying perioperative hypotension. Finally, patients with rheumatoid arthritis can benefit from the inflammation-resolving effects of octreotide and the therapeutic effect can be evaluated by scintigraphy of the involved joints.

### **Clinical usage and adverse effects of octreotide<sup>[2]</sup>**

Octreotide can be delivered *via* intravenous and subcutaneous routes, but is mostly administered as a subcutaneous injection at present. Dosage ranges from 50 µg to 200 µg, 3 times a day, with the most common being 100 µg, 3 times a day. For advanced tumor cases, 500 µg to 1000 µg or 2000 µg, 3 times a day for 6 months has been used safely, but usage of the larger doses is more rare at present. The most common adverse effects include nausea, diarrhea, flatulence, abdominal cramps, and malabsorption of fat, all of which usually subside spontaneously in 10 to 12 d regardless of continuance of treatment.

Long-term treatment with octreotide (> 1 mo) is associated with an increased incidence of cholesterol gallstones, with rates ranging from 20% to 30%. This effect seems to be related to dosage and duration of the octreotide treatment. In most cases, the gallstones remain asymptomatic and the management should be similar to that applied to patients with non-octreotide-induced gallstones. The process of gallstone formation during octreotide therapy probably

involves inhibition of gallbladder emptying and reduction of bile flow. Discontinuation of octreotide treatment tends towards gallstone resolution. A smaller dose, such as 100 µg, 3 times a day for less than one month, may present less to no risk of developing this problem. In the case of long-term octreotide treatment, however, intermittent usage may prevent gallbladder formation.

## FUTURE PROSPECTIVES

Octreotide has widespread therapeutic effects on various diseases, and hundreds of articles on octreotide treatment are published annually. The reasons why this agent is capable of exerting such a broad range of effects remains unknown and further investigation is necessary. For patients with acromegaly, a long-acting preparation of 20 mg to 30 mg administered intramuscularly is capable of controlling growth hormone hypersecretion for 28 to 42 d as effectively as multiple daily subcutaneous injections. A long-acting intramuscular formulation of lanreotide (another SS analogue) can also maintain therapeutic effects for 2 wk.

Development of novel SS analogues and further development of their diverse preparations may speed up the progression of both basic and clinical research for the wide of array of disease conditions they are related to. Identification and characterization of the various subtypes of SS receptors in different organs and lesions, and of their specific ligands, will improve diagnosis and treatment as well. A current limitation of SS analogues is that they are expensive. When their costs are lowered in the future, they will be used more extensively and their theoretical basis and practical value will become more apparent as well. It can be predicted that octreotide will become a common drug in clinically use when it is able to be administered orally and for lower, more affordable cost.

## REFERENCES

- 1 Patel PC. General aspects of biology and function of somatostatin. In: Weil C, Miller EE, Thorner MO, eds. Somatostatin. BERLIN: Springer Sandoz 1992: 1-16
- 2 Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ. Octreotide. *N Engl J Med* 1996; **334**: 246-254 [PMID: 8532003]
- 3 Harris AG. Somatostatin and somatostatin analogues: pharmacokinetics and pharmacodynamic effects. *Gut* 1994; **35**: S1-S4 [PMID: 7911441 DOI: 10.1136/gut.35.3\_Suppl.S1]
- 4 Kvols LK, Reubi JC, Horisberger U, Moertel CG, Rubin J, Charboneau JW. The presence of somatostatin receptors in malignant neuroendocrine tumor tissue predicts responsiveness to octreotide. *Yale J Biol Med* 1992; **65**: 505-518; discussion 531-536 [PMID: 1364090]
- 5 Krenning EP, Kooij PP, Pauwels S, Breeman WA, Postema PT, De Herder WW, Valkema R, Kwakkeboom DJ. Somatostatin receptor: scintigraphy and radionuclide therapy. *Digestion* 1996; **57** Suppl 1: 57-61 [PMID: 8813472 DOI: 10.1159/000201398]
- 6 Faiss S, Scherübl H, Bäder M, Fett U, Koppenhagen K, Wiedenmann B, Riecken EO. [Significance of false-positive findings of somatostatin receptor scintigraphy in diagnosis of neuroendocrine tumors of the gastroenteropancreatic system]. *Z Gastroenterol* 1994; **32**: 243-246 [PMID: 7915450]
- 7 Huang XQ. Octreotide and its clinical applications. In: Huang XQ, eds. Therapeutics of gastrointestinal disease. Tianjin: Tianjin Science and Technology Publication 1996: 60-76
- 8 Eriksson B, Janson ET, Bax ND, Mignon M, Morant R, Opolon P, Rougier P, Oberg KE. The use of new somatostatin analogues, lanreotide and octastatin, in neuroendocrine gastro-intestinal tumours. *Digestion* 1996; **57** Suppl 1: 77-80 [PMID: 8813476 DOI: 10.1159/000201402]
- 9 Weckbecker G, Raulf F, Tolcsvai L, Bruns C. Potentiation of the anti-proliferative effects of anti-cancer drugs by octreotide in vitro and in vivo. *Digestion* 1996; **57** Suppl 1: 22-28 [PMID: 8813462 DOI: 10.1159/000201388]
- 10 Pollak M. Enhancement of the anti-neoplastic effects of tamoxifen by somatostatin analogues. *Digestion* 1996; **57** Suppl 1: 29-33 [PMID: 8813463 DOI: 10.1159/000201389]
- 11 Albers AR, O'Dorisio MS. Clinical use of somatostatin analogues in paediatric oncology. *Digestion* 1996; **57** Suppl 1: 38-41 [PMID: 8813466 DOI: 10.1159/000201392]
- 12 Stolz B, Smith-Jones P, Albert R, Tolcsvai L, Briner U, Ruser G, Mäcke H, Weckbecker G, Bruns C. Somatostatin analogues for somatostatin-receptor-mediated radiotherapy of cancer. *Digestion* 1996; **57** Suppl 1: 17-21 [PMID: 8813461 DOI: 10.1159/000201387]
- 13 Camilleri M. Effects of somatostatin analogues on human gastrointestinal motility. *Digestion* 1996; **57** Suppl 1: 90-92 [PMID: 8813481]
- 14 Farthing MJ. The role of somatostatin analogues in the treatment of refractory diarrhoea. *Digestion* 1996; **57** Suppl 1: 107-113 [PMID: 8813486 DOI: 10.1159/000201412]
- 15 Scarpignato C. The place of octreotide in the medical management of the dumping syndrome. *Digestion* 1996; **57** Suppl 1: 114-118 [PMID: 8813487 DOI: 10.1159/000201413]

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Advances and applications of enteroscopy for small bowel

Dian-Yuan Zhou, Bo Jiang, Xi-Shan Yang

Dian-Yuan Zhou, Bo-Jiang, Xi-Shan Yang, Institute of PLA for Digestive Diseases, Nanfang Hospital, First Military Medical University, Guangzhou 510515, Guangdong Province, China

Dian-Yuan Zhou, Professor and Director of Institute of PLA for Digestive Diseases, Gastroenterologist, having over 100 papers and 7 books published.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dian-Yuan Zhou, Professor and Director of Institute of PLA for Digestive Diseases, Nanfang Hospital, First Military Medical University, Guangzhou 510515, Guangdong Province, China

Received: June 13, 1996

Revised: July 7, 1996

Accepted: August 13, 1996

Published online: December 15, 1997

**Key words:** Gastrointestinal; Endoscopy; Small intestine; Intestinal diseases; Gastrointestinal hemorrhage; Intestinal neoplasms

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Zhou DY, Jiang B, Yang XS. Advances and applications of enteroscopy for small bowel. *World J Gastroenterol* 1997; 3(4): 205-207 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/205.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.205>

### INTRODUCTION

Visual inspection of the entire small intestine is difficult and time-consuming, due to its anatomical location. While the usual techniques of endoscopy facilitate visualization of the most proximal and distal ends of the gut, only limited views of the terminal ileum (only about 5 cm to 25 cm) can be obtained by intubation of the ileocecal valve after total colonoscopy. The proximal jejunal mucosa (20 cm to 50 cm) can be inspected by standard colonoscopy or use of related modified instrumentation. Therefore, investigation of this region is dependent upon enteroclysis and angiography. The diagnostic efficacy of these procedures, however, is relatively low, and confirmative diagnosis has frequently been made only after laparotomy. Achievement of successful endoscopy for other parts of the gastrointestinal tract has allowed for further improvements to the enteroscopy technique. Since the 1970s, vigorous efforts have been made to produce three types of enteroscopes, namely the push type, sonde type, and ropeway type. The recent development of video endoscopes, including a new prototype video enteroscope for small bowel, has benefited clinical practice. In this paper, we describe the recent advances in enteroscopy, including its devices and applications.

### ENTEROSCOPE INSERTION TECHNIQUE FOR THE DIFFERENT DEVICE TYPES

#### *Push type enteroscope*<sup>[1-4]</sup>

Push type enteroscopes, such as the SIF-RP, SIF-B and SIF-10, are long forward-viewing fiberscope devices. At present, SIF-10 is the most widely used for examination of the upper small intestine<sup>[1-5]</sup>. The insertion technique for the push type enteroscopes can be described as follows: The patient should fast for at least for 12 h prior to the procedure. On the procedure day, a local anesthesia (such as lidocaine) is sprayed over the patient's pharynx. The patient is placed on the fluoroscopic table in the left lateral decubitus position. The enteroscope is swiftly introduced into the duodenum, with minimal distention of the stomach. As the instrument is advanced, the patient is moved into various positions to assist in endoscope passage; meanwhile, abdominal pressure helps to minimize or prevent loop formation in the stomach and, simultaneously, withdrawal of the enteroscope can also straighten the formed loop and allow for paradoxical advancement of the instrument tip. When the entire length of the insertion tube is passed, glucagon (1.0 mg) should be administered to inhibit peristalsis. Full insufflation of the bowel with air reduces the height of the intestinal folds. The endoscope may be withdrawn slowly as the bowel is carefully inspected. Usually, endoscopists are able to advance the instrument to an average depth of 35 cm to 60 cm beyond the ligament of Treitz with a mean 50 min procedure time; biopsy is also possible at this step. However, the extent of insertion is limited to the proximal jejunum at 20 cm to 60 cm due to the crooked nature of the duodenum, which is embedded in the retroperitoneum and which makes the transmission of propelling force difficult. As a result, a large loop tends to form in the stomach, consuming the effective length of the scope. In order to overcome this problem, the new push type enteroscope SIF-10L was developed. This type of scope is equipped with a stiffening tube that is specially made as an auxiliary device. Shimizu *et al*<sup>[6]</sup> described the insertion technique for the SIF-10L. When the scope is advanced to the duodenojejunal junction and its position has been confirmed by fluoroscopy, the distal tip is maximally flexed for fixation at the duodenojejunal junction and the scope can be pulled until it is straight from the descending portion of the duodenum. After that, the stiffening tube, which was lubricated prior to the procedure, is gently advanced over the shaft into the descending portion of the duodenum. When the insertion of the sliding tube is completed, it is fixed on the mouthpiece by the stopper to protect against its being pulled out. The scope is then pushed into the deeper part of the jejunum with concurrent observation of the intestinal lumen. When the maximal working length is achieved, the distal tip is again fixed and the sliding tube is gently advanced beyond the duodenojejunal junction to prevent loop formation in the horizontal portion of the duodenum. The passage of the stiffening tube is observed by fluoroscopy. At this point, the scope is again advanced to its full working length. Insertion extent is determined by measuring the length of the scope from the distal

end of the sliding tube, with measurements made on an X-ray film. It has been reported that the time for insertion can range from 7 min to 30 min, with a mean of 11.7 min and the range of observable length being 60 cm to 120 cm from the distal end of the stiffening tube which could be inserted beyond the duodenojejunal junction, at a mean insertion length of  $105.8 \pm 17.5$  cm. By introducing the stiffening tube as an auxiliary device, the insertion extent can be increased remarkably.

#### **Sonde type enteroscope**

Several types of sonde scopes are currently available and in clinical use<sup>[7-11]</sup>. The sonde type small intestinal fiberscope (SSIF-VII) is one among the series of prototype fiberscopes; its features include a 5 mm diameter, 2790 mm length, forward-viewing capacity and 90-degree angle view. It can be passed transnasally and migrates distally, responding to peristaltic activity. The essential step of the procedure is a technique called "piggy backing" in which a long endoscope is introduced into the stomach carrying the enteroscope through the pylorus and beyond the ligament of Treitz, which is facilitated by grasping with forceps and pulling into place. The balloon at the tip of the instrument advances the enteroscope in the small bowel when the endoscope is removed. Metoclopramide is then administered to stimulate small bowel motility. Mucosal inspection is performed when the scope has reached the distal ileum. Glucagon is administered to arrest small bowel activity. The whole procedure lasts 3-8 h, with an average duration of 4.3-6 h<sup>[7-9]</sup>. However, it is estimated that only approximately 50% of the lumen view is visible during the controlled withdrawal due to the absence of tip deflection, the relatively small angle of view, and occasional rapid withdrawal of the instrument through loops of small intestine or migration to the colon. The another disadvantage of this scope and its application technique is that biopsy is impossible. The newest sonde type enteroscope (SSIF-type 10) that has been developed features a 2 mm biopsy channel, which makes target biopsy possible even in distal parts of the small intestine<sup>[9-10]</sup>. Finally, a new prototype video enteroscope (ESI-2000, Pentax) has been developed and successfully applied in clinic<sup>[11-13]</sup>.

#### **Ropeway type enteroscope<sup>[15-19]</sup>**

Several types of ropeway enteroscopes, such as FIS-II, SIE-RP and SIE-2C, are currently available. The key step for application of the ropeway enteroscopes, during the examination, is insertion of a long intestinal teflon string that is advanced perorally and discharged from the anus. Completion of this step, however, may require at least 24 h. Once this step is finished, the ropeway enteroscope can be pulled through the gastrointestinal tract with the aid of the Teflon string relatively quickly (within 10 min). Observation and biopsy throughout the entire intestine are possible. Use of this method causes the intestine to shorten, like an accordion, which precludes complete observation; moreover, stenotic and diffuse lesions in the small bowel disallow free passage of the string and limit the effectiveness of this device. Finally, excessive traction on the string augments the risk of instrument-induced perforation. From a physical standpoint, this procedure may cause discomfort to the patient and requires general anesthesia. Thus, application of ropeway enteroscope is limited in clinical practice.

## **APPLICATIONS IN SMALL BOWEL DISEASES**

### **Obscure gastrointestinal bleeding**

Obscure gastrointestinal bleeding is a troublesome medical problem. Although a wide range of investigational tools are available to aid clinicians in localizing sites of gastrointestinal blood loss, approximately 5% of the patients with GI bleeding continue to bleed from an unidentified source. In recent years, some authors have reported their experiences in use of small bowel enteroscope for detecting the source and cause of obscure bleeding. For nearly 26%-77% of those patients, correct diagnosis or identification of the site of occult bleeding was achieved<sup>[2,20-22]</sup>. Moreover, these results indicated that the following specific lesions could cause obscure bleeding: arteriovenous malformations (AVMs), hemangiomas, Meckel's diverticulum, jejunal diverticulum, jejunal leiomyoma,

leiomyosarcoma, ulcerative jejunitis, Peutz-Jeghers syndrome, and Crohn's disease. Of the known causes of obscure gastrointestinal bleeding, AVMs appear to be the most common source. Lewis and Waye<sup>[7]</sup> employed the SSIF-VII sonde type enteroscope to investigate the cause of chronic gastrointestinal obscure bleeding in 60 patients and found that 33% ( $n = 20$ ) had blood loss within the small bowel, with 80% ( $n = 16$ ) of those being caused by AVMs and 15% ( $n = 3$ ) by ulcers; for only 1 of the 20 patients with small bowel blood loss was the bleeding foci not found, despite the presence of fresh blood. AVMs were limited to the proximal jejunum in 7 patients, distal small intestine in 3 patients, and diffuse small bowel in 6 patients. Foutch *et al.*<sup>[2]</sup> described a group of patients with gastrointestinal bleeding of obscure origin and reported finding a definite cause of bleeding for 15 cases by using the push type enteroscope, 12 of which were diagnosed as AVMs. Similar results reported by another group support the general finding that AVMs are the main cause of obscure gastrointestinal bleeding<sup>[20]</sup>.

### **Detection of small bowel tumor**

Small bowel tumors are difficult to detect using conventional diagnostic techniques. Imaging examinations, such as barium X-ray, ultrasonography and angiography, often produce false negative test results, which can reportedly delay diagnosis for 12 mo or more in nearly one-quarter of patients with primary small bowel neoplasms<sup>[23]</sup>. At present, enteroscopy is considered to play a precise role in the diagnosis of small bowel disease. Kawai and colleagues published their 11-year experience of detecting and identifying lesions using three types of enteroscope<sup>[16]</sup>, in which a total of 19 lesions were detected and identified as cancer ( $n = 3$ ), malignant lymphoma ( $n = 5$ ), leiomyosarcoma ( $n = 1$ ), leiomyoma ( $n = 5$ ), hemangiopericytoma ( $n = 1$ ) and Peutz-Jeghers polyp ( $n = 5$ ). The authors described the endoscopic appearances of the tumors, which were classified among three types: type I, sessile tumor; type II, semipedunculated or pedunculated tumor; type III, large tumor with central depression. Type III was further classified into two subgroups: type IIIa, tumors partially encircling the bowel; type IIIb, tumors completely encircling the bowel. Malignant tumors showed various appearance ranging from type II to type IIIb, but benign tumors never showed type IIIb. Shinya and Mc Sherry<sup>[19]</sup> also presented their experience with push type and sonde type enteroscopes, and reported that among the 3 cases total 2 were diagnosed as duodenal adenocarcinoma and one as a jejunal hemangiolymphangioma.

### **Application in familial adenomatous coli (FAC)**

Upper gastrointestinal lesions have been considered rare in patients with FAC or Gardner's syndrome, although recent studies have indicated that FAC and Gardner's syndrome are the same disease and affect the upper as well as the lower gastrointestinal tract<sup>[24-26]</sup>. Iida *et al.*<sup>[25]</sup> used the push type enteroscope and found that jejunal polypoid lesions were detected in 9 of 10 patients (90%) with FAC or Gardner's syndrome. The lesions were located in the upper jejunum, particularly within about 20 cm below the duodenojejunal junction. The number of polyps varied from 1 to 20, and their sizes were 3 mm or less in diameter. Endoscopically, the polypoid lesions appeared sessile and whitish. Histologic findings of the lesion biopsy specimens revealed a tubular adenoma with mild to moderate epithelial atypia in all 9 cases. Other authors reported 3 cases of jejunal carcinoma in patients with FAC/Gardner's syndrome<sup>[28,29]</sup>. However, such a situation is quite rare, since the jejunal lesions are usually very small, asymptomatic, and adenomatous in histology. Therefore, as treating physicians we should pay attention to related changes and careful follow-up of patients is needed with endoscopic removal of larger polyps, whenever feasible.

### **Application in other small bowel diseases**

Correct diagnosis of small intestine diseases requires not only intestinal visualization but also biopsy of lesions. Barkin *et al.*<sup>[4]</sup> examined 31 patients with clinically suspected small bowel disease using a PCF-135 flexible pediatric colonoscope perorally and 14 patients were confirmed by biopsy, including celiac disease ( $n = 5$ ), Whipple's disease ( $n = 2$ ), lymphoma ( $n = 1$ ), adenocarcinoma ( $n$

= 1), Crohn's disease ( $n = 1$ ), tropical sprue ( $n = 1$ ), amyloidosis ( $n = 1$ ), enteritis ( $n = 1$ ) and cytomegalovirus infiltration ( $n = 1$ ). Such diseases as Whipple's disease, tropical sprue and amyloidosis could not be diagnosed if biopsies were not available. Tada *et al* examined 15 cases of Crohn's disease using the sonde type enteroscope, and the presence of small intestinal lesions was confirmed in 13 cases, while the small intestine was not involved in the remaining 2. The characteristic findings of Crohn's disease were observed, including longitudinal ulcers, circular ulcers, irregular ulcers, aphthoid ulcers, cobble-stone appearance, inflammatory polyps, pseudo-diverticular formation, and stenosis. The author considered that the enteroscope device was superior to X-ray instrumentation for detecting tiny lesions, and that enteroscopy plays an important role in diagnosis of small bowel disease; further developments and improvements in enteroscopy will support its more widespread use clinically. Different types of enteroscopes have different characteristics, for instance, the push type enteroscope is suitable to detect a lesion on the upper jejunum or obtain a biopsy in the case of a diffusely spreading lesion. The ropeway type enteroscope is helpful for observation of the entire intestine, whereas the sonde type is useful for the examination of emergency cases of gastrointestinal bleeding, in cases of poor general condition, or when a stenosing lesion is located at the distal parts of the small intestine where neither the push type scope nor the ropeway type scope can be introduced. Thus, we can observe small intestinal diseases sufficiently when the suitable enteroscope is employed. However, the questions remain as to how to best utilize the various enteroscopes for pathophysiological research of the small intestine and how to improve the insertion techniques so as to make it easier in technical operation and for patient tolerance.

## REFERENCES

- 1 Shimizu S, Tada M, Kawai K. Clinical evaluation of a new enteroscope (SIF-10). *Gastroenterol Endosc*, 1986; **28**: 659-663
- 2 Foutch PG, Sawyer R, Sanowski RA. Push-type enteroscopy for diagnosis of patients with gastrointestinal bleeding of obscure origin. *Gastrointest Endosc* 1990; **36**: 337-342 [DOI: 10.1016/S0016-5107(90)71060-7]
- 3 Tada M, Kawai K. Small-bowel endoscopy. *Scand J Gastroenterol Suppl* 1984; **102**: 39-52 [PMID: 6591374]
- 4 Barkin JS, Schonfeld W, Thomsen S, Manten HD, Rogers AI. Enteroscopy and small bowel biopsy--an improved technique for the diagnosis of small bowel disease. *Gastrointest Endosc* 1985; **31**: 215-217 [PMID: 4007443 DOI: 10.1016/S0016-5107(85)72050-0]
- 5 Ueno K, Wada J, Tsuboi M, Shinzawa H, Ishikawa M. A new technique for intubation of small intestinal fiberscope (SIF-B) into the deep jejunum by using stiffening tube. *Gastroenterol Endosc* 1980; **22**: 47-55
- 6 Shimizu S, Tada M, Kawai K. Development of a new insertion technique in push-type enteroscopy. *Am J Gastroenterol* 1987; **82**: 844-847 [PMID: 3631030]
- 7 Lewis BS, Wayne JD. Chronic gastrointestinal bleeding of obscure origin: role of small bowel enteroscopy. *Gastroenterology* 1988; **94**: 1117-1120 [PMID: 3258259]
- 8 Lewis BS, Wayne JD. Total small bowel enteroscopy. *Gastrointest Endosc* 1987; **33**: 435-438 [PMID: 3443262 DOI: 10.1016/S0016-5107(87)71682-4]
- 9 Tada M, Shimizu S, Kawai K. Small bowel endoscopy with a new transnasal sonde-type fiberscope (SSIF-type 10). *Endoscopy* 1992; **24**(Suppl): 631
- 10 Van Gossom A, Adler M, Cremer M. Chronic gastrointestinal bleeding of obscure origin: role of small bowel enteroscopy. *Endoscopy* 1992; **24**(Suppl): 631
- 11 Tada M, Misaki F, Kawai K. Pediatric enteroscopy with a sonde-type small intestinal fiberscope (SSIF-type VI). *Gastrointest Endosc* 1983; **29**: 44-47 [PMID: 6826009 DOI: 10.1016/S0016-5107(83)72503-4]
- 12 Dabezies MA, Fisher RS, Krevsky B. Video small bowel enteroscopy: early experience with a prototype instrument. *Gastrointest Endosc* 1991; **37**: 60-62 [PMID: 2004685 DOI: 10.1016/S0016-5107(91)70624-X]
- 13 Lee DE, Gitkind M. Video small enteroscopy for chronic GI bleeding of obscure origin. *Am J Gastroenterol* 1990; **85** (Suppl): A1266
- 14 Sato N, Tamegai Y, Yamakawa T, Hiratsuka H. Clinical applications of the small intestinal video endoscope. *Endoscopy* 1992; **24**(Suppl): 631
- 15 Tada M, Tanaka Y, Kawai K. Clinical evaluation of a new type of enteroscope, SIF-RP. *Gastroenterol Endosc* 1983; **25**: 136-140
- 16 Kawai K, Takemoto T, Hiratsuka H, Ohi I, Tada M. Present status of enteroscopy for the diagnosis of small intestinal tumors. *Stomach and Intestine* 1981; **16**: 991-997
- 17 Tada M, Shimizu S, Okada H, Iwasaku J, Yoshinaka M. Recent advances in enteroscopy. *Stomach and Intestine* 1985; **20**: 723-731
- 18 Foutch PG, Sanowski RA, Kelly S. Enteroscopy: a method for detection of small bowel tumors. *Am J Gastroenterol* 1985; **80**: 887-890 [PMID: 3876761]
- 19 Shinya H, McSherry C. Endoscopy of the small bowel. *Surg Clin North Am* 1982; **62**: 821-824 [PMID: 6981858]
- 20 Lewis BS, Wenger JS, Wayne JD. Small bowel enteroscopy and intraoperative enteroscopy for obscure gastrointestinal bleeding. *Am J Gastroenterol* 1991; **86**: 171-174 [PMID: 1992630]
- 21 Gostout CJ, Schroeder KW, Burton DD. Small bowel enteroscopy: an early experience in gastrointestinal bleeding of unknown origin. *Gastrointest Endosc* 1991; **37**: 5-8 [PMID: 2004683 DOI: 10.1016/S0016-5107(91)70612-3]
- 22 Bowden TA. Endoscopy of the small intestine. *Surg Clin North Am* 1989; **69**: 1237-1247 [PMID: 2688152]
- 23 Norberg KA, Emås S. Primary tumors of the small intestine. *Am J Surg* 1981; **142**: 569-573 [PMID: 7304812 DOI: 10.1016/0002-9610(81)90428-1]
- 24 Iida M, Yao T, Ohsato K, Itoh H, Watanabe H. Diagnostic value of intraoperative fiberscopy for small-intestinal polyps in familial adenomatosis coli. *Endoscopy* 1980; **12**: 161-165 [PMID: 7398597]
- 25 Iida M, Yao T, Itoh H, Watanabe H, Matsui T, Iwashita A, Fujishima M. Natural history of duodenal lesions in Japanese patients with familial adenomatosis coli (Gardner's syndrome). *Gastroenterology* 1989; **96**: 1301-1306 [PMID: 2703115]
- 26 Iida M, Yao T, Itoh H, Ohsato K, Watanabe H. Endoscopic features of adenoma of the duodenal papilla in familial polyposis of the colon. *Gastrointest Endosc* 1981; **27**: 6-8 [PMID: 7215747 DOI: 10.1016/S0016-5107(81)73132-8]
- 27 Iida M, Matsui T, Itoh H, Mibu R, Fujishima M. The value of push-type jejunal endoscopy in familial adenomatosis coli/Gardner's syndrome. *Am J Gastroenterol* 1990; **85**: 1346-1348 [PMID: 2171327]
- 28 Ross JE, Mara JE. Small bowel polyps and carcinoma in multiple intestinal polyposis. *Arch Surg* 1974; **108**: 736-738 [PMID: 4829791 DOI: 10.1001/archsurg.1974.01350290098018]
- 29 No authors listed. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 47-1978. *N Engl J Med* 1978; **299**: 1237-1245 [PMID: 714081 DOI: 10.1056/NEJM197811302992208]

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Clinical significance of CD44v mRNA detection by PCR in peripheral blood of patients with hepatocellular carcinoma

Peng-Fei Liu, Meng-Chao Wu, Han Cheng, Guang-Xiang Qian, Ji-Liang Fu

Peng-Fei Liu, Meng-Chao Wu, Han Cheng, Guang-Xiang Qian, East Institute of Hepatobiliary Surgery, Second Military Medical University, Shanghai 200433, China

Ji-Liang Fu, Department of Biology, Second Military Medical University, Shanghai 200433, China

Peng-Fei Liu, Associate Professor, MD, PhD, having 22 papers published.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Peng-Fei Liu, MD, PhD, Associate Professor, East Institute of Hepatobiliary Surgery, Second Military Medical University, Shanghai 200433, China  
Telephone: +86-21-565347018-77439

Received: February 20, 1997

Revised: April 14, 1997

Accepted: May 6, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To study the clinical significance of detecting the expression of CD44v mRNA in the blood of patients with hepatocellular carcinoma (HCC).

**METHODS:** The expression of CD44v mRNA was detected in blood with RT and diploid PCR, and the clinical significance was discussed based on the result of pathological examination and follow-up.

**RESULTS:** CD44v mRNA was detected in the blood of 10/15 patients, giving a positive rate of 66.67%. In the 13 patients who showed response in the follow up period, the CD44v mRNA expression was positive in 9 and negative in 4. Recurrence rate was higher in the patients with positive CD44v mRNA expression than in those with negative CD44v mRNA expression, and the clinical pathological indices were also higher in the former than in the latter.

**CONCLUSION:** Detection of the expression level of CD44v mRNA in blood of the patients with HCC can be used as an adjuvant means for differential diagnosis, prediction and monitoring of HCC recurrence.

**Key words:** CD44; Liver neoplasms; mRNA; Hepatocellular carcinoma; Polymerase chain reaction

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Liu PF, Wu MC, Cheng H, Qian GX, Fu JL. Clinical significance of CD44v mRNA detection by PCR in peripheral blood of patients with hepatocellular carcinoma. *World J Gastroenterol* 1997; 3(4): 208-209 Available from: URL:

<http://www.wjgnet.com/1007-9327/full/v3/i4/208.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.208>

### INTRODUCTION

CD44 is an adhesive molecule located on the cell surface, where it participates in cell-cell and cell-matrix interactions<sup>[1,2]</sup>. "Standard" CD44 can be modified by the insertion of transcripts from at least five extra exons<sup>[3]</sup>, after which splice variants of CD44 (CD44v) can be produced. A single cell may contain two or more variant mRNAs from the same gene simultaneously. CD44v may play an important role in tumor growth and metastasis. The human homologue of rat metastasis-associated CD44v has been isolated from the cell lines of human non-small cell lung carcinoma, colon carcinoma, and breast carcinoma<sup>[4]</sup>. Furthermore, CD44v expression has been detected in fresh human tumor tissues of the breast, colon<sup>[7]</sup>, and gastrointestinal tract<sup>[8]</sup>, as well as in melanoma<sup>[9]</sup> and metastases in brain<sup>[10]</sup>.

Gene expression of a single tumor cell in 10<sup>7</sup> white blood cells can be detected by sensitive RT-PCR<sup>[7]</sup>. Thus, it is possible to detect the gene expression of a free tumor cell in the blood of patients with malignant diseases. In the study described herein, we explored an auxiliary method for the diagnosis and monitoring of recurrence of malignant tumors by detecting CD44v mRNA expression in the peripheral blood of patients with hepatocellular carcinoma (HCC).

### MATERIALS AND METHODS

#### Experimental specimens

On a Ficoll-Hypaque gradient, free tumor cells were collected from blood samples obtained from the peripheral vein of 15 patients with HCC prior to surgical intervention. Meanwhile, tumor tissue samples were obtained from the resected HCC specimens and control blood samples were obtained from 16 patients with benign diseases (including chronic cholecystitis, chronic abscess of liver, chronic hepatitis B, cirrhosis, inflammatory pseudotumor of liver, and cavernous hemangioma of liver). All samples were stored at -196 °C and kept frozen until use.

#### RNA isolation and RT-PCR

RNA was extracted from the free tumor cells and the tumor tissue samples according to the method of Chomzynski and Sacchi<sup>[11]</sup>. RT was carried out on the total RNA sample using an AMV reverse transcriptase kit (Promega Co., USA). First-strand cDNA was synthesized at 42 °C for 60 min in 20 μL reaction solution. The first round of PCR was carried out with the synthesized cDNA in 50 μL reaction solution (upstream primer: 5'-GACAGACACCTCAGTTTTTCTGGA-3'; downstream primer: 5'-TTCCTTCGTGTGGTAATGAGA-3') for 30 cycles. The conditions of PCR were 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.5 min. The second round of PCR was carried out under the same conditions as the first round (upstream primer: 5'-GACAGACACCTCAGTTTTTCTGGA-3'; downstream primer: 5'-TTCCTTCGTGTGGTAATGAGA-3') for 30 cycles. PCR products

Table 1 Follow-up data of 15 detected patients with primary liver cancer

CD44v mRNA in blood	Detected, <i>n</i>	Positive AFP	Followed up, <i>n</i>	Responders, <i>n</i>	Recurrence, <i>n</i>	Recurrence rate, %	Survivors, <i>n</i>	Survival rate, %
+	10	8	10	9	9	100.00	1	11.11
-	5	3	5	4	1	25.00	3	75.00
Total	15	11	15	13	10	76.92	4	30.77

Table 2 Clinicopathological data of the patients who responded during follow-up

CD44v mRNA in blood	Followed up, <i>n</i>	Responders, <i>n</i>	DTC	PTC	TEPV	DTN
+	10	9	8	2	9	7
-	5	4	1	1	2	1
Total	15	13	9	3	11	8

Note: DTC: deficiency of tumor capsule; PTC: penetration of tumor capsule; TEPV: tumor embolus in portal vein observed by microscopy; DTN: daughter tumor nodules observed by microscopy.  $\chi^2_{DTC} = 5.7778, P < 0.025$ ;  $\chi^2_{PTC} = 0.0164, P > 0.9$ ;  $\chi^2_{TEPV} = 5.3182, P < 0.025$ ;  $\chi^2_{DTN} = 4.9661, P < 0.05$ .

(20  $\mu$ L) were separated on an ethidium bromide-stained 1.5% agarose gel and analyzed.

### Pathological indices for metastasis

All tumor samples were observed under microscopy to determine whether the tumor capsule was intact or deficient, whether it had been penetrated by tumor cells, whether tumor cells had migrated into the portal vein of normal liver tissues, and whether daughter tumor nodules appeared in normal liver tissues near the main mass. Follow-up after tumor resection was carried out for 20-22 mo and was conducted by questionnaire.

### Statistical analysis

Statistical analysis was carried out using the chi-square test.

## RESULTS

All the tumor tissue samples and 10 of the 15 blood samples obtained from the patients with HCC expressed CD44v mRNA. No CD44v mRNA expression was detected in the control blood samples (Tables 1 and 2, Figure 1).

In the patients with positive CD44v mRNA expression in blood, the recurrence rate was higher and the survival rate was lower than in the patients with negative CD44v mRNA expression ( $\chi^2 = 8.775, P < 0.005$  and  $\chi^2 = 5.7778, P < 0.025$ ).

## DISCUSSION

Free tumor cells can appear in the peripheral vein of patients with malignant tumors of the digestive system<sup>[12]</sup>. Tumor cells can enter into the vein through the short path involving the microartery vein and elastic blood capillaries<sup>[13]</sup>. Our study has shown that liver cancer cells may enter into the peripheral vein via 1) collateral circulation of the portal caval vein, or 2) liver vein  $\rightarrow$  inferior caval vein  $\rightarrow$  capillary of lung  $\rightarrow$  artery  $\rightarrow$  blood capillary of tissue  $\rightarrow$  peripheral vein of body.

It is possible to detect the CD44v mRNA expression of free tumor cells in blood by RT-PCR. This method may be an auxiliary indicator for diagnosis of malignant tumors. CD44v can confer metastatic potential on tumor cells, which was supported by the findings of our study<sup>[14]</sup>. In this study, the metastatic data were more robust in patients with positive CD44v mRNA expression in blood than in patients with negative CD44v mRNA expression; additionally, the recurrence rate was higher and the survival rate was lower in the former than in the latter. Thus, CD44v expression in blood may indicate metastatic potential of cancer and become an auxiliary means for monitoring recurrence.

Although detection of alpha-fetoprotein (AFP) is considered the most effective method for HCC diagnosis, there are still about 10% to 30% of patients with HCC who present with negativity for AFP. In the current study, we found that the CD44v mRNA expression in blood of patients with HCC was not related to AFP, suggesting that this method can be a complementary indicator for HCC diagnosis.

However, CD44v mRNA expression in blood cannot be a



Figure 1 Electropherogram of PCR products.

specific diagnostic index for primary hepatic carcinoma because the specificity of CD44v in different tumors was not clear. Yet, it may be an auxiliary method for differential diagnosis of malignant and benign diseases (*i.e.*, HCC and non-typical abscess of liver, inflammatory pseudotumor of liver, *etc.*).

The reasons for CD44v mRNA expression being positive in only 10 of 15 blood samples of HCC in the current study, but being positive in all of the 15 tumor tissue samples, may be as follows: (1) none of tumor cells were collected from blood or there may have been no free tumor cells in the blood; (2) the experimental method used was not optimal or sufficient; and (3) the expression level of CD44v mRNA in tumors was low. Therefore, the detection method for CD44v mRNA expression in blood should be further improved.

## REFERENCES

- Haynes BF, Telen MJ, Hale LP, Denning SM. CD44—a molecule involved in leukocyte adherence and T-cell activation. *Immunol Today* 1989; **10**: 423-428 [PMID: 2695102 DOI: 10.1016/0167-5699(89)90040-6]
- Haynes BF, Liao HX, Patton KL. The transmembrane hyaluronate receptor (CD44): multiple functions, multiple forms. *Cancer Cells* 1991; **3**: 347-350 [PMID: 1721518]
- Hofmann M, Rudy W, Zöller M, Tölg C, Ponta H, Herrlich P, Günthert U. CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. *Cancer Res* 1991; **51**: 5292-5297 [PMID: 1717145]
- Smith CW, Patton JG, Nadal-Ginard B. Alternative splicing in the control of gene expression. *Annu Rev Genet* 1989; **23**: 527-577 [PMID: 2694943 DOI: 10.1146/annurev.ge.23.120189.002523]
- Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Hausmann I, Matzku S, Wenzel A, Ponta H, Herrlich P. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell* 1991; **65**: 13-24 [PMID: 1707342 DOI: 10.1016/0092-8674(91)90403-L]
- Reber S, Matzku S, Günthert U, Ponta H, Herrlich P, Zöller M. Retardation of metastatic tumor growth after immunization with metastasis-specific monoclonal antibodies. *Int J Cancer* 1990; **46**: 919-927 [PMID: 2228320 DOI: 10.1002/ijc.2910460528]
- Matsumura Y, Tarin D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet* 1992; **340**: 1053-1058 [PMID: 1357452 DOI: 10.1016/0140-6736(92)93077-Z]
- Guo YJ, Liu G, Wang X, Jin D, Wu M, Ma J, Sy MS. Potential use of soluble CD44 in serum as indicator of tumor burden and metastasis in patients with gastric or colon cancer. *Cancer Res* 1994; **54**: 422-426 [PMID: 7506122]
- Birch M, Mitchell S, Hart IR. Isolation and characterization of human melanoma cell variants expressing high and low levels of CD44. *Cancer Res* 1991; **51**: 6660-6667 [PMID: 1742741]
- Li H, Hamou MF, de Tribolet N, Jaufeerally R, Hofmann M, Diserens AC, Van Meir EG. Variant CD44 adhesion molecules are expressed in human brain metastases but not in glioblastomas. *Cancer Res* 1993; **53**: 5345-5349 [PMID: 7693337]
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159 [PMID: 2440339 DOI: 10.1006/abio.1987.9999]
- Cole WH, McDonald G, Roberts SS. Dissemination of cancer, prevention and therapy. New York: Appleton-Century-Crofts, Inc, 1961: 155
- Cole WH, McDonald G, Roberts SS. Dissemination of cancer, prevention and therapy. New York: Appleton-Century-Crofts, Inc, 1961: 137
- Liu PF, Wu MC, Cheng H, Qian GX, Fu JL. Expression of splice variants of CD44 and significance in human liver carcinoma. *Jiefangjun Yixue Zazhi* 1996; **21**: 189-190

## Analysis of two constitutive forms of microsomal heme oxygenase in different rat tissues

Zhen-Wei Xia, Yun-Zhu Li, Shun-Nian Chen, Qing-Xiang Shen, Xiao-Ming Ben, Shan-Chang Yu

Zhen-Wei Xia, Yun-Zhu Li, Shun-Nian Chen, Xiao-Ming Ben, Shan-Chang Yu, Department of Pediatrics, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China

Qing-Xiang Shen, Shanghai Institute of Cell Biology, Academia Sinica, Shanghai 200031, China

Zhen-Wei Xia, male, born in 1963, graduated from Shanghai 2<sup>nd</sup> Medical University with MD of pediatrics, now attending doctor.

Author contributions: All authors contributed equally to the work.

Supported by the National Natural Science Foundation of China, No. 39170767.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Zhen-Wei Xia, MD, Department of Pediatrics, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China

Received: April 8, 1997

Revised: May 21, 1997

Accepted: June 14, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To isolate and purify the heme oxygenase (HO) isoform in microsomal fractions of Sprague-Dawley rat liver and brain in order to understand the characteristics of the two constitutive forms and the mechanism of the occurrence of hyperbilirubinemia.

**METHODS:** After induction by hematin and phenylhydrazine, the rat liver and brain microsomal fractions were isolated and purified by DEAE-Sephacel and hydroxyapatite. Activity and the apparent molecular weight of the two isoforms [heme oxygenase 1 (HO-1) and heme oxygenase-2 (HO-2)] were measured. Kunming mice were used to prepare antiserum against purified liver HO-2. Rat liver HO-1 and brain HO-2 preparations were analyzed by the western immunoblotting technique.

**RESULTS:** Two isoforms were purified and identified in the treated rat liver, and HO-1 was the predominant form with a ratio of 2:1. In the native state, HO-2 activity was detectable but HO-1 activity was increased in response to hematin and phenylhydrazine, while HO-2 activity was fully refractory to these agents. The apparent molecular weights of HO-1 and HO-2 were about Mr 30000 and Mr 36000 under reducing conditions, respectively. In the untreated liver and treated brain, only one peak of HO activity exhibiting an elution profile similar to that of HO-2 of the treated liver was detected. The presence of an activity peak was not found in the elution profile at the region where the inducible isoform of HO (HO-1) was expected. The apparent molecular weight in treated brain preparation was identical to that of the purified liver HO-2. Cross-reactivity of HO-2 in the brain microsomal preparation was established, but a reactivity

of HO-1 in the liver was not observed by western immunoblotting analysis when antiserum to liver HO-2 was applied.

**CONCLUSION:** Two constitutive forms of HO, designated as HO-1 and HO-2, exist in the treated rat liver. HO-1 is an inducible enzyme. In the treated rat brain only HO-2 exists and is a molecular entity similar to that found in liver. The two constitutive forms were different in molecular weight and in inducibility and immunochemical properties.

**Key words:** Heme oxygenase; Liver; Brain; Hyperbilirubinemia

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Xia ZW, Li YZ, Chen SN, Shen QX, Ben XM, Yu SC. Analysis of two constitutive forms of microsomal heme oxygenase in different rat tissues. *World J Gastroenterol* 1997; 3(4): 210-212 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/210.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.210>

### INTRODUCTION

Oxidative degradation of heme (protoheme, Fe protoporphyrin IX, heme b) to the open tetrapyrrole, biliverdin, is catalyzed by the microsomal enzymes heme oxygenase (HO) and NADPH-cytochrome C (P-450) reductase. Although the exact mechanism of heme degradation is not yet fully understood, it is generally accepted that the role of HO is to bind the substrate in a specific orientation, such that upon degradation the predominant end product is the IX-*α*-isomer of biliverdin; the dual function of the reductase is to activate molecular oxygen and to promote the reduction of heme iron to the ferrous state. In mammals, the cytosolic enzyme, biliverdin reductase, catalyzes the conversion of biliverdin to bilirubin<sup>[1-6]</sup>. The purpose of the present study was, therefore, to verify the possibility of the existence of multiple forms of HO in the rat liver and brain, and if existing to compare the properties of these enzymatic isoforms in order to understand their mechanisms of action.

### MATERIALS AND METHODS

#### Materials and animals

Hematin was purchased from Sigma. Sodium cholate and DTT were purchased from Serva. Sprague-Dawley (S-D) rats (weight range: 180-220 g) and Kunming mice were purchased from the Shanghai Institute of Cell Biology, Academia Sinica.

#### Preparation of tissue

The S-D rats were given ad libitum access to food and water. Hypoxia was induced in the rats by exposure to a gas mixture of 5%-7% oxygen and 93%-97% nitrogen for 60 min to increase the

**Table 1** Levels of sIL-2R, ALT, and HBV DNA in the sera of patients with chronic HBV infection (mean  $\pm$  SD)

Purified fractions	Total protein, mg	Total activity, $\times 10^3$ U	Specific activity, U/mg	Recovery, %	Purification, -fold
HO-1					
Solubilized microsomes	82	68.6 (64.0-73.2)	837.5 (781.4-893.6)	100	1.0
DEAE-Sephacel	8.7	18.3 (16.6-20.0)	2097.3 (1902.9-2291.7)	10.7	2.5
Hydroxyapatite	1.2	5.1 (5.0-5.2)	4301.1 (3692.8-4909.3)	1.5	5.1
HO-2					
Solubilized microsomes	82	68.6 (64.0-73.2)	837.5 (781.4-893.6)	100	1.0
DEAE-Sephacel	4.9	7.7 (7.3-8.0)	1574 (1504.5-1643.6)	6.0	1.9
Hydroxyapatite	3.1	6.7 (5.3-8.0)	2146.3 (1708.5-2584.0)	3.8	2.6

blood brain barrier permeability, after which injections of 40  $\mu$ mol/kg hematin (pH 7.4, intraperitoneally) and 100 mg/kg phenylhydrazine (intravenously) were given. The control animals (untreated, hypoxic) received saline injections. The animals were euthanized 20 h after treatment for analysis.

### Resolution and isolation of HO isoforms

All operations were carried out below 4  $^{\circ}$ C. The rat tissues were homogenized in 2.0 volumes of 20.0 mmol/L potassium phosphate buffer (pH 7.4) containing 0.1 mmol/L EDTA and 135.0 mmol/L KCl; the resulting homogenate was centrifuged at 10000  $\times g$  for 20 min. The supernatant fractions were collected and subsequently centrifuged at 150000  $\times g$  for 1 h. Washing, resuspension, and solubilization of the microsomes were performed according to Maines' method<sup>[1,4]</sup>. The solubilized microsomes were diluted with 1.0 volume of 0.05 mmol/L DTT solution (pH adjusted to 8.0) and loaded onto a DEAE-Sephacel column (2.5 cm  $\times$  13.6 cm) that was previously equilibrated with 20.0 mmol/L Tris HCl buffer (pH 7.5) containing 0.05 mmol/L EDTA, 0.5% Triton X-100, 0.1% sodium cholate and 0.05 mmol/L DTT. The column was eluted with concurrent linear gradients of KCl (0-0.4 mol/L) and Triton X-100 (0.5%-0.9%) prepared with equilibration buffer (75 mL). Fractions (3.0-4.0 mL) were collected and analyzed for HO activity.

### Purification of HO-1 and HO-2

The pooled DEAE-Sephacel fractions containing the first and second peaks of HO activity were dialyzed against distilled water for 24 h at temperature below 4  $^{\circ}$ C, and then concentrated and respectively loaded onto a hydroxyapatite column equilibrated with 10.0 mmol/L potassium phosphate buffer (pH 7.5). The column was washed with 10.0 mmol/L potassium phosphate buffer (pH 7.2) containing 0.05 mmol/L EDTA, 0.4% Triton X-100 and 0.1% sodium cholate, followed by the same solution supplemented with 20% glycerol. The column was eluted with a linear gradient of 10.0-260.0 mmol/L potassium phosphate in the above buffer, and the fractions containing HO activity were collected.

### Preparation of biliverdin reductase from rat liver

Each rat liver sample was homogenized in 2.0 volumes of 0.1 mol/L potassium phosphate buffer (pH 7.4). The resulting homogenate was centrifuged at 9000  $\times g$  for 20 min. The supernatant containing biliverdin reductase was prepared by additional centrifugation of that supernatant fraction at 105000  $\times g$  for 1 h. The protein concentration of the supernatant fraction was adjusted to 10.0 mg/mL. Biliverdin reductase activity was determined by measuring the increase in spectra absorption due to the production of bilirubin.

### Assay of HO isoforms activities

HO isoform activities were routinely determined according to the previously described methods in<sup>[1]</sup> and<sup>[4]</sup>. One unit of HO activity was defined as the amount of enzyme catalyzing the formation of 1 nmol of bilirubin in 1 h.

### SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of HO isoforms

Electrophoretic procedures were performed according to Laemmli's method in order to identify HO isoforms.

### Production of antiserum

The purified rat liver HO-2 fraction (69  $\mu$ g) was mixed with 100  $\mu$ L of 0.9% NaCl solution and injected intraperitoneally into Kunming mice (10-15 g). These mice were then boosted with antigen (69  $\mu$ g) every second week, for a total of three times. The mice were bled at 10 days after the final booster and sera were collected and stored at -20  $^{\circ}$ C.

### Western blotting

Western blotting was performed as follows. Protein samples, rat liver HO-1 and rat brain HO-2 fractions, were subjected to SDS-PAGE in 1.5 mm slab gels according to the procedure of Laemmli. The separated polypeptides were then transferred electrophoretically from the gel to a nitrocellulose membrane using a transfer buffer consisting of 48 mmol/L Tris base, 39 mmol/L glycine, 20% (v/v) methanol and 0.037% (w/v) SDS (pH 8.3). Following electroblotting, the nitrocellulose blot was stained with Ponceau S for protein detection. Destaining was performed with distilled water. For immunostaining, the nitrocellulose blot was incubated at 37  $^{\circ}$ C for 1 h with bovine serum albumin in 20.0 mmol/L Tris-HCl buffer (pH 7.5) containing 0.5 mol/L sodium chloride, in order to saturate all protein binding sites. The nitrocellulose blot was then incubated with antiserum against rat liver HO-2 at 37  $^{\circ}$ C for 1 h, washed several times with PBS-Tween-20 solution and then incubated for 1 h at 37  $^{\circ}$ C with peroxidase anti-mouse IgG diluted with PBS solution. The blot was stained with diaminobenzidine (DAB) solution, washed with distilled water, and air-dried.

## RESULTS

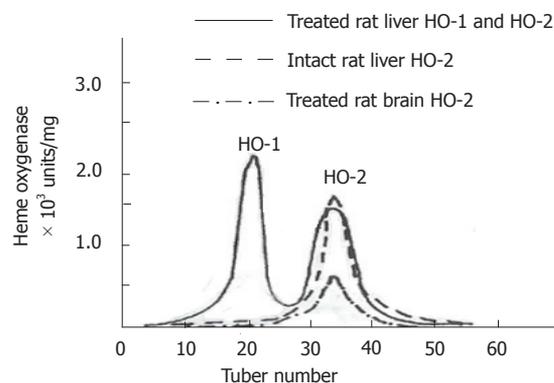
### Purification and identification of the two forms of HO in two tissues

Rat liver microsomal fractions were loaded for DEAE-Sephacel chromatography, and 3.0-4.0 mL fractions were collected and analyzed for HO activity. Two peaks exhibiting HO activity were eluted (Figure 1). These peaks were designated as HO-1 and HO-2 according to the sequence of their elution from the column. The pooled DEAE-Sephacel fractions containing HO-1 and HO-2 activities were loaded onto hydroxyapatite column, and the final purified HO-1 and HO-2 preparations exhibited only single bands, respectively, when visualized by staining with Coomassie blue following SDS-PAGE. The final HO-1 and HO-2 preparations showed specific activities of up to 4300 nmol of bilirubin/mg of protein $\cdot$ h and 2150 nmol of bilirubin/mg of protein $\cdot$ h, respectively (Table 1).

The treated rat brain and untreated rat liver microsomal fractions were also solubilized and respectively subjected to ion exchange chromatography of DEAE-Sephacel. The chromatographic elution patterns of HO activity were compared with those of treated rat liver. In the treated rat brain and untreated rat liver, only one peak of HO activity was detected. An activity peak was not present in the eluate corresponding to the region where the inducible isoform of HO, HO-1, was detected (Figure 1). Tables 2 and 3 show the procedures and the activity for purification of treated rat brain HO-2

**Table 2** Purification of HO-2 from treated rat brain microsomal fractions

Purified fractions	Total protein, mg	Total activity, × 10 <sup>3</sup> U	Specific activity, U/mg	Recovery, %	Purification, -fold
HO-2					
Solubilized microsomes	56.0	24 (16.9-31.1)	428.7 (302.7-554.7)	100	1.0
DEAE-Sephacel	20.1	12.2 (10.3-14.1)	607.7 (512.0-703.4)	35.9	1.4
Hydroxyapatite	1.6	10.6 (10.0-11.2)	6478.1 (6102.4-6853.8)	2.9	15.1



**Figure 1** DEAE-Sephacel chromatography of rat liver and brain solubilized microsomes. Solubilized microsomal fractions were loaded onto a DEAE-Sephacel column (2.5 cm × 13.6 cm) equilibrated with 20.0 mmol/L Tris-HCl buffer (pH 7.5) containing 0.05 mmol/L EDTA, 0.5% Triton X-100, 0.1% sodium cholate, and 0.05 mmol/L DTT. The column was eluted with concurrent linear gradients of KCl (0-0.4 mol/L) and Triton X-100 (0.5%-0.9%) in the same buffer. The fractions (3.0-4.0 mL) were collected and analyzed for enzyme activities.

and untreated liver HO-2, respectively.

### Characteristics of HO-1 and HO-2

Two forms of HO from the treated rat liver were subjected to electrophoresis and two distinct bands exhibiting HO activity were detected, with the migration patterns of HO-1 and HO-2 being different. This differential separation pattern suggested the presence of distinct molecular properties associated with HO-1 and HO-2. The purified HO-2 after SDS-PAGE treatment displayed a higher monomeric molecular weight. The apparent molecular weights for HO-1 and HO-2 were Mr 30000 and Mr 36000, respectively. The apparent molecular weight in treated brain microsomal preparation was identical to the purified liver HO-2 (Mr 36000). The HO-1 activity was increased in response to hematin and phenylhydrazine, while that of HO-2 was fully refractory to these agents. The blot was treated with anti-rat liver HO-2 serum followed by anti-mouse IgG-peroxidase conjugate and then stained for peroxidase activity. As expected, rat brain HO-2 preparation gave a reddish brown band in the region of molecular weight Mr 36000. However, the rat HO-1 preparation failed to show a stained band (Figure 2).

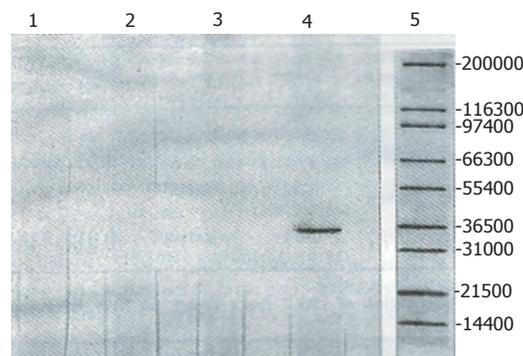
## DISCUSSION

We have purified and identified two constitutive forms of HO from rat liver and brain, and provided evidence for decidedly different characteristics of the two forms. These two forms are named HO-1 and HO-2. The results indicate that in the liver of treated rats, there exist two different molecular types of HO. It appears that HO-1 and HO-2 substantially differ in their molecular composition and structure as indicated by their chromatographic behavior and electrophoretic migration pattern. Differences in amino acid composition or sequence usually result in such observations. It is rather curious that in the untreated rat liver only the HO-2 isoform exists, and the HO-1 form of the enzyme could not be detected. This finding suggests that the amount of the HO-1 form is too low to be detected. The present findings also show that the activity of HO-1 in the treated rat liver apparently surpasses that of HO-2, and the HO-2 activity is almost refractory, indicating that only the activity of HO-1 is inducible.

With the same procedure, only HO-2 could be clearly detected in the brain, and the HO-1 form was absent, after the brain barrier permeability had been increased by hypoxia in order to induce the

**Table 3** Purification of HO-2 from intact rat liver microsomal fractions

Purified fractions	Total protein, mg	Total activity, × 10 <sup>3</sup> U	Specific activity, U/mg	Recovery, %	Purification, -fold
HO-2					
Solubilized microsomes	662.7	394.7 (351.8-437.6)	595.6 (530.9-660.3)	100	1
DEAE-Sephacel	10.2	16 (13.8-18.2)	1574.4 (1358.7-1789.5)	1.5	2.6
Hydroxyapatite	2.4	8.2 (7.3-9.0)	3381.6 (3020.1-3743.2)	0.4	5.7



**Figure 2** Western immunoblot of rat liver HO-1 and brain HO-2 preparations. Preparations of HO-1 and HO-2 were subjected to SDS-polyacrylamide slab gel electrophoresis, electroblotted onto nitrocellulose membranes and visualized as described in the "experimental procedures". 1 and 3, controls; 2, liver HO-1; 4, brain HO-2; 5, molecular weight markers.

brain microsomal HO isoforms with hematin and phenylhydrazine. This finding, however, does not suggest that this isoform is absent in the organ; rather, it may reflect inability of the presently used procedures to detect the exceedingly low level of the isoform and expression of the HO-1 isoform in the brain being suppressed under various conditions. Surprisingly, the brain displayed a higher level of HO-2 activity than the liver, possibly reflecting a major biological adaptation<sup>[2]</sup>. HO-1 and HO-2 preparations were analyzed by the western immunoblotting technique. As expected, the rat brain HO-2 preparation exhibited immunological reactivity with antibody to rat liver HO-2, but the rat HO-1 preparation did not, indicating that these two HO preparations are antigenically different.

The present studies further suggested that the liver and brain HO-2 are similar protein entities, according to the following criteria: (1) similarity in molecular weight; (2) cross-reactivity with antiserum to rat liver HO-2; and (3) similarity in chromatographic behavior on a DEAE-Sephacel column.

Collectively, this study showed that the two constitutive forms differ in molecular weight and in inducibility and immunochemical properties and that these differences are highly important for understanding the occurrence of hyperbilirubinemia, particularly in the premature newborn.

## REFERENCES

- 1 Maines MD, Trakshel GM, Kutty RK. Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible. *J Biol Chem* 1986; **261**: 411-419 [PMID: 3079757]
- 2 Trakshel GM, Kutty RK, Maines MD. Resolution of the rat brain heme oxygenase activity: absence of a detectable amount of the inducible form (HO-1). *Arch Biochem Biophys* 1988; **260**: 732-739 [PMID: 3124761 DOI: 10.1016/0003-9861(88)90503-6]
- 3 Braggins PE, Trakshel GM, Kutty RK, Maines MD. Characterization of two heme oxygenase isoforms in rat spleen: comparison with the hematin-induced and constitutive isoforms of the liver. *Biochem Biophys Res Commun* 1986; **141**: 528-533 [PMID: 3099789 DOI: 10.1016/S0006-291X(86)80205-4]
- 4 Trakshel GM, Kutty RK, Maines MD. Purification and characterization of the major constitutive form of testicular heme oxygenase. The noninducible isoform. *J Biol Chem* 1986; **261**: 11131-11137 [PMID: 3525562]
- 5 Ishikawa K, Sato M, Yoshida T. Expression of rat heme oxygenase in *Escherichia coli* as a catalytically active, full-length form that binds to bacterial membranes. *Eur J Biochem* 1991; **202**: 161-165 [PMID: 1935972 DOI: 10.1111/j.1432-1033.1991.tb16357.x]
- 6 Ishikawa K, Sato M, Ito M, Yoshida T. Importance of histidine residue 25 of rat heme oxygenase for its catalytic activity. *Biochem Biophys Res Commun* 1992; **182**: 981-986 [PMID: 1540195 DOI: 10.1016/0006-291X(92)91828-E]

## Effects of endotoxin on expression of *ras*, *p53* and *bcl-2* oncoprotein in hepatocarcinogenesis induced by thioacetamide in rats

Jin-Ming Yang, De-Wu Han, Quan-Chen Liang, Jia-Li Zhao, Su-Yuan Hao, Xue-Hui Ma, Yuan-Chang Zhao

Jin-Ming Yang, De-Wu Han, Su-Yuan Hao, Xue-Hui Ma, Yuan-Chang Zhao, The Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China

Quan-Chen Liang, Jia-Li Zhao, Shanxi Second People's Hospital, Taiyuan 030012, Shanxi Province, China

Jin-Ming Yang, Associate Professor in Toxicology, having 10 papers published, PhD student in hepatic pathophysiology, specializing in hepatocarcinogenesis.

Author contributions: All authors contributed equally to the work.

Supported by the Overseas Scholarship Grant (No. 96025) from the Shanxi Provincial Committee of Science and Technology.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: De-Wu Han, Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China  
Telephone: +86-351-4135067

Received: January 12, 1997

Revised: May 25, 1997

Accepted: July 11, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To evaluate the relationship between expression of *ras*, *p53* and *bcl-2* gene products and hepatocarcinogenesis since the endotoxemia produced from lipopolysaccharide administration and/or the hypophagocytic state of splenectomy significantly accelerated hepatocarcinogenesis induced by thioacetamide.

**METHODS:** The hepatocarcinoma model was induced by 6-mo oral intake of 0.03% thioacetamide. During the hepatocarcinoma modeling process, rats were additionally treated with splenectomy and/or lipopolysaccharide administration. The techniques of flow cytometry, immunohistochemistry and immunoelectronmicroscopy were applied for quantitative analysis of the expression of oncogene proteins.

**RESULTS:** In this model system, overexpression of *ras* p21 protein mainly occurred in the precancerous cell population or in cells in the early stage of hepatocyte transformation. The levels of *ras* p21 declined when nuclear DNA aneuploidy increased. Expression of *bcl-2* protein slowly and steadily rose, with more hepatocytes staying in S+G2M phases, as the hepatocarcinoma became more malignant. *p53* was moderately expressed during hepatocarcinogenesis. There was no statistical correlation between endotoxemia levels and the changes in levels of *ras*, *p53* and *bcl-2* gene products.

**CONCLUSION:** Overexpression of oncogene *ras* p21 was considered likely to be a precursor of premalignant hepatocytes and possibly

as responsible for the initiation of hepatocarcinogenesis. *Bcl-2* protein expression is proportional to the severity of malignancy in hepatocarcinogenesis. *p53* may be involved in a key pathway underlying the transformation and development processes of hepatocarcinoma. This study confirmed the hypothesis that there are multiple genes and multiple steps involved in hepatocarcinogenesis. Expression of oncogene proteins reflects the properties of the premalignant and malignant cells, but is not directly related to endotoxemia statistically.

**Key words:** Genes, *ras*; Genes, *p53*; Oncogene proteins; Gene expression; Liver neoplasms; Thioacetamide

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Yang JM, Han DW, Liang QC, Zhao JL, Hao SY, Ma XH, Zhao YC. Effects of endotoxin on expression of *ras*, *p53* and *bcl-2* oncoprotein in hepatocarcinogenesis induced by thioacetamide in rats. *World J Gastroenterol* 1997; 3(4): 213-217 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/213.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.213>

### INTRODUCTION

Chemically-induced hepatocarcinogenesis in rats is widely used to assess the carcinogenic risk of chemicals to humans and to study the molecular pathogenesis in order to improve hepatoma prevention and treatment strategies for humans. Based on our previous observations that endotoxin can enhance hepatocarcinogenesis in rats induced by oral intake of thioacetamide (TAA), we hypothesized that endotoxin is responsible for, at least some of, the changes in levels of oncogenes and tumor suppressor genes that occur during the development of TAA-induced hepatocarcinoma.

A multitude of studies have provided evidence that members of the *ras* oncogene family (Ha-*ras*, Ki-*ras* and N-*ras*, activated by point mutation in codons 12, 13 and 61 respectively) are responsible for cell transformation<sup>[1]</sup>. NIH-373 cell transfection and immunohistochemical analysis, in particular, have yielded data that demonstrates the presence of a dominant activated N-*ras* gene in premalignant and malignant cells, which also appears to play a key role in the initiation of hepatocarcinogenesis<sup>[2-4]</sup>.

Mutations in the *p53* tumor suppressor gene are frequently detected in many human and animal cancers (approximately 50%), and studies of its consequent misexpression have provided clues to the etiology and molecular pathogenesis of neoplasia<sup>[5,6]</sup>. In addition to its role in neoplastic transformation, the *p53* protein plays an important role in normal cell function and its encoding gene has been found to be highly conserved among vertebrates, allowing extrapolation of data from animal models<sup>[7]</sup>.

The *bcl-2* gene was first discovered in non-Hodgkin's B-cell lymphomas and its presence in tumors is due to a *bcl-2* gene

**Table 1** Oncogene *ras* p21 expression and DNA content in the various study groups

Group	<i>n</i>	FI, $\bar{x} \pm s$	% cells labeled, $\bar{x} \pm s$	Cases with positive <i>ras</i> p21 expression	DNA index, $\bar{x} \pm s$
Sham	5	1	17 ± 4	1	1
TAA control	6	1.29 ± 0.085	18 ± 5	6	1.15 ± 0.21
TAA + LPS	6	1.60 ± 0.071 <sup>a</sup>	24 ± 7 <sup>a</sup>	6	1.24 ± 0.25 <sup>a</sup>
TAA + ST	6	1.52 ± 0.0105 <sup>a</sup>	15 ± 3 <sup>b</sup>	6	1.44 ± 0.015 <sup>a,b</sup>
TAA + ST + LPS	6	1.32 ± 0.061 <sup>b,c</sup>	49 ± 9 <sup>a,b,c</sup>	6	1.56 ± 0.07 <sup>a,b,c</sup>
CBRH-7919 cells	1	1.54	42	1	1.34

<sup>a</sup>*P* < 0.02 vs TAA control group; <sup>b</sup>*P* < 0.02 vs TAA + LPS group; FI: fluorescence index; <sup>c</sup>*P* < 0.02 vs TAA + ST group.

CBRH-7919: rat hepatocarcinoma cell line; DNA index: ratio of mean nuclear DNA content of the treated rat to mean DNA content of normal rats.

translocation from 18q21 to cis-configuration. However, high levels of *bcl-2* protein production in a variety of human solid tumors have been observed in the without translocation or alterations in the structure of the *bcl-2* gene<sup>[8]</sup>. It is generally accepted that the *bcl-2* protein contributes to the process of neoplastic cell expansion by blocking normal physiological cell death<sup>[9]</sup>; in addition, gene transfer-mediated elevation in *bcl-2* protein levels have been shown to render tumor cells relatively more resistant to induction of apoptosis by chemotherapeutic drugs<sup>[10]</sup>. Therefore, *bcl-2* has been theorized to play a significant role in the origins of cancer and in its therapy.

Considering the important contributions of *ras*, *p53* and *bcl-2* genes to carcinogenesis, the aim of this study was to explore the relations between expression of oncoproteins and malignity or incidence of hepatocarcinoma using a TAA-treated model system coupled with splenectomy (ST) and/or endotoxin (lipopolysaccharide, LPS) treatment.

## MATERIALS AND METHODS

### Hepatoma models and treatment

The protocol course of TAA-induced modeling encompassed 4 mo for cirrhosis and 6 mo for liver tumor. Female Wistar rats (provided by the Experimental Animals Center of Shanxi Medical University), weighing 125 ± 9 g, were housed in wire-bottom cages under a 12 h light/dark cycle and fed with a balanced pet diet ad libitum. Animals were randomly assigned to the following five groups: sham group (*n* = 5), which was the untreated control group that received tap water ad libitum; TAA control group (*n* = 6), which was given 0.03% w/v TAA (purity > 99%; Shanghai Central Chemical Factory) in drinking water; TAA + ST group (*n* = 6), which was splenectomized 1 week before commencement of the experiment; for the final two groups, mice first treated as the TAA + LPS group (*n* = 6) or the TAA + ST + LPS group (*n* = 6) were given 0.8 mg LPS (*Escherichia coli* serotype 055:B5; Sigma) in drinking water containing 0.03% TAA for the last 2 months of the modeling course. The sham group, without splenectomy, underwent midline laparotomy and spleen manipulation as the sham surgery procedure. All surgeries were carried out under light ether anesthesia and all procedures were performed under sterile conditions. At the end of the 6 mo modeling course, all rats were euthanized by over-anesthetization with ethyl ether and the liver was excised for the following preparations.

### Immunofluorescence flow cytometry

Expression of oncogene proteins was quantitatively determined as described by Zuo *et al.*<sup>[11]</sup>. Briefly, ~2 g of excised liver tissue was immediately minced in 0.05% collagenase (type IV; Sigma) and filtered through a 200 mesh stainless steel filter. A single-cell suspension was prepared and fixed in 70% ethanol. After washing with PBS, the cells were resuspended in a solution containing 0.1% Triton X-100 and 5% goat serum, in order to increase the permeability of the cell membrane and to block the non-specific binding sites of IgG. Antibodies, including pan *ras* (F132, a mouse monoclonal IgG2b antibody), *p53* (CM1, a rabbit polyclonal antibody) and *bcl-2* (N19, a rabbit polyclonal antibody) were purchased from Santa Cruz Biotechnology Inc., United Kingdom. They were diluted at 1:100 and incubated with 10<sup>5</sup> cells respectively at 37 °C for 30 min. After washing twice, the cells were incubated with 100 μL of species-specific secondary-FITC-IgG (Lot 9609; Military Medical Academy) at 37 °C for 30 min. The negative control (omission of the primary antibody) and the positive control (the rat hepatocarcinoma

cell line CBRH-7919, provided by Shanghai Institute of Cell Biology) were generated using the same procedure described above. For flow cytometric analysis, 10000 cells from each sample were passed through a 400 mesh filter and then quantitatively examined on the Fluorescence Activated Cell Sorter FACS-420 (Becton Dickinson, United States). Data were analyzed on an IBM PC-compatible computer with HP-300 Consort 30 software. The quantitative expression of oncogene product was calculated according to Morker *et al.*<sup>[12]</sup>, as follows:

Fluorescence index (FI) =

$$\frac{\text{Mean channels of the treated rat} - \text{Mean channels of negative controls}}{\text{Mean channels of the sham group}}$$

The criteria for oncoprotein expression was as follows: FI value > 1, positive; FI ≤ 1, negative.

In addition, liver cells were stained with propidium iodide reagents (50 mg/L propidium iodide, 20 mg/L RNase, and 1% Triton X-100) at 4 °C for 30 min, and the nuclear DNA content was determined by flow cytometry.

### Immunohistochemistry

Immunohistochemical staining was conducted using the streptavidin/peroxidase kit (SP-TM; Zymed, United States) and the biotin-streptavidin method<sup>[13]</sup> with slight modification. Briefly, all liver sections were fixed with neutral buffered 10% formalin and embedded in paraffin. The paraffin-embedded sections were deparaffinized through an alcohol series graded with distilled water. The resultant hydrated sections were then incubated in 0.3% hydrogen peroxide for 10 min to quench the endogenous peroxidase activity. Liver sections were then incubated in a salt buffer (pH 6.0) at 92–98 °C for 10 min to restore antigenicity, followed by incubation in 5% normal blocking serum for 20 min to suppress non-specific binding of IgG. The preparations were then incubated sequentially with primary antibodies (diluted 1:50 in PBS) at 4 °C for overnight, followed by sequential incubation with the biotinylated secondary antibodies (diluted 1:100 in 1% BSA-PBS) at 37 °C for 10 min and streptavidin horseradish peroxidase (diluted 1:100) at 37 °C for 20 min. The colored reaction product was developed with diaminobenzidine (DAB). The sections were lightly counterstained with hematoxylin. Immunostaining by replacing primary antibody with PBS was also conducted as a negative control.

### Immunoelectronmicroscopy

Anti-mouse IgG/colloidal gold (10 nm) was obtained from Beijing Zhongshan Biotechnology Co. LTD. The liver ultrathin sections were sequentially incubated with pan *ras* p21 antibody and the secondary immunogold antibody using the procedures described by Yin *et al.*<sup>[14]</sup>.

### Statistical analysis

All results are expressed as the  $\bar{x} \pm s$ . Data were analyzed by Student's *t*-test and multiple regression. *P* values < 0.05 were considered statistically significant.

## RESULTS

Expression of oncogene *ras* p21 was quantitatively detected by flow cytometry. The fluorescence index indicated the potentiality of gene product expression. Table 1 shows that the TAA-treated group with 2-mo administration of LPS presented the highest expression of *ras* p21 despite their relatively low DNA content, reflecting the malignity of hepatocarcinoma to a great extent. However, the group with the

**Table 2** p53 expression and hepatocarcinoma rate in the various study groups

Group	n	FI, x ± s	% cells labeled, x ± s	Cases with positive ras p21 expression	DNA index, x ± s
Sham	5	1	32	1	0
TAA control	6	1.15 ± 0.067	22 ± 12	6	17
TAA + LPS	6	1.32 ± 0.024 <sup>a</sup>	20 ± 8.2	6	33
TAA + ST	6	1.22 ± 0.088 <sup>a</sup>	25 ± 12	6	50
TAA + ST + LPS	6	1.29 ± 0.083 <sup>a</sup>	30 ± 14	6	67
CBRH-7919 cells	1	1.35	9	1	

<sup>a</sup>*P* < 0.01 vs TAA control group; <sup>b</sup>*P* < 0.05 vs TAA + LPS group; <sup>c</sup>*P* < 0.01 vs TAA + ST group. CBRH-7919: rat hepatocarcinoma cell line.

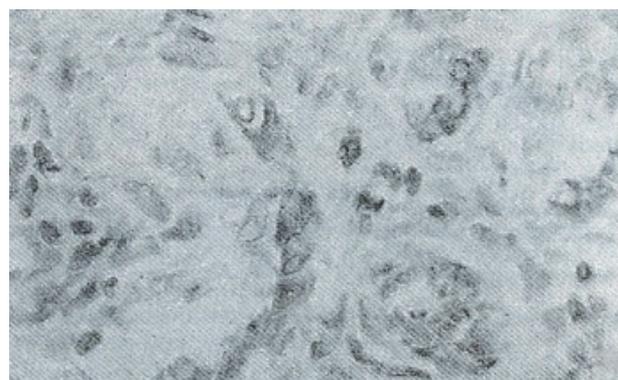
**Table 3** Expression of *bcl-2* protein and percentage of cells in S and G<sub>2</sub>M phases

Group	N	FI, x ± s	% cells labeled, x ± s	Cases with positive ras p21 expression	DNA index, x ± s
Sham	5	1	23	1	25 ± 3.2
TAA control	6	0.96 ± 0.14	23 ± 6	4	33 ± 4.7
TAA + LPS	6	1.06 ± 0.07	37 ± 14	6	35 ± 4.5
TAA + ST	6	1.12 ± 0.11	28 ± 14	6	35 ± 7.7
TAA + ST + LPS	6	1.30 ± 0.09 <sup>a, b, c</sup>	25 ± 6	6	45 ± 3.9 <sup>a, b, c</sup>
CBRH-7919 cells	1	1.56	15	1	30

<sup>a</sup>*P* < 0.05 vs TAA control group; <sup>b</sup>*P* < 0.05 vs TAA + LPS group; <sup>c</sup>*P* < 0.05 vs TAA + ST group. CBRH-7919: rat hepatocarcinoma cell line.



**Figure 1** Immunostaining of a rat malignant nodule with anti-p21 monoclonal IgG2b antibody detected by the biotin-streptavidin method. Magnification × 10.



**Figure 3** Immunostaining of a rat malignant foci with anti-bcl-2 polyclonal antibody detected by the biotin-streptavidin method. Magnification × 40.



**Figure 2** Immunostaining of a rat malignant foci with anti-p53 polyclonal antibody detected by the biotin-streptavidin method. Magnification × 40.



**Figure 4** Immunostaining of ultrastructure of a rat normal hepatocyte with anti-p21 IgG/colloidal gold (φ 10 nm) antibody. Magnification × 2000

highest DNA content had an average value of fluorescence index that was significantly decreased (*P* < 0.01) but a percentage of labeled cells that was obviously increased (*P* < 0.01).

Similar to the results of *ras* p21 expression, the fluorescence index of the *p53* tumor suppressor gene product was highest in the TAA + LPS group (*P* < 0.01) and relatively higher in the TAA + ST + LPS group (*P* < 0.05). However, the alteration in expression did not correlate with the hepatocarcinoma rate (Table 2).

Unlike the results of *p53* and *ras* p21, the expression of the *bcl-2* protein was consistently correlated with DNA index (*r* = 0.93, *P* < 0.01) and the percentage of cells in S plus G<sub>2</sub>M phases (*r* = 0.86, *P* < 0.05). The rat hepatocarcinoma cell line CBRH-7919 was used as a positive control of expression of oncogene product when the samples of hepatocytes were analyzed by flow cytometry (Table 3).

Immunohistochemical analyses showed that immunostaining for the *ras* p21 oncogene was mainly localized to the cytoplasm in malignant cells and in dysplastic hepatocytes in the hyperplastic

nodules. It was noticed that expression of *ras* p21 was more apparent in hepatocytes adjacent to neoplastic lesions than in hepatic tumor tissues (Figure 1). The hyperexpression of *p53* was present in the nuclei of malignant hepatocytes (Figure 2), and *bcl-2* protein was moderately expressed in some cytoplasm of neoplastic foci (Figure 3).

Immunoelectronmicroscopy showed that in contrast to the data presented in Figure 4, overexpression of *ras* p21 dominantly occurred on the increased nuclear heterochromatin of malignant hepatoma and secondarily on the endoplasmic reticulum adjacent to the nuclear membrane (Figure 5).

## DISCUSSION

The proto-oncogene *ras* gene family (Ha-*ras*, Ki-*ras* and N-*ras*) commonly exists in normal mammalian cells and its products are involved in cell proliferation, metabolism, differentiation and various signaling pathways. Here, the mutation-activated *ras* gene

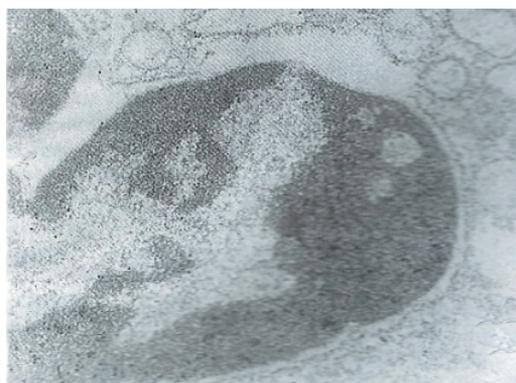


Figure 5 Immunostaining of ultrastructure of a rat malignant hepatocyte with anti-p21 IgG/colloidal gold ( $\phi$  10 nm) antibody. Magnification  $\times$  2000.

was found to be associated with hepatocarcinogenesis. It has been reported that mutation of Ki-ras in codon 13 is present in liver angiosarcoma of humans as well as animals exposed to vinyl chloride<sup>[4]</sup>. Activated Ha-ras has also been detected in spontaneous hepatoma of the B6C3F1 mouse<sup>[15]</sup> and 2 amino 3 methylimidazo quinolone-induced hepatocarcinoma in rat<sup>[16]</sup>. However, the mutation-activated N-ras oncogene has been demonstrated frequently in hepatocarcinomas.

The p21 *ras* gene product plays a key role in cell transformation, as indicated by its strong expression in preneoplastic cells<sup>[17]</sup>; thus, *ras* p21 may be a biomarker for tumor diagnosis and prognosis. Our study, along with others<sup>[17,18]</sup>, demonstrate oncogene *ras* p21 expression as a early event of hepatocarcinogenesis, since its expression levels do not correspond with nuclear DNA content. In contrast, we found that p21 expression declines when the DNA content is increased in the premalignant and malignant liver. Therefore, the inverse correlation between the levels of *ras* p21 production and the severity of liver cancerous lesions indicates that *ras* p21 is a potent trigger for transformation of the hepatocyte phenotype and it is not crucial for maintenance of the transformed cell phenotype. It is generally accepted that members of the *ras* gene family, especially N-ras, are "transforming genes" involved in initiation of carcinogenesis<sup>[1,2,17,18]</sup>. Therefore, although we observed a high content of p21 protein in the model system that occurred in response to additional administration of LPS purified from *E. coli*, it is questionable whether LPS itself induces overexpression of *ras* p21 or the property of preneoplastic hepatocytes. The increased heterochromatin in the malignant hepatocytes showed overexpression of *ras* p21, which may imply some mechanism of hepatocarcinogenesis.

The 17-y history of research into the p53 tumor suppressor gene has indicated that p53 protein is involved in gene transcription, DNA synthesis and repair, genomic plasticity, and programmed cell death. So it is not surprising that p53 is a functional component of signaling pathways central to human carcinogenesis. Deactivation of p53 causes loss of tumor suppressor function and gain of oncogenic activity, and this dynamic represents one of the explanations for pathogenesis of cell transformation. The genetic mutation of p53 and of some viral oncoproteins binding to the p53 protein can lead to deactivation of p53 functions; for example, aflatoxin B1 induces hepatocellular carcinoma by mutation at codon 249 of p53<sup>[19]</sup> and HBV X protein binding to the p53 protein mediates p53 inactivation and remains an important possible mechanism of primary hepatocellular carcinoma lacking a p53 mutation<sup>[20]</sup>. In addition, some small carcinogen-DNA adducts, such as O6 methylguanine, may cause DNA polymerase to misread the base pairing, and bulky DNA adducts may render the bases unreadable, thereby increasing errors during DNA replication<sup>[21]</sup>.

The wild-type p53 protein exists in a very small quantity and has a very short half-life in the cell nucleus, and these two features complicate its detection by routine immunological methods. Missense mutations often increase the half-life and quantity of the p53 protein by 20-fold, so p53 overexpression is a surrogate marker for missense mutation. Previous studies have noted that alteration of the function of the p53 pathway (*via* mutation or epigenetic inactivation) are associated with aneuploidy and increased proliferative rates in ovarian, colorectal

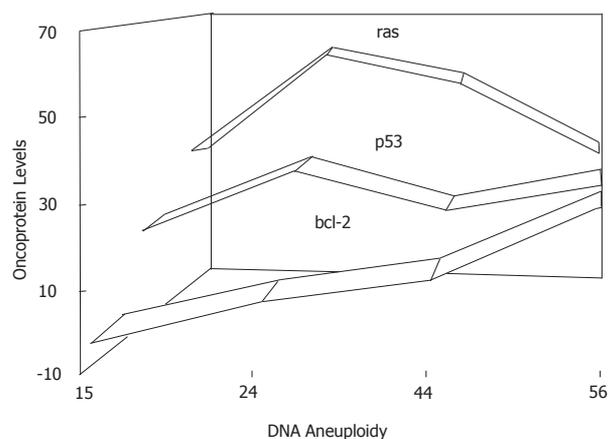


Figure 6 Relationship between hepatocarcinoma malignancy and expression of oncogene proteins detected by immunofluorescence flow cytometry.

and gastric cancers, features which are also considered precursors for tumor progression and poor prognosis<sup>[22]</sup>; however, some authors have reported that changes in the levels of p53 protein are not related to tumor differentiation<sup>[23]</sup>. Our study showed that a high content of p53 protein, as detected by flow cytometry and immunohistochemistry, was present in the model system with LPS treatment or in the hyperplastic nodules and malignant liver lesions, but neither specifically related to endotoxemia levels nor to malignancy. These findings suggested that the overexpression of p53 protein was relatively associated with LPS challenge and to both the early and late events of hepatocarcinogenesis, but the precise mechanism remains unclear.

The *bcl-2* proto-oncogene on chromosome 18 may normally exert a regulatory function towards avoidance of cell apoptosis (programmed cell death), mainly in tissues where apoptosis may represent a specific control mechanism of cell turnover. Bcl-2 protein localized in mitochondria, endoplasmic reticulum and nuclear membrane has been shown to contribute to neoplastic cell expansion by blocking programmed cell death<sup>[24]</sup>. Bcl-2 expression has been detected in hepatocarcinoma and other solid tumors but appears to be absent in normal and dysplastic hepatocytes<sup>[25]</sup>. In normal liver, the majority of hepatocytes exist in a state of proliferative quiescence (G0 phase), but can enter a cell renewal compartment upon appropriate stimulation<sup>[26]</sup>. Dysplastic hepatocytes and regenerating hepatocytes in cirrhotic nodules have failed to show *bcl-2* protein expression, but some types of dysplasia represent a precancerous lesion<sup>[27]</sup>. Thus, *bcl-2* expression seems to be a relatively late event in hepatocarcinogenesis. In our study, the data from flow cytometry analysis with an antibody specific for *bcl-2* protein indicated that expression of *bcl-2* protein is significantly correlated with malignancy of hepatocarcinoma ( $r = 0.93$ ,  $P < 0.01$ ), which is in agreement with findings from other studies<sup>[25-27]</sup>. On the other hand, the *bcl-2* protein was also found to be related to the percentage of hepatocytes in S and G2M phases ( $r = 0.86$ ,  $P < 0.05$ ). Therefore, inhibition of apoptosis may be one of the mechanisms of carcinogenesis as little is known about the intracellular mechanisms underlying programmed cell death. Unlike other oncogene proteins described in the literature so far, *bcl-2* acts in both S and G2M transitions and the potential impact of *bcl-2* functions seem to, in addition to protecting cells from apoptosis, play a more complex role in morphogenesis, differentiation and homeostasis.

It is generally accepted that there are multiple genes and steps involved in carcinogenesis. The quantitatively analyzed data obtained in the current study showing elevations of *ras*, p53, and *bcl-2* proteins in a mass of normal, premalignant and malignant cells in rat liver, together with the immunohistochemistry data, confirm the hypothesis that *ras* mainly contributes to neoplastic transformation and *bcl-2* to malignant progression, and with p53 contributing to several pathways during hepatocarcinogenesis. Although endotoxemia was found to be significantly correlated with the malignancy or incidence of rat hepatocarcinoma, it was not directly related to the overexpression of *ras*, p53 or *bcl-2* proteins (maximum:  $r = 0.78$ ,  $P > 0.05$ ). Thus, it is suggested

that expression of oncogene proteins may represent some of the properties of the hepatocarcinoma itself (Figure 6).

## REFERENCES

- 1 **Barbacid M.** *ras* genes. *Annu Rev Biochem* 1987; **56**: 779-827 [PMID: 3304147 DOI: 10.1146/annurev.bi.56.070187.004023]
- 2 **Notario V.** A common mechanism for the malignant activation of *ras* oncogenes in human neoplasia and in chemically induced animal tumors. In: V Woude, ed. *Cancer cells*. Vol.2, New York: Cold Spring Harbor Laboratory, 1984
- 3 **Jagirdar J,** Nonomura A, Patil J, Paronetto F. Activated *ras* oncogene p21 expression in hepatocellular carcinoma and HBsAg-positive liver cells. *Hepatology* 1985; **5**: 1055
- 4 **Froment O,** Boivin S, Barbin A, Bancel B, Trepo C, Marion MJ. Mutagenesis of *ras* proto-oncogenes in rat liver tumors induced by vinyl chloride. *Cancer Res* 1994; **54**: 5340-5345 [PMID: 7923162]
- 5 **Levine AJ,** Momand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991; **351**: 453-456 [PMID: 2046748 DOI: 10.1038/351453a0]
- 6 **Harris CC.** p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science* 1993; **262**: 1980-1981 [PMID: 8266092 DOI: 10.1126/science.8266092]
- 7 **Soussi T,** Caron de Fromental C, May P. Structural aspects of the p53 protein in relation to gene evolution. *Oncogene* 1990; **5**: 945-952 [PMID: 2142762]
- 8 **Reed JC,** Meister L, Tanaka S, Cuddy M, Yum S, Geyer C, Pleasure D. Differential expression of *bcl2* protooncogene in neuroblastoma and other human tumor cell lines of neural origin. *Cancer Res* 1991; **51**: 6529-6538 [PMID: 1742726]
- 9 **Reed JC.** Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 1994; **124**: 1-6 [PMID: 8294493 DOI: 10.1083/jcb.124.1.1]
- 10 **Miyashita T,** Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* 1993; **81**: 151-157 [PMID: 8417786]
- 11 **Zuo LF,** Hu JL, Lin JH, Guo JW, Gao GD. Quantitative Study of oncogene *ras* p21 expression in carcinomas and non-cancer lesion of the stomach. *Prog Biochem Biophys* 1995; **22**: 146-149
- 12 **Morker O,** Laerum OD. Flow cytometric measurement of quantitative expression of oncogene product. *Cytometry* 1991; **12**: 138
- 13 **Iwaki T,** Miyazono M, Hitosumatsu T, Tateishi J. An immunohistochemical study of tissue transglutaminase in gliomas with reference to their cell dying processes. *Am J Pathol* 1994; **145**: 776-781 [PMID: 7524329]
- 14 **Yin GH.** Immuno-gel-gold technique. In: Yin Guohua, ed. *Microscopic technology of medical biology and cellular ultrastructure*. vol.5. Hong Kong: Modern Press 1992: 114-116
- 15 **Reynolds SH,** Stowers SJ, Maronpot RR, Anderson MW, Aaronson SA. Detection and identification of activated oncogenes in spontaneously occurring benign and malignant hepatocellular tumors of the B6C3F1 mouse. *Proc Natl Acad Sci USA* 1986; **83**: 33-37 [PMID: 3510430 DOI: 10.1073/pnas.83.1.33]
- 16 **Ishikawa F,** Takaku F, Nagao M, Ochiai M, Hayashi K, Takayama S, Sugimura T. Activated oncogenes in a rat hepatocellular carcinoma induced by 2-amino-3-methylimidazo[4,5-f]quinoline. *Jpn J Cancer Res* 1985; **76**: 425-428 [PMID: 3926575]
- 17 **Wang S,** Ying HJ. A study of relationship of p21 expression and DNA ploidy in preneoplastic and neoplastic lesion of stomach. *Practical J Cancer* 1996; **11**: 79
- 18 **Wang Z,** Liao TJ, Yong WC. A study of *ras* p21 expression and DNA ploidy in bladder tumors. *Zhongguo Aizheng Zazhi* 1991; **13**: 245
- 19 **Bressac B,** Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991; **350**: 429-431 [PMID: 1672732 DOI: 10.1038/350429a0]
- 20 **Feitelson MA,** Zhu M, Duan LX, London WT. Hepatitis B x antigen and p53 are associated in vitro and in liver tissues from patients with primary hepatocellular carcinoma. *Oncogene* 1993; **8**: 1109-1117 [PMID: 8386823]
- 21 **Yuspa SH,** Poirier MC. Chemical carcinogenesis: from animal models to molecular models in one decade. *Adv Cancer Res* 1988; **50**: 25-70 [PMID: 3287845 DOI: 10.1016/S0065-230X(08)60434-0]
- 22 **Kihana T,** Tsuda H, Teshima S, Okada S, Matsuura S, Hirohashi S. High incidence of p53 gene mutation in human ovarian cancer and its association with nuclear accumulation of p53 protein and tumor DNA aneuploidy. *Jpn J Cancer Res* 1992; **83**: 978-984 [PMID: 1429209 DOI: 10.1111/j.1349-7006.1992.tb02010.x]
- 23 **Campo E,** de la Calle-Martin O, Miquel R, Palacin A, Romero M, Fabregat V, Vives J, Cardesa A, Yague J. Loss of heterozygosity of p53 gene and p53 protein expression in human colorectal carcinomas. *Cancer Res* 1991; **51**: 4436-4442 [PMID: 1868464]
- 24 **Reed JC.** Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 1994; **124**: 1-6 [PMID: 8294493 DOI: 10.1083/jcb.124.1.1]
- 25 **Zhao M,** Zhang NX, Economou M, Blaha I, Laissue JA, Zimmermann A. Immunohistochemical detection of bcl-2 protein in liver lesions: bcl-2 protein is expressed in hepatocellular carcinomas but not in liver cell dysplasia. *Histopathology* 1994; **25**: 237-245 [PMID: 7821891 DOI: 10.1111/j.1365-2559.1994.tb01323.x]
- 26 **Ma XH.** Hepatocyte regeneration and regulation. In: Han Dewu, ed. *Hepatic pathophysiology*. Taiyuan: Shanxi United Universities Press, 1992: 91-96
- 27 **Borzio M,** Bruno S, Roncalli M, Mels GC, Ramella G, Borzio F, Leandro G, Podda M. Liver cell dysplasia and risk of hepatocellular carcinoma in cirrhosis: a preliminary report. *BMJ* 1991; **302**: 1312 [PMID: 1647827 DOI: 10.1136/bmj.302.6788.1312]

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Effects of somatostatin analog on splanchnic hemodynamics and plasma glucagon level in portal hypertensive rats

Zhi-Yong Wu, Xiao-Jie Zhao, Zhe Jiao, Zhi-Ping Chen, Yao-Ling Kuang

Zhi-Yong Wu, Xiao-Jie Zhao, Zhe Jiao, Zhi-Ping Chen, Yao-Ling Kuang, Department of Surgery, Renji Hospital Shanghai Second Medical University, Shanghai 200001, China

Zhi-Yong Wu, MD, a Postdoctoral Fellow in Louisiana State University Medical Center, Shreveport, USA between July 1, 1991 and June 30, 1994, now Professor and Vice Director of the Department of Surgery, having more than 40 papers published.

Author contributions: All authors contributed equally to the work.

Supported by the National Natural Science Foundation of China (No. C38970703).

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Zhi-Yong Wu, MD, Department of Surgery, Renji Hospital Shanghai Second Medical University, Shanghai 200001, China

Received: April 27, 1997

Revised: May 22, 1997

Accepted: June 14, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To investigate the effects of somatostatin analog on splanchnic hemodynamics and plasma glucagon level in portal hypertensive rats.

**METHODS:** Twenty-eight male Sprague-Dawley rats were equally divided into a intrahepatic portal hypertension (IHPH) model group ( $n = 14$ , established by injection of  $\text{CCl}_4$ ) and a prehepatic portal hypertension (PHPH) model group ( $n = 14$ , established by stenosis of the portal vein). Animals in each group were subdivided into an octreotide treatment (injection) group and a control (normal saline injection) group. Seven age-matched unmodeled/untreated normal rats served as controls. The mean systemic arterial pressure (MSAP) and free portal venous pressure (FPP) were measured. The splanchnic blood flow was detected by injection of toad blood red cell labelled with  $^{51}\text{Cr}$  and  $^{125}\text{I}\cdot\text{T}_3$ . The concentration of plasma glucagon was determined by radioimmunoassay.

**RESULTS:** All rats with portal hypertension showed significantly decreased splanchnic blood flow and FPP in response to octreotide treatment, as well as markedly increased splanchnic vascular and portal venous resistance. The octreotide treatment did not appear to significantly lower the plasma glucagon levels in either the peripheral or the portal veins.

**CONCLUSION:** Octreotide induces a decrease in splanchnic blood flow in rats with portal hypertension, and this effect results primarily from direct vasoconstriction and to a lesser extent from decreased plasma glucagon level.

**Key words:** Portal hypertension; Octreotide; Glucagon; Splanchnic

hemodynamics; Somatostatin analog

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wu ZY, Zhang XJ, Jiao Z, Chen ZP, Kuang YL. Effects of somatostatin analog on splanchnic hemodynamics and plasma glucagon level in portal hypertensive rats. *World J Gastroenterol* 1997; 3(4): 218-220 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/218.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.218>

### INTRODUCTION

Somatostatin has been widely used as treatment for variceal bleeding in patients with portal hypertension. However, the collective research on the mechanisms of somatostatin therapeutic action have not definitely determined whether the decreased splanchnic blood flow and free portal venous pressure (FPP) result from splanchnic vasoconstriction or decreased concentration of plasma glucagon or both<sup>[1]</sup>. The present study was designed to observe the effects of a commonly used somatostatin analog, octreotide, on splanchnic hemodynamics and concentration of plasma glucagon using rat model systems of both intrahepatic portal hypertension (IHPH) and prehepatic portal hypertension (PHPH) in order to investigate the underlying mechanism.

### MATERIALS AND METHODS

Thirty-five male Sprague-Dawley rats, weighing  $31.7 \pm 15.8$  g, were used in the study. All animals were housed in an environmentally controlled vivarium and allowed free access to a standard pellet diet and water.

#### IHPH modeling

Rats were given a subcutaneous injection of  $\text{CCl}_4$  (60% vol/vol in mineral oil, at a dose of 0.3 mL/100 g body weight) every 4 days for a total of 20 times. During the full modeling course, the rats were allowed to drink 10% alcohol.

#### PHPH modeling

Rats were put under ether anesthesia and after surgically isolating the portal vein a 7-gauge needle was placed alongside it. A ligature was then tied snugly to the needle and the vein at a location between the portal hepatic vein and the coronary vein. The needle was then removed to yield a calibrated stenosis of the portal vein.

#### Experimental and control treatment groups

Fourteen of the rats used in the IHPH and PHPH modeling ( $n = 7$  each) were divided into two groups: octreotide (injection) treatment and untreated (normal saline injection) control. In addition, 7 age-matched unmodeled/untreated rats served as normal controls.

#### Hemodynamics study

According to the method described by Zang *et al*<sup>[2]</sup>, toad red

**Table 1** Effects of octreotide on splanchnic hemodynamics

	Normal Cont	IHPH Cont	IHPH Thera	PHPH Cont	PHPH Thera
THBF, mL/min/100 g BW	6.30 ± 0.5	5.86 ± 0.9	4.53 ± 0.9 <sup>c</sup>	2.80 ± 0.3 <sup>a</sup>	2.12 ± 0.4 <sup>c</sup>
THBF, mL/min/g LW	2.50 ± 0.3	1.61 ± 0.3 <sup>a</sup>	1.37 ± 0.3 <sup>a</sup>	1.17 ± 0.2 <sup>ac</sup>	0.09 ± 0.2 <sup>ac</sup>
PVI, mL/min/100 g BW	4.85 ± 0.4	7.03 ± 0.7 <sup>a</sup>	5.85 ± 0.6 <sup>c</sup>	7.86 ± 0.4 <sup>ac</sup>	5.38 ± 0.6 <sup>ca</sup>
SVR, mmHg min/mL 100 g BW	26.68 ± 2.1	15.86 ± 2.3 <sup>a</sup>	20.64 ± 2.8 <sup>c</sup>	12.84 ± 1.0 <sup>a</sup>	19.88 ± 2.4 <sup>ca</sup>
PVR, mmHg min/mL 100 g BW	1.72 ± 0.2	2.01 ± 0.3 <sup>a</sup>	1.95 ± 0.2 <sup>a</sup>	1.76 ± 0.1 <sup>c</sup>	2.20 ± 0.3 <sup>c</sup>
PSS, %	1.46 ± 0.3	34.29 ± 11.7 <sup>a</sup>	38.77 ± 11.2 <sup>a</sup>	94.4 ± 1.3 <sup>ac</sup>	87.7 ± 4.5

<sup>a</sup>*P* < 0.05 vs unmodeled/untreated normal controls; <sup>c</sup>*P* < 0.05 vs control group; <sup>a</sup>*P* < 0.05 vs the IHPH control group.

Cont: Control; Thera: Therapy; THBF: Total hepatic blood flow; PVI: Portal venous inflow; SVR: Splanchnic vascular resistance; PVR: Portal venous resistance; PSS: Portosystemic shunt.

blood cells labelled with <sup>51</sup>Cr and <sup>125</sup>I-T<sub>3</sub> were prepared for use in hemodynamics study. The study was carried out at 2 wk after creation of the portal hypertension. Briefly, the modeled rats were fasted, but allowed access to water, for 18 h prior to experimental use. Following anesthetization with pentobarbital sodium (30 mg/kg, intra-abdominal injection), the rats were placed on a heated surgical table in a supine position with rectal temperature maintained at 37 ± 0.5 °C. The left femoral vein was dissected and cannulated with a PE-50 catheter for octreotide and normal saline injection. The right femoral artery was dissected and cannulated with a PE-50 catheter for mean systemic arterial pressure (MSAP) measurement and blood collection. A PE-50 catheter was also introduced into the right carotid artery and then into the left ventricle for injecting toad red blood cell labelled with radioisotope. Ten minutes after all procedures were completed, MSAP was measured. The zero reference point was placed 1 cm above the operating table, and the PE-50 catheter was connected to a transducer for continuous monitoring of MSAP. The octreotide treatment group of rats received an injection of 25 µg/kg body weight in 0.2 mL normal saline into the femoral vein followed by a 30-min infusion delivered at a rate of 0.02 mL/min. The untreated control group received an injection of normal saline that was administered intravenously. MSAP was measured at 1 and 30 min after the octreotide or normal saline administration. Subsequently, 0.3 mL of toad red blood cells labelled with <sup>51</sup>Cr was injected into the left ventricle over a duration of 20 s. A reference blood sample was drawn from the femoral artery at a rate of 1 mL/min for a duration of 10 s prior to and at 60 s after the injection of the radioactive toad red blood cells. A midline incision was then made and the spleen was gently taken out of the abdomen and injected with 0.5 mL of toad red blood cells labelled with <sup>125</sup>I-T<sub>3</sub> over a duration of 10 sec. After the puncture point bleeding was controlled by local pressure, the spleen was placed back into the abdominal cavity. Five minutes later, the main trunk of the portal vein was dissected. FPP was measured by inserting a heparin solution-filled 4-gauge needle connected to a catheter going directly into the portal vein and which was connected to an IVAC560 transducer for venous pressure measurement. Blood samples (1.5 mL) were drawn from both the portal vein and the carotid artery to determine the concentrations of plasma glucagon. Animals were then sacrificed by an intra-arterial bolus of saturated 10% KCl solution. All abdominal organs including the liver, spleen, pancreas, stomach, small and large intestine, and mesentery, as well as the lung, were removed and weighed. These organs were cut into small fractions for measurement of radioactivity.

#### Determination of plasma glucagon concentrations

The blood samples were placed into chilled collection tubes containing EDTA (25 g/L) and aprotinin (5 × 10<sup>5</sup> U/L) and immediately centrifuged (2400 × *g* for 20 min) at 4 °C; the separated plasma was stored at -40 °C until use for determination of glucagon concentration by radioimmunoassay with a specific antibody for glucagon (DPC, United States).

#### Statistical analysis

All values are expressed as  $\bar{x} \pm s$ . Variance analysis and Student's *t*-test were conducted to assess the significance of between and within group differences using SAS statistical software. Statistical significance was indicated by *P* < 0.05.

## RESULTS AND DISCUSSION

### Effects of octreotide on MSAP and FPP

The initial MSAP in PHPH and IHPH rats was lower than that in the unmodeled/untreated normal control rats (111.1 ± 2.6 mmHg and 126.9 ± 6.6 mmHg vs 137.3 ± 9.8 mmHg). In addition, the initial MSAP of the PHPH rats was significantly lower than that of the IHPH rats (*P* < 0.05). Octreotide treatment had no effects on MSAP. FPP in IHPH and PHPH rats was higher than that in the unmodeled/untreated normal control rats (14.01 ± 0.56 mmHg and 13.79 ± 0.31 mmHg vs 8.37 ± 0.10 mmHg). Octreotide treatment decreased FPP in both the IHPH rats (11.29 ± 0.64 mmHg) and PHPH rats (11.70 ± 0.36 mmHg).

### Effects of octreotide on splanchnic hemodynamics

Table 1 presents the effects of octreotide on splanchnic hemodynamics. Portal venous inflow (PVI) of the IHPH and PHPH rats was significantly increased as compared with that of the unmodeled/untreated normal rats, indicating hyperhemodynamics in portal hypertension. Octreotide markedly reduced PVI in both the IHPH and PHPH rats (*P* < 0.05). Splanchnic vascular resistance (SVR) in the IHPH and PHPH rats was much lower than that in unmodeled/untreated normal rats (*P* < 0.05), and octreotide increased SVR in both; however, the changes in SVR in the IHPH rats were not significantly different from the SVR in the unmodeled/untreated normal rats while the SVR in the PHPH rats was still lower than that of unmodeled/untreated normal ones. Portal venous resistance (PVR) was significantly increased in the IHPH rats (*P* < 0.05) but showed no change in the PHPH rats, fitting with the observation of the magnitude of portosystemic shunt (PSS) being much higher in the PHPH rats than in the IHPH rats. However, FPP of the PHPH rats was still higher than that of the unmodeled/untreated normal rats, possibly related to both the increased PVI and collateral resistance observed in the PHPH rats. The octreotide treatment led to significantly increased PVR in the PHPH rats (*P* < 0.05). PSS was 34.29% ± 11.72% and 94.4% ± 1.3% in the IHPH and PHPH rats, respectively. The magnitude of PSS was much greater in the PHPH rats than in the IHPH rats. However, the octreotide treatment produced no effects on PSS in either the IHPH or the PHPH rats.

### Effects of octreotide on splanchnic blood flow

Table 2 presents the effects of octreotide treatment on splanchnic blood flow. Blood flow of the stomach, small and large intestines, mesentery and pancreas was markedly increased in both the IHPH and PHPH rats as compared with the unmodeled/untreated normal rats. Furthermore, the splanchnic blood flow was increased to a greater extent in the PHPH rats than in the IHPH rats. Due to splenomegaly in the IHPH and PHPH rats, the blood flow/1 g spleen weight was not significantly different in the IHPH rats, the PHPH rats and the unmodeled/untreated normal rats, and the hepatic artery flow (HAF)/1 g liver weight in the IHPH rats was lower than that in the unmodeled/untreated normal rats (*P* < 0.05). In the PHPH rats, HAF was greater than that in the unmodeled/untreated normal rats because of the hepatotrophy in the former. The ratio of liver weight to body weight in the PHPH rats was only decreased by 5.2% when compared with that of the unmodeled/untreated normal rats (2.40% ± 0.21% vs 2.53% ± 0.11%). However, the HAF/1 g liver weight was increased by 70.7% in PHPH rats when compared with that in the unmodeled/untreated normal rats (0.99

**Table 2** Effects of octreotide on splanchnic blood flow (mL•g•min)

	Normal Cont	IHPH Cont	IHPH Thera	PHPH Cont	PHPH Thera
Stomach	0.59 ± 0.1	0.76 ± 0.2 <sup>a</sup>	0.59 ± 0.1 <sup>c</sup>	0.71 ± 0.1 <sup>a</sup>	0.53 ± 0.1 <sup>c</sup>
Intestine and mesentery	1.36 ± 0.1	1.65 ± 0.1 <sup>a</sup>	1.11 ± 0.1 <sup>bc</sup>	1.83 ± 0.2 <sup>a</sup>	1.21 ± 0.1 <sup>bc</sup>
Pancreas	0.84 ± 0.1	0.97 ± 0.1 <sup>a</sup>	0.62 ± 0.1 <sup>c</sup>	1.27 ± 0.2 <sup>a</sup>	1.01 ± 0.2 <sup>c</sup>
Spleen	0.98 ± 0.4	0.78 ± 0.2	0.46 ± 0.2 <sup>bc</sup>	0.96 ± 0.2	0.72 ± 0.2 <sup>c</sup>
Liver	0.58 ± 0.1	0.38 ± 0.1 <sup>a</sup>	0.28 ± 0.1 <sup>bc</sup>	0.99 ± 0.1 <sup>bc</sup>	0.62 ± 0.1 <sup>c</sup>

<sup>a</sup>*P* < 0.05 vs unmodeled/untreated normal controls; <sup>b</sup>*P* < 0.05 vs control group; <sup>c</sup>*P* < 0.05 vs the IHPH control group.

**Table 3** Effects of octreotide on plasma glucagon levels (ng/L)

	Peripheral Vein	Portal Vein
Normal Cont	19.28 ± 2.5	44.25 ± 6.1
IHPH Cont	49.37 ± 10.6 <sup>a</sup>	180.25 ± 31.2 <sup>a</sup>
IHPH Thera	40.12 ± 14.6 <sup>a</sup>	140.96 ± 41.9 <sup>a</sup>
PHPH Cont	69.82 ± 17.2 <sup>a</sup>	143.60 ± 25.6 <sup>a</sup>
PHPH Thera	50.08 ± 7.6 <sup>a</sup>	106.33 ± 37.9 <sup>a</sup>

<sup>a</sup>*P* < 0.05 vs unmodeled/untreated normal controls.

± 0.14 mL vs 0.58 ± 0.12 mL). These results indicate that, due to the decrease of portal blood flow, HAF compensatorily increased in the PHPH rats. The octreotide treatment significantly decreased PVI in both the IHPH and PHPH rats, and decreased the HAF/1 g liver weight by 26.3% in the IHPH rats (0.28 ± 0.1 mL vs 0.38 ± 0.7 mL) and by 37.4% in the PHPH rats (0.99 ± 0.14 mL vs 0.62 ± 0.14 mL).

#### Effects of octreotide on the concentration of plasma glucagon

Table 3 presents the effects of octreotide on the concentrations of plasma glucagon. The IHPH and PHPH rats showed significantly higher concentrations of plasma glucagon in both the peripheral and portal veins as compared with that in the unmodeled/untreated normal rats (*P* < 0.05), indicating hyperglucagonemia as part of

the portal hypertension condition. However, the octreotide-induced decrease in plasma glucagon levels in the peripheral and portal veins did not reach statistical significance for either the IHPH rats or the PHPH rats.

In summary, the results of the present study demonstrate that octreotide significantly decreases PVI and HAF and increases SVR and PVR in rats with IHPH and PHPH. However, the octreotide-induced reduction in plasma glucagon concentrations in both the peripheral and portal veins did not reach statistical significance. These data suggest that the reduction of splanchnic blood flow produced by octreotide predominantly results from direct vasoconstriction, whereas the decrease of plasma glucagon levels is less important. However, the octreotide treatment was unable to produce a decrease in either PVI or FPP to normal levels in the portal hypertensive rat models, indicating that other factors in addition to glucagon may be responsible for the increased splanchnic blood flow in portal hypertension.

#### REFERENCES

- 1 **Morgan JS**, Groszmann RJ. Somatostatin in portal hypertension. *Dig Dis Sci* 1989; **34**: 405-475 [DOI: 10.1007/BF01536044 PMID: 2563965]
- 2 **Zang DL**, Zheng DS, Huang DJ, Huang MX, Yuan JM. Measuring the regional blood flow with a 51aaCr labelled formalized toad red blood cell. *Zhonghua Xinxueguanbing Zazhi* 1986, **14**: 243-246

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Overproduction of nitric oxide inhibits vascular reactivity in portal hypertensive rats

Xi-Ru Li, Jin-Sheng Wu, Ze-Sheng He, Qing-Jiu Ma, De-Ming Gao

Xi-Ru Li, Jin-Sheng Wu, Ze-Sheng He, Qing-Jiu Ma, De-Ming Gao, Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, Shaanxi Province, China

Xi-Ru Li, male, born on 1966-01-15 in Liuyang City, Hunan Province, Han nationality, graduated from the First Military Medical University in 1989, currently Surgeon in Charge, Master of General Surgery, having 5 papers published.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Xi-Ru Li, Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, Shaanxi Province, China

Telephone: +86-29-3524578-77131

Fax: +86-29-3513140-77199

Received: November 2, 1996

Revised: January 25, 1997

Accepted: March 5, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To evaluate the relationship between nitric oxide (NO) and hyperdynamic circulatory status in portal hypertension.

**METHODS:** Twenty male Sprague Dawley rats (weighing  $200 \pm 20$  g) were randomized into two groups: portal hypertension group ( $n = 12$ ) and sham-operated control group ( $n = 8$ ). The portal hypertensive model was established by means of graded constriction of the portal vein. The concentrations of nitrite ( $\text{NO}_2^-$ ) in the portal vein and peripheral blood were measured by fluorometric assay to reflect NO levels. The reactivity of isolated abdominal aortic rings from rats with partial portal vein constriction and controls was determined by assessing response to administration of potassium chloride (KCl) ( $10\text{--}80$  mmol/L) and phenylephrine ( $10^{-9}\text{--}10^{-4}$  mol/L) with or without preincubation with NO synthase inhibitor N $\omega$ -nitro-L-arginine (L-NNA).

**RESULTS:** Serum concentrations of  $\text{NO}_2^-$  in the portal vein blood ( $0.766 \pm 0.097$   $\mu\text{mol/L}$ ) and peripheral blood ( $0.687 \pm 0.092$   $\mu\text{mol/L}$ ) were elevated in portal hypertensive rats, as compared with the concentrations in controls ( $0.613 \pm 0.084$   $\mu\text{mol/L}$  and  $0.591 \pm 0.045$   $\mu\text{mol/L}$  respectively, both  $P < 0.01$ ). In addition, the rates of  $\text{NO}_2^-$  in portal vein blood were markedly higher than those in peripheral blood ( $P < 0.05$ ) in the portal hypertensive rats. Abdominal aortic rings from rats with portal vein constriction exhibited significantly impaired contractility to phenylephrine and KCl, as compared with the control rats. The  $\text{EC}_{50}$  values of KCl were markedly higher in the portal hypertensive rings ( $26.5 \pm 0.9$  mmol/L) than in the control rings ( $22.3 \pm 1.7$  mmol/L,  $P < 0.01$ ), as were the  $\text{EC}_{50}$  values of

phenylephrine ( $37.2 \pm 0.4$  nmol/L vs control rings:  $28.1 \pm 0.2$  nmol/L,  $P < 0.01$ ). After preincubation of rings with L-NNA, the difference in  $\text{EC}_{50}$  values between portal hypertensive and control rings was no longer statistically significant for either KCl ( $20.18 \pm 0.8$  mmol/L vs  $19.4 \pm 1.2$  mmol/L,  $P > 0.05$ ) or phenylephrine ( $22.4 \pm 1.8$  nmol/L vs  $21.8 \pm 1.4$  nmol/L,  $P > 0.05$ ). However, the maximal concentrations of KCl and phenylephrine for inducing contractions were still significantly lower in the portal hypertensive rings ( $1.08 \pm 0.1$  g and  $1.43 \pm 0.14$  g) than in the control rings ( $1.21 \pm 0.11$  g and  $1.72 \pm 0.11$  g respectively, both  $P < 0.05$ ). Thus, addition of the NO synthase inhibitor L-NNA could partially restore contractile responses to KCl and phenylephrine in portal hypertensive rings.

**CONCLUSION:** NO overproduction inhibits the vascular reactivity to vasoconstrictors, and it might be one of the main causes of vasodilatation and hyperdynamic circulatory status in portal hypertension.

**Key words:** Portal hypertension; Nitric oxide; Vascular reactivity; Hyperdynamic circulatory status; Vasodilatation

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Li XR, Wu JS, He ZS, Ma QJ, Gao DM. Overproduction of nitric oxide inhibits vascular reactivity in portal hypertensive rats. *World J Gastroenterol* 1997; 3(4): 221-224 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/221.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.221>

### INTRODUCTION

Mechanisms of portal hypertension are complicated. In addition, circulatory abnormalities may affect the portal venous systemic circulation regardless of the presence or absence of defective liver structure and function. Hyperdynamic circulation can perpetuate and aggravate portal hypertension, possibly resulting in a series of grave cardiovascular and non-cardiovascular complications<sup>[1]</sup>. In recent years, nitric oxide (NO), a newly identified neurotransmitter, cell-killing factor and cellular messenger molecule, has attracted the attention of biologists and medical workers. Evidence has shown that NO plays an important role in cardiovascular function and is involved in the physiology of vascular tone, blood flow velocity and vascular resistance. Recent studies have also indicated that NO is involved in the pathophysiology of portal hypertension<sup>[2]</sup>.

In order to evaluate the relationship between NO and hyperdynamic circulation in portal hypertension, we used rats with partial portal vein constriction and rats that were sham-operated (controls) to investigate the reactivity of isolated vascular rings to potassium chloride (KCl) and to phenylephrine with or without pre-exposure to the NO synthetic inhibitor N $\omega$ -nitro-L-arginine (L-NNA). We determined that the serum concentrations of  $\text{NO}_2^-$  detected by

**Table 1** Baseline values in rats with portal hypertension and sham-operated control rats

Parameter	Portal hypertension ( <i>n</i> = 11)	Control ( <i>n</i> = 8)
Body weight (g)	208 ± 17	201 ± 21
PVP (kPa)	1.644 ± 0.142 <sup>b</sup>	1.034 ± 0.113
Serum Na <sup>+</sup> (mmol/L)	135 ± 1 <sup>b</sup>	141 ± 2
Serum K <sup>+</sup> (mmol/L)	4.1 ± 0.2	4.3 ± 0.2
ALT (U/L)	147.6 ± 38.4	141.7 ± 53.3
Albumin (g/L)	16.43 ± 4.12	17.21 ± 3.84
A/G	0.64 ± 0.04	0.66 ± 0.08

<sup>b</sup>*P* < 0.01 vs control.

fluorometric analysis reflect NO levels. The results of this study provide new insights that may help towards developing new treatments of portal hypertension.

## MATERIALS AND METHODS

### Animal model

Twenty male Sprague-Dawley rats (weighing 200 ± 20 g) were randomized into two groups: the portal hypertension model group (*n* = 12) and the sham-operated control group (*n* = 8). The portal hypertensive model was established by means of graded constriction of the portal vein. Specifically, after the rats were anesthetized with ketamine hydrochloride (0.15 mg/100 g, intra-abdominal), the portal vein was isolated and a stenosis was created by means of a single ligature (3-0 silk) placed around both the portal vein trunk and a needle (blunt, external diameter 0.8 mm). Removal of the needle facilitated production of a consistent extrahepatic portal hypertension. The sham operation consisted of dissection and visual inspection of the portal vein.

### Sample preparation

Two weeks after the surgeries, the animals were re-anesthetized and portal venous pressure was measured. Blood samples from the portal vein and abdominal vein (2.0-3.0 mL) were obtained by direct puncture and centrifuged at 3000 rpm for 10 min to separate the serum. The serum samples were then stored at -40 °C for subsequent detection of NO<sub>2</sub><sup>-</sup>. Whole blood samples (1.5 mL) were also obtained for other assays to detect serum electrolytes and markers of liver function. The abdominal aorta was dissected, placed in oxygenated Krebs solution, stripped of all fatty tissues and adventitia, and then cut into 3 mm rings. The length and external diameter of which were measured.

### Observation of vascular reactivity

The prepared rings from both groups of animals were mounted horizontally between two stainless-steel stirrups in individual organ bath chambers filled with 10 mL of modified Krebs solution (mmol/L concentrations: NaCl 118.3, KCl 14.7, NaHCO<sub>3</sub> 25, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.5, glucose 11.1, and EDTA 0.026, pH 7.4) and constantly bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 37 °C. After 60 min of equilibration, the rings were allowed to adjust gradually with 30 mmol/L KCl until the optimal resting tension was obtained. All the experiments were performed on the rings in the optimal resting tension, which was approximately 1.5 g. The contraction responsiveness to cumulative additions of KCl (10, 20, 30, 40, 50, 60, 70 and 80 mmol/L) and phenylephrine (10<sup>-9</sup>, 10<sup>-8</sup>, 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> and 10<sup>-4</sup> mol/L) were measured with force displacement transducers (JZ-BK) and recorded with a multichannel paper recorder (XWTD464). The rings were then washed and equilibrated for 30 min, and then preincubated for 30 min with 10<sup>-5</sup> mol/L of the specific NO biosynthesis inhibitor L-NNA. Contraction responses were evoked and measured with the range of cumulative concentrations of KCl and phenylephrine listed above in the presence of L-NNA. At the end of the experiments, the rings were weighed.

### Quantification of NO<sub>2</sub><sup>-</sup>

In principle, NO<sub>2</sub><sup>-</sup> can react with 2, 3-diaminonathalen (DAN) in acid solution and produce a fluorescent substance, the intensity of which

**Table 2** Serum levels of NO<sub>2</sub><sup>-</sup> in rats with portal hypertension and sham-operated control rats ( $\bar{x} \pm s$ , × 10<sup>-6</sup> mol/L)

Group	<i>n</i>	Portal vein	Peripheral vein
PH	11	0.766 ± 0.097 <sup>ac</sup>	0.687 ± 0.092 <sup>a</sup>
Control	8	0.613 ± 0.084	0.591 ± 0.045

<sup>a</sup>*P* < 0.05 vs control; <sup>c</sup>*P* < 0.05 vs peripheral vein blood.**Table 3** Physical characteristics of aortic rings from rats with portal hypertension and sham-operated control rats ( $\bar{x} \pm s$ )

Parameter	Portal hypertension	Control
ED (mm)	1.51 ± 0.04	1.49 ± 0.03
Length (mm)	3.09 ± 0.04	3.05 ± 0.07
Weight (mg)	1.59 ± 0.08	1.54 ± 0.08

ED: External diameter.

can be measured by a spectrofluorometer and used to indicate NONO<sub>2</sub> content. To quantitate NO<sub>2</sub><sup>-</sup>, we first made a standard curve by putting 100 μL aliquots of 0, 3.2 × 10<sup>-7</sup>, 6.4 × 10<sup>-7</sup>, 1.28 × 10<sup>-6</sup>, 1.92 × 10<sup>-6</sup>, 2.56 × 10<sup>-6</sup>, 3.2 × 10<sup>-6</sup> and 3.84 × 10<sup>-6</sup>-mol/L NaNO<sub>2</sub> into 5 mL test tubes, and then adding 10 μL of DAN-HCl solution, mixing well and allowing to react for 10 min at 20 °C. The reaction was stopped by adding 5 μL of 2.8 mol/L NaOH. Double-distilled water was then added to bring the final volume up to 3 mL, and the fluorescence intensity was measured at Ex365nm, Em420nm (RF-5000 Spectrofluorometer, Japan). Finally, the standard curve of fluorescence intensity versus the concentration of NaNO<sub>2</sub> was generated. To measurement the concentration of serum NO<sub>2</sub><sup>-</sup>, 200 μL aliquots of serum were placed in test tubes and mixed with 200 μL chloroform. After standing for 5 min, each tube was centrifuged for 15 min at 3500 r/min. The resultant supernatant was then mixed with 20% of its volume of 20% sodium sulfosalicylate to remove proteins. After centrifuging for 15 min at 3500 r/min, 100 μL of the resultant supernatant was mixed with 50 μL of 0.01 mol/L EDTA and then 40 μL of DAN-HCl solution was added. After reacting at 20°C for 10 min, 5 μL of 2.8 mol/L NaOH was added to stop the reaction. Double-distilled water was added up to a final volume of 3 mL, and the fluorescence intensity was measured. The concentration of NO<sub>2</sub><sup>-</sup> was calculated from the standard curve.

### Statistical analysis

Dose-response contraction curves were made with the experimental data. The EC<sup>50</sup> values and the Rmax were calculated from each dose-response contraction curve by means of a non-linear regression method with a curve-fitting program. Rmax and EC<sup>50</sup> values were averaged and the results were assessed statistically using either the paired or unpaired Student's t-test as appropriate. The data of NO<sub>2</sub><sup>-</sup> were analyzed with Student's t-test, and the results are presented as mean ± SEM. Statistical significance was set at a *P* level of 0.05 or less.

## RESULTS

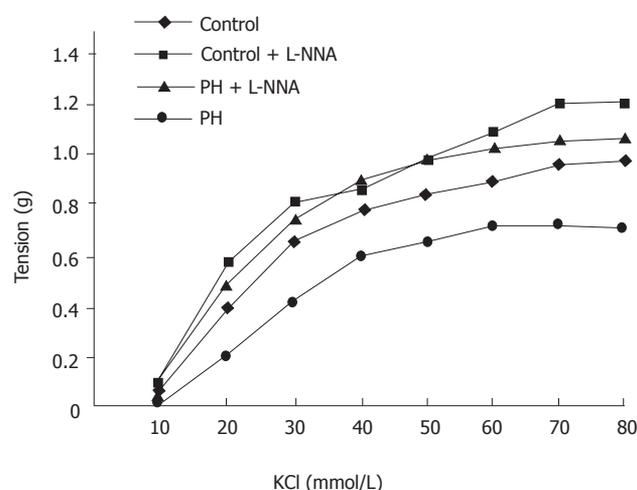
All the animals survived the modeling and sham operations and subsequent experimental course, except for one. No rats in the control or portal hypertensive model group showed any appreciable alteration in hepatic histology. Body weight, age, serum electrolytes, liver function and portal venous pressure are shown in Table 1. No differences were found between the partial portal vein constricted and control rats for the parameters of body weight, age, serum potassium, alanine aminotransferase (ALT) and albumin. However, compared with the controls, the portal hypertensive rats showed marked hyponatremia and elevated portal venous pressure (both *P* < 0.01).

Serum NO<sub>2</sub><sup>-</sup> levels are shown in Table 2. Serum concentrations of NO<sub>2</sub><sup>-</sup> in portal vein blood (0.766 ± 0.097 μmol/L) and peripheral blood (0.687 ± 0.092 μmol/L) were markedly elevated in the portal hypertensive rats, as compared with the levels in controls (0.613 ± 0.084 μmol/L and 0.591 ± 0.045 μmol/L respectively, *P* < 0.05). The level of NO<sub>2</sub><sup>-</sup> in portal vein blood of portal hypertensive rats

**Table 4** Rmax and EC<sup>50</sup> values for KCl and phenylephrine in aortic rings from rats with portal hypertension and sham-operated control rats with and without L-NNA treatment ( $\bar{x} \pm s_x$ )

Agent	EC <sup>50</sup>		Maximal contractions (g)	
	Untreated	L-NNA	Untreated	L-NNA
KCl (mmol/L)				
PH	26.5 ± 0.9 <sup>ba</sup>	20.2 ± 0.8	0.72 ± 0.05 <sup>ba</sup>	1.08 ± 0.1 <sup>b</sup>
Control	22.3 ± 1.7 <sup>a</sup>	19.4 ± 1.2	0.98 ± 0.05 <sup>a</sup>	1.21 ± 0.11
PE (× 10 <sup>-8</sup> mol/L)				
PH	3.72 ± 0.4 <sup>ba</sup>	2.24 ± 0.18	1.04 ± 0.08 <sup>ba</sup>	1.43 ± 0.14 <sup>a</sup>
Control	2.81 ± 0.2 <sup>b</sup>	2.18 ± 0.14	1.34 ± 0.39 <sup>a</sup>	1.72 ± 0.11

<sup>b</sup>*P* < 0.01 vs control; <sup>a</sup>*P* < 0.05 vs L-NNA.



**Figure 1** Dose response contraction curves for KCl in the presence of 10<sup>-5</sup> mol/L L-NNA in control and portal hypertensive abdominal aortic rings.

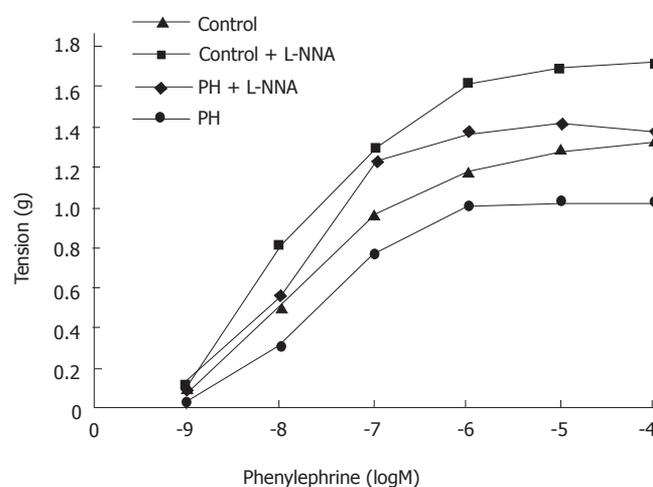
was significantly higher than that in peripheral blood (*P* < 0.05). The physical parameters of the aortic rings are shown in Table 3. The aortic rings of the portal hypertensive rats and the control rats showed no differences in external diameter, length or weight (*P* > 0.05).

#### Contractile responses of abdominal aortic rings to KCl

Contractile responses to KCl were attenuated in rings from the portal hypertensive rats, as compared with those from the control rats, as evidenced by the dose response curves being shifted to the right (Figure 1). The EC<sup>50</sup> values were markedly higher in the aortic rings from the portal hypertensive rats (26.5 ± 0.9 mmol/L) than in those of the control rats (22.3 ± 1.7 mmol/L, *P* < 0.01). Maximal contraction was significantly diminished in the aortic rings of the portal hypertensive rats (0.72 ± 0.05 g), as compared with those of the control rats (0.98 ± 0.05 g, *P* < 0.01). After preincubation of the rings with L-NNA (10<sup>-5</sup> mol/L), the KCl-induced contractile curves were shifted to the left (compared with those made in the absence of L-NNA) for both the portal hypertensive and control rings. The difference in EC<sup>50</sup> values of the aortic rings was no longer statistically significant between the portal hypertensive rats (20.18 ± 0.8 mmol/L) and the control rats (19.4 ± 1.2 mmol/L, *P* > 0.05). However, the maximal KCl-induced contractions were still lower in the aortic rings from the portal hypertensive rats (1.08 ± 0.18 g) than in those from the control rats (1.21 ± 0.11 g, *P* < 0.05) (Table 4).

#### Contractile responses of abdominal aortic rings to phenylephrine

Phenylephrine induced contractile curves were shifted to the right for the aortic rings from the portal hypertensive rats (Figure 2). The EC<sub>50</sub> values were markedly higher for the aortic rings from the portal hypertensive rats (37.2 ± 4 nmol/L) than those from the control rats (28.1 ± 2 nmol/L, *P* < 0.01). Maximal contractions were markedly lower in the aortic rings from the portal hypertensive rats (1.04 ± 0.08 g) than in those from the controls (1.34 ± 0.39 g, *P* < 0.01). After the rings were preincubated with L-NNA (10<sup>-5</sup> mol/L), the phenylephrine-induced contractile curves were shifted to the left for both portal hypertensive and control rings (Figure 2). The difference in EC<sub>50</sub> values of the aortic rings was no longer statistically significant between the portal hypertensive rats (22.4 ± 1.8 nmol/L)



**Figure 2** Dose-response contraction curves for phenylephrine in the presence of 10<sup>-5</sup> mol/L L-NNA in control and portal hypertensive abdominal aortic rings.

and the control rats (21.8 ± 1.4 nmol/L, *P* > 0.05). The maximal contractions were still lower in the portal hypertensive rings (1.43 ± 0.14 g) than in the control rings (1.72 ± 0.11 g, *P* < 0.05) (Table 4).

## DISCUSSION

NO is synthesized from L-arginine by the enzyme NO synthase (NOS). There are two different types of NOS; the constitutive from (cNOS) is dependent on calcium and calmodulin for its activity, while the inducible from (iNOS) has no calcium or calmodulin requirement. cNOS is continuously present in the internal surface of the endothelial cell membrane, but iNOS represents a newly synthesized enzyme that is expressed in response to specific stimuli of endotoxin and cytokines in serial cell types, including vascular endothelium, vascular smooth muscle cells, hepatocytes and macrophages<sup>[3]</sup>.

To date, no ideal technique is available to detect the NO content in tissues. The metabolic products of NO<sup>-3</sup>/NO<sup>-2</sup> are used to assess the NO level indirectly. The Griess reaction has been widely applied to measure NO<sup>-2</sup>, and it is relatively simple but its sensitivity is low<sup>[4]</sup>. In the present study, a new fluorometric analysis assay was developed and used to detect serum content of NO<sup>-2</sup>. Because NO<sup>-2</sup> plus 2, 3-diaminonaphthalene make a kind of fluorescent substance of 1-(H)-naphthotriazole, and the detection of the fluorescence intensity can reflect NO<sup>-2</sup> levels, the sensitivity of detection was markedly improved. The results of this study showed that serum levels of NO<sup>-2</sup>, especially those in portal vein blood, were elevated in rats with partial portal vein constriction, as compared with those in sham-operated controls. These findings indicated that there was an increased production of NO in portal hypertensive rats.

NO is an important regulator of systemic and local hemodynamics in physiological conditions, and is involved in the physiology of vascular tone, blood flow, velocity and vascular resistance. The mechanisms underlying these processes involve NO diffusion into the vascular smooth muscle cell, where it activates soluble guanylate cyclase and generates a subsequent increase in cGMP to cause relaxation of the vascular smooth muscle by binding of free intracellular calcium. Patients and experimental animals with portal hypertension reportedly present with hyperdynamic circulatory dysfunction characterized by splanchnic and systemic

arterial vasodilation<sup>[5]</sup>. The pathogenesis of arterial vasodilation correlated with NO overproduction in portal hypertension. These findings collectively suggest that NO is an important mechanism of the hyperkinetic circulation associated with prehepatic portal hypertension.

L-NNA is an L-arginine analog, and competitively inhibits the activity of both iNOS and cNOS, subsequently attenuating NO release<sup>[6]</sup>. Our study found reduced contractile responses to both KCl and phenylephrine in abdominal aortic rings from partial portal vein constricted rats as compared with those from the sham-operated controls. Addition of the NOS inhibitor L-NNA ( $10^{-5}$  mol/L) significantly increased the contractility to KCl and phenylephrine in both the control and portal hypertensive rings, but the contractile responses increased proportionally more in the portal hypertensive aortic rings than in the control rings in the presence of L-NNA. However, the difference in EC<sub>50</sub> values between the portal hypertensive and the control groups lost significance. These results suggest that vascular endothelium produces and releases some NO to maintain vasodilation under normal circumstances, while overproduction of NO in portal hypertensive vascular endothelium inhibits the vascular contractile response to KCl and phenylephrine. However, although the maximal KCl and phenylephrine-induced contractions were increased in rings from both portal hypertensive rats and control rats in the presence of L-NNA, the maximal contractions were still significantly lower in the portal hypertensive rings than those of the control rings. Thus, other factors, such as alteration in vascular smooth muscle, may play an important role in the pathogenesis of vasodilation and hyperdynamic circulation in portal hypertension.

The mechanism of increased vascular production of NO in rats with partial portal vein constriction cannot be shown from this study. The likely explanation is up-regulation of cNOS in the vascular endothelium, because iNOS is not expressed in isolated vessels which are not able to be stimulated by endotoxin and cytokines. In fact, increased cNOS activity has been reported by measuring NOS activity directly in homogenized blood vessels

in animal models of portal hypertension with and without cirrhosis<sup>[7,8]</sup>. Whether the up-regulation of cNOS and subsequent NO overproduction is a primary or secondary event in the arteriolar vasodilation in portal hypertension could not be ascertained. Shear stress has been shown to be a powerful stimuli of cNOS<sup>[3]</sup>; therefore, overproduction of NO could be consequent to shear stress and other factors related to the hyperkinetic circulation. NO overproduction by endothelial cells would amplify and perpetuate arteriolar vasodilation. The effective inhibition of cNOS may reverse hyperdynamic circulation.

## ACKNOWLEDGEMENTS

We are indebted to Dr. Ma Jin for his skillful technical assistance.

## REFERENCES

- 1 **Groszmann RJ**. Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. *Hepatology* 1994; **20**: 1359-1363 [PMID: 7927273 DOI: 10.1002/hep.1840200538]
- 2 **Bomzon A**, Blendis LM. The nitric oxide hypothesis and the hyperdynamic circulation in cirrhosis. *Hepatology* 1994; **20**: 1343-1350 [PMID: 7927270 DOI: 10.1002/hep.1840200535]
- 3 **Moncada S**, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43**: 109-142 [PMID: 1852778]
- 4 **Wang W**, Keller K, Chadee K. Entamoeba histolytica modulates the nitric oxide synthase gene and nitric oxide production by macrophages for cytotoxicity against amoebae and tumour cells. *Immunology* 1994; **83**: 601-610 [PMID: 7533135]
- 5 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157 [PMID: 2971015 DOI: 10.1002/hep.1840080532]
- 6 **Ishii K**, Chang B, Kerwin JF, Huang ZJ, Murad F. N omega-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur J Pharmacol* 1990; **176**: 219-223 [PMID: 2155799]
- 7 **Cahill PA**, Wu YP, Sitzmann JV. Nitric oxide synthase activity in portal hypertension. *Hepatology* 1993; **18**: 141A
- 8 **Ros J**, Jimenez W, Lamas S, Claria J, Arroyo V, Rodes J. Nitric oxide synthase activity in aortic segments of rats with cirrhosis and ascites. *J Hepatol* 1993; **18**(Suppl 1): 14S

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Plasma D (-)-lactate as a new marker for diagnosis of acute intestinal injury following ischemia-reperfusion

Yong-Ming Yao, Yan Yu, Ye Wu, Lian-Rong Lu, Zhi-Yong Sheng

Yong-Ming Yao, Yan Yu, Ye Wu, Lian-Rong Lu, Zhi-Yong Sheng, Department of Immunology, Burn Research Institute, Chinese PLA 304<sup>th</sup> Hospital, Beijing 100037, China

Ye Wu, Department of Surgery, Chinese PLA 223<sup>rd</sup> Hospital, Yanji 133000, Jilin Province, China

Yong-Ming Yao, was granted post doctor certification in 1995, Austria, Member of the New York Academy of Sciences, European Shock Society, and International Society for Burn Injuries, having 58 papers in Chinese and 51 articles in English published.

Author contributions: All authors contributed equally to the work.

Supported by Grants from the Medical Science Council of Chinese PLA (No. 96Q116) and from the Lorenz Boehler Fund, Austria

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Yong-Ming Yao, Department of Immunology, Burn Research Institute, Chinese PLA 304<sup>th</sup> Hospital, Beijing 100037, China  
Telephone: +86-10-66813129-41394

Received: March 6, 1997

Revised: May 8, 1997

Accepted: June 23, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To observe the kinetics of D (-)-lactate alteration in both portal and systemic circulation systems, and its relationship with intestinal injury in rats subjected to acute intestinal ischemia-reperfusion.

**METHODS:** Anesthetized rats underwent a 75-min superior mesenteric artery occlusion followed by a 6-h reperfusion. Plasma D (-)-lactate levels were measured by an enzymatic spectrophotometric assay.

**RESULTS:** Intestinal ischemia for 75 min resulted in a significant elevation of D (-)-lactate levels in the portal vein, as compared with the baseline values ( $P < 0.05$ ). Plasma D (-)-lactate levels had a tendency to further increase after reperfusion, up to 6 h. Similar alterations in D (-)-lactate were also found in systemic circulation, and there were no significant differences between the portal and systemic circulations at any time point. Moreover, the macropathological evaluation scores were significantly correlated to the portal D (-)-lactate levels in animals at various time points ( $r = 0.415$ ,  $P < 0.01$ ). In addition, there was a remarkable rise of endotoxin concentration within the portal vein at the end of the 75-min ischemic period ( $P < 0.05$ ), reaching a peak at 2 h post-reperfusion.

**CONCLUSION:** Acute intestinal ischemia is associated with failure of

the mucosal barrier resulting in increased plasma D (-)-lactate levels in both portal and systemic blood. The subsequent reperfusion might further increase D (-)-lactate levels, which are correlated to the macropathological alterations. Plasma D (-)-lactate may be a useful marker of intestinal injury following both ischemia and reperfusion insults.

**Key words:** D (-)lactate; Endotoxin; Intestinal injury; Reperfusion injury

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Yao YM, Yu Y, Wu Y, Lu LR, Sheng ZY. Plasma D (-)-lactate as a new marker for diagnosis of acute intestinal injury following ischemia-reperfusion. *World J Gastroenterol* 1997; 3(4): 225-227 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/225.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.225>

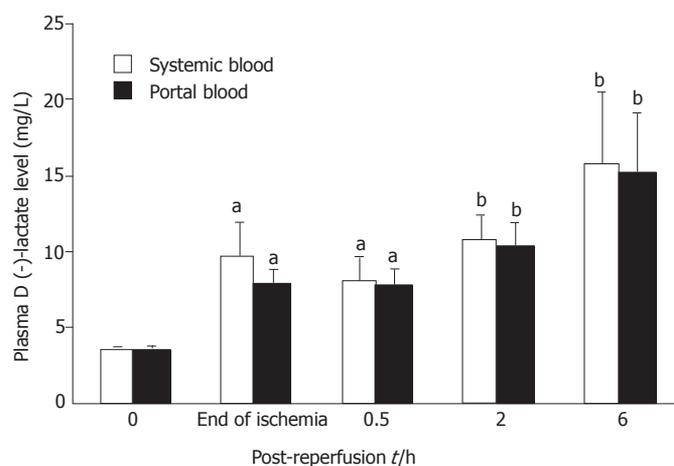
### INTRODUCTION

Major trauma and shock may initiate a cascade of events leading to sepsis with subsequent multiple organ failure. Gut mucosal barrier dysfunction is assumed to play an important role in causing the septic process<sup>[1]</sup>. From our and other studies, it has been suggested that enteric organisms and/or their toxins might translocate across the intestinal mucosa, entering the systemic circulation *via* the lymphatic or portal systems and resulting in the development of remote organ damage in animals and patients with shock following hemorrhage, trauma, or burns<sup>[1-3]</sup>.

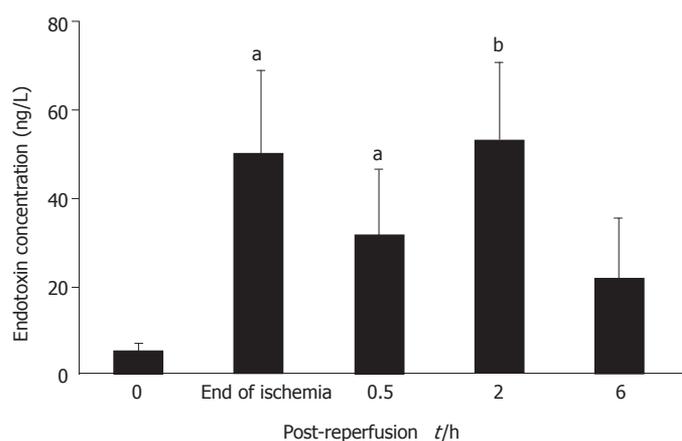
The intestine is one of the most sensitive tissues to ischemia-reperfusion injury. Reperfusion of the intestine is often associated with increased mucosal permeability and ulceration. Mucosal injury has been assessed either morphologically or by measuring the permeability of the mucosal barrier to small or large solutes; however, there exists no reliable serum marker for the early diagnosis of acute intestinal insult in clinical practice.

D (-)-lactate is produced by indigenous bacteria found in the gastrointestinal tract, and mammals do not possess the enzyme systems to rapidly metabolize it<sup>[4]</sup>. Therefore, an increase in D (-)-lactate might reflect an efflux of bacteria and/or their products into circulation as a result of mucosal injury. Recently, a report was published showing that serum D (-)-lactate was increased in animal models of acute intestinal ischemia and simple obstruction<sup>[5]</sup>; yet, it remains unclear whether this process is associated with a significant dysfunction in mucosal barrier following reperfusion.

The present study was designed to determine the kinetics of plasma D (-)-lactate changes, and to examine whether D (-)-lactate levels are correlated to intestinal damage in rats caused by acute intestinal ischemia-reperfusion injury.



**Figure 1** Kinetics of plasma D (-)-lactate alteration in portal and systemic circulation systems. Six to nine animals were assessed for each time point. Data are expressed as  $\bar{x} \pm s_x$ . <sup>a</sup> $P < 0.05$  and <sup>b</sup> $P < 0.01$  vs baseline values.



**Figure 3** Changes in plasma endotoxin concentration in portal vein following acute intestinal ischemia-reperfusion injury. Six to nine animals were assessed for each time point. Data are expressed as  $\bar{x} \pm s_x$ . <sup>a</sup> $P < 0.05$  and <sup>b</sup> $P < 0.01$  vs baseline values.

## MATERIALS AND METHODS

### Animal model

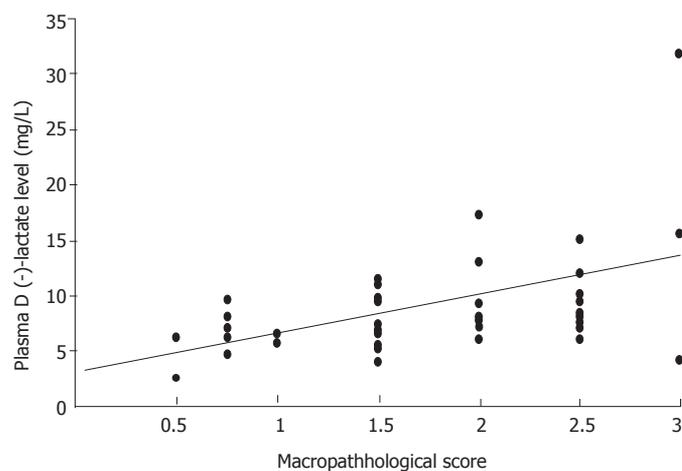
Adult male Sprague-Dawley rats purchased from Charles River Wiga GmbH (Sulzfeld, Germany) were used in this study. Following anesthesia (ketamine/xylazine: 112/15 mg/kg), the right femoral artery was cannulated under aseptic conditions using a polyethylene catheter connected to a blood pressure monitor. After a midline laparotomy was performed, the superior mesenteric artery (SMA) was isolated from the surrounding connective tissues near its aortic origin. The SMA was then occluded with an atraumatic clip applied to the root of the mesentery. After 75 min of ischemia, the arterial clip was removed, allowing reperfusion. Reperfusion was confirmed by the return of pulsations to the mesenteric vascular arcade<sup>[6]</sup>. The laparotomy incision was closed, and anesthetized animals were monitored during an additional 6 h of reperfusion.

### Study design

Six to nine animals were sacrificed at each of following time points: before occlusion, at end of ischemia, and at 0.5 h, 2 h and 6 h after release of the clamp. Portal blood samples were obtained by portal vein puncture before the animals were sacrificed at each time point, and the collection was limited to once per animal to avoid potential contamination. Plasma was separated by centrifugation and stored at  $-30^{\circ}\text{C}$  until analysis.

### Plasma D (-)-lactate measurement

Plasma D (-)-lactate levels were measured by an enzymatic spectrophotometric assay with slight modification using a centrifugal analyzer (Cobas-Fara)<sup>[7]</sup>. For analysis, the supernatant plasma without deproteinization was added to tubes and mixed with carbonate buffer containing 4.6 mmol/L of  $\text{NAD}^+$ . The reaction was initiated by adding 50  $\mu\text{L}$  of D-lactic dehydrogenase (D-LDH; Sigma Chemical Co., St. Louis, MO, USA) to each tube and the absorbance



**Figure 2** Correlation between the macropathological evaluation scores of intestine and the portal D (-)-lactate levels in animals subjected to intestinal ischemia-reperfusion injury.

change at 340 nm was measured against the reagent blank tubes with 50  $\mu\text{L}$  water instead of D-LDH. Serial concentrations of D-lactate (D-lactic acid; Sigma Chemical Co.), from 3.125 to 50 mg/L, were analyzed in order to prepare the standard curve.

### Plasma endotoxin determination

The endotoxin content of plasma samples was assayed by the Limulus amoebocyte lysate test with a kinetic modification of the test kit procedure<sup>[2]</sup>. The method has a detection limit of 0.75 ng/L in 1:10 plasma dilution.

### Morphological evaluation

In a blind manner, a postmortem examination was performed together with a macropathological evaluation based on a pre-established four-grade scoring system<sup>[8]</sup>. For histological examination, tissue samples from small intestine were taken and fixed in 10% buffered formalin for analysis as light microscopic sections stained with hematoxylin-eosin.

## RESULTS

As shown in Figure 1, intestinal ischemia for 75 min resulted in a significant elevation in D (-)-lactate levels in portal vein as compared with the baseline values ( $P < 0.05$ ). Plasma D (-)-lactate levels had a tendency to further increase after reperfusion, up to 6 h. Similar alterations in D (-)-lactate were also found in systemic circulation (Figure 1), there were no significant differences between the portal and systemic circulations at any time point.

The animals were found to have no abnormal changes in bowel functions before the induction of intestinal ischemia. SMA occlusion for 75 min caused marked intestinal damage, which was much worse upon reperfusion. The macropathological evaluation scores were  $2.0 \pm 0.2$ ,  $2.2 \pm 0.2$  and  $2.7 \pm 0.1$  at 0.5, 2, and 6 h after reperfusion. The macropathological evaluation scores of intestine were significantly correlated to portal D (-)-lactate levels in animals at various time points ( $r = 0.415$ ,  $P < 0.01$ ; Figure 2).

The endotoxin concentration within portal vein rose remarkably in the control animals, being 9.5-fold over the baseline values at the end of 75-min ischemia ( $P < 0.05$ ). It reached a maximum at 2 h after clamp release, and decreased gradually up to the end of the observation period (Figure 3).

## DISCUSSION

In the present experiment, we observed that ischemia-reperfusion of the small bowel resulted in a significant elevation in D (-)-lactate levels in both portal and systemic blood. This was potentially associated with failure of the intestinal mucosal barrier leading to the subsequent escape of indigenous endotoxin/bacteria to the circulation systems. Our data also suggested that an early and sustained rise in systemic D (-)-lactate might occur during mesenteric ischemia, even without further reperfusion injury<sup>[5]</sup>.

The normal gut epithelium provides an anatomical and

physiological barrier to the toxic intestinal contents. Conditions which lead to mesenteric hypoperfusion, such as those seen in hemorrhage and other shock states, have been shown to cause breakdown of the intestinal mucosal barrier<sup>[1-3,9]</sup>. In this study, the status of the mucosal barrier was assessed quantitatively using a plasma marker, namely D (-)-lactate. D (-)-lactate is strictly a product of bacterial fermentation, and it is known to be produced by many species of bacteria found in the gut flora<sup>[4,5]</sup>, while mammalian tissue does not produce D-lactic acid, which may not or only slowly be metabolized. D-lactic acid accumulating in the blood can generally be considered as a result of active bacterial metabolism due to systemic infections or some gastrointestinal disorders. In fact, D-lactate acidosis has been described in humans with short bowel syndrome, as well as bacterial infections<sup>[10]</sup>. More recently, in a clinical study, Murray *et al.*<sup>[11]</sup> demonstrated that patients found to have mesenteric ischemia at laparotomy had significantly elevated D (-)-lactate levels in peripheral blood as compared with the patients with other acute or normal abdominal conditions. Thus, circulating D (-)-lactate could aid in diagnosing acute mesenteric ischemia<sup>[5]</sup>. From the current experiment, it is clearly revealed that marked elevation in plasma D (-)-lactate occurred during the occlusive episode, which had a tendency to further increase after release of the clamp and was correlated with a progressive disintegration in the histology of the small bowel in response to reperfusion injury. Therefore, plasma D (-)-lactate appears to be a useful marker reflecting gut barrier damage in the setting of acute ischemia-reperfusion insult.

The mechanisms by which intestinal ischemia-reperfusion leads to a sustained elevation in plasma D (-)-lactate are not entirely understood from this experiment; although, it is possible that acute intestinal insult might cause substantial mucosal injury and increase permeability, thereby inducing an efflux of bacteria and their metabolic products into the portal circulation. In this study, the pathological examination showed marked intestinal lesions during ischemia, which became worse upon reperfusion. Another possible explanation would be that an increased gut permeability might attribute to release of inflammatory mediators, particularly tumor necrosis factor, which is considered to be induced by gut-derived endotoxin/bacteria, as well as hypoxia. These mediators may directly damage the mucosal barrier function<sup>[6]</sup>. In addition, it has been reported that ischemia-reperfusion injury to the intestine is associated with overgrowth of the residing microbial flora, and normally low plasma D (-)-lactate levels could be elevated markedly as a result of increased release of D-lactate by bacterial proliferation concomitant to increased mucosal permeability. Our previous observation that the overgrowth of Gram-negative bacteria in the intestinal tract was associated with an increase in intraluminal bacterial products, such as endotoxin following hemorrhage and resuscitation<sup>[3]</sup>, gives further support to this opinion. Finally, since D-lactate is not metabolized in the liver; persistent elevation of D-lactate level in both portal and systemic circulation systems following intestinal ischemia-reperfusion injury resulted in similar D

(-)-lactate concentration in the peripheral and portal vein throughout the observation period. Therefore, our data might be of potential importance, serving as the experimental basis for the favorable clinical use of D (-)-lactate in the diagnosis of acute intestinal disorders related to ischemia-reperfusion injury.

In summary, these data suggest that acute intestinal ischemia is associated with failure of the mucosal barrier resulting in increased plasma D (-)-lactate levels in both portal and systemic blood, and it is also remarkably enhanced by the subsequent reperfusion. Plasma D (-)-lactate may be a useful marker of intestinal injury following both ischemia and reperfusion insult.

## ACKNOWLEDGMENTS

The authors express their gratitude to all colleagues who have been of considerable help in the realization of this study, in particular to Dr. Zhou Bao-Tong, Dr. S Bahrami, and Dr. G Schlag.

## REFERENCES

- 1 Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. *World J Surg* 1996; **20**: 411-417 [PMID: 8662128 DOI: 10.1007/s002689900065]
- 2 Yao YM, Bahrami S, Leichtfried G, Redl H, Schlag G. Pathogenesis of hemorrhage-induced bacteria/endotoxin translocation in rats. Effects of recombinant bactericidal/permeability-increasing protein. *Ann Surg* 1995; **221**: 398-405 [PMID: 7726676 DOI: 10.1097/0000658-199504000-00011]
- 3 Yao YM, Sheng ZY, Tian HM, Wang YP, Yu Y, Fu XB, Lu LR, Wang DW, Ma YY. Gut-derived endotoxemia and multiple system organ failure following gunshot wounds combined with hemorrhagic shock: an experimental study in the dog. *J Trauma* 1995; **38**: 742-746 [PMID: 7760402 DOI: 10.1097/0005373-199505000-00011]
- 4 Smith SM, Eng RH, Bucchini F. Use of D-lactic acid measurements in the diagnosis of bacterial infections. *J Infect Dis* 1986; **154**: 658-664 [PMID: 3528318 DOI: 10.1093/infdis/154.4.658]
- 5 Murray MJ, Barbose JJ, Cobb CF. Serum D(-)-lactate levels as a predictor of acute intestinal ischemia in a rat model. *J Surg Res* 1993; **54**: 507-509 [PMID: 8361176 DOI: 10.1006/jsre.1993.1078]
- 6 Yao YM, Bahrami S, Redl H, Schlag G. Monoclonal antibody to tumor necrosis factor-alpha attenuates hemodynamic dysfunction secondary to intestinal ischemia/reperfusion in rats. *Crit Care Med* 1996; **24**: 1547-1553 [PMID: 8797630 DOI: 10.1097/00003246-199609000-00020]
- 7 Brandt RB, Siegel SA, Waters MG, Bloch MH. Spectrophotometric assay for D(-)-lactate in plasma. *Anal Biochem* 1980; **102**: 39-46 [PMID: 7356162 DOI: 10.1016/0003-2697(80)90314-0]
- 8 Schlag G, Redl H, van Vuuren CJ, Davies J. Hyperdynamic sepsis in baboons: II. Relation of organ damage to severity of sepsis evaluated by a newly developed morphological scoring system. *Circ Shock* 1992; **38**: 253-263 [PMID: 1292889]
- 9 Haglund U. Gut ischaemia. *Gut* 1994; **35**: S73-S76 [PMID: 8125397 DOI: 10.1136/gut.35.1\_Suppl.S73]
- 10 Marcos MA, Vila J, Gratacos J, Brancos MA, Jimenez de Anta MT. Determination of D-lactate concentration for rapid diagnosis of bacterial infections of body fluids. *Eur J Clin Microbiol Infect Dis* 1991; **10**: 966-969 [PMID: 1794370 DOI: 10.1007/BF02005455]
- 11 Murray MJ, Gonze MD, Nowak LR, Cobb CF. Serum D(-)-lactate levels as an aid to diagnosing acute intestinal ischemia. *Am J Surg* 1994; **167**: 575-578 [PMID: 8209931]

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Establishment and application of an experimental model of human fetal hepatocytes for investigation of the protective effects of silybin and polyporus umbellalus polysaccharides

Mao-Rong Wang, Mei-Zhao Le, Jia-Zhang Xu, Chang-Lun He

Mao-Rong Wang, Mei-Zhao Le, Jia-Zhang Xu, Chang-Lun He, Institute of Liver Diseases, 81<sup>st</sup> Hospital, PLA, Nanjing 210002, Jiangsu Province, China

Mao-Rong Wang, male, born on Dec. 17, 1962 in Fanchang, Anhui Province, graduated from the Department of Medicine at Wannan Medical College, currently Physician-in-Charge, engaged in the study of viral hepatitis, having 30 papers published.

Author contributions: All authors contributed equally to the work.

Supported by the Military Scientific Foundation for Youth Scientists, No. 91D049-0300.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Mao-Rong Wang, Institute of Liver Diseases, 81<sup>st</sup> Hospital, PLA, Nanjing 210002, Jiangsu Province, China  
Telephone: +86-25-4419662

Received: August 3, 1996  
Revised: August 26, 1996  
Accepted: September 13, 1996  
Published online: December 15, 1997

### Abstract

**AIM:** To establish a new experimental model system of human fetal hepatocytes to study the mechanisms underlying the protective effect of silybin and polyporus umbellalus polysaccharides (PSP) on the cellular ultrastructure.

**METHODS:** Human fetal hepatocytes were obtained from the liver of a human fetus that resulted from a medically necessary induced labor; the mother provided informed consent for sampling, experimental use and publication of findings. The hepatocytes were cultured and then pretreated with silybin or PSP or without either (control), after which the treated cells were exposed to CCl<sub>4</sub> for 4 h. Changes in cellular ultrastructure were observed by scanning electron microscopy and transmission electron microscopy, and changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and superoxide dismutase (SOD) were assayed.

**RESULTS:** Levels of ALT and AST were significantly decreased, and level of SOD was elevated in the two pretreatment groups following CCl<sub>4</sub> exposure, as compared to the control group. The cellular integrity and ultrastructure were well preserved in the two pretreatment groups but were seriously damaged in the control group.

**CONCLUSION:** The CCl<sub>4</sub>-induced hepatotoxic cell model system of human fetal hepatocytes is an effective tool for studying the hepatoprotective effect of drugs and may be applicable for studies to

screen medicines for treatment of hepatitis.

**Key words:** Fetal hepatocytes; Experimental model; Silybin; Polyporus umbellalus polysaccharides

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Wang MR, Le MZ, Xu JZ, He CL. Establishment and application of an experimental model of human fetal hepatocytes for investigation of the protective effects of silybin and polyporus umbellalus polysaccharides. *World J Gastroenterol* 1997; 3(4): 228-230 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/228.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.228>

### INTRODUCTION

The protective effects of drugs against hepatic injury, such as that induced by viruses or chemical substances, were traditionally studied by using animal model systems. The animals — most frequently dogs or rats — were pretreated with the drugs under investigation either by oral administration or injection and then administered hepatotoxic substances — usually CCl<sub>4</sub> or D-galactosamine<sup>[1]</sup>. The protective effects of the drug were then determined by biochemical assay of serum and the pathological changes observed in the liver. However, there are some drawbacks to the animal-based approach, including a long experimental period and complicated procedure and inconsistent results due to the inherent individual differences of the animals and animal-human interspecies differences. Effects of a drug on an animal's liver may not precisely or comprehensively reflect effects on the human liver. Moreover, the animal models are limited in their ability to indicate whether any protective effects of a drug result from a direct or an indirect action, specifically because the protective effects of a drug on liver can be produced by stimulating the humoral factors. In order to overcome each of these challenges of the animal model system approach, we established a model system based on primary culture of human fetal hepatocytes and the applied it to the study of the protective effects of silybin and polyporus umbellalus polysaccharides (PSP) on liver using scanning and transmission electron microscopy (SEM and TEM, respectively).

### MATERIALS AND METHODS

#### Isolation and culture of hepatocytes

Hepatocytes were obtained from the liver of a human fetus that had resulted from a medically necessary induced labor; the mother provided written informed consent for sampling, experimentation and publication of the related findings. The hepatocytes were isolated by means of the modified collagenase method<sup>[2]</sup>. The viability of the final parenchymal cell suspension was determined by

**Table 1** Levels of alpha-fetoprotein and transaminase in culture medium at different culture times ( $\bar{x} \pm s$ )

	<i>n</i>	Activity (U)		
		1 h	16 h	24 h
aFP/ng·L <sup>-1</sup>	3			18.3 ± 2.0
ALT/IU·L <sup>-1</sup>	3	8.2 ± 2.3	10.0 ± 1.6	12.0 ± 2.1
AST/IU·L <sup>-1</sup>	3	10.3 ± 1.6	12.4 ± 1.8	13.5 ± 1.5

**Table 2** Alanine aminotransferase and aspartate aminotransferase activity in culture medium of hepatocytes ( $\bar{x} \pm s$ )

Group	<i>n</i>	ALT activity (U)			AST activity (U)		
		1 h	4 h	16 h	1 h	4 h	16 h
Control	3	40 ± 3.1	63 ± 2.5	71 ± 3.0	108 ± 5.6	165 ± 4.5	178 ± 2.4
PSP (0.5 g·L <sup>-1</sup> )	3	48 ± 2.2	35 ± 2.5	27 ± 3.4	95 ± 2.8	126 ± 2.3	101 ± 3.1
Silybin (0.5 g·L <sup>-1</sup> )	3	40 ± 2.5	46 ± 3.0	34 ± 3.3	93 ± 3.2	143 ± 4.0	123 ± 3.8

**Table 3** Superoxide dismutase level in the culture medium of hepatocytes ( $\bar{x} \pm s$ )

Group	<i>n</i>	SOD (nmol/L)		
		1 h	4 h	16 h
Control	3	2.3 ± 0.8	3.0 ± 0.6	3.3 ± 1.2
PSP (0.5 g·L <sup>-1</sup> )	3	3.1 ± 1.8	4.8 ± 1.6	7.9 ± 2.1
Silybin (0.5 g·L <sup>-1</sup> )	3	2.4 ± 1.6	3.7 ± 1.1	5.9 ± 0.7

trypan blue exclusion assay, and found to be > 90% with a < 5% contamination of non-parenchymal cells. The cells were seeded at a density of  $1 \times 10^{12} \cdot L^{-1}$  into 24-well plates (Nuclon) and cultured at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in 2 mL RPMI-1640 (pH 7.0, Gibco). Each well was then supplemented with 4 mmol·L<sup>-1</sup>-L-glutamine, 10% fetal calf serum, penicillin (at 100 IU/mL) and streptomycin (at 100 µg/mL).

#### Determination of bioactivity of the cultured hepatocytes

The medium was exchanged at 4 h after plating, and levels of alpha-fetoprotein (aFP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the medium were measured at 1, 4 and 16 h of culture.

#### Exposure of hepatocytes to CCl<sub>4</sub>

Ten hours after the first medium change, a co-culture of silybin or PSP (or normal saline, for a control group) was started with hepatocytes at a final concentration of 0.5 g·L<sup>-1</sup>. The hepatotoxic injury was then induced at 1, 4 and 16 h by addition of 1 mol·L<sup>-1</sup> CCl<sub>4</sub> directly into the medium, with hepatocytes at a final concentration of 20 µg·L<sup>-1</sup>. After 4 h, the levels of ALT, AST and superoxide dismutase (SOD) in culture medium were measured and the cells were collected for SEM and TEM.

#### Preparation of hepatocytes for electron microscopy

The primarily cultured hepatocytes were collected for SEM and TEM observation after 4 h exposure to CCl<sub>4</sub> for the groups pretreated with silybin or PSP and the normal saline control group. The methods for the hepatocyte preparation were carried out as previously described<sup>[3]</sup>. The changes of hepatocytes were observed under scanning (Philips SEM-50) and transmission electron (Japanese H-300) microscopes.

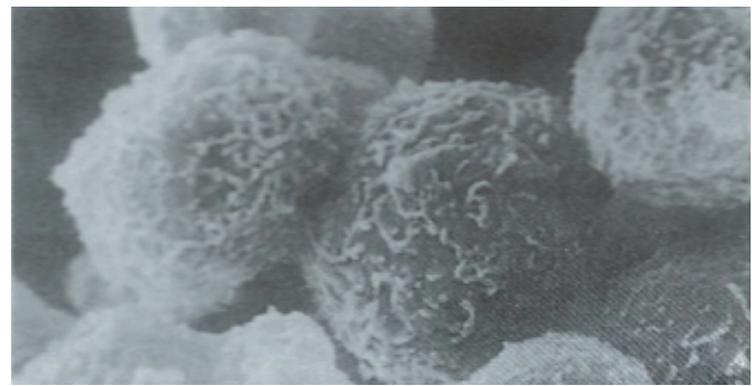
## RESULTS

#### Bioactivities of isolated hepatocytes

The isolated human fetal hepatocytes possessed good bioactivities, as evidenced by the detection of aFP in medium after 24 h of culture. The levels of ALT and AST were lower in the culture medium (Table 1) and the hepatocytes were less damaged than in the control group, and showed normal integrity of cellular membrane under SEM (Figures 1 and 2).

#### Activities of transaminases and SOD in culture medium

As shown in Tables 2 and 3, the activity of ALT and AST in the

**Figure 1** Normal human fetal hepatocytes. The cellular membrane is intact and microvilli can be clearly seen. × 7500**Figure 2** Normal human fetal hepatocytes. × 4500

medium was significantly decreased and the level of SOD was elevated in the silybin and PSP pretreatment groups as compared with the control. The effects peaked at 16 h for the cultured hepatocytes pretreated with silybin and PSP.

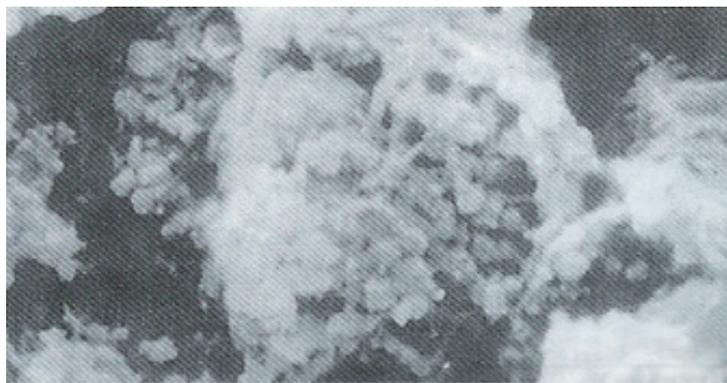
#### Ultrastructural changes of human fetal hepatocytes

Ultrastructure of the human fetal hepatocytes that had been pretreated with silybin or PSP remained well preserved following exposure to CCl<sub>4</sub>, as compared with that of the control group. The control group showed a remarkable amount of dead cells and irregularly shaped hepatocytes (Figure 3), with the cellular membranes showing mesh-like appearance. In the silybin (Figure 4) and PSP pretreatment groups (Figure 5), the surface structure of the cellular membrane was slightly damaged and the morphological integrity was indistinguishable from that of the normal fetal hepatocytes (Figure 1). In the control group, most hepatocytes showed necrosis and serious damage to the cellular membranes (Figure 6). In contrast, the hepatocytes pretreated with silybin or PSP appeared generally similar to the normal fetal hepatocytes (Figure 2), with only a few showing swelling of the mitochondria, increased chromatin, reduced glycogen granules and slight dilatation of endoplasmic reticulum (Figures 7 and 8).

## DISCUSSION

In this study, the hepatoprotective effects of silybin and PSP were demonstrated using the newly established experimental model system based on human fetal hepatocytes with toxic injury induced by CCl<sub>4</sub> exposure. In particular, pretreatment with silybin or PSP protected the ultrastructure of the hepatocytes CCl<sub>4</sub>-induced damage. Moreover, the pretreated cells showed lower transaminases and higher SOD in the culture medium. Thus, these two drugs were able to effectively protect the cultured primary human fetal hepatocytes from chemical injury of CCl<sub>4</sub>. The lower transaminase activity in the culture medium may have been due to the preserved integrity of the cellular membrane system and/or of the mitochondria. The preserved integrity itself may have been brought about by the elevated SOD, which normally acts to prevent cellular membrane damage caused by free radicals.

To date, there has been no report of an experimental model system using primary cultured human fetal hepatocytes to study



**Figure 3** Hepatocytes in the control group following exposure to CCl<sub>4</sub>. The cellular membrane appears mesh-like. × 10500



**Figure 4** Hepatocytes pretreated with silybin and exposed to CCl<sub>4</sub>. The changes of the cellular membrane are similar to Figure 5. × 8000.



**Figure 5** Hepatocytes pretreated with PSP and exposed to CCl<sub>4</sub>. The cellular membrane is intact and microvilli swelling is present. × 7500

the hepatoprotective effects of drugs using assessment by SEM and TEM. The design of this model system was thought to be reasonable. The parenchymal hepatocytes were isolated from the liver of a human fetus by using collagenase digestion, and this process provided a high purity and good bioactivity, with sensitivity to CCl<sub>4</sub>. Cell systems offer many advantages as compared to whole animal model systems. The experimental time in the cell system is shorter, the procedures are simpler, and the cost is lower. Additionally, there exists no cellular individual difference between the hepatocytes used in experimental group and control group because they are of the same origin; thus, the results are more reliable. Moreover, the results of silybin and PSP treatment of the human fetal hepatocytes in this study were similar to those observed with silybin and PSP treatment of whole animals<sup>[4,5]</sup>. Therefore, this model system will be useful for studying the effects of drugs on human liver cells.

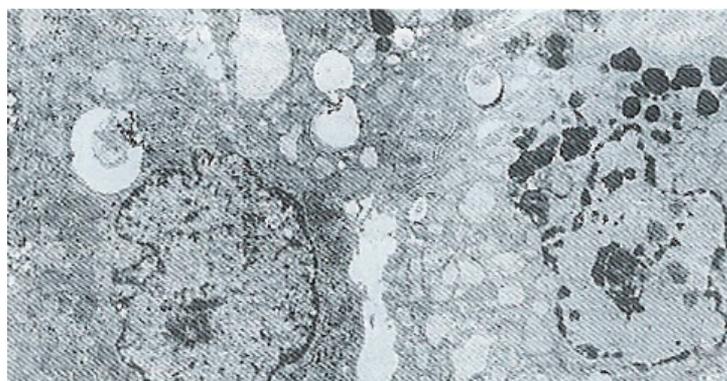
The model system established in this study could also be applied to the following studies: comparative analysis of several drugs on human hepatocytes at the same time, due to large amounts of hepatocytes being obtained from a single liver from a human fetus; study of the mechanisms and the localization of the action of medicines against hepatitis by observation of the ultrastructural changes of hepatocytes combined with biochemical



**Figure 6** Hepatocytes in the non-pretreated control group after exposure to CCl<sub>4</sub>. × 4500



**Figure 7** Hepatocytes in the PSP pretreatment group. The ultrastructure is well preserved. × 4500



**Figure 8** Hepatocytes in the silybin pretreatment group. The changes are similar to those in Figure 7. × 4500.

and enzymological changes in culture medium; and screening of new drugs against viral hepatitis or alcoholic hepatitis or cirrhosis<sup>[2]</sup>.

## ACKNOWLEDGMENTS

The authors thank Prof. Huang JS for providing the scanning electron microscope and Prof. Zhou XJ for the preparation of hepatocytes for transmission electron microscopy.

## REFERENCES

- 1 **Ju JL**, Xu JZ, Ding Q, Liu GZ, Zhang DQ, Wang MR. Therapeutic effects of BHGF on acute hepatic failure induced by D-galactosamine in rats. *Zhongguo Yaolixue Tongbao* 1991; **7**: 222-224
- 2 **Wang MR**, Jia KM. Umbilicus vein perfusion enhancing the purity of human fetal hepatocytes isolated by trypsin digestion. *Wannan Yixueyuan Xuebao* 1992; **11**: 153-155
- 3 **Wang MR**, Le MZ, Xu JZ, He CL, Xia TY, Wang GX, et al. Foundation and application of model system for sieving drugs protecting hepatocytes with human fetal hepatocytes injured by CCl<sub>4</sub>. *Zhongguo Yaolixue Tongbao* 1996; **12**: 55-59
- 4 **Qiu ZY**. Clinical study of Silybin on chronic viral hepatitis. *Beifang Yaoxue Zazhi* 1984; **4**: 43-44
- 5 **Liu YF**. Protective effects of polyporus umbellatus polysaccharides on the liver of mice injured by CCl<sub>4</sub>. *Zhongguo Yiyao Zazhi* 1988; **6**: 345-347

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Comparative study of different interventional therapies for primary liver cancer

Qi Liu, Yu-Chen Jia, Jian-Ming Tian, Zhen-Tang Wang, Hua Ye, Ji-Jin Yang, Fei Sun

Qi Liu, Yu-Chen Jia, Jian-Ming Tian, Zhen-Tang Wang, Hua Ye, Ji-Jin Yang, Fei Sun, Department of Radiology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

Qi Liu, MD, PhD, currently Attending Doctor of Radiology, and having 7 papers published.

This paper was presented at the 19<sup>th</sup> International Congress of Radiology in Beijing.

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Qi Liu, MD, PhD, Department of Radiology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China  
Telephone: +86-21-65490018

Received: March 19, 1997

Revised: May 2, 1997

Accepted: June 16, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To compare the therapeutic effect of three types of interventional management for primary liver cancer.

**METHODS:** A total of 468 patients with primary liver cancer were randomly allocated to the following three groups: 138 cases treated with chemotherapy alone using mitomycin C, adriamycin and 5-FU (group A); 158 cases treated with chemoembolization using lipiodol (group B); and 172 cases with chemoembolization using lipiodol and gelfoam (group C). All patients were angiographically and sonographically followed-up.

**RESULTS:** In group C, 67.5% patients had AFP value decreased by > 50%, which was much higher than the 43.3% in group B and 32.2% in group A. Tumor size reduction by  $\geq$  50% occurred in 20.3% of patients in group A, 41.2% of patients in group B and 44.8% of patients in group C. The intergroup differences between group A and group B or C were significant ( $P < 0.01$ ). The 1-year and 3-year survival rates were  $20.5\% \pm 3.6\%$  and  $1.9\% \pm 2.4\%$  for group A,  $51.3\% \pm 4.4\%$  and  $10.1\% \pm 4.9\%$  for group B, and  $63.0\% \pm 2.4\%$  and  $13.9\% \pm 5.0\%$  for group C, respectively. The differences between all three groups were significant ( $P < 0.05$ ). The mean survival time for patients in groups A, B and C were 9.6 mo, 16.1 mo and 17.9 mo, respectively.

**CONCLUSION:** Chemoembolization with lipiodol and gelfoam was the most effective therapy for primary liver cancer in this study. The position of the embolization should be far and middle sections of the hepatic artery, and the proximal section should be reserved as the

route of the next intra-arterial chemoembolization.

**Key words:** Liver neoplasms/therapy; Chemoembolization; Therapeutic; Mitomycin; Adriamycin; Lipiodol; 5-fluorouracil gelfoam

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Liu Q, Jia YC, Tian JM, Wang ZT, Ye H, Yang JJ, Sun F. Comparative study of different interventional therapies for primary liver cancer. *World J Gastroenterol* 1997; 3(4): 231-233 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/231.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.231>

### INTRODUCTION

In recent years, interventional therapies have been widely applied to the treatment of inoperable patients with middle and late stage liver cancer. These therapies include, but are not limited to, simple intra-arterial drug infusion, lipiodol embolization, gelfoam embolization, and microsphere chemoembolization. The literature has not provided definitive evidence as to which modalities are most efficacious. In order to summarize the experience in this field, we analyzed and compared our data as follows.

### MATERIALS AND METHODS

Over the past 4 years, we have treated nearly 1500 cases of liver cancer with 2764 transcatheter chemotherapies. In order to analyze the results of these collective cases more reliably, the cases from the most recent 1-year period were excluded and only cases with complete data were included. A total of 468 cases with complete data were analyzed comparatively; among these, 73 had been confirmed by surgery, 17 by puncture biopsy, and all others diagnosed by findings from AFP testing and/or B-ultrasound, CT and angiography examination. The 468 cases were divided randomly into the following three groups: 138 cases treated with simple intra-arterial drug infusion (TAI, group A), 158 cases treated with TAI combined with lipiodol embolization (TAI-Lp TAE, group B), and 172 cases treated with TAI-Lp TAE plus gelfoam embolization (TAI-Lp Gs-TAE, group C). In group C, 22 patients received microcapsule embolization (containing drugs). The three groups of patients were similar in clinical characteristics.

Celiac angiography was first performed according to Seldinger's method for all patients to determine the arterial distribution and tumor location, size and type. Then, the catheter was inserted into the feeding artery and infusion or embolization was performed. All patients were treated with ADM 30-50 mg, MMC 20 mg and 5-FU 1000 mg simultaneously. Occasionally, CP 200 mg was used instead of the MMC. Lipiodol 10-30 mL mixed with anti-cancer drugs was infused. Finally, 1-2 mm<sup>3</sup> particles of gelfoam sponge were added to the contrast medium and infused slowly.

**Table 1** Changes in AFP level *n*(%)

Group	Positive	No. of comparison	Decreased		No change	Increased
			≥ 50%	25%-50%		
A	89 (64.5)	59 (66.3)	19 (32.2)	9 (15.3)	15 (25.4)	16 (27.1)
B	102 (46.6)	79 (77.5)	34 (43.0)	12 (15.2)	10 (12.7)	23 (29.1)
C	104 (60.5)	83 (79.8)	56 (67.5)	10 (12.1)	4 (4.8)	13 (15.7)

**Table 2** Changes in tumor size after treatment *n*(%)

Group	Reduced		No change	Increased > 25%
	≥ 50%	25%-50%		
A	28 (20.3)	14 (10.2)	72 (52.2)	24 (17.4)
B	54 (41.2)	28 (17.7)	58 (36.7)	18 (11.4)
C	77 (44.8)	34 (20.0)	57 (33.1)	4 (2.3)

**Table 3** Survival rate ± standard error *n*(%)

Year	Group A	Group B	Group C
1	20.5 ± 3.6	51.4 ± 4.4	63.0 ± 3.9
2	5.6 ± 2.9	18.1 ± 4.5	26.6 ± 4.2
3	2.0 ± 2.4	10.1 ± 4.9	13.9 ± 4.7

$P < 0.01$ , group A vs groups B and C;  $P < 0.05$ , group B vs group C.

## RESULTS

Angiographic results allowed for tumor types to be classified as single nodules, multiple nodules, massive (5-10 cm), gigantic (>10 cm), gigantic plus multiple nodules, and diffuse types. There was no obvious difference among the groups.

The criteria of improvement after the treatment of liver cancer were decreased AFP value, reduced tumor size, and prolonged survival. The therapeutic effects for the 3 groups are shown in Tables 1-3. The mean survival period of group A was 9.6 mo, of group B was 16.1 mo and of group C was 17.3 mo. The difference was statistically significant between group A and group B or C ( $P < 0.01$ ), but not between group B and group C.

## DISCUSSION

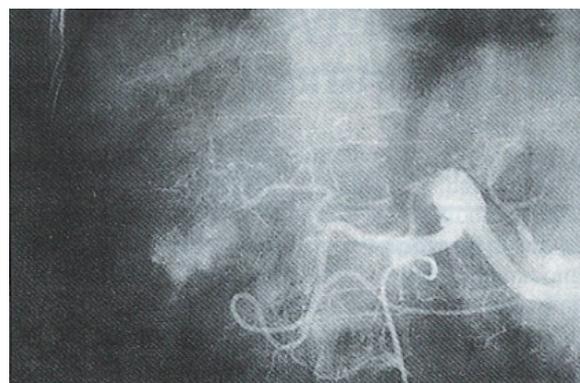
Interventional treatment of liver cancer in our hospital has advanced remarkably over the most recent decades. Early on, the fear of serious complications related to the embolization procedure and a lack of practical skill in application of the technique meant that most patients were offered only intra-arterial infusion chemotherapy. No patient with artery to portal vein fistula or cancer embolus in the portal vein was treated by embolization. With accumulated practical experience, however, the scope of embolization grew and our practitioners became comfortable with learning and adopting new methods. As a result, curative outcomes were increased obviously.

### Changes of AFP value

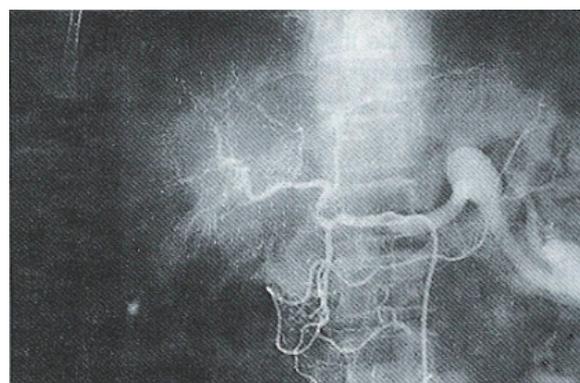
Serum AFP value is one of the most important standards for evaluating the therapeutic effects. The positive rates among the three groups in the current study were 60%-65%. Among the cases receiving more than 2 times of treatment, the cases whose AFP value decreased by > 50% represented 67.5% of group C, which was much higher than the 43.0% of group B and 32.2% of group A. AFP levels reduced < 25% or increased < 25% were regarded as unchanged, cases of which were the lowest in group C (4.8%) and the highest in group A (25.4%). The total rate of cases with decreased was 47.5% in group A, 58.2% in group B, and 79.5% in group C, which was in general agreement with the results previously reported for our home country<sup>[1,2]</sup> and abroad<sup>[3]</sup>. High or low value of AFP was not compatible with the reduction of tumors. Four cases were found in our series in which the tumors decreased obviously but the AFP increased rapidly in a short period of time after embolization.

### Changes of tumor size and necrosis rate

Generally, all tumors show a reduction or necrosis (to varying



**Figure 1** Primary liver cancer of the right lobe, with a hypervascular area diameter of 3 cm before treatment.



**Figure 2** The same case as in Figure 1, showing the tumor stain having disappeared after the 6<sup>th</sup> treatment.

degrees) after embolization chemotherapy. The effective rates of therapy for groups A, B and C were 20.3%, 41.2% and 44.8% respectively, which were similar to the results reported by Imaeda *et al*<sup>[3]</sup> in which 6% of the TAI group and 36% of the TAE group had tumors reduced by > 50%. There were only three cases with tumors disappearing completely (complete remission, CR) in our series and all the tumors were single nodule and had diameters of < 5 cm (Figures 1 and 2). It is uncommon for massive or gigantic tumors (> 5 cm in diameter) to disappear completely after treatment. In our study, these tumors generally showed necrosis of the central area and reduced tumor blood vessels (Figures 3 and 4). The scope of necrosis varied greatly among the different therapies. Among the 22 cases examined pathologically in our hospital<sup>[4]</sup>, the tumor necrosis rate was 30.0% ± 28.3% for the 7 cases with simple MMC TAI, and necrosis of > 50% accounted for 28.6% of these; the rates in the other groups were 81.4% ± 28.3% and 85.7% respectively for the 7 cases with MTXmc TAE and 25%-75% for the 8 cases with MMC-TAI-Lp-TAE. Takayasu *et al*<sup>[5]</sup> also compared the effects of different therapies and reported that simple Lp TAE in 6 cases had almost no treatment significance, but showed value for position diagnosis, ADM TAI-Lp-TAE in 15 cases had some effects but not obvious, ADM TAI-Lp Gs TAE in 10 cases gave a complete necrosis rate of 83% for the main tumors and 53% for the daughter nodules. The results showed that lipiodol itself was not a good embolization agent but was a carrier that delayed drug excretion, thereby enhancing therapeutic effects. Therefore, gelfoam or microcapsule chemoembolization was the most effective therapy.

### Survival rate and period

Therapeutic effects are closely related with treatment methods. Hirai *et al*<sup>[6]</sup> reported that the 1-year survival rates for 191 cases with TAI and 187 cases with TAI-Lp TAE were 22.0% and 66.2% respectively. In the report by Ohishi *et al*<sup>[7]</sup>, the 1-year survival rates for 181 cases with TAI-Gs-TAE and 547 cases with TAI-Lp Gs-TAE were 44.0% and 60.4% respectively. In our series, the 1-year survival rates for groups A, B and C were 20.5%, 51.3% and 63.0%. Imaoka *et al*<sup>[8]</sup> reported that 146 patients with liver cancer tumors of < 3.6 ± 2.3 cm in diameter who received interventional treatment before operation had a 4-year survival rate of 53% in the TAI group,

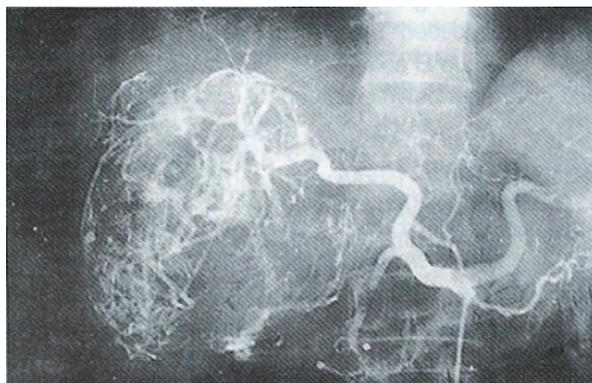


Figure 3 Celiac angiogram of gigantic nodular HCC with 12.5 cm diameter in the right lobe before treatment.

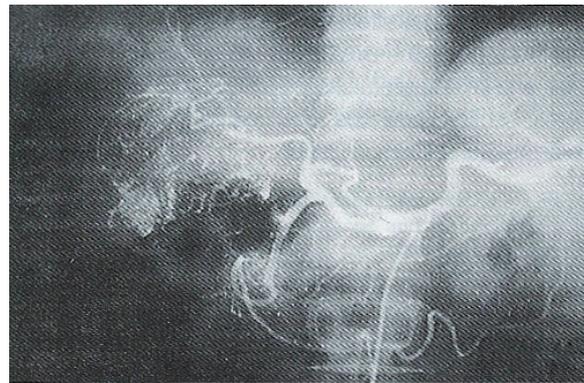


Figure 4 The same case as in Figure 3 after four treatments, showing necrosis in the center of the tumor and reduced presence of new vessels.

66% in the TAI-Lp Gs-TAE group and 83% in the sandwich therapy group. The survival period of the group with simple TAI was about 6 mo. Imaeda *et al.*<sup>[3]</sup> reported that the mean survival period of the TAI group was  $8 \pm 0.6$  mo and of the TAE group was  $13.7 \pm 0.7$  mo, while the mean survival period of groups A, B and C in our series were 9.6, 16.1 and 17.9 mo respectively, with the difference being significant ( $P < 0.01$ ).

The above data shows that the survival rate of patients treated with intra-arterial drug infusion is higher than that of patients treated with systemic chemotherapy (1-year survival rate of 5.4%), that the TAI-Lp TAE is better than simple TAI, that the effect of TAI-Lp Gs-TAE is better than the former methods, and that much better effects are achieved when sectional sandwich therapy is used, i.e. embolization of the peripheral liver artery with Lp, infusion of the anti-cancer drugs and embolization with Lp and gelfoam. Why are compound embolization chemotherapy and the sandwich method most effective? To answer this question, it is first necessary to explain it in terms of the microcirculation and physiological anatomy of liver<sup>[9]</sup>. The hepatic artery and portal vein are parallel and divided into two branches. The segment below the 8th class is segmentulum and the 12th class is the terminal hepatic arteriole (THA) of the classic lobule. The inner diameter of the THA is 20-50  $\mu\text{m}$ , and that of the terminal portal vein (TPV) is 35  $\mu\text{m}$ . The pressure of THA is 30-35 mmHg, which is 8 times that of TPV. Because the pressure difference between THA and TPV is high, drugs and Lp fill the THA and sinusoid, and flow reversely into the TPV when they are used at a volume exceeding a certain level, so both the THA and TPV are chemoembolized. Gelatin sponge particles are also injected into the small arteries to achieve the synergic effect of microtumor necrosing completely.

Traditionally, embolization was divided into the central and peripheral type. Arteries of the two far sections belonged to the peripheral type, and peripheral arteries constituted over 10 sections. We consider that the range of the peripheral type is too extensive, unfavorably limiting embolization. We suggest then a classification for far, middle and proximal sections, with class 7 and above being

the far section (peripheral type), classes 3-6 being the middle section, and classes 1 and 2 being the proximal section or central type. In treatment of liver cancer, the better effect can be achieved if Lp or drug microcapsules are used first for embolization of the far section and then if gelfoam particles are used for embolization of the middle section. Large arteries of the proximal section are regarded as the route of intra-arterial chemoembolization and not appropriate for embolization.

## REFERENCES

- 1 Jia YC, Liu Q, He J, Wang ZT, Wang F, Chen D, et al. Analysis of prognostic factors using Cox's model in hepatocellular carcinoma treated by interventional therapy. *Zhonghua Fangshexue Zazhi* 1996; **30**: 80-84
- 2 Jia YC, Wang ZT, Liu Q, Tian JM, Yie H, Wang F, et al. Comparative study on therapeutic effects of transcatheter arterial chemoembolization and intra-arterial infusion of anti-cancerous agents for hepatic carcinoma. *Zhonghua Fangshexue Zazhi* 1991; **25**: 197-199
- 3 Imaeda T, Yamawaki Y, Hirota K, Suzuki M, Goto H, Seki M, Asada S, Sone Y, Iinuma G, Doi H. [Comparative studies on the therapeutic effects of transcatheter arterial chemo-embolization and intra-arterial one-shot infusion of anticancer drugs for unresectable hepatocellular carcinoma]. *Rinsho Hoshasen* 1987; **32**: 807-813 [PMID: 2824883]
- 4 Hao NX, Jia YC. Methotrexate microspheres in the preoperative treatment of liver tumor by intrahepatic arterial infusion. *Zhongguo Linchuang Yixue Yingxiang Zazhi* 1991; **2**: 129-130
- 5 Takayasu K, Shima Y, Muramatsu Y, Moriyama N, Yamada T, Makuuchi M, Hasegawa H, Hirohashi S. Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. *Radiology* 1987; **163**: 345-351 [PMID: 3031724 DOI: 10.1148/radiology.163.2.3031724]
- 6 Hirai K, Kawazoe Y, Yamashita K, Fujimitsu R, Nakamura T and Nozaki Y, et al. Arterial chemotherapy and TAE therapy for nonresectable hepatic primary cancer. *CCP* 1989; **23**: 37-41
- 7 Ohishi H, Yoshimura H, Sakaguchi H, Iwamoto S, Konno T, Iwai K, et al. TAE using iodized oil mixed with an anticancer drug for the treatment for hepatic cellular carcinoma. *CCP* 1989; **23**: 33-36
- 8 Imaoka M, Sasagi H, Ohashi I, Masutani S, Furukawa H, Fukuda I, et al. Surgical therapy and interventional radiology. *Jpn J Clin Radiol* 1991; **36**: 559-563
- 9 OKUDAIRA M. [VASCULAR STRUCTURE OF THE LIVER]. *Saishin Igaku* 1965; **20**: 254-263 [PMID: 14322711]

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Anti-human AFP variant monoclonal antibody in radioimmunodetection of primary hepatocellular carcinoma

Yang Liu, Meng-Chao Wu, Han Chen, Bai-He Zhang, Guang-Xiang Qian, Wen-Zhou Pan, Mei-Yu Qiang

Yang Liu, Meng-Chao Wu, Han Chen, Bai-He Zhang, Guang-Xiang Qian, Wen-Zhou Pan, Mei-Yu Qiang, Eastern Hospital of Hepatobiliary Surgery, Second Military Medical University, Shanghai 200433, China

Yang Liu, MD, male, born on July 16, 1963 in Xi'an, graduated from the Department of Medicine at Xi'an Medical University, currently Associated Professor, engaged in research of liver neoplasms, and having 10 papers published.

Author contributions: All authors contributed equally to the work.

Supported by the 8·5 Military Medical Research Foundation of China, No. 91A018-0052.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Yang Liu, MD, Eastern Hospital of Hepatobiliary Surgery, Second Military Medical University, Shanghai 200433, China  
Telephone: +86-21-65564166-72882

Received: May 3, 1997

Revised: June 11, 1997

Accepted: July 17, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To investigate the affinity of AFP-R-LCA monoclonal antibody (AFP-R-LCA McAb) for AFP-positive primary hepatocellular carcinoma (HCC) cells.

**METHODS:** AFP-R-LCA McAb was labeled by  $^{131}\text{I}$ . Eleven cases of HCC with AFP positivity, 6 with AFP negativity, and 4 with hepatitis B-related cirrhosis were investigated by radioimmunodetection.

**RESULTS:** The  $^{131}\text{I}$ -AFP-R-LCA McAb immunoreacted with 9 of the HCC AFP-positive cases (9/11), but with none of the 6 AFP negative HCC cases or of the 4 cirrhosis patients.  $^{131}\text{I}$ -AFP-R-LCA McAb at a small dose ( $7.4 \times 10^7 \text{ Bq}/300 \mu\text{g}$ ) was associated with no side effects as determined by the liver function test, prothrombin time (Pt) test and thyroid gland function test ( $P > 0.05$ ). Two cases of AFP-positive HCC were not imaged because of large tumor size (diameter  $> 10 \text{ cm}$ ) and higher AFP concentration in serum ( $20000 \mu\text{g}/\text{L}$ ).

**CONCLUSION:** AFP-R-LCA McAb has a strong and special affinity to AFP-positive HCC cells and may be useful as a carrier for radioimmunodetection and radioimmunotherapy.

**Key words:** Liver neoplasms; AFP; McAb; Radioimmunodetection

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Liu Y, Wu MC, Chen H, Zhang BH, Qian GX, Pan WZ, Qiang MY. Anti-human AFP variant monoclonal antibody in radioimmunodetection of primary hepatocellular carcinoma. *World J Gastroenterol* 1997; 3(4): 234-235 Available

from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/234.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.234>

### INTRODUCTION

Radioimmunodetection (RAID) and radioimmunotherapy using antibodies against tumor-associated antigens (TAA) are hot topics of tumor targeting research. Since the 1980s, several articles describing such anti-tumor monoclonal antibodies (McAb) have been published. Successful RAID and radioimmunotherapy with the anti-human AFP variant McAb (AFP-R-LCA McAb) against human hepatocellular carcinoma (HCC) were carried out in our laboratory using nude mice xenografts<sup>[1]</sup>. Based on our findings from those animal experiments, we next used the AFP-R-LCA McAb labeled by  $^{131}\text{I}$  to immunodetect 17 human cases of HCC and 4 cases of hepatitis B-related liver cirrhosis.

### MATERIALS AND METHODS

#### Cases and groups

HCC was diagnosed according to clinical findings from CT or MRI, B ultrasonography or AFP concentration in serum, and pathological examinations (after operation). Liver cirrhosis was diagnosed according to clinical findings from B ultrasonography, CT or MRI, liver function test, etc. The pre-RAID clinical characteristics are shown in Table 1. All patients included in this study had been admitted to our hospital between March 1, 1995 and September 1, 1996.

#### Preparation of $^{131}\text{I}$ -AFP-R-LCA McAb

The purification procedure for AFP-R-LCA McAb from ascites fluid was adopted from an earlier study<sup>[1]</sup>. Briefly, the monoclonal immunoglobulin IgG-containing ascites fluid was precipitated by saturated ammonium sulfate and the purified IgG was separated with a DEAE-Sephacel column. The monoclonal IgG was radioiodinated to a high specific activity with  $^{131}\text{I}$  using the chloramine-T method. The product of  $^{131}\text{I}$ -AFP-R-LCA McAb was isolated by a Sephadex-G column and then passed through a  $0.22 \mu\text{m}$  filter to remove any bacterial contaminants. After cultivation and Limulus testing to ascertain the absence of bacteria and pyrogens, the product was prepared for clinical use. The labeling rates were 51% to 60%, and the specific activities were 0.11 to 0.33 GBq/mg.

#### Liver function, thyroid gland function, and prothrombin time (Pt)

Periphery vein blood was collected at 1-3 d before and 2 wk after the RAID.

#### Methods of RAID

Seven days before and 1 d before the RAID, patients were administered a compound iodine solution to block the thyroid gland. Thirty min before the RAID, patients were injected with 25 mg of phenergan intramuscularly. The radioiodine-labeled monoclonal antibodies,  $^{131}\text{I}$ -AFP-R-LCA McAb, were injected intravenously through peripheral veins ( $3.7\text{-}7.4 \times 10^7 \text{ Bq}/300 \mu\text{g}$ ). The upper abdomen was scanned using emission CT (ECT ILC 3700; Siemens, Germany) and photographs were taken (Omega 500  $\gamma$  camera) at 24 h, 48 h, 72 h, 120 h and 144 h later.

**Table 1** Pre-radioimmunodetection clinical characteristics of hepatocellular carcinoma and control groups

Parameter	HCC group	Control group	
		AFP-negative HCC	Liver cirrhosis
Case	11	6	4
Sex, male/female	11/0	6/0	4/0
Median age in years	52	54	54
Age range in years	35-65	35-65	35-65
Tumor mass of ≤ 10 cm	9	6	
Tumor mass of > 10 cm	2		
AFP of 2000-10000 µg/L	9		0
AFP of > 10000 µg/L	2		0

**Table 2** Differences in liver function and Prothrombin time of patients with hepatocellular carcinoma or liver cirrhosis, before and after intravenous injection of <sup>131</sup>I-McAb ( $x \pm s$ )

Group	n	Liver function				Pt, t/s			
		TB, cB/µmol·L <sup>-1</sup>		ALT, λB/nmol·S <sup>-1</sup> ·L <sup>-1</sup>		Before	After		
		Before	After	Before	After			A/G	
AFP-positive HCC	11	8.1 ± 2.3	8.2 ± 2.1	756 ± 108	748 ± 112	1.4 ± 0.3	1.4 ± 0.2	11.0 ± 2.3	11.0 ± 2.2
AFP-negative HCC	6	6.2 ± 2.0	6.5 ± 2.1	781 ± 116	768 ± 105	1.4 ± 0.2	1.4 ± 0.4	13.0 ± 2.5	13.0 ± 2.6
Liver cirrhosis	4	7.1 ± 2.5	7.5 ± 2.4	914 ± 152	926 ± 146	1.3 ± 0.4	1.3 ± 0.5	12.0 ± 3.1	12.0 ± 3.2

ALT, alanine aminotransferase; HCC, Hepatocellular carcinoma; Pt, Prothrombin time; TB, Total bilirubin.

### Statistical analysis

All the clinical parameters are expressed as  $x \pm s$ , and the Student's *t*-test was used to determine statistical significance of differences.

## RESULTS

The <sup>131</sup>I-AFP-R-LCA McAb (<sup>131</sup>I-McAb) was detected in human HCC of 9 of 11 patients with positive AFP status. The imaging of tumors began at 72 h after intravenous infusion of <sup>131</sup>I-McAb. After 120 h, the tumor image became clear, but disappearing gradually at 144 h. Besides the detection in the tumor tissues, high concentrations of <sup>131</sup>I-McAb were detected in tissues of the heart and spleen. Two patients with AFP-positive HCC were not imaged, as 1 had a large tumor (diameter > 10 cm) and the other had an excessively high concentration of AFP in serum (> 100000 µg/L). There was no positive detection in any of the 6 HCC cases with AFP-negative status or in any of the 4 cases of liver cirrhosis.

### Liver function and Pt

There were no obvious differences in liver function or Pt when the levels of before and after intravenous injection of <sup>131</sup>I-McAb were compared (Table 2).

### Thyroid gland function

There were no obvious differences in thyroid gland function when the levels from before and after intravenous injection of <sup>131</sup>I-McAb were compared (Table 3).

The relationship between AFP level and radioimmunodetection findings of the patients with HCC is presented in Table 4.

## DISCUSSION

Radiolabeled antibodies against TAA are gaining acceptance as tools for the detection of neoplasms using the technologies of external scintigraphy and RAID<sup>[2,3]</sup>. Many reports of RAID for human liver neoplasms, carried out using radioiodine-labeled monoclonal antibodies<sup>[4,5]</sup>, have appeared in the literature. Here, we describe our most recent study that successfully used <sup>131</sup>I-AFP-R-LCA McAb for RAID of human liver neoplasms.

We selected AFP-R-LCA McAb labeled by <sup>131</sup>I with intravenous delivery. After 72 h, tumor images were obtained, but the findings were more distinct after 120 h. Radioactivity of the tumor tissue and other organs in the area scanned by ECT showed that the tumor was more radioactive than the other tissues (with exception of the heart and spleen). Thus, AFP-R-LCA McAb showed a strong and special affinity for AFP-positive HCC cells. AFP-R-LCA McAb was detected in local area of tumors, with increased intensity over time (within 120 h).

**Table 3** Differences in serum FT3 and FT4, before and after intravenous injection of <sup>131</sup>I-McAb ( $x \pm s$ , cB/pmol·L<sup>-1</sup>)

Group	n	FT3		FT4	
		Before	After	Before	After
AFP-positive HCC	11	5.2 ± 1.3	5.1 ± 1.2	15.2 ± 3.2	15.1 ± 3.4
AFP-negative HCC	6	5.6 ± 1.5	5.5 ± 1.4	16.2 ± 3.1	16.4 ± 3.0
Liver cirrhosis	4	5.4 ± 1.2	5.3 ± 1.3	15.4 ± 4.5	15.4 ± 4.1

HCC, Hepatocellular carcinoma.

**Table 4** Relationship between AFP level and radioimmunodetection findings of hepatocellular carcinoma patients

AFP, ρB/µg·L <sup>-1</sup>	n	Positive imaging, n	Positive percentage, %
2000-10000	6	6	100
10000-100000	3	2	66.7
> 100000	2	1	50

The heart and spleen have a large blood supply, and the blood and spleen belong to the reticuloendothelial system and have a strong non-specific affinity to <sup>131</sup>I-AFP-R-LCA McAb. Therefore, the heart and the spleen showed densely under ECT imaging.

Two of the AFP-positive HCC cases in the current study were not imaged because of large tumor size (diameter > 10 cm) with poor blood supply or related necrosis and higher serum AFP concentration (200000 µg/L); these features can thwart <sup>131</sup>I-AFP-R-LCA McAb competitively to produce a large amount of immune complexes. The immune complex, itself, can hinder <sup>131</sup>I-AFP-R-LCA McAb from getting into the tumor area. Thus, a clear image depends not only on the TAA concentration in serum but also on the tumor's blood supply.

Goldenberg *et al.*<sup>[6]</sup> reported that the serum concentration of AFP may have no influence on RAID, possibly because AFP on the surface of HCC cells is different from AFP circulating in the blood. This conclusion, however, is not consistent with ours.

Successful localization of a radioisotope-labeled monoclonal antibody to a tumor *in vivo* depends not only on the affinity of the antibody for the target cells but also on the speed with which the immunoconjugate passes through the physiological barriers (*i.e.* resistance of the blood vessel wall and phagocytosis by histiocytes) and the speed of its entering into the tumor tissues, as well as the antigen concentration in serum<sup>[7,8]</sup>. It is a generally accepted practice that tumor images be obtained at 72 h after intravenous infusion of the antibody ligand, but that more distinct images are found within 120 h. Our experimental result agreed with this.

In conclusion, AFP-R-LCA-McAb has a strong and special affinity to AFP-positive HCC cells. The detection of AFP-R-LCA-McAb in tumor tissues of HCC suggests its potential as a carrier for RAID and radioimmunotherapy.

## REFERENCES

- Liu Y, Wu MC, Qian GX, Zhang BH, Chen CS. Anti-human AFP variant McAb in radioimmunodetection and radioimmunotherapy for human hepatocellular carcinoma model in nude mice. *Dier Junyi Daxue Xuebao* 1994; 15: 446-451
- Goldenberg DM. Introduction to the second conference on radioimmunodetection and radioimmunotherapy of cancer. *Cancer Res* 1990; 50(Suppl): 778s-779
- Pauwels EK, van Kroonenburgh MJ. Prospects for radioimmunodetection and radioimmunotherapy in oncology? *Nucl Med Commun* 1988; 9: 867-869 [PMID: 3251174]
- Goldenberg DM, Goldenberg H, Higginbotham-Ford E, Shochat D, Ruoslahti E. Imaging of primary and metastatic liver cancer with <sup>131</sup>I monoclonal and polyclonal antibodies against alpha-fetoprotein. *J Clin Oncol* 1987; 5: 1827-1835 [PMID: 2445933]
- Markham N, Ritson A, James O, Curtin N, Bassendine M, Sikora K. Primary hepatocellular carcinoma localised by a radiolabelled monoclonal antibody. *J Hepatol* 1986; 2: 25-31 [PMID: 3005388 DOI: 10.1016/S0168-8278(86)80005-8]
- Goldenberg DM, Kim EE, Deland F, Spremulli E, Nelson MO, Gockerman JP, Primus FJ, Corgan RL, Alpert E. Clinical studies on the radioimmunodetection of tumors containing alpha-fetoprotein. *Cancer* 1980; 45: 2500-2505 [PMID: 6155193 DOI: 10.1002/1097-0142(19800515)45:10<2500::AID-CNCR2820451006>3.0.CO;2-J]
- Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. *Cancer Res* 1990; 50: 814s-819s [PMID: 2404582]
- Vaughan AT, Anderson P, Dykes PW, Chapman CE, Bradwell AR. Limitations to the killing of tumours using radiolabelled antibodies. *Br J Radiol* 1987; 60: 567-572 [PMID: 3620814 DOI: 10.1259/0007-1285-60-714-567]

## Comparative study on proliferation activity in small hepatocellular carcinoma related to hepatitis virus B and C

Shao-Jun Yu

Shao-Jun Yu, Tumor Hospital of Gansu Province, Lanzhou 730050, Gansu Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Shao-Jun Yu, Tumor Hospital of Gansu Province, Lanzhou 730050, Gansu Province, China

Received: September 23, 1996

Revised: December 30, 1996

Accepted: February 6, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To compare the proliferation activity of small hepatocellular carcinoma (HCC) related to hepatitis B virus (HBV) and hepatitis C virus (HCV).

**METHODS:** Sixty liver biopsy specimens were obtained from patients with small HCC ( $\leq 3$  cm in diameter) and examined immunohistochemically using anti-proliferating cell nuclear antigen monoclonal antibody. Of the 60 specimens, 30 were HBV-related and 30 were HCV-related. The 60 patients providing the samples for study were matched by sex and morphologic features of the HCC specimens.

**RESULTS:** The labeling index of proliferating cell nuclear antigen was 7.9% in the HBV-related HCC specimens and 12.5% in the HCV-related HCC specimens. There was no statistically significant difference between the two groups ( $P > 0.05$ ).

**CONCLUSION:** In the early phase, or small stage, of HCC, HBV-related HCC shows similar proliferating activity to that of HCV-related HCC; this finding suggests that in the early phase, HBV-related HCC has similar malignancy to HCV-related HCC.

**Key words:** Liver neoplasms; Carcinoma; Hepatocellular; Hepatitis B virus; Proliferating cell nuclear antigen

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Yu SJ. Comparative study on proliferation activity in small hepatocellular carcinoma related to hepatitis B and C. *World J Gastroenterol* 1997; 3(4): 236-237 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/236.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.236>

### INTRODUCTION

Human hepatocellular carcinoma (HCC) is one of the most common

malignant tumors and its development is known to be closely associated with hepatitis B virus (HBV) infection and hepatitis C virus (HCV) infection<sup>[1,2]</sup>. However, no reports in the literature have yet described a comparative analysis of the tumor behaviors of these two kinds of HCCs.

Proliferating cell nuclear antigen (PCNA) is a nuclear protein related to cell proliferative activity<sup>[3]</sup>, and its immunohistochemical detection is a useful adjunct to morphologic features, providing insight into the understanding of tumor behavior. In this study, we compared the proliferation activity of HBV-related small HCC with that of HCV-related small HCC by means of immunohistochemical staining using a monoclonal antibody against PCNA to provide information about their clinical features.

### MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded liver biopsy specimens were obtained from the archives of the Second Department of Internal Medicine at Kurume University School of Medicine, Japan. These specimens included 30 HBV-related HCCs (male:female ratio of 25:5; mean age of 57.6 years) and 30 HCV-related HCCs (matched by sex ratio and morphologic features; mean age of 60.1 years). The size of the tumors was  $< 3$  cm in diameter and the number of tumors per patient ranged from 1 to 4. Histopathological diagnoses were made according to the General Rules for Cancer of the Liver (Liver Cancer Study Group of Japan)<sup>[4]</sup>. Of the 60 cases involved in this study, 32 were well-differentiated, 24 were moderately differentiated, and 4 were poorly differentiated.

#### Immunohistochemistry

Three  $\mu$ m thick sections were prepared from paraffin blocks and deparaffinized by soaking in a graded ethanol series, after which the processed sections were treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Normal sheep serum was then applied for 20 min to reduce non-specific antibody binding. Monoclonal antibody to PCNA (clone 5A10) was applied as a 1:250 dilution and allowed to incubate for 1 h in a moist chamber. Biotinylated sheep anti-mouse IgG was then applied as the linker molecule and allowed to incubate for 30 min, followed by application of a streptavidin-horseradish peroxidase complex (Vector) and incubation for 1 h. All steps were carried out at room temperature and followed by washing in phosphate buffered saline (PBS). Diaminobenzidine-hydrogen peroxide was used as a chromogen and a hematoxylin counterstain was made. Sections were then processed in an alcohol gradient series, cleared in xylene, and mounted in DPX. A negative control was generated by replacing the primary antibody with PBS.

#### Assessment of PCNA

PCNA labeling indices were calculated after counting PCNA-positive nuclei per 500 nuclei in 2-5 fields of each HCC case at high power ( $\times 200$ ).

**Table 1** Clinical parameters associated with prognosis

Parameter	HBV-related HCC	HCV-related HCC	P value
AFP in $\mu\text{g/L}$ , mean	80.22	99.04	0.743
Survival duration in days, mean	1353.4	1341	0.947

**Other clinical parameters**

Alpha-fetoprotein (AFP) concentration and survival duration were analyzed retrospectively. Statistical analysis was made by Student's *t*-test.

**RESULTS****Proliferating cell nuclear antigen LI**

PCNA staining was confined to the nuclei. All identifiable staining was considered positive, regardless of the staining intensity. The PCNA LI was 7.9% in HBV-related HCCs and 12.5% in HCV-related HCCs. There was no statistically significant differences between these two groups ( $P = 0.33 > 0.05$ ).

**Other clinical parameters (Table 1)****DISCUSSION**

HBV-related HCC and HCV-related HCC are clinically distinct. In the early phase, or small stage, of the HCC tumor development, these two kinds of HCC show similar growth rates, survival rates and prognosis, whereas in the advanced stage, the HBV-related HCC has poorer prognosis than that of HCV-related HCC (unpublished data). In this study, we retrospectively analyzed the survival duration of patients with small HCC who were treated with percutaneous ethanol injection therapy (PEIT) and confirmed that the survival duration was similar for the two forms.

Proliferation activity of tumors that is defined by PCNA immunohistochemical analysis has been reported to be related to metastatic potential, recurrence and overall prognosis<sup>[5,6]</sup>. Therefore, PCNA LI expression in the tumor reflects, at least partially, the degree of malignancy. The current study showed no significant difference in the expression of PCNA LI in patients with HBV-related

small HCC and HCV-related small HCC; this finding suggests that in the early phase HBV-related HCC has similar malignancy to HCV-related HCC. It may therefore help to understand the clinical features of these two kinds of HCCs. Additionally, it has been previously reported that serum AFP values are not only of diagnostic value but also of prognostic significance in patients with HCC<sup>[7]</sup>. In the current study, we found that the serum AFP values were not significantly different between HBV-related small HCC and HCV-related small HCC. This finding is also in accordance with the clinical features of HBV-related HCC and HCV-related HCC in the early stage.

In conclusion, our results indicate that, in the early stage, the proliferation activity of HBV-related HCC is similar to that of HCV-related HCC. This information provides a better understanding of the clinical features of these two kinds of HCC.

**REFERENCES**

- 1 **Beasley RP.** Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* 1988; **61**: 1942-1956 [PMID: 2834034]
- 2 **Nishioka K, Watanabe J, Furuta S, Tanaka E, Iino S, Suzuki H, Tsuji T, Yano M, Kuo G, Choo QL.** A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. *Cancer* 1991; **67**: 429-433 [PMID: 1845946 DOI: 10.1002/1097-0142(19910115)67:2<429::AID-CNCR2820670218>3.0.CO;2-#]
- 3 **Bravo R, Frank R, Blundell PA, Macdonald-Bravo H.** Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature* 1987; **326**: 515-517 [PMID: 2882423 DOI: 10.1038/326515a0]
- 4 **The Liver Cancer Study Group of Japan.** The general rules for clinical and pathological study of primary liver cancer (in Japanese). Tokyo, Kanahara, 1992
- 5 **Kitamoto M, Nakanishi T, Kira S, Kawaguchi M, Nakashio R, Suemori S, Kajiyama G, Asahara T, Dohi K.** The assessment of proliferating cell nuclear antigen immunohistochemical staining in small hepatocellular carcinoma and its relationship to histologic characteristics and prognosis. *Cancer* 1993; **72**: 1859-1865 [PMID: 8103417 DOI: 10.1002/1097-0142(19930915)72:6<1859::AID-CNCR2820720612>3.0.CO;2-A]
- 6 **Ng IO, Lai EC, Fan ST, Ng M, Chan AS, So MK.** Prognostic significance of proliferating cell nuclear antigen expression in hepatocellular carcinoma. *Cancer* 1994; **73**: 2268-2274 [PMID: 7513246]
- 7 **Nomura F, Ohnishi K, Tanabe Y.** Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. Analysis of 606 patients. *Cancer* 1989; **64**: 1700-1707 [PMID: 2477133]

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## Effect of garlic on micronuclei frequency in peripheral blood lymphocytes of rats with N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinoma and precancerous lesions

Qi Su, Zhao-Yang Luo, Yang-Gui Ou, Yi-Qin Li, Jian-Guo Zhou, Dan Zhang

Qi Su, Zhao-Yang Luo, Yang-Gui Ou, Yi-Qin Li, Jian-Guo Zhou, Dan Zhang, Institute of Oncology, Hengyang Medical College, Hengyang 421001, Hunan Province, China

Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. Qi Su, Institute of Oncology, Hengyang Medical College, Hengyang 421001, Hunan Province, China

Received: March 13, 1997

Revised: April 22, 1997

Accepted: June 18, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To investigate the effects of garlic on micronuclei frequency (MNF) in peripheral blood lymphocytes (PBLs) of Wistar rats with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinoma (GC) and precancerous lesions (PLs).

**METHODS:** Wistar rats were exposed to MNNG at 1.25 mg/5 mL per day for 10 mo to induce GC and PLs (MNNG group,  $n = 30$ ); rats not exposed to MNNG served as unmodeled controls (control group,  $n = 16$ ). The MNNG rats were randomly divided into a prevention treatment group ( $n = 30$ ; receiving 10 mL of a 10% garlic solution per day) and an untreated model control group ( $n = 20$ ; receiving tap water). MNF in PBLs were detected at experiment months 10 and

16 mo by the microculture technique.

**RESULTS:** The MNNG group had similar MNF levels at months 10 and 16. Compared to the control group, the MNNG, prevention and untreated model control groups had remarkably higher MNF levels ( $P < 0.01$ ). The level of PLs was significantly lower in the prevention treatment group than in the untreated model control group ( $P < 0.01$ ). The prevention group showed significantly lower MNF than the MNNG group ( $P < 0.01$ ), and the MNF level was reduced in month 16 compared to month 10 ( $P < 0.01$ ). However, the difference in MNF levels in groups given prevention or treatment was not significant.

**CONCLUSION:** MNNG exposure exerted continuous mutagenicity and carcinogenicity properties on PBLs, and garlic exerted a remarkable anti-mutagenic and anti-carcinogenic effect. MNF in PBL may be a novel marker of early-stage GC.

**Key words:** Garlic/Pharmacology; Stomach neoplasms/zhongyi-yaoliaofa; Adenocarcinoma/Zhongyiyao liaofa; Precancerous conditions/zhongyiyao liaofa; Lymphocytes/Drug effects; Micronuclei/Drug effects

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Su Q, Luo ZY, Ou YG, Li YQ, Zhou JG, Zhang D. Effect of garlic on micronuclei frequency in peripheral blood lymphocytes of rats with N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinoma and precancerous lesions. *World J Gastroenterol* 1997; 3(4): 237 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/237.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.237>

S- Editor: A L- Editor: Filipodia E- Editor: Li RF

## A multicenter randomized study on Me-CCNU, 5-FU and ADM versus ACNU, 5-FU and ADM for treatment of advanced gastric cancer

Shu-Dong Xiao, De-Hua Li, De-Zhong Zhang, Mou-Ji Shen, Xiao-Ting Zhu, Gui-Fen He, Ti-Ping Zhao, Le-Ping Li, Xing-Cun Deng, Min Wang, Xiu-Ling Wang, Qiang Chen, Yong-Ping Zhang, Cui-Ling Yao, Ji-Gui Bao, Guo-Wei Tong, Liang-Fa Zhu, Hao Jiang, Kurihara Minoru

Shu-Dong Xiao, De-Hua Li, De-Zhong Zhang, Mou-Ji Shen, Xiao-Ting Zhu, Shanghai Institute of Digestive Disease, Shanghai Second Medical University, Shanghai 200001, China

Gui-Fen He, Ti-Ping Zhao, Le-Ping Li, Shanghai Cancer Hospital, Shanghai Medical University, Shanghai 200032, China

Xing-Cun Deng, Min Wang, The 9<sup>th</sup> People's Hospital, Shanghai Second Medical University, Shanghai 200011, China

Xiu-Ling Wang, Qiang Chen, Yong-Ping Zhang, Xin-Hua Hospital, Shanghai Second Medical University, Shanghai 200092, China

Cui-Ling Yao, Ji-Gui Bao, Guo-Wei Tong, Yang Pu Cancer Hospital, Shanghai 200082, China

Liang-Fa Zhu, Hao Jiang, Rui Jin Hospital, Shanghai Second Medical University, Shanghai 200025, China

Kurihara Minoru, Toyosu Hospital, Showa University School of Medicine, Tokyo, Japan

Dr. Shu-Dong Xiao, Professor of Medicine, Director, Shanghai Institute of Digestive Disease, and Director of the Key Laboratory of Digestive Diseases, Health Ministry of People's Republic of China, Shanghai Ren-Ji Hospital, Shanghai Second Medical University, 145 Shan Dong Zhong Road, Shanghai 200001, China

Author contributions: All authors contributed equally to the work.

Correspondence to: Dr. Shu-Dong Xiao, Director, Shanghai Institute of Digestive Disease, Shanghai Second Medical University, Shanghai 200001, China

Received: March 10, 1997

Revised: April 12, 1997

Accepted: September 22, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To compare the efficacy of a combined chemotherapy regimen of 5-fluouracil (5-FU) and adriamycin (ADM) with nimustine hydrochloride (ACNU; brand name Nidran), a new nitrosourea agent, or with methyl-CCNU for advanced gastric cancer.

**METHODS:** One-hundred-and-three cases of advanced gastric cancer were randomly allocated into Group A (Me-CCNU, 5-FU and ADM combination) and Group B (ACNU, 5-FU and ADM combination). The quality of life (QOL) questionnaire, composed of 11 ordinal categorical items, was used to collect data from these patients.

**RESULTS:** Group A had no case of complete remission (CR) or partial remission (PR), while Group B had no CR but 8 PR (8/46 cases), for a response rate of 0% in Group A and 17.4% in Group B. The median survival time in Group A was 108 d and in Group B was 112 d. Both groups tolerated the treatment well and there were no serious adverse effects. QOL evaluations showed better psychological and physical feelings of tiredness for Group B than for Group A, and scores based on facial scaling showed a more pleasant inclination for the former.

**CONCLUSION:** ACNU combination is superior to the Me-CCNU combination for advanced gastric cancer patients.

**Key words:** Stomach neoplasms; Adriamycin; 5-fluouracil; Mitomycin C; Nimustine hydrochloride

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Xiao SD, Li DH, Zhang DZ, Shen MJ, Zhu XT, He GF, Zhao TP, Xi LP, Deng XC, Wang M, Wang XL, Chen Q, Zhang YP, Yao CL, Bao JG, Tong GW, Zhu LF, Jiang H, Minoru K. A multicenter randomized study on Me-CCNU, 5-FU and ADM versus ACNU, 5-FU and ADM for treatment of advanced gastric cancer. *World J Gastroenterol* 1997; 3(4): 238-241 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/238.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.238>

### INTRODUCTION

In the 1960s, combined chemotherapy regimens with 5-fluouracil (5-FU), adriamycin (ADM) and mitomycin C, known as FAM, and with 5-FU, ADM and methyl (Me)-CCNU, known as FAME, were regarded as effective for the treatment of advanced gastric cancer. However, later massive serial studies of FAM showed low response rates, namely 33% for FAM<sup>[1]</sup> and 18% for FAME<sup>[2]</sup>.

ACNU or nimustine hydrochloride (brand name Nidran, manufactured by Sankyo Co. Ltd., Japan) is a new nitrosourea anti-tumor agent applicable to various malignant tumors. It becomes even more effective when used in combination with other chemical agents. The Shanghai nimustine hydrochloride (ACNU) Cooperative Study Group carried out a randomized controlled study on Me-CCNU, 5-FU and ADM combination versus ACNU, 5-FU and ADM combination for advanced gastric cancer, and evaluated the efficacies by observing response rates, toxicities, adverse effects, and quality of life (QOL).

Table 1 General data of evaluable patients in Groups A and B

Parameter	Group A	Group B
No. of cases	47	46
Sex, M	36	32
Sex, F	11	14
Age (yr), <40	4	7
41-50	9	4
51-60	8	14
61-70	16	17
71-80	10	4
Performance status grade 1	1	0
Performance status grade 2	5	11
Performance status grade 3	5	5
Performance status grade 4	35	26
Unclear	1	4
Histology		
Papillary adenocarcinoma	3	1
Tubular adenocarcinoma 1	4	8
Tubular adenocarcinoma 2	17	12
Low differentiated adenocarcinoma	13	8
Mucocellular carcinoma	2	1
Signet ring cell carcinoma	0	2
Others	8	14

## MATERIALS AND METHODS

### General data

A study on ACNU combination therapy versus Me-CCNU combination therapy was carried out by the Shanghai ACNU Cooperative Study Group from October 1992 through April 1994, using 103 patients with advanced gastric cancer. Among them, 102 cases were eligible and 93 of them had complete records; therefore, the participation rate was 90.3%. The patient data for age, sex, performance status (PS), and histological classification are presented in Table 1.

### Patient population

Patients in the study had primary gastric cancers that were histologically confirmed by biopsy and no patient had received chemotherapy and/or radiotherapy prior to study enrollment. All patients had unresectable or recurrent primary foci with metastatic lesions. The age of the patients ranged from 35-75 years and had PS of 1-4. All of these patients had no active duplicate cancers, no severe disturbance in bone marrow, hepatic, renal or cardiac functions, and were able to be treated with oral medication.

### Methods

**Allocation** Patients meeting the requirements of the study from Zeach were enrolled and their data obtained and recorded using standardized forms and telephone interview conducted by the cooperative study group office. After all the reported details were checked for comprehensiveness, the patient participants were randomly allocated into either treatment Group A or Group B.

**Treatment methods** Group A (control group) consisted of patients who received Me-CCNU 100 mg/d p.o. on day 1, ADM 30 mg/m<sup>2</sup> i.v. on day 1, and 5-FU 300 mg/m<sup>2</sup> i.v. from days 1-5. Group B (treatment group) consisted of patients who received ACNU 50-75 mg/d i.v. on day 1, ADM 30 mg/m<sup>2</sup> i.v. on day 1, and 5-FU 300 mg/m<sup>2</sup> i.v. from days 1-5. Thus, 5 d were taken as one course and successive courses were repeated at an interval of 4-5 wk. The total ADM doses did not exceed 500 mg/m<sup>2</sup>.

**QOL questionnaire**<sup>[3]</sup> Measurement of QOL was made by a self-assessment report by the patients. The patients themselves, their relatives, or care nurses filled in the QOL questionnaire once a week at regular intervals. Doctors were forbidden from filling in the questionnaire in order to avoid confounding by subjective assumption. The QOL questionnaire was composed of 11 ordinal categorical items, consisting of 3 basic items covering mental feelings (appetite, psychology and sleepiness), physical feelings (tiredness, pain and worry) and social relationships (family, friends or co workers).

### QOL Questionnaire

Question 1. How good is your appetite?

a. Very good

Table 2 Courses of treatment in Groups A and B patients

Groups	No. of courses								Total
	1	2	3	4	5	6	7	8	
Group A	16	20	5	2	0	1	0	0	47
Group B	8	23	6	2	0	0	1	1	46
Total	24	43	11	4	0	1	1	1	93

b. Pretty good

c. OK

d. Not too good

e. Not good at all

Question 2. How well do you sleep?

Question 3. How often do you feel tired (psychological)?

Question 4. How often do you feel tired (physical)?

Question 5. How much and how often do you feel pain?

Question 6. How much does your family understand and support you?

Question 7. What is your relationship with your family and co-workers?

Question 8. How often do you worry about your disease?

Question 9. Would you like to continue the current therapy?

Question 10. How much help do you need to accomplish your daily activity?

Question 11. Please rate your overall condition today by marking 0 around the number.

**Observations** Blood cell counting was performed every week. Hepatic and renal functions were tested every 2 wk. Imaging examination, including barium X-ray surveys or endoscopic and ultrasonic B and/or CT scanning, were performed every 4-8 wk. Patients who had considerable adverse effects and could not proceed to a second course were given a barium X-ray check-up 4 wk after the first course.

Details of all examinations, as well as of any adverse effects, were carefully recorded by means of standardized forms. Each patient was followed-up until death.

**Evaluation of efficacy** A clinical evaluation committee was organized to estimate the following 4 items periodically in accordance with the Criteria of Evaluating the Efficacy of Chemotherapy for Solid Tumors proposed by the Japanese Research Society for Gastric Cancer: Qualification and completion of materials; extent of development of lesions and response to the treatment; survival time; and quality of life.

### Statistics

The data of treatment efficacy was compared using the Chi-square test and Fisher's exact test. Principal component analysis and correlation analysis were used to assess the quality of life data.

## RESULTS

### Completion of courses

Each case underwent at least one course of combination therapy. In Group A 44 cases underwent a total of 85 courses, and in Group B 41 cases received a total of 95 courses (Table 2).

### Efficacy

Complete response (CR) and partial response (PR) were regarded as effective. No CR occurred in either groups. As shown in Table 3, there was no PR in Group A (0/47 cases) and 8 in Group B (8/46; for a response rate of 17.4%,  $P < 0.01$ ). The anti-tumor efficacy of the treatments in the two groups is shown in Table 4. The X-ray barium examinations of 2 PR cases before and after ACNU combination therapy are shown in Figures 1-4.

### Survival time

In group A the median survival time from the beginning of the treatment was 108 d. Three out of 47 cases in Group A, survived > 1 year, with the longest survival time being 740 d (Case No. 36). In

**Table 3** Data on partial remission cases

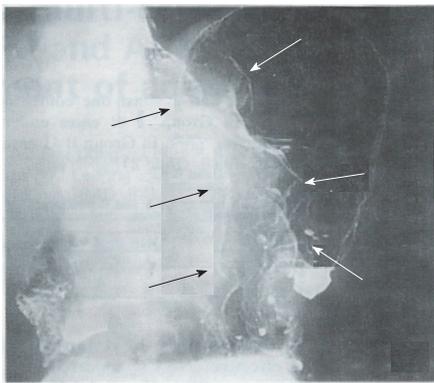
Case No.	Group	Sex	Age	Performance status	Pathohistology	No. of courses	Effective site
22	B	M	54	4	Tubular adenocarcinoma	4	Liver
35	B	M	46	4	Tubular adenocarcinoma	3	Stomach
45	B	M	65	4	Low differentiated adenocarcinoma	7	Liver
67	B	F	60	4	Low differentiated adenocarcinoma	4	Spleen
71	B	F	66	3	Tubular adenocarcinoma	3	Stomach
82	B	F	72	2	Low differentiated adenocarcinoma	2	Liver spleen
83	B	M	74	4	Tubular adenocarcinoma	8	Stomach
100	B	M	65	4	Tubular adenocarcinoma	3	Liver

**Table 4** Anti-tumor effect in Group A and B patients

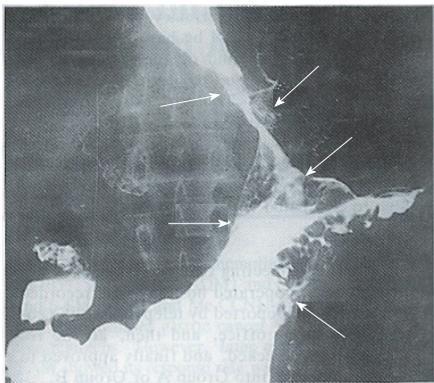
Group	A	B
Partial response (%)	0 (0%)	8 (17.4%)
No change (moderate response)	16 (1)	17 (2)
Progressive lesion	31	21
95% reliability	0, 6.2%	6.4%, 28.4%
Fisher's test	$P > 0.01$	$P > 0.01$
Median survival time	108 d	112 d
Average course	1.9	2.3

**Table 5** Adverse effects in Group A and B patients

	Group A		Group B	
	Cases	%	Cases	%
Loss of appetite	3	6.5	4	8.7
Nausea	3	6.5	3	6.5
Vomiting	9	19.6	8	17.4
Fatigue	1	2.2	1	2.2
Muscular disability	4	8.7	3	8.7
Alopecia	4	8.7	3	6.5
Fever	1	2.2	2	4.3
EKG abnormality	1	2.2	1	2.2
Leukopenia	2	4.3	5	10.9
Decrease of hemoglobin	1	2.2	0	0.0
Elevation of GPT	1	2.2	2	4.3
Total No. of side effects	30		33	
Total cases with side effects	23	48.9	22	47.8
Total cases without side effects	24	50.1	24	52.2



**Figure 1** Huge filling defect found between the angulus and fundus.



**Figure 2** The lesion became remarkably smaller 3 mo after receipt of the combination chemotherapy with nimustine hydrochloride.

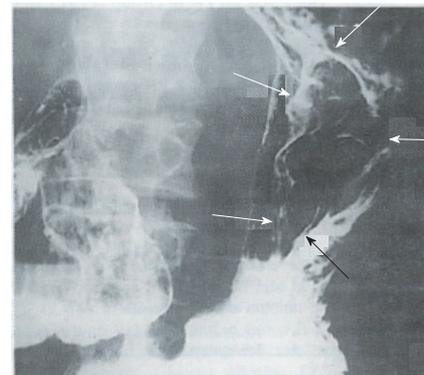
Group B the median survival time was 112 d. Of the 46 total cases, 6 survived > 1 year, with the longest survival time being 690 d (Case No. 53).

**Toxicity**

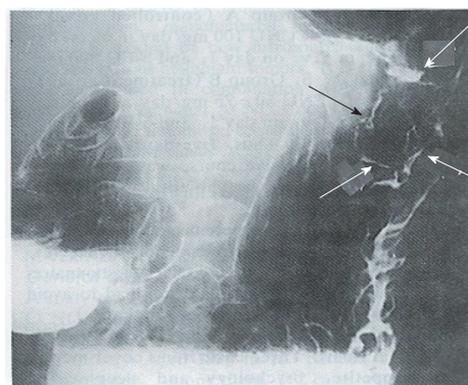
Both groups tolerated the respective treatment well and very few adverse effects were seen and those occurred only in the early days of chemotherapy. No remarkable leukopenia, thrombopenia or decreased hemoglobin occurred, and there were no serious impairments of hepatic, renal or cardiac functions (Table 5).

**QOL**

An overall investigation into appetite, sleepiness, tiredness (psychological and physical), pain, social relationships, mood, daily activities and facial expressions showed that the reports of psychological tiredness (Q3), physical tiredness (Q4) and facial expression (Q11) were better for Group B than for Group A (Figure 5).



**Figure 3** Filling defect in the upper part of the gastric corpus.



**Figure 4** Nine months after combination chemotherapy, the lesion was much smaller.

**DISCUSSION**

ACNU is a newly developed nitrosourea anti-cancer agent. Different from other kinds of nitrosourea agents, it is water-soluble, capable of being administered through the vein and artery. Moreover, it is able to penetrate the blood brain barrier to reach into brain cancerous tissues and exert a full anti-tumor action. The mechanism of ACNU acts strongly across a broad spectrum against various tumors

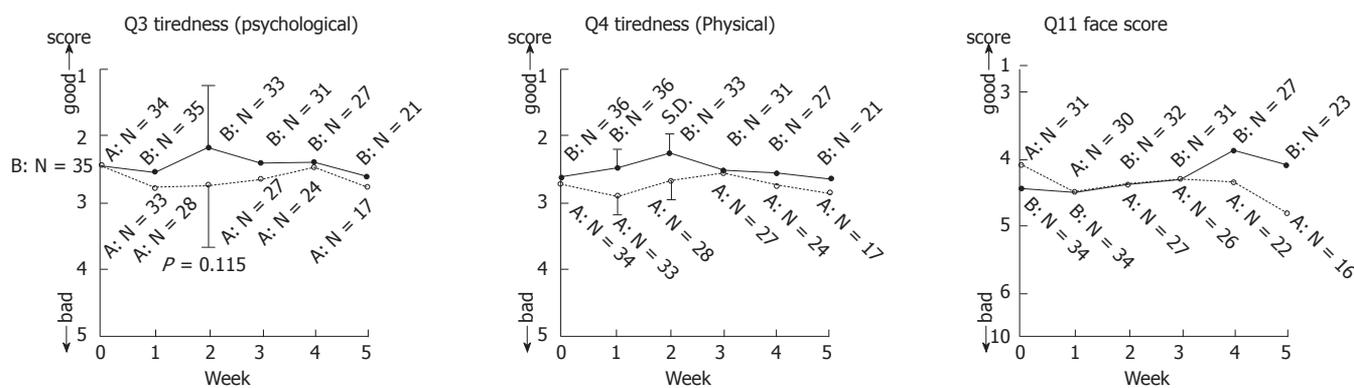


Figure 5 Comparison of psychological tiredness (Q3), physical tiredness (Q4), and face scores (Q11) between Groups A and B.

by way of alkylation of cellular DNA, which causes depolymerization of DNA and results in inhibition of DNA synthesis. Laboratory experiments showed that it could rapidly distribute systemically to organs including brain. Administration of ACNU to mice solid FM3A tumors inhibited tumor growth and reduced tumor size<sup>[5]</sup>.

Our clinical study on advanced gastric cancer showed that the Group B treatment (ACNU combination) was significantly superior to the Group A treatment (Me-CCNU combination) in terms of efficacy, QOL, survival time, etc. As for survival time, 6 cases in Group B survived > 1 year, but only 3 cases did so in Group A. The median survival time was 112 d for patients in Group B, but 108 d for those in Group A. However, the survival time in neither group was long, which might be related to the fact that 70% of the cases had performance status of Grade 4 upon enrollment.

As to dosage of ACNU, 14 cases were given 50 mg/d, and 2 cases reached PR, accounting for a response rate of 14.3%. When the dosage was increased to 75 mg/d for 32 cases, 6 reached PR, accounting for a response rate of 18.3%. Neither dosage caused significant adverse effects. Therefore, we suggest that 75 mg/d should be used at the very beginning or at least on condition that no significant adverse effects appear, dosage could be adjusted to 75 mg/d. Moreover, an even higher dosage could be considered as an approach to yield more ideal results.

Recently QOL has become accepted worldwide as an impor-

tant assessment tool for use in clinical practice. It is also regarded as an important factor for evaluating anti-cancer agents. The QOL questionnaire that we used had been proven clinically at the Tokyo Women's Medical College for its practicability, suitability and reliability. The current study proved that the overall QOL features of Group B were better than those of Group A, which is consistent with the results of efficacy of ACNU.

## REFERENCES

- 1 **Chen Q.** The present status and advances of chemotherapy for gastric cancer. *Zhonghua Xiaohua Zazhi* 1994; **14**: 187-189
- 2 **Lacave A,** Wils J, Bleiberg H, Diaz-Rubio E, Duez N, Dalesio O. An EORTC Gastrointestinal Group phase III evaluation of combinations of methyl-CCNU, 5-fluorouracil, and adriamycin in advanced gastric cancer. *J Clin Oncol* 1987; **5**: 1387-1393 [PMID: 3305795]
- 3 **Ishihara Y,** Nagai A and Kagawa J. Development of a core questionnaire on the quality of life for advanced cancer patients in palliative therapy. *Biotherapy* 1992; **6**: 1467-1473
- 4 **Kurhara M,** Izumi T. Evaluation of the effect of chemotherapy in gastric cancer patients with an intact primary tumor according to the criteria of the Japanese Research Society for Gastric Cancer. *J Jpn Soc Cancer Therapy* 1991; **26**: 644-654
- 5 **Shimizu F,** Imonata T, Mizuno H, Arakawa M, Ogata T and Okawa T. Effects of combined treatment with Nimustine hydrochloride and radiation on solid FM3A tumor in mice. *Jpn J Cancer Res (Gann)* 1987; **78**: 756-762

L- Editor: Filipodia E- Editor: Liu WX



Published by **Baishideng Publishing Group Inc**  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>



ISSN 1007 - 9327



## Comparison of preoperative TN staging of gastric carcinoma by endoscopic ultrasonography with CT examination

Wen Guo, Ya-Li Zhang, Guo-Xing Li, Dian-Yuan Zhou, Wan-Dai Zhang

Wen Guo, Ya-Li Zhang, Dian-Yuan Zhou, Wan-Dai Zhang, PLA Research Institute for Digestive Diseases, Department of General Surgery, Nanfang Hospital, Guangzhou 510515, Guangdong Province, China

Guo-Xing Li, Department of General Surgery, Nanfang Hospital, Guangzhou 510515, Guangdong Province, China

Wen Guo, female, born on 1967-01-30, in Hunan, graduated from the Department of Medicine, 1<sup>st</sup> Military Medical University in 1988, awarded the MD in 1997, currently lecturer and attending physician specializing in ultrasonography, with 10 papers published.

Author contributions: All authors contributed equally to the work.

Correspondence to: Wen Guo, MD, Department of General Surgery, Nanfang Hospital, Guangzhou 510515, Guangdong Province, China

Received: March 26, 1997  
Revised: May 28, 1997  
Accepted: September 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To assess the accuracy and limitations of endoscopic ultrasonography (EUS) in the preoperative staging of gastric carcinoma in comparison with computed tomography (CT).

**METHODS:** According to the new (1987) TN staging, 62 patients with gastric carcinomas were examined preoperatively by EUS and the results compared with those of postoperative pathological TN staging. CT of abdomen was performed before surgery for 32 of the patients.

**RESULTS:** The overall accuracy of T staging was 83.9% for EUS and 28.1% for CT. For the detection of regional lymph node metastases, EUS accuracy was 79.0%, sensitivity 80.0% and specificity 87.5%, versus 50.0% accuracy for CT. The coincidence of perigastric infiltration was 90.0% for EUS and 41.2% for CT. The most frequent causes of misdiagnosis by EUS were microscopic tumor invasion and peritumorous inflammatory or fibrous changes.

**CONCLUSION:** EUS is a reliable method for the clinical evaluation of locoregional extension of gastric cancer and more accurate than CT in the preoperative staging of gastric carcinoma.

**Key words:** Stomach neoplasms/radiography; Stomach neoplasms/ultrasonography; Neoplasm staging; Tomography, X-ray computed; Endoscopy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All

rights reserved.

Guo W, Zhang YL, Li GX, Zhou DY, Zhang WD. Comparison of preoperative TN staging of gastric carcinoma by endoscopic ultrasonography with CT examination. *World J Gastroenterol* 1997; 3(4): 242-245 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/242.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.242>

### INTRODUCTION

Gastric carcinoma is one of the most common malignant human tumors and carries a very poor prognosis with a 5-year survival rate of 6%-25%, depending on the tumor stage<sup>[1]</sup>. Surgical resection of the tumor and the involved lymph nodes remains the only historically proven curative treatment. An accurate preoperative staging of the most significant prognostic factors of gastric cancer, such as depth of invasion (T staging) and involvement of lymph nodes (N staging), would allow better planning of appropriate treatment. The decision as to which patients should either undergo primary operation or prior to chemotherapy, or should be treated by only palliative methods is greatly influenced by the results of preoperative tumor staging. The overall goal is to prevent under or overtreatment, to minimize the inherent morbidity and mortality rates.

Conventional endoscopy permits an examination of only the surface of the stomach, and important data, such as infiltration of the wall, local spread and lymph node involvement, are not provided by this technique. Computed tomography (CT) is currently used in the preoperative staging of gastric cancer, but the results are not completely satisfactory. The major difficulty encountered with CT is its inherent incapability for correct staging of the depth of tumor penetration of the gastric wall and its capacity for correct staging of regional lymph node involvement is also limited.

The purpose of our study was to assess the role of endoscopic ultrasonography, a relatively new modality, in the preoperative TN staging of gastric cancer.

### MATERIALS AND METHODS

From February 1996 to February 1997, 62 patients (35 men and 27 women; from 27 to 76 years of age, with mean age of 61-year-old) with gastric cancer diagnosed by upper gastrointestinal endoscopy and biopsy underwent preoperative evaluation by endoscopic ultrasonography (EUS). CT of abdomen was performed before surgery for 32 of the patients. Tumors were located predominantly in the fundus and cardia region ( $n = 20$ ), the body ( $n = 16$ ) and antrum ( $n = 23$ ), and finally throughout the whole stomach ( $n = 3$ ). Fourteen of the patients had linitis plastica. All of the patients

**Table 1 Relationship between endoscopic ultrasonography and anatomic layers in the normal gastric wall**

EUS	Histology
1 <sup>st</sup> hyperechoic layer	Water interface and superficial mucosa
2 <sup>nd</sup> hypoechoic layer	Deeper mucosa
3 <sup>rd</sup> hyperechoic layer	Submucosa
4 <sup>th</sup> hypoechoic layer	Muscularis propria
5 <sup>th</sup> hyperechoic layer	Subserosa, serosa and the interface echo

EUS: Endoscopic ultrasonography.

**Table 2 TNM classification**

T <sub>1</sub>	Invasion of mucosa or submucosa
T <sub>2</sub>	Invasion of muscularis propria or subserosa
T <sub>3</sub>	Invasion of serosa
T <sub>4</sub>	Invasion of adjacent structures
N <sub>0</sub>	No lymph node metastasis
N <sub>1</sub>	Metastasis in perigastric lymph node (s) within 3 cm of the edge of the tumor
N <sub>2</sub>	Metastasis in perigastric lymph node (s) > 3 cm from the edge of the primary, or in lymph node along the left gastric, common hepatic, splenic or celiac arteries
M <sub>0</sub>	No distant metastasis
M <sub>1</sub>	Distant metastasis

were operated on within 3 wk after the findings upon EUS and CT examination. No complication was encountered during this study.

CT was carried out with a Somatom Plus (Siemens Corp, Germany). Scanning of abdomen was done with 10 mm sections using intravenous and oral contrast.

EUS studies were made using an echoendoscope GF-UM20/EUM20 (Olympus Corp., Japan), which had a rotating 360° transducer with switchable frequencies of 7.5 MHz and 12 MHz. The patients were examined in a left lateral position after oropharyngeal anesthesia and given premedication with 5 mg diazepam and 20 mg 654.2 intramuscularly. The ultrasonic endoscope was introduced and advanced into the stomach. The stomach was filled with 300-500 mL de-gassed water and the balloon filled with water. The transducer was pulled back from the pylorus to the cardia, moving the tip of the endoscope along all parts of the stomach to detect the tumor, its contiguous structures and perigastric lymph nodes. The findings were recorded with a Polaroid camera.

The normal gastric wall is made up of five ultrasonographic layers of different echogenicities (Table 1). Tumors were identified by thickening and disruption of the typical five-layered configuration of the wall appearing as a hypoechoic mass. The assessment of tumor infiltration depth was based on the generally accepted five-layered structure of the gastric wall. Lymph nodes with a homogeneous or inhomogeneous hypoechoic echo pattern and sharply delineated boundaries were considered malignant, whereas hyperechoic lymph nodes with indistinct contours were classified as benign<sup>[2]</sup>.

The findings of EUS and CT in terms of the diagnosis of depth of invasion by the primary tumor, local invasion, and lymph node metastasis were compared with postoperative pathological determination according to the new (1987) TNM staging system (Table 2)<sup>[3]</sup>.

## RESULTS

A total of 62 patients underwent endoscopic ultrasonography for gastric cancer staging. All tumors were diagnosed as adenocarcinomas by histologic examination. The depth of infiltration of the tumor was displayed by EUS in all cases (Table 3). A T<sub>1</sub> carcinoma was correctly diagnosed with EUS as a hypoechoic pattern limited to the mucosa in 1 out of 2 patients (M type), or to the submucosa (SM-type) in all 3 patients. Overstaging occurred in 1 patient as a result of inflammatory changes surrounding the lesion, which was suggested as M-type or SM-type tumor sonographically, but all the 5 patients were eventually correctly diagnosed as having stage T<sub>1</sub>. Nine of 11 T<sub>2</sub> tumors were correctly staged, whereas 2 cases were overstaged as stage T<sub>3</sub>. A T<sub>3</sub> carcinoma was correctly diagnosed with EUS in 18 out of 24 patients. Understaging occurred

**Table 3 Accuracy of endoscopic ultrasonography in the preoperative T staging in 62 patients with gastric carcinoma**

Histopathological T stage	n	EUS T stage (n)				EUS accuracy (%)	EUS overstaging (%)	EUS understaging (%)
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>			
T <sub>1</sub>	5	5	0	0	0	100.0	0.0	0.0
T <sub>2</sub>	11	0	9	2	0	81.8	18.2	0.0
T <sub>3</sub>	24	0	2	18	4	75.0	16.7	8.3
T <sub>4</sub>	22	0	0	2	20	90.9	0.0	10.0
Total	62					83.9	9.7	6.5

EUS: Endoscopic ultrasonography; T staging: Depth of invasion.

**Table 4 Accuracy in the preoperative determination of the N stage in 62 patients with gastric carcinoma**

Histopathological N stage	n	EUS N stage (n)			EUS accuracy (%)
		N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	
N <sub>0</sub>	32	28	4	0	87.5
N <sub>1</sub>	18	5	13	0	72.2
N <sub>2</sub>	12	1	3	8	66.7
Total	62				79.0

EUS: Endoscopic ultrasonography; N staging: Involvement of lymph nodes.

in 2 patients, and overstaging in 4. For stage T<sub>4</sub>, EUS diagnosed 20 out of 22 lesions correctly and understaged the other 2.

The staging accuracy of EUS was 100% for T<sub>1</sub> tumors, 81.8% for T<sub>2</sub>, 75% for T<sub>3</sub>, and 90.9% for T<sub>4</sub>. The overall accuracy rate was 83.9% (52/62). Understaging occurred in 6.5% (4 cases), and overstaging in 9.7% (6 cases). The total mis-staged rate was 16.1%. The coincidence of perigastric infiltration was 90.9% (20/22).

Thirty-two patients were examined by CT, including 5 cases of T<sub>2</sub>, 10 cases of T<sub>3</sub> and 17 cases of T<sub>4</sub> diagnosed by post-operative pathologic examination. CT correctly predicted 2 out of the 10 T<sub>3</sub> and 7 of the 17 T<sub>4</sub> lesions according to findings of thickening of gastric wall or accompanying infiltration of the pancreas or the adjacent liver lobe; however, the examinations failed to assess the depth of wall penetration in the remaining T<sub>2</sub>-T<sub>4</sub> lesions. The overall accuracy rate of CT for T staging was 28.1% (9/32). The coincidence of perigastric infiltration was 41.2% (7/17).

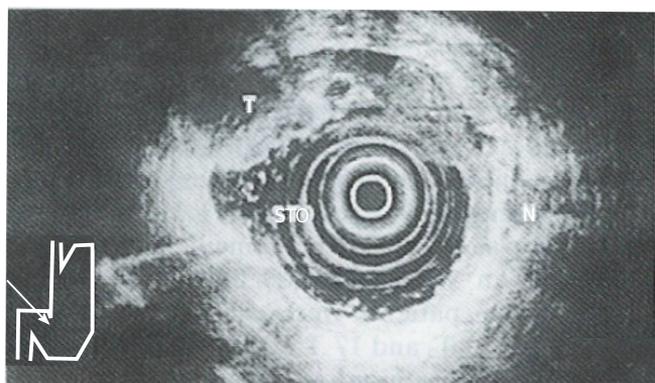
Table 4 summarizes the results of EUS and histopathology for assessing perigastric lymph node involvement. EUS was able to display enlarged lymph nodes of > 3 mm in diameter. The display rate of enlarged lymph nodes was 88.9% (40/45). N<sub>0</sub> stage was correctly diagnosed with EUS in 28 (87.5%) of 32 patients; EUS misdiagnosed benign lymph nodes as metastatic lesions in 4 (12.5%) patients. N<sub>1</sub> was correctly diagnosed with EUS in 13 of 18 (72.2%) patients; metastasis was misinterpreted as benign lesions in the other 5 patients (27.8%). N<sub>2</sub> was correctly predicted in 8 of 12 (66.7%) patients and misinterpreted as N<sub>1</sub> in 3 (25.0%) patients and as N<sub>0</sub> in 1 (8.3%) patient. The sensitivity and specificity of EUS in determining the n stage was 80.0% and 87.5%, respectively. The accuracy of EUS in staging lymph nodes was 87.5% for N<sub>0</sub>, 72.2% for N<sub>1</sub>, and 66.7% for N<sub>2</sub>. The overall accuracy rate was 79.0% (49/62).

CT correctly predicted 9 out of 19 patients with cancer positive lymph nodes, for an accuracy rate of 47.4%, and correctly diagnosed the absence of nodal spread in 7 patients that were deemed N<sub>0</sub> histologically. The overall accuracy rate was 50.0%.

## DISCUSSION

This prospective study showed that EUS was not only highly accurate in determining the depth of invasion of gastric carcinoma, but also in detecting regional lymph node metastases, and that EUS was more accurate than CT for T staging (83.9% vs 28.1%) and n staging (79.0% vs 50.0%). This is in accordance with the results of previous studies<sup>[4]</sup>.

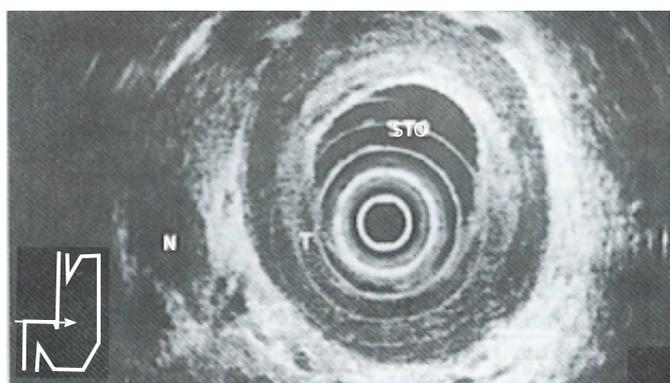
The ability of EUS to image the gastrointestinal tract wall in detail provides a great advantage for staging the depth of tumor invasion (*i.e.* T). The high resolution of the individual layers of the



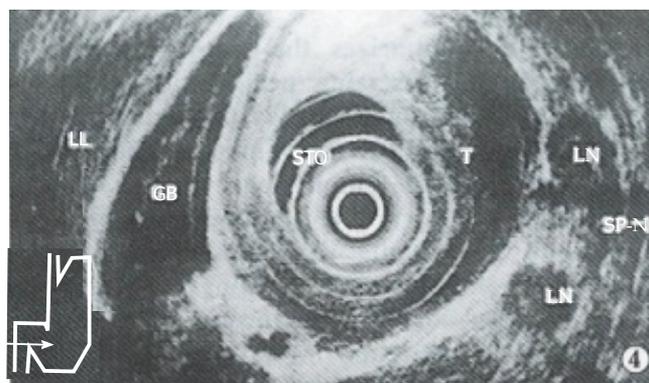
**Figure 1 Endoscopic ultrasonography-T<sub>2</sub> cancer.** Endoscopic ultrasonography shows a hypoechoic tumor (T) adjacent to an ulcerative lesion (U) invading the muscularis propria (Mp).



**Figure 3 Endoscopic ultrasonography-T<sub>4</sub> cancer.** Endoscopic ultrasonography shows a transmurular hypoechoic tumor with penetration into adjacent structures.



**Figure 2 Endoscopic ultrasonography-T<sub>3</sub> cancer.** Endoscopic ultrasonography findings in linitis plastica. Hypoechoic diffuse wall thickening is shown, with preservation of layers as distinctive structures. Note invasion of all layers but absence of invasion of adjacent organs.



**Figure 4 Peritumorous lymph node metastasis.**

gastrointestinal wall seen with EUS is a direct result of the relatively high frequency transducers used (7.5 MHz and 12 MHz). The degree of tumor penetration into each layer can be documented as a focal disruption. Early gastric cancer (*i.e.* T<sub>1</sub>) is visualized as a lesion with a hypoechoic echo pattern limited to the mucosa (*i.e.* M type) and/or submucosa (*i.e.* SM type). Advanced cancer is visualized as a structure with a hypoechoic pattern penetrating into (*i.e.* T<sub>2</sub>) (Figure 1) and/or through the muscularis propria (*i.e.* T<sub>3</sub>) (Figure 2) and/or into the adjacent structures (*i.e.* T<sub>4</sub>) (Figure 3). Endosonographically, early carcinoma can be distinguished from advanced carcinoma. With respect to CT, the size and location of a gastric mass can be documented as areas of gastric wall thickening, with the normal thickness in a distended stomach defined as less than 8 mm. Furthermore, a lack of the fat layer between the gastric mass and an adjacent organ indicates direct invasion. On the other hand, CT cannot differentiate between single layers of the gastrointestinal wall. This limits the use of CT in separating early (*i.e.* T<sub>1</sub>) from advanced tumor stages (*i.e.* T<sub>2</sub>/T<sub>3</sub>).

In our study, 16.1% carcinomas were mis-staged by EUS. Whereas microscopic tumor invasion was the most common cause of understaging, overstaging was mainly due to peritumorous inflammation or scar tissue which could not be distinguished from the tumor. In our experience, an association of the carcinoma with scar tissue should be suspected when a fusion of the 2<sup>nd</sup> and 4<sup>th</sup> layer is seen on EUS. Sometimes, overstaging in gastric T<sub>2</sub> carcinoma is due to the fact that in patients without ascites, differentiation between serosa (T<sub>3</sub>) and subserosal (T<sub>2</sub>) infiltration is not possible. Furthermore, not all the parts of the stomach are covered by serosa; it is absent in some areas, such as the lesser curvature and the anterior wall of the antrum. On the other hand, in T<sub>4</sub> cancers, EUS did not detect organ invasion (especially in the colon), leading to understaging. In addition, anatomic limitations inhibit perfect endosonographic examinations of two regions of the stomach: The prepyloric antral area and the proximal portion of the lesser curvature distal to the cardia.

In staging nodal diseases, EUS is also superior to CT, with an accuracy rate of 79.0% versus 50.0%. Lymph node metastases were determined by CT as enlarged lymph nodes of > 8 mm in

diameter. We found the size of imaged lymph nodes to be an unreliable criterion of malignancy. Malignant nodes as small as 3 mm can be imaged with EUS, and our error in overstaging nodes by EUS occurred in nodes > 2 cm in diameter. The echo pattern and borders of lymph nodes seen on EUS were found to be reliable features for evaluating metastatic lymph node involvement. We have found that malignant nodes (Figure 4) tend to be rounded, sharply defined and hypoechoic compared to benign nodes that tend to be elongated, hazier in outline and more echogenic. Inflammatory changes and micrometastases were the most important causes of false positive and false negative results. These shortcomings can probably be resolved by using the echoendoscope equipped for EUS-guided cytological aspiration<sup>[5]</sup>.

EUS is limited in evaluation of distant metastasis (*i.e.* M staging). Due to its limited depth of penetration, high frequency EUS can be used to scan the left liver lobe but only a portion of the right lobe, and it is unable to evaluate pulmonary metastases and distant peritoneal metastases, such as retroperitoneal and mesenteric nodes below the level of the superior mesenteric artery. CT is superior to EUS in this regard. In our experience in staging gastric cancer, the greatest accuracy for overall staging is achieved with combination of CT and EUS, namely using CT for M, and EUS for T and N.

In summary, our study has shown that EUS is superior to CT in staging T and N categories of gastric carcinoma. EUS is a reliable method for the clinical evaluation of locoregional extension of gastric cancer, and will be helpful in guiding management decisions, selecting appropriate surgical procedures and facilitating research aimed towards improving prognosis for this highly lethal disease. Therefore, EUS should become an important, if not essential, diagnostic procedure for clinical TNM staging.

## REFERENCES

- 1 Maruyama K. The most important prognostic factors for gastric cancer patients. A study using univariate and multivariate analyses. *Scand J Gastroenterol* 1987; **22**: 63-68 [DOI: 10.3109/00365528709091021]
- 2 Grimm H, Binmoeller KF, Hamper K, Koch J, Henne-Bruns D, Soehendra N. Endosonography for preoperative locoregional staging of esophageal and gastric cancer. *Endoscopy* 1993; **25**: 224-230 [PMID: 8519241 DOI: 10.1055/s-2007-1010297]
- 3 Tio TL. The TNM staging system. *Gastrointest Endosc* 1996; **43**: S19-S24 [PMID:

8929802 DOI: 10.1016/S0016-5107(96)81509-4]

- 4 **Rösch T.** Endosonographic staging of gastric cancer: a review of literature results. *Gastrointest Endosc Clin n Am* 1995; **5**: 549-557 [PMID: 7582581]

- 5 **Vilmann P.** Endoscopic ultrasonography-guided fine-needle aspiration biopsy of lymph nodes. *Gastrointest Endosc* 1996; **43**: S24-S29 [PMID: 8929803 DOI: 10.1016/S0016-5107(96)81510-0]

L- Editor: Filipodia E- Editor: Liu WX

## Radioimmunoassay-detected basal level of epidermal growth factor in gastric juice of 86 healthy Chinese volunteers

Li Zhang, Ming-Liang Zhang, Yue-Qing Yan, Da-Xing Liang

Li Zhang, Ming-Liang Zhang, Yue-Qing Yan, Da-Xing Liang, Department of Digestive Research, Second Affiliated Hospital of Hengyang Medical College, Hengyang 421001, Hunan Province, China

Li Zhang, female, born on July 12, 1964 in Yongxing County, Hunan Province, graduated from the Department of Medicine in Hengyang Medical College, currently Physician in Charge engaged in the diagnostic and therapeutic study of *Helicobacter pylori* infection, having 7 papers published.

Author contributions: All authors contributed equally to the work.

Supported by The Health and Scientific Foundation of Hunan Province, No. 93015.

Correspondence to: Dr. Li Zhang, Department of Digestive Research, Second Affiliated Hospital of Hengyang Medical College, Hengyang 421001, Hunan Province, China  
Telephone: +86-734-8223251-8405

Received: October 10, 1996  
Revised: November 10, 1996  
Accepted: June 28, 1997  
Published online: December 15, 1997

**Key words:** Gastric juice; Epidermal growth factor analysis; Radioimmunoassay

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhang L, Zhang ML, Yan YQ, Liang DX. Radioimmunoassay-detected basal level of epidermal growth factor in gastric juice of 86 healthy Chinese volunteers. *World J Gastroenterol* 1997; 3(4): 245 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/245.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.245>

### INTRODUCTION

Epidermal growth factor (EGF) is a low molecular weight polypeptide consisting of 53 amino acid residues<sup>[1]</sup>. EGF is primarily produced by the submaxillary glands and Brunner's glands, but its distribution pattern spans the gastrointestinal tract with a particularly high concentration in gastric juice. To date, however, the precise quantitative value of EGF in gastric juice of healthy subjects has not been reported for a study cohort of Chinese. In the study presented herein, we used radioimmunoassay (RIA) to measure the basal level of EGF in 86 healthy Chinese volunteers of various age.

### MATERIALS AND METHODS

#### Subjects

Eighty-six healthy volunteers, including 24 children (male/female, 18/6; age range: 8-9 years) 28 young adults (male/female, 16/12; age range: 18-20 years) and 34 adults over the age of 40 (male/female, 20/14).

#### Methods

Special capsules designed for collecting minute quantities of gastric juice were swallowed by each of the 86 subjects under basal condition (*i.e.* before 8 am in the morning, representing the fasting state). After 20 min in the stomach, the capsules were manually extracted; the volume of gastric juice in each ranged from 0.3 mL to 0.5 mL. Each specimen was preserved individually by freezing at -40 °C until use. In preparation for measurement and analysis, the specimen was centrifuged at 2000 r for 30 min at 4 °C. A total of 0.1 mL of the resulting supernatant was used to measure EGF by RIA, according to the method described by Lu *et al*<sup>[2]</sup>.

### RESULTS

The average EGF concentration in the gastric juice of the 86 healthy volunteers under basal condition was  $0.62 \pm 0.15 \mu\text{g/L}$ . The values of EGF by age group were as follows: Children,  $0.61 \pm 0.14 \mu\text{g/L}$ ; young adults,  $0.65 \pm 0.14 \mu\text{g/L}$ ; older adults,  $0.59 \pm 0.13 \mu\text{g/L}$ . The EGF level among males in the study ( $n = 52$ ) was  $0.61 \pm 0.14 \mu\text{g/L}$  and among females was  $0.62 \pm 0.15 \mu\text{g/L}$ . No statistically significant differences were noted between the different age and sex groups.

### DISCUSSION

The primary sources of EGF are the submaxillary glands and Brunner's glands of the duodenum. It is believed that EGF can inhibit secretion of gastric acid and stimulate DNA synthesis, and that it plays a role in protection of gastrointestinal mucosa<sup>[3]</sup>. Although, EGF is known to be of higher concentration in the gastric juice, the exact concentration of EGF in normal gastric juice had not yet been defined, partially due to the inconvenience of collecting specimens. Conventional gastric tubing and gastroscopy causes discomfort, but the newly developed capsule (taken orally) can more easily obtain minute specimens of gastric juice from both children and adults. These capsules are a promising innovation for research science since EGF level might be an important variable in evaluating health status. The study described herein indicates that the capsules will be useful for future investigations into various benign and malignant diseases of the gastrointestinal tract.

### REFERENCES

- 1 Savage CR, Cohen S. Epidermal growth factor and a new derivative. Rapid isolation procedures and biological and chemical characterization. *J Biol Chem* 1972; **247**: 7609-7611 [PMID: 4636326]
- 2 Lu GJ, Hou X, Cheng YF, Huan BR, Chai LW. Radioimmunoassay of human epidermal growth factor (h-EGF) and characterization of domestically produced antiserum against h-EGF by genetic engineering. *Zhonghua Heyixue Zazhi* 1993; **13**: 22-24
- 3 Konturek SJ. Role of growth factors in gastroduodenal protection and healing of peptic ulcers. *Gastroenterol Clin North Am* 1990; **19**: 41-65 [PMID: 1970337]

L- Editor: Filipodia E- Editor: Liu WX

## Treatment of cancerous ascites and radical gastrectomy with intraperitoneal hyperthermic double-distilled water and cis-diaminodichloro-platinum perfusion

Zhi-Xing Chen, Jia-Ping Chen, Zhong Chen, De-Shu Peng, Ji-Xiang Zhen, Jian-San Tan

Zhi-Xing Chen, Jia-Ping Chen, Zhong Chen, De-Shu Peng, Ji-Xiang Zhen, Department of Surgery, First Affiliated Hospital, West China University of Medical Sciences, Chengdu 610041, Sichuan Province, China

Jian-San Tan, Department of Pathology, Fourth Affiliated Hospital, West China University of Medical Sciences, Chengdu 610041, Sichuan Province, China

Dr. Zhi-Xing Chen, male, born on 1961-12-25 in Langzhong City, Sichuan Province, graduated from the West China University of Medical Sciences as a post-graduate in 1993, currently Attending Surgeon, mainly devoted to the studies of diagnosis and treatment of gastroenteric tumors, having 10 papers published.

Author contributions: All authors contributed equally to the work.

Supported by The Sichuan Provincial Health Bureau (94F0132).

Correspondence to: Dr. Zhi-Xing Chen, Department of Surgery, First Affiliated Hospital, West China University of Medical Sciences, Chengdu 610041, Sichuan Province, China  
Telephone: +86-28-5551255-26808

Received: July 20, 1996  
Revised: September 9, 1997  
Accepted: October 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To study the therapeutic effect of intraperitoneal hyperthermic double-distilled water and cis-diaminodichloro-platinum (DDP) perfusion for cancerous ascites and radical gastrectomy.

**METHODS:** LACA mice were injected peritoneally with H<sub>22</sub> cancer cells ( $2 \times 10^7$  tumor cells). Five days later, the mice received treatments with either intraperitoneal perfusion of 37 °C isotonic fluid (group I), or 43 °C simple hyperthermic double-distilled water (group II), isotonic fluid (group III), DDP (group IV) or a combination of the hyperthermic double-distilled water with DDP (group V). A clinical experiment with intraperitoneal hyperthermic double-distilled water perfusion with DDP was carried out from September 1991 through September 1993 with 32 advanced gastric cancer patients who had undergone radical gastrectomy.

**RESULTS:** In comparison with the untreated control group of cancer cell-bearing LACA mice, the mice in all treatment groups showed near complete obliteration of cancer cells in the peritoneal cavity, markedly reduced ascites, prolonged survival times, and reduced growth of peritoneal cancerous nodes. In the clinical experiment, all 32 patients with advanced carcinoma had achieved satisfactory results at the 1-year follow-up, but had unsatisfactory results at the

2-year follow-up.

**CONCLUSION:** The intraperitoneal hyperthermic double-distilled water perfusion with DDP inhibited the occurrence of ascites in LACA mice bearing cancer cells, and prolonged the lifetime of patients with gastric cancer who had undergone radical gastrectomy.

**Key words:** Gastrectomy; Stomach neoplasms; Ascites; Cis-diaminodichloro-platinum; Perfusion

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen ZX, Chen JP, Chen Z, Peng DS, Zhen JX, Tan JS. Treatment of cancerous ascites and radical gastrectomy with intraperitoneal hyperthermic double-distilled water and cis-diaminodichloro-platinum perfusion. *World J Gastroenterol* 1997; 3(4): 246-248 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/246.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.246>

### INTRODUCTION

The five-year survival rate of gastric carcinoma after radical gastrectomy is about 30%-40%. Among the various causes of death, peritoneal metastasis recurrence accounts for about 50%. Recent reports from abroad<sup>[1-4]</sup> have demonstrated the therapeutic efficacy of intraperitoneal hyperthermic isotonic anticarcinogen fluid perfusion, but the long-term outcome has not yet been reported. In this study, we investigated the treatment of intraperitoneal hyperthermic double-distilled water and cis-diaminodichloro-platinum (DDP; the cisplatin chemotherapy drug) perfusion to prevent peritoneal metastasis and treat cancerous ascites in a mouse model and in patients with gastric cancer who had undergone radical gastrectomy.

### MATERIALS AND METHODS

#### Materials

LACA mice and ascites tumor cells (liver cancer cell ascites, H<sub>22</sub>) were provided by the Cancer Institute of West-China University of Medical Sciences. Double-distilled water (DDW) and physiological saline were provided by our pharmaceutical department. DDP (2 mL/tube, containing 10 mg DDP) were procured from the manufacturer, Gejiu Biochemical Pharmaceutical Factory (Yunnan, China).

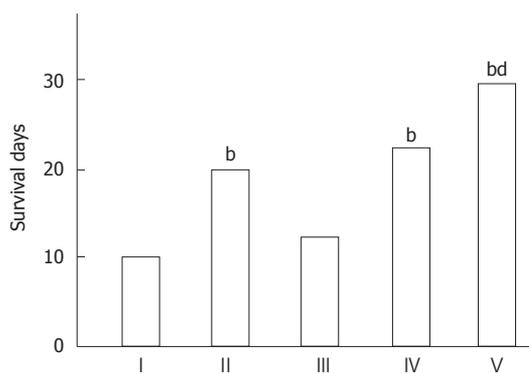
#### Methods

Seventy mice were given an intraperitoneal injection of  $2 \times 10^7$  tumor cells; the male: female ratio was 35:35 and the range of

**Table 1** Management strategies for ascites cancer cells in LACA mice

Management	Group I	Group II	Group III	Group IV	Group V
Injection of tumors cells	+	+	+	+	+
37 °C	+	-	-	-	-
43 °C	-	+	+	+	+
0.9% normal saline	+	-	+	+	-
DDW	-	+	-	-	-
DDP	-	-	-	+	+

+: Management; -: Without management. DDP: double-distilled water and cis-diaminodichloro-platinum; DDW: Double-distilled water.



**Figure 1** Survival days for all groups. <sup>b</sup>*P* < 0.01 vs group I, <sup>a</sup>*P* < 0.01 vs groups II, IV and V.

body weight was 22-24 g. The injected mice were randomly divided into the following 5 groups for treatment at day 5 post-injection: Physiological saline at 1.5 mL<sup>[5]</sup> (group I, control group); hyperthermic double-distilled water (group II); isotonic fluid (group III); DDP (2.5 mg/kg)<sup>[6]</sup> (group IV); and hyperthermic double-distilled water combined with DDP (group V). All treatments were delivered as intraperitoneal perfusion, and the various features of the perfusions are presented in Table 1.

### Observation and analysis of indexes

**Average survival days of mice and quantity of ascites** The exact time of death was recorded, after which a small incision was made in the abdominal wall in order to remove the ascitic fluid by suction. The collected fluid was used in subsequent analysis as described below.

**Peritoneal cancer nodes examination** An autopsy was performed to observe the distribution, number and size of the cancer nodules. All data were recorded for subsequent statistical analysis.

**Number of living tumor cells** Samples of the collected ascitic fluid from each mouse were diluted with physiological saline and stained with TaiPan blue to distinguish the living tumor cells. The total number were counted and recorded.

**Tumor cells under optical and electron microscopic observation** A smear was made using the precipitate obtained by centrifugation. After ultra-thin sectioning and staining with hematoxylin-eosin and counterstaining with uranyl, acetate and lead citrate, the sections were observed under both optical and electronic microscopy. *F* and *Q* tests were used for statistical comparison.

## RESULTS

### Ascites tumor cells and survival rate of tumor cells

In the control group, ascites were obvious within 1 wk, while the managed groups (groups II-V) presented a slower appearance of ascites and a reduced amount of ascitic fluid. Four mice in group V developed no ascites. Managed groups II, IV and V, with the exception of group III, showed a significantly low number of tumor cells (*P* < 0.01 vs control group I). When group V was compared with groups II and IV, the tumor cells were found to be drastically

**Table 2** Ascites, tumor cells and survival rate of tumor cells for all groups

Group	<i>n</i>	Ascites (mL)	Tumor cells ( × 10 <sup>7</sup> /mL)	Survival rate of tumor cells (%)
I	14	22.13 ± 3.16	21.43 ± 3.42	89.42 ± 3.21
II	14	10.61 ± 2.85 <sup>b</sup>	8.20 ± 3.41 <sup>b</sup>	69.21 ± 7.22 <sup>b</sup>
III	14	17.29 ± 2.93	18.26 ± 3.12	87.86 ± 1.68
IV	14	10.86 ± 1.86 <sup>b</sup>	6.54 ± 3.75 <sup>b</sup>	75.14 ± 6.89 <sup>b</sup>
V	14	5.36 ± 4.28 <sup>bd</sup>	4.57 ± 2.90 <sup>bd</sup>	45.14 ± 22.64 <sup>bd</sup>

<sup>b</sup>*P* < 0.01 vs group I, <sup>d</sup>*P* < 0.01 vs groups II and III.

**Table 3** Detected peritoneal cancerous nodes for all groups

	Group I	Group II	Group III	Group IV	Group V
Proportion of peritoneal cancerous nodes	7/14	3/14	5/14	4/14	1/14 <sup>a</sup>
Incidence of peritoneal cancerous nodes	50.00	21.43	35.71	28.57	7.64 <sup>a</sup>

<sup>a</sup>*P* < 0.05 vs group I.

lower in the former and the survival rate to be markedly improved (Table 2).

### Survival days of mice

With the exception of group III, the management strategies were associated with significantly improved survival (*P* < 0.05 vs control group I). When group V was compared with groups II, III and IV, the former showed significantly greater prolonged survival (Figure 1).

The data for effects of the different management strategies on growth of peritoneal cancerous nodes are presented in Table 3.

### Light and electron microscopy observation of ascites tumor cells

Under the light microscope, the tumor cells of group I were found to be larger in number, gathered in clots, and presenting with large and deeply-stained nuclei. The tumor cells of group III were found to be reduced in number. The tumor cells of groups II and IV showed a portion with water-like degeneration and necrosis. The tumor cells of group V showed a sharp sharply reduction in number, with the present cells showing necrotic pathological changes, cytoplasm dissolution and nucleus disappearance. Under the electron microscope, the tumor cells of group V showed no complete structures, while those of groups II and IV showed degeneration in various degrees, with distension of mitochondria and nucleoplasm.

## CLINICAL APPLICATION

The clinical experiment was conducted from September 1991 to September 1993 and involved 60 cases of advanced gastric cancer. The patients, once enrolled, were divided randomly into the following two groups. The first group was a control (28 cases) and was treated with operation only; the second group was experimental (32 cases) and was treated with an intraperitoneal perfusion of 43 °C double-distilled water combined with DDP.

For each patient, the abdominal cavity was opened for perfusion and washing with 200 mL of 37 °C normal saline, which was then sucked out in order to search for the detached tumor cells. Patients allocated to the second group received the following additional treatment: Before closure of the abdomen, an intraperitoneal perfusion of hyperthermic (44-46 °C) sterile double-distilled water (2500-3000 mL) was delivered by circulating perfusion for 30 min, after which 700-1000 mL of perfusate was allowed to remain in the abdomen, and 300 mg of DDP (200 mg/m<sup>2</sup>) was added<sup>[7]</sup>; the abdomen was then closed with a tube in place to facilitate post-operative drainage. After postoperative clamping for 4 h, the tube was opened for drainage. At the same time of the perioperative perfusion, a rapid (20 min) *i.v.* drip of 12 g of sodium thiosulfate was administered, followed by a gradual (8 h) *i.v.* drip of 24 g of sodium thiosulfate in 1000 mL of 5% G.S.<sup>[8]</sup>.

In the second group (prevention group), 13 (48.1%) of the 27 patients were positive for detached tumor cells, and all 13 became negative after treatment. In the first group (control group), 9 patients were positive and 6 remained positive after the operation.

The prevention group had a 1-year survival rate of 96.9%

(31/32), which was significantly higher than that of the control group (46.1%, 13/28;  $P < 0.01$ ), but a 2-year survival rate that was similar to the control group (75.0%, 24/32 vs 42.9%, 12/28;  $P > 0.05$ ).

The incidence of complications of intraperitoneal perfusion was 65.6% (21/32) and included nausea (43.8%, 14 patients) and vomiting (25.0%, 8 patients). These cases were mild, and easily relieved by paspertin or ondansetron zofran. The levels of serum creatinine and urea nitrogen were higher after perfusion than before the operation, but still within normal limits. Six patients showed a slight decrease in white blood cell count after perfusion, but in all cases the count was above  $3.0 \times 10^9$ . All patients showed normal findings from liver function tests; there were no instances of peritoneal nerve injury, delayed healing of the incision, anastomotic leakage, or intestinal adhesion.

## DISCUSSION

When the serosa of the gastric tumor is invaded, the tumor cell may detach spontaneously or a result of manipulation during operation, thereby entering the free peritoneal cavity or the pelvic fossae<sup>[9]</sup>. Kaibara<sup>[3]</sup> reported that the 5-year survival rate in patients free from peritoneal metastasis was 51.4% but only 18.7% for their counterparts with peritoneal metastasis. Thus, it is important to prevent and treat peritoneal metastasis.

Thermochemotherapy is able to eliminate recurrence at the excision site and helps to avoid peritoneal seeding. Moreover, Kaibara<sup>[3]</sup> reported that it might raise the 5-year survival rate. In our experiment, on day 5 after intraperitoneal injection of H22 cancer cells into the peritoneal cavity of LACA mice, the tumor cells presented with continuous division and proliferation activity, and seeding was present. When the treatment of intraperitoneal perfusion of hyperthermic (43 °C) hypotonic (double-distilled water) fluid combined with

DDP was compared with perfusion of normal temperature hypotonic fluid, isotonic fluid and DDP, the former showed the best effect, probably due to the synergic action of the agents when used as a combination therapy.

## REFERENCES

- 1 **Fujimoto S**, Shrestha RD, Kokubun M, Ohta M, Takahashi M, Kobayashi K, Kiuchi S, Okui K, Miyoshi T, Arimizu N. Intraperitoneal hyperthermic perfusion combined with surgery effective for gastric cancer patients with peritoneal seeding. *Ann Surg* 1988; **208**: 36-41 [PMID: 3133994 DOI: 10.1097/00000658-198807000-00005]
- 2 **Koga S**, Hamazoe R, Maeta M, Shimizu N, Murakami A, Wakatsuki T. Prophylactic therapy for peritoneal recurrence of gastric cancer by continuous hyperthermic peritoneal perfusion with mitomycin C. *Cancer* 1988; **61**: 232-237 [PMID: 3121165 DOI: 10.1002/1097-0142 (19880115)61:2 < 232::AID-CNCR2820610205 > 3.0.CO; 2-U]
- 3 **Kaibara N**, Hamazoe R, Iitsuka Y, Maeta M, Koga S. Hyperthermic peritoneal perfusion combined with anticancer chemotherapy as prophylactic treatment of peritoneal recurrence of gastric cancer. *Hepatogastroenterology* 1989; **36**: 75-78 [PMID: 2499527]
- 4 **Fujimura T**, Yonemura Y, Fushida S, Urade M, Takegawa S, Kamata T, Sugiyama K, Hasegawa H, Katayama K, Miwa K. Continuous hyperthermic peritoneal perfusion for the treatment of peritoneal dissemination in gastric cancers and subsequent second-look operation. *Cancer* 1990; **65**: 65-71 [PMID: 2104572 DOI: 10.1002/1097-0142 (19900101)65:1 < 65::AID-CNCR2820650115 > 3.0.CO; 2-L]
- 5 **Xu SY**. Pharmacological experimental methodology, 2nd ed. Beijing: People's Health Publishing House 1991: 1424-1430
- 6 **Trave F**, Rustum YM, Boransen T. Synergistic antitumor activity of cisplatin (DDP) and 5-fluorouracil (Fura 0 in mice bearing leukemic L1210 cells. *Proc Annual Meeting of Association of Cancer Am Res* 1985; **26**: 322
- 7 **Xu SY**. Clinical guidance of administration drug. 1st ed. Hefei: Anhui Publishing House of Science and Technology 1989: 8-10
- 8 **Howell SB**, Pfeifle CE, Wung WE, Olshen RA. Intraperitoneal cis-diamminedichloroplatinum with systemic thiosulfate protection. *Cancer Res* 1983; **43**: 1426-1431 [PMID: 6681730]
- 9 **Iitsuka Y**, Kaneshima S, Tanida O, Takeuchi T, Koga S. Intraperitoneal free cancer cells and their viability in gastric cancer. *Cancer* 1979; **44**: 1476-1480 [PMID: 498022 DOI: 10.1002/1097-0142 (197910)44:4 < 1476::AID-CNCR2820440442 > 3.0.CO; 2-R]

L- Editor: Filipodia E- Editor: Liu WX

## Pancreatitis associated with herpes zoster: A case report

Hong-Guang Wang, Gui-Zhi Yang, Heng-Yan Li

Hong-Guang Wang, Gui-Zhi Yang, Heng-Yan Li, Department of Gastroenterology, Railway Central Hospital of Jilin, Jilin Province 132001, China

Author contributions: All authors contributed equally to the work.

Correspondence to: Hong-Guang Wang, Chief Physician, Director of the Department of Gastroenterology, Railway Central Hospital of Jilin, Jilin Province 132001, China

Received: November 26, 1996  
Revised: June 3, 1997  
Accepted: June 28, 1997  
Published online: December 15, 1997

**Key words:** Pancreatitis/Herpes zoster

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Wang HG, Yang GZ, Li HY. Pancreatitis associated with herpes zoster: A case report. *World J Gastroenterol* 1997; 3(4): 248 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/248.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.248>

### INTRODUCTION

The etiology of acute pancreatitis has been well studied and is known to be diverse, but pancreatitis caused by herpes zoster (HZV) has rarely been reported. We encountered a case of pancreatitis associated with HZV in our hospital and report it below.

### CASE REPORT

A 28-year-old woman was admitted to our hospital with complaints of anorexia, nausea and epigastric pain that had lasted for 6 d. The patient denied recent alcohol ingestion, previous food intolerance, jaundice or abdominal pain, and she had no history of peptic ulcer disease, hyperlipidemia, abdominal trauma or recent use of medication. Physical examination revealed umbilicated vesicles on

an erythematous base at the right hypogastric region and extending dorsally along the right ribs. There was marked tenderness in the epigastric region, and auscultation revealed diminished bowel sounds. No abnormal mass or enlargement of the liver or spleen was detected by palpation. Both the Grey-Turner's sign and Cullen's signs were absent. Pelvic examination revealed no tenderness of the cervix or cul-de-sac. Initial laboratory tests included a white blood cell count of  $7.7 \times 10^9/L$ , hemoglobin level of 123 g/L, serum amylase of 603 U/L (normal, < 180 U/L), urine amylase of 3864 U/L (normal, < 1200 U/L), serum lipase of 2780 U/L (normal, < 100 U/L) and positive serum HZV (by PCR).

Abdominal ultrasound showed a normal liver, gallbladder, and pancreas. Bile ducts were not dilated. There was a small amount of fluid detected in the peritoneal cavity. The serum potassium level was 2.3 mmol/L, but the serum levels of calcium, cholesterol and triglycerides were normal. The diagnostic impression was acute pancreatitis and HZV. Treatment included intravenous fluids, electrolyte replacement, interferon and nasogastric suction. A week later, the patient's abdominal pain disappeared gradually and she resumed eating. Repeated abdominal B-ultrasonography showed no fluid in the peritoneal cavity. The vesicles presence resolved.

### DISCUSSION

Acute pancreatitis is a process of autodigestion of the pancreas caused by premature activation of zymogens, leading them to become active proteolytic enzymes. The etiology of acute pancreatitis is diverse and reported causes include the mumps virus, rubella virus, Coxsackie B virus, Epstein Barr virus and hepatitis A virus; however, the association between HZV and pancreatitis has rarely been reported.

The pathogenic mechanism underlying HZV-related pancreatitis remains unknown. One plausible mechanism for virus-associated acute pancreatitis is inflammation and destruction of pancreatic acinar cells directly induced by the virus. In the case described herein, HZV was detected in the serum by PCR and other coincidental etiologies, such as alcohol abuse, gallstone disease, drug-induced pancreatitis, trauma, hyperlipidemia, and ulcer diseases, were excluded by clinical examination and history taking.

L- Editor: Filipodia E- Editor: Liu WX

## Pharmacokinetics of four 5-FU preparations administered rectally to rats and rabbits

Xiang Zhang, Chong-Shu Wang, Gong-Zhu Wu, Bao-Dong Ling, Ru-Huai Liang, Xue-Sheng Fang, Tian-Yong Xin

Xiang Zhang, Bao-Dong Ling, Department of Pharmacology, North Sichuan Medical College, Nanchong 637007, Sichuan Province, China

Chong-Shu Wang, Xue-Sheng Fang, Tian-Yong Xin, Surgical Department, Affiliated Hospital, North Sichuan Medical College, Nanchong 637000, Sichuan Province, China

Gong-Zhu Wu, Ru-Huai Liang, Medicamentous Department, the Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, Sichuan Province, China

Author contributions: All authors contributed equally to the work.

Supported by The Educational Committee of Sichuan Province, No. 56, 1990.

Correspondence to: Xiang Zhang, Department of Pharmacology, North Sichuan Medical College, Nanchong 637007, Sichuan Province, China  
Telephone: +86-817-2226611-2060

Received: November 24, 1996  
Revised: January 6, 1997  
Accepted: April 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To compare the pharmacokinetic characteristics of four preparations of fluorouracil (5-FU) administered rectally using a rat model.

**METHODS:** Concentrations of 5-FU were measured in plasma, the rectal wall and mesentery lymph tissues of rats and rabbits by high performance liquid chromatography. Differences between the main pharmacokinetic parameters were compared by statistical analysis.

**RESULTS:** The 5-FU concentrations in the rectal wall and mesenteric lymph tissues were significantly higher than the concentration in blood following rectal administration for all four of the preparations ( $P < 0.01$ ). The drug level in the rectal wall was higher in the animals received delivery of an emulsion, compared to those who received delivery as a suppository ( $P < 0.05$ ). Moreover, the animals who received a lipophil-based suppository had lower plasma level of drug than those who received a hydrophil-based suppository, and the animals who received the simple (o/w) emulsion had lower plasma level than those who received the complex (w/o/w) emulsion. The differences found in the rat model were confirmed in rabbits ( $P < 0.01$ ).

**CONCLUSION:** The lipophil-based suppository and the simple emulsion of 5-FU might be more suitable for rectal administration for treatment of rectal cancers.

**Key words:** Fluorouracil/pharmacokinetics; Rectal administration; Rectal neoplasms/drug therapy

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhang X, Wang CS, Wu GZ, Ling BD, Liang RH, Fang XS, Xin TY. Pharmacokinetics of four 5-FU preparations administered rectally to rats and rabbits. *World J Gastroenterol* 1997; 3(4): 249-250 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/249.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.249>

### INTRODUCTION

Fluorouracil (5-FU) is one of the most effective medicines for chemotherapy of large bowel cancer; unfortunately, intravenous administration does not allow for a sufficiently high concentration to reach the cancer tissues and is associated with frequent serious toxic reactions and side effects<sup>[1]</sup>. However, a higher 5-FU concentration in cancer tissues accompanied by a relative lower level in plasma could be obtained if the drug is administered rectally<sup>[2]</sup>; as such, this strategy would also reduce the risk of adverse reactions, such as arrest of bone marrow functions.

We generated four different preparations of 5-FU for rectal administration, and compared their pharmacokinetic characteristics in the rat and rabbit whole animal systems.

### MATERIALS AND METHODS

#### Animals

Female and male rats (weight range: 150-200 g) and rabbits (weight range: 2-3 kg) were provided by the animal facility of North Sichuan Medical College.

#### Compounds

5-FU raw drug (lot number: 910732) was procured from the manufacturer, Shanghai 12<sup>th</sup> Pharmaceutical Factory. All reagents used in the study were of AR grade.

#### Preparations

Crystalline grain (o.d. 10  $\mu$ m) was generated by the solvent-transformation method. The hydrophil-based (polyethylene glycols) suppositories (HBS) and lipophil-based (semi-synthetic fatty glyceride) suppositories (LBS), each containing 250 mg 5-FU (for rabbits) or 25 mg (for rats), were generated by the heating-melt method. Two kinds of emulsions containing 5-FU (5%) (wt/vol) were generated using an aqueous solution, namely the simple (o/w) emulsion (SE)

**Table 1** Comparison of tissue concentrations in rats among the four 5-FU preparations ( $\bar{x} \pm s, n = 5$ )

Group	Concentration		
	Plasma (mg/L)	Rectal wall (mg/g)	Mesenteric lymph nodes (mg/g)
HBS	27.10 ± 15.06 <sup>c</sup>	71.97 ± 21.64 <sup>b</sup>	75.92 ± 21.81 <sup>b</sup>
LBS	16.60 ± 15.03	46.82 ± 20.84 <sup>b</sup>	41.93 ± 17.22 <sup>b</sup>
SE	14.39 ± 9.40	154.11 ± 46.54 <sup>a,b</sup>	41.29 ± 15.30 <sup>b</sup>
CE	19.47 ± 8.71 <sup>c</sup>	156.60 ± 42.31 <sup>a,b</sup>	46.45 ± 13.41 <sup>b</sup>

<sup>b</sup> $P < 0.01$  vs plasma level; <sup>a</sup> $P < 0.05$  SE group vs HBS group, CE group vs LBS group; <sup>c</sup> $P > 0.05$  HBS group vs LBS group, CE group vs SE group. HBS: hydrophil-based (polyethylene glycols) suppositories; LBS: Lipophil-based (semi-synthetic fatty glyceride) suppositories SE: Simple (o/w) emulsion; CE: Complex (w/o/w) emulsion.

and the complex (w/o/w) emulsion (CE).

### Instruments and requirements of assay

High performance liquid chromatography (HPLC) was carried out with the chromatographic column-Zorbax ODS (Gilson Corporation, France) using the following parameters: 4.6 mm × 150 mm; graininess 5 μm; mobile phase, phosphate buffer solution (0.025 mol/L, pH 3.0); and flow rate, 1 mL/min.

### Experiments

Twenty rats were randomly divided into four groups, with total weight balanced in each. The rats in the four groups were rectally administered 5-FU as HBS, LBS, SE or CE at a dose of 25 mg. In order to prevent leakage of the drug solution from the anus, the orifice was clipped closed after the drug delivery. At 1 h post-delivery, blood samples (2 mL each) were drawn from the tail vein and the rats were sacrificed for immediate tissue harvesting (rectal and mesenteric lymph nodes). The rectal tissues were first cleaned by distilled water and blotted on filter paper, then 30 mg were weighed out for the subsequent analyses. The blood samples (containing heparin) were centrifuged and 1 mL plasma was collected for analysis. The 5-FU concentrations in the tissues and plasma were determined by HPLC assay as previously described but with slight modification<sup>[3]</sup>.

Twelve rabbits were randomly divided into four groups and rectally administered 5-FU as HBS, LBS, SE or CE at a dose of 250 mg. At 0.5, 1, 1.5, 2, 4 and 6 h post-delivery, blood samples (1.5 mL) were drawn from the posterior auricular arteries and processed for analysis as described above.

## RESULTS

Data for the main pharmacokinetic parameters are presented in Tables 1 and 2.

## DISCUSSION

5-FU is one of the most common anticancer drugs used in clinical practice today, and its strong killing effects on cancer cells are

**Table 2** Main pharmacokinetic parameters in rabbits after rectal administration of four 5-FU preparations ( $\bar{x} \pm s, n = 3$ )

Group	T1/2 (h)	Vd (L)	AUC [(mg·g)/L]	Cmax (mg/L)	Tmax (h)
HBS	1.19 ± 0.52	2.25 ± 1.01	95.41 ± 45.21 <sup>b</sup>	39.56 ± 19.02	0.84 ± 0.41
LBS	1.92 ± 0.80	16.90 ± 7.44	20.44 ± 9.82	8.56 ± 3.70	0.55 ± 0.29
SE	4.54 ± 1.75	22.98 ± 10.51	35.60 ± 15.03	9.95 ± 3.97	0.40 ± 0.17
CE	2.10 ± 0.98	3.00 ± 1.42	126.19 ± 73.35 <sup>b</sup>	30.48 ± 15.24	0.99 ± 0.49

<sup>b</sup> $P < 0.01$  HBS group vs LBS group, CE group vs SE group. HBS: hydrophil-based (polyethylene glycols) suppositories; LBS: Lipophil-based (semi-synthetic fatty glyceride) suppositories SE: Simple (o/w) emulsion; CE: Complex (w/o/w) emulsion.

well recognized. Specifically, 5-FU damages proliferating cells, thereby reducing the tumor mass in size and preventing tumor cells from spreading and undergoing metastasis. However, if the drug is administered intravenously, its curative effects are inadequate because it achieves a lower concentration in the rectal wall and mesenteric lymph nodes and instead has a relatively higher concentration in blood<sup>[4]</sup>. High blood concentrations lead to serious side effects that preclude the patients' ability to tolerate the treatment.

In this study, the 5-FU concentrations in the rectal wall and mesenteric lymph nodes were significantly higher than that in blood at 1 h after administration to rats, for all four of the different preparation types ( $P < 0.01$ ). Comparison of the four types of preparations showed that the emulsions provided higher levels of 5-FU in the rectal wall than did the suppositories ( $P < 0.05$ ). The drug concentrations in the blood was higher in the rats given HBS than in those given LBS, and higher for CE than for SE ( $P > 0.05$ ). The differences were confirmed in the rabbit system as well ( $P < 0.01$ ).

Thus, LBS and SE provided higher 5-FU concentrations in the rectal wall and mesenteric lymph nodes, and a lower concentration in blood. Rectal administration can reduce toxic and side effects and increase anticancer effects; therefore, the two preparations, LBS and SE, are more suitable for clinical application.

## ACKNOWLEDGEMENTS

We thank You-Zhi Zhen and Zhi-Li Tong of Central Hospital of Nanchong City for their help with performing the HPLC assay.

## REFERENCES

- 1 Woley PV. Chemotherapy of colorectal carcinoma. *Zhonghua Zongliu Zazhi* 1976; **3**: 415-418
- 2 Luo JM. Observation under electronic microscope on the effects of 5-FU suppository on rectal cancers. *Hunan Yiyao Zazhi* 1990; **7**: 215-217
- 3 Zhang HF, Yan BX, Zhang ZS, Lu SM, Zhang TL. The investigation of disposition of 5-fluorouracil *in vivo* after three routes of administration. *Zhongguo Linchuang Yaoxixue Zazhi* 1990; **6**: 92-99
- 4 Zhou XG, Wang YC, Yu BM, Shen YX, Jiang JT, Zhang DS, Ding QG, Xia ZQ, Xie GP, Liu Y. [Route and preparation of 5-Fu administration as preoperative adjuvant chemotherapy in rectal cancer. I. Concentration and distribution of 5-Fu in tissues monitored by 14C-isotopically tagged 5-Fu]. *Zhonghua Zongliu Zazhi* 1988; **10**: 81-84 [PMID: 3208659]

L- Editor: Filipodia E- Editor: Liu WX

## Influence of diet intake on liver function test

Shu-Quan Cheng, Ji-Fang Zhang, Zi-Fu Zhang, Mei-Yan Qian, Xiao-Ling Guo, Wen-Zhang Shang, Duo-Jing Li

Shu-Quan Cheng, Ji-Fang Zhang, Zi-Fu Zhang, Mei-Yan Qian, Xiao-Ling Guo, Wen-Zhang Shang, Duo-Jing Li, Department of Medical Laboratory, Central Hospital of Jiaozuo Coal-Mine Administration, Jiaozuo 454150, Henan Province, China

Received: November 24, 1996  
Revised: January 6, 1997  
Accepted: April 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To study whether dietary intake influences liver function test.

**METHODS:** Blood samples were obtained from patients liver diseases ( $n = 100$ ) and controls (without liver diseases;  $n = 100$ ) first at 07: 00 in the morning (fasting state) and then 2 h after a meal (fed state). The Hitachi-7150 automatic biochemistry analyzer was used to assess the following liver function indexes: Serum bilirubin, thymol turbidity test, alanine aminotransferase, aspartate

aminotransferase, alkaline phosphatase, lactate dehydrogenase, gamma glutamyl transferase, SP, A and G. Statistical significance of differences between inter-group values was determined using SAS software.

**RESULTS:** None of the indexes showed statistically significant differences between the fasting state and the fed state ( $P = 0.476-0.978$ ).

**CONCLUSION:** Liver function test can be performed after a meal.

**Key words:** Dietary proteins; Dietary fats; Liver function tests

© **The Author(s) 1997.** Published by Baishideng Publishing Group Inc. All rights reserved.

Cheng SQ, Zhang JF, Zhang ZF, Qian MY, Guo XL, Shang WZ, Li DJ. Influence of diet intake on liver function test. *World J Gastroenterol* 1997; 3(4): 250 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/250.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.250>

L- Editor: Filipodia E- Editor: Liu WX

## Determination of $\beta$ -glucuronidase in human colorectal carcinoma cell lines

Shu Feng, Jin-Dan Song

Shu Feng, Jin-Dan Song, Key Laboratory of Cell Biology, Ministry of Public Health of China, China Medical University, Shenyang 110001, Liaonin Province, China

Dr. Shu Feng, female, born on 1965-02-09 in Hailin, Heilongjiang Province, graduated from Jiamusi Medical Collage in 1986, currently lecturer and post-graduate, having 9 papers published.

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 3904005.

Correspondence to: Dr. Shu Feng, Key Laboratory of Cell Biology, Ministry of Public Health of China, China Medical University, Shenyang 110001, Liaonin Province, China  
Telephone: +86-24-3916243

Received: March 8, 1997  
Revised: May 2, 1997  
Accepted: October 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To study the relationship between  $\beta$ -glucuronidase and the invasiveness of human colorectal carcinoma cell lines.

**METHODS:** Six colorectal carcinoma cell lines, including three well-differentiated (CX1, CCL187, and CCL229) and three poorly differentiated ones (CCL227, CCL228, and Clone A), were analyzed by Fischman's method to determine the concentration of  $\beta$ -glucuronidase in the medium.

**RESULTS:** Low levels of  $\beta$ -glucuronidase (activity range: 1.29 to 1.96  $\mu\text{g}/10^6$  cells $\cdot\text{h}$ ) were associated with poor invasiveness. This finding was in contrast to the elevated levels of the enzyme (2.46-3.37  $\mu\text{g}/10^6$  cells $\cdot\text{h}$ ) detected in the medium derived from the more aggressively invasive cells (CCL 227, CCL 228, Clone A, and CCL 229).

**CONCLUSION:** Highly invasive colorectal carcinoma cells secreted higher levels of  $\beta$ -glucuronidase than the poorly invasive cells. Determination of secreted  $\beta$ -glucuronidase might represent a useful in vitro measurement tool to assess the invasiveness of colorectal carcinoma.

**Key words:** Colorectal neoplasms;  $\beta$ -glucuronidase invasiveness; Cell lines

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Feng S, Song JD. Determination of  $\beta$ -glucuronidase in human colorectal carcinoma cell lines. *World J Gastroenterol* 1997; 3(4): 251-252 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/251.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.251>

### INTRODUCTION

The major cause of death in patients with malignant neoplasms is metastasis. The spread of tumor cells from the primary site to other organs involves a sequence of complex biologic events. Improving our knowledge about the mechanisms of metastasis, at a biologic level, may enable us to detect the pre-metastatic state and inhibit tumor invasion. The following multistep cascade contributes to the initiation of tumor metastasis: (1) tumor cells attach to the extracellular matrix by means of cell-surface receptors; (2) tumor cells degrade the matrix by secreting specific degradative enzymes; and (3) tumor cells migrate through the degraded matrix, facilitating metastasis<sup>[1]</sup>. Matrix degradative enzymes, such as urokinase, type IV collagenase,  $\beta$ -glucuronidase and Cathepsin B, play important roles in this process.

$\beta$ -glucuronidase, a lysosomal acid enzyme that acts to degrade proteoglycan, the major component of basement membrane, participates in the processes of tumor invasion and metastasis. In this study, an attempt has been made to establish a relationship between  $\beta$ -glucuronidase secretion and the primitive nature of *in vitro* colorectal carcinoma. Accordingly, the  $\beta$ -glucuronidase secretory capacities of six colorectal carcinoma cell lines, representing varying degrees of differentiation and invasiveness, were compared. The results indicated that the poorly and highly invasive cells could be separated on the basis of  $\beta$ -glucuronidase secretion. This biochemical marker may prove useful in assessing the primitive characteristics of cultured colorectal carcinoma cells.

### MATERIALS AND METHODS

#### Materials

Human colorectal carcinoma cell lines that had originated from tumor tissues and were well established in serial subcultures were used in this study. The cell lines CX1, CCL187, CCL227, CCL228, CCL229, and Clone A were generous gifts from the Dana Farber Cancer Institute of Harvard Medical School, USA.

#### Methods

The cell lines were maintained in Dulbecco's modified Eagle's medium (GIBCO, BRL) supplemented with 10% calf serum and 2 U/mL gentamycin. Cell cultures were incubated at 37 °C in a humidified atmosphere of 95% air and 5% carbon dioxide.

The culture medium was collected as follows. Colorectal carci-

**Table 1** Conditions for measuring  $\beta$ -glucuronidase in culture medium

Medium (mL)	Water (mL)	Acetate buffer (mL)	pH	Substrate final	Molarity (mL)	Incubation time at 37 °C (h)	Alkalinizing reagent (mL)	Final pH	Coloremeter wavelength (nm)
0.2	0.4	0.2	4.5	0.2	0.006	18	2 <sup>1</sup> + 3 mL H <sub>2</sub> O	10.2	540

<sup>1</sup>Glycine-Duportal reagent: 15.01 g of glycine dissolved in 900 mL of H<sub>2</sub>O and brought to pH 11.7 by addition of 50% NaOH solution. Duportal (sodium lauryl sulfate) was added to produce a final concentration of 0.2% and water was added to achieve a final volume of 1 L.

**Table 2** Activity of secreted  $\beta$ -glucuronidase in culture medium of six colorectal carcinoma cell lines

Cell line	Differentiation degree <sup>[3]</sup>	Invasiveness <sup>[3,4]</sup>	n	$\beta$ -glucuronidase activity, $\bar{x} \pm s$
CX1	Good	Low	6	1.29 $\pm$ 0.17
CCL187	Good	Low	6	1.96 $\pm$ 0.28
CCL229	Good	High	6	3.37 $\pm$ 0.34 <sup>b</sup>
CCL227	Poor	High	6	2.46 $\pm$ 0.18 <sup>b</sup>
CCL228	Poor	High	6	2.73 $\pm$ 0.19 <sup>b</sup>
Clone A	Poor	High	6	3.22 $\pm$ 0.38

<sup>b</sup>*P* < 0.001 vs CX1, CCL187.

carcinoma cells were seeded in 100 mL flasks ( $2.5 \times 10^5$  cells/mL). After 3 d of culture, the medium was refreshed completely. After an additional day of culturing, the medium was harvested and the cells enumerated. The collected medium was condensed (mL/5  $\times 10^6$  cells) and stored at 4 °C for future use.  $\beta$ -glucuronidase activity levels in the collected medium was determined by Fischman's method<sup>[2]</sup>. Phenolphthalein standard curve was set up in a range of 0 mg/L to 40 mg/L. The substrate was phenolphthalein mono- $\beta$ -D glucosiduronic acid sodium salt. The conditions for measuring medium levels of  $\beta$ -glucuronidase are shown in Table 1. One enzyme activity unit equated to 1  $\mu$ g of released phenolphthalein/10<sup>6</sup> cells·h. The results were analyzed by Student's *t*-test.

## RESULTS

The medium from each cell line was analyzed for activity of  $\beta$ -glucuronidase. The well differentiated and poorly invasive cell lines CX1 and CCL187 were found to be low secretors of the enzyme (activity range: 1.29-1.96  $\mu$ g/10<sup>6</sup> cells·h). In contrast, the poorly differentiated and highly invasive cell lines CCL227, CCL228 and Clone A, as well as the well differentiated CCL229 with high invasiveness<sup>[3]</sup>, were relatively more active in this respect, with  $\beta$ -glucuronidase activities ranging between 2.46  $\mu$ g/10<sup>6</sup> cells·h and 3.37  $\mu$ g/10<sup>6</sup> cells·h (Table 2).

## DISCUSSION

Recent studies have highlighted the association of matrix degradative enzymes with malignant tumors, and have suggested that these enzymes may play a role in tumor invasion and metastasis. Although a lot of work has been done to investigate the effects of urokinase and type (WTBZ) IV (WTB1) collagenase on tumor invasion and metastasis<sup>[5,6]</sup>, there are few reports about  $\beta$ -glucuronidase in this respect, especially in regards to colorectal carcinoma.

$\beta$ -glucuronidase, a lysosomal acid enzyme that can degrade proteoglycan, the major component of basement membrane, is known to participate in the process of tumor invasion and metastasis. Poole<sup>[7]</sup> reported that  $\beta$ -glucuronidase activity was high in experimental rat tumors and that the enzyme was present in the matrix ahead of the invading tumor. Dai *et al.*<sup>[8]</sup> reported that the  $\beta$ -glucuronidase activity level in stomach cancer was higher than that in non-cancerous tissues. Nicolson *et al.*<sup>[9]</sup> confirmed that highly metastatic melanoma cells secreted higher levels of  $\beta$ -glucuronidase and degraded sub-endothelial basement membrane at a higher rate than poorly metastatic melanoma cells. All these findings have supported the hypothesis that  $\beta$ -glucuronidase is closely related to tumor metastasis.

In order to illustrate the relationship between  $\beta$ -glucuronidase secretion and invasiveness of human colorectal carcinoma, we analyzed the culture medium from six cell lines to determine the activity of  $\beta$ -glucuronidase within. The results indicated that the highly invasive cell lines secreted higher levels of  $\beta$ -glucuronidase than the poorly invasive ones, supporting the notion that  $\beta$ -glucuronidase might contribute to colorectal carcinoma invasion and metastasis. Moreover, determination of secreted  $\beta$ -glucuronidase might represent a useful measurement tool for the invasiveness of *in vitro* colorectal carcinoma.

## REFERENCES

- 1 Liotta LA. Cancer cell invasion and metastasis. *Sci Am* 1992; **266**: 54-9, 62-3 [PMID: 1373003 DOI: 10.1038/scientificamerican0292-54]
- 2 Fishman WH, Kato K, Anstiss CL, Green S. Human serum beta-glucuronidase; its measurement and some of its properties. *Clin Chim Acta* 1967; **15**: 435-447 [PMID: 6034420 DOI: 10.1016/0009-8981(67)90008-3]
- 3 Lee EC, Woo HJ, Korzelius CA, Steele GD, Mercurio AM. Carbohydrate-binding protein 35 is the major cell-surface laminin-binding protein in colon carcinoma. *Arch Surg* 1991; **126**: 1498-1502 [PMID: 1842179 DOI: 10.1001/archsurg.1991.01410360072011]
- 4 Sun BD, Song JD. Inhibition of invasiveness and expression of epidermal growth factor receptor in human colorectal carcinoma cells induced by retinoic acid. *Cell Res* 1995; **5**: 135-142 [DOI: 10.1038/cr.1995.13]
- 5 Murphy G. Matrix metalloproteinases and their inhibitors. *Acta Orthop Scand Suppl* 1995; **266**: 55-60 [PMID: 8553862]
- 6 Zheng MH, Fan Y, Panicker A, Smith A, Robertson T, Wysocki S, Robbins P, Papadimitriou JM, Wood DJ. Detection of mRNAs for urokinase-type plasminogen activator, its receptor, and type 1 inhibitor in giant cell tumors of bone with in situ hybridization. *Am J Pathol* 1995; **147**: 1559-1566 [PMID: 7495280]
- 7 Poole AR. Invasion of cartilage by an experimental rat tumor. *Cancer Res* 1970; **30**: 2252-2259 [PMID: 4195911]
- 8 Dai DQ, Chen JQ, Ren CS, Zhang WF. The relation of  $\beta$ -glucuronidase, acid phosphatase and lactic dehydrogenase with gastric cancer. *Zhongguo Yike Daxue Xuebao* 1991; **20**: 23-28
- 9 Nicolson GL, Nakajima M, Herrmann JL, Menter DG, Cavanaugh PG, Park JS, Marchetti D. Malignant melanoma metastasis to brain: role of degradative enzymes and responses to paracrine growth factors. *J Neurooncol* 1994; **18**: 139-149 [PMID: 7964976 DOI: 10.1007/BF01050420]

L- Editor: Filipodia E- Editor: Liu WX

## AgNOR and rasp21 expression in gastric mucosal lesions caused by *Helicobacter pylori* infection

Heng-Jun Gao, Xiu-Zhen Lu, Xiao-Yong Zhang, Zhi-Quan Zhao

Heng-Jun Gao, Xiu-Zhen Lu, Xiao-Yong Zhang, Zhi-Quan Zhao, Department of Gastroenterology, First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Received: March 8, 1997  
Revised: May 2, 1997  
Accepted: October 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To investigate AgNOR and rasp21 expression levels in gastric mucosal lesions caused by *Helicobacter pylori* (Hp) infection in order to gain insight into the related biological processes (i.e. tumor-like behavior) and possible underlying mechanism supporting Hp pathogenesis.

**METHODS:** Hp infection was diagnosed in using the standard Campylobacter-like organism test along with Wathin-Starry staining. The expression of AgNOR was detected by the silver colloid staining technique. The expression of rasp21 was detected by monoclonal antibody and immunohistochemical staining using the ABC method. The study included a total of 278 patients with endoscopically- and pathologically-confirmed gastric mucosal lesions, representing chronic superficial gastritis (CSG), chronic atrophic gastritis (CAG), intestinal metaplasia, dysplasia, and gastric cancer. Among these, 146 of the

patients were Hp-positive and 132 were Hp-negative.

**RESULTS:** The Hp-positive group of patients showed significantly greater AgNOR in the gastric mucosal lesions than the Hp-negative group, with the exception of the CSG sub-group ( $P < 0.05$  or  $P < 0.01$ ). The positive rate of rasp21 expression in gastric mucosal lesions in the Hp-positive group was also significantly higher than that in the Hp-negative group, with the exception of the CSG and CAG sub-groups ( $P < 0.05$ ).

**CONCLUSION:** Hp-positive gastric mucosal lesions show biological behaviour of tumors. Hp may act as a promoter to activate the ras gene and to stimulate cell over-proliferation.

**Key words:** *Helicobacter pylori*/pathogenicity; Gastric mucosal/pathology; Stomach diseases/microbiology; Stomach neoplasms/microbiology; Nucleoproteins/metabolism; Proto-oncogene protein P21/ (RAS)

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Gao HJ, Lu XZ, Zhang XY, Zhao ZQ. AgNOR and rasp21 expression in gastric mucosal lesions caused by *Helicobacter pylori* infection. *World J Gastroenterol* 1997; 3(4): 252 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/252.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.252>

L- Editor: Filipodia E- Editor: Liu WX

## Clinical significance of CA19-9 in diagnosis of digestive tract tumors

Ji-Zong Zhao, Bo-Heng Wu

Ji-Zong Zhao, Bo-Heng Wu, First Affiliated Hospital, Sun-Yat Sen University of Medical Sciences, Guangzhou 510080, Guangdong Province, China

Dr. Ji-Zong Zhao, male, born on January 22, 1945 in Guangzhou, graduated from the Department of Laboratory Medical Sciences at Guangzhou Medical College, currently Laboratorian in Charge, engaged in laboratory diagnostic study of clinical chemistry and immunology, and having 15 papers published.

Author contributions: All authors contributed equally to the work.

Correspondence to: Dr. Ji-Zong Zhao, First Affiliated Hospital, Sun-Yat Sen University of Medical Sciences, Guangzhou 510080, Guangdong Province, China  
Telephone: +86-20-87755766

Received: September 6, 1996  
Revised: October 5, 1996  
Accepted: October 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To evaluate the clinical value of CA19-9 in diagnosing and differentiating gastrointestinal tumors and in monitoring patients treated surgically.

**METHODS:** Patients with gastric cancer ( $n = 70$ ), colorectal cancer ( $n = 90$ ), pancreatic cancer ( $n = 7$ ), esophageal cancer ( $n = 10$ ) and benign disorders ( $n = 30$ ), and normal adults ( $n = 111$ ; used as healthy controls), were studied. Fasting blood samples were obtained from each study participant. The serum CA19-9 concentration was measured with radioimmunoassay.

**RESULTS:** The mean CA19-9 level was significantly higher in patients with gastric cancer ( $170.69 \pm 91.45$  kU/L) and patients with colorectal cancer ( $87.21 \pm 39.55$  kU/L) than in the healthy controls ( $11.254 \pm 6.00$  kU/L). Compared with the healthy controls, the CA19-9 level was also much higher in patients with pancreatic cancer ( $1266.58 \pm 521.31$  kU/L) ( $P < 0.01$ ). However, the CA19-9 concentrations in patients with non-recurrent gastric cancer ( $12.63 \pm 3.62$  kU/L), colorectal cancer ( $14.14 \pm 3.26$  kU/L) and benign disorders ( $14.23 \pm 2.60$  kU/L) were statistically similar to those in the healthy controls ( $P > 0.05$ ). The demarcation value of CA19-9 between negative and positive was  $< 31.0$  kU/L. The sensitivity of CA19-9 for gastric, colorectal, pancreatic and esophageal cancers and for gastrointestinal benign disorders was 47.3%, 50.0%, 83.3%, 20.0% and 0%, respectively. The specificity of CA19-9 for digestive system malignant diseases was 100% for all.

**Key words:** Digestive system neoplasms; CA19-9; Tumor-related antigen; Stomach neoplasms; Colorectal neoplasms; Pancreatic neoplasms; Esophageal neoplasms

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All

rights reserved.

Zhao JZ, Wu BH. Clinical significance of CA19-9 in diagnosis of digestive tract tumors. *World J Gastroenterol* 1997; 3(4): 253-254 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/253.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.253>

### INTRODUCTION

The CA<sub>19-9</sub> antigen isolated by Koprowski and colleagues<sup>[1]</sup> in 1979 is a lacto-N-fucopentaose II-like substance and one of the tumor-associated antigens present in serum in the mucin fraction. Close attention has been paid to the role of CA<sub>19-9</sub> in the diagnosis of digestive tract tumors<sup>[2,3]</sup>. After strictly inspecting the quality of a commercially available CA<sub>19-9</sub> antigen kit, we assayed serum CA<sub>19-9</sub> levels in 207 patients with gastrointestinal diseases and in 111 normal adults (for comparative analysis as healthy controls). These data was used to evaluate the clinical significance of CA<sub>19-9</sub> in diagnosis of digestive tract tumors.

### MATERIALS AND METHODS

#### Experimental equipment

The CA<sub>19-9</sub> McAb solid phase IRMA kit (Tianjin SYTRON Biotech Inc.) and the FT-613 automatic counter of <sup>125</sup>I radioimmunoassay (Beijing) were used.

#### Normal CA<sub>19-9</sub> value

CA<sub>19-9</sub> concentration was determined in the sera of 111 normal adults from the Guangzhou area (55 males, 50 females; mean age of 47.7 years) and used as the normal referential values for the study.

#### Patients

Serum samples were obtained from 207 patients with malignant and benign diseases of the digestive system. All diagnoses were confirmed by clinical, laboratory and pathological examinations. Of the 207 cases, 70 were gastric cancer, 90 were colorectal cancer, 7 were pancreatic cancer and 10 were esophageal cancer; a total of 30 of the cases were benign disorders, including 6 cases of chronic superficial gastritis, 7 of antral gastritis, 5 of gastric ulcers, 10 of duodenal ulcers, and 2 of acute appendicitis. The patients consisted of 148 males and 59 females, with age range of 22 years to 85 years (average age: 52.9 years).

### RESULTS

The mean serum CA<sub>19-9</sub> concentration in the 111 healthy controls from the Guangzhou area was  $11.254 \pm 6.006$  kU/L. The differ-

**Table 1 Serum CA19-9 concentration in patients and healthy controls**

	<i>n</i>	$\bar{x} \pm s$	Range	> 31.0 kU/L	PR, %
Gastric cancer					
Preoperation	46	170.69 ± 91.45 <sup>a</sup>	10.3-3220.0	19	41.3
Postoperative stability	15	12.62 ± 3.26	0.2-22.8	0	0.0
Postoperative recurrence	9	393.17 ± 3.804	9.0-2843.8	7	77.8
Colorectal cancer					
Preoperation	50	152.69 ± 76.39 <sup>a</sup>	0.2-3261.0	20	40.0
Postoperative stability	24	14.15 ± 2.25	0.0-31.0	0	0.0
Postoperative recurrence	16	87.21 ± 39.55 <sup>a</sup>	0.5-657.8	11	68.7
Pancreatic cancer	7	1266.58 ± 521.31 <sup>b</sup>	11.0-3220.0	6	83.3
Esophageal cancer	10	19.94 ± 6.31	0.0-53.0	2	20.0
Benign disorders	30	14.23 ± 2.60	0.0-27.2	0	0.0
Healthy controls	111	11.25 ± 0.57	0.0-27.5	0	0.0

<sup>a</sup>*P* < 0.05 vs healthy controls, <sup>b</sup>*P* < 0.01 vs healthy controls; PR, positive rate.

ence of CA<sub>19-9</sub> value among the healthy controls was not statistically significant among groups divided by age or sex (*P* > 0.05). The demarcation value of CA<sub>19-9</sub> between the negative and positive was < 31.0 kU/L in our laboratory. The serum CA<sub>19-9</sub> concentrations in patients with malignant and benign diseases are listed in Table 1. Table 2 shows the evaluational indexes of CA<sub>19-9</sub> for diagnosing some of the gastrointestinal tumors.

## DISCUSSION

We have determined the normal referential value of CA<sub>19-9</sub> (11.254 ± 6.006 kU/L) in 111 healthy controls from the Guangzhou area using the solid phase IRMA kit. This result was similar to the results obtained by testing with solid phase radioimmunoassay kits manufactured by the Abbott Company and ORIS Company (France). The demarcation value of CA<sub>19-9</sub> between the negative and positive was < 31.0 kU/L. This value was slightly lower than that reported in the kit's accompanying documentation (< 34.0 kU/L).

The results of our study showed that serum CA<sub>19-9</sub> levels in patients with gastric cancer, colorectal cancer and some postoperatively recurrent cancers were significantly higher than those detected in the healthy controls. The sensitivity of CA<sub>19-9</sub> in diagnosing gastric and colorectal cancers was 47.7% and 50.0%, and the specificity and positive predictive value were both 100% for all. None of the 30 patients with benign disorders of the gastrointestinal tract had a higher serum CA<sub>19-9</sub> level than the normal referential value. The sensitivity and specificity of CA<sub>19-9</sub> for benign disorders were 0% and 100%, respectively, indicating that CA<sub>19-9</sub> is a highly specific tumor marker for diagnosing gastric and colorectal cancers and suggesting its potential to play an important role in the differentiation of benign and malignant diseases of digestive tract.

It is worth noting here that of the 39 patients with gastric cancer or colorectal cancer in this study who had no recurrent tumor postoperatively, none had a higher CA<sub>19-9</sub> level than the normal referential value. The mean value of CA<sub>19-9</sub> of the 39 patients was similar to the normal referential value. However, the serum levels of CA<sub>19-9</sub> in the 9 patients with recurrent gastric cancer and in the 16 patients with recurrent colorectal cancer postoperatively were significantly higher than the normal referential value. The positive rate of CA<sub>19-9</sub> in recurrent gastric and colorectal cancers was 77.8% and 68.7%, respectively. The CA<sub>19-9</sub> level reached 2500 kU/L in a few of the patients with recurrent tumors. These collective results suggest that CA<sub>19-9</sub> is a good index for evaluating the effect of treatment and predicting the prognosis of gastric and colorectal cancers postoperatively.

**Table 2 Evaluational indexes for CA19-9-based diagnosis of gastrointestinal benign and malignant diseases**

Disease	<i>n</i>	Positive	Se, %	Sp, %	Ac, %	+PV, %	-PV, %
Gastric cancer							
Preoperation and recurrence	55	26	47.3	100.0	65.9	100.0	50.8
Colorectal cancer							
Preoperation and recurrence	66	33	50.0	100.0	65.6	100.0	47.6
Esophageal cancer	10	2	20.0	100.0	80.0	100.0	78.9
Pancreatic cancer	7	6	83.3	100.0	97.3	100.0	96.8
GI benign disorders	30	0	0.0	100.0	78.7		78.7

Se, Sensitivity; Sp, Specificity; Ac, Accuracy; +PV, Positive predictive value; -PV, Negative predictive value.

Elevated serum CA<sub>19-9</sub> levels have been observed in many different malignant diseases. The test seems especially promising for detection of pancreatic cancer, as more than 80% of these patients have been reported to show increased serum CA<sub>19-9</sub> concentration<sup>[4,5]</sup>; therefore, CA<sub>19-9</sub> may be a good tumor marker for diagnosing pancreatic cancer and monitoring patients treated surgically<sup>[6]</sup>. Here, we assayed the CA<sub>19-9</sub> levels in patients with pancreatic cancer, and the serum CA<sub>19-9</sub> concentration was found to be > 85 kU/L in 6 of the patients, > 110 kU/L in 5, > 1900 kU/L in 3, and 3200 kU/L in 1. The sensitivity, specificity, accuracy, positive predictive value and negative predictive value were 83.3%, 100%, 97.3%, 100% and 96.8%, respectively.

The incidence of pancreatic cancer is increasing worldwide. In 1987, the Japanese Cancer Registries reported the 5-year survival rates for 177 resected cases of T<sub>1</sub> stage, 783 resected cases of T<sub>2</sub> stage, 463 resected cases of T<sub>3</sub> stage and 304 resected cases of T<sub>4</sub> stage cancer as being 39.8%, 21.7%, 14.3% and 13.4%, respectively<sup>[7]</sup>. These results indicate that it is important to diagnose early stage pancreatic cancer, so as to improve prognosis. However, using the current diagnostic methods, it is difficult to diagnose pancreatic cancer in the early stage and to distinguish it from benign conditions that resemble pancreatic cancer in many aspects. Although only 7 patients with pancreatic cancer received the CA<sub>19-9</sub> test in the current study, the results still showed that the CA<sub>19-9</sub> test seems to be a useful additional tool in the diagnosis of pancreatic cancer.

## REFERENCES

- Koprowski H**, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Colorectal carcinoma antigens detected by hybridoma antibodies. *Somatic Cell Genet* 1979; **5**: 957-971 [PMID: 94699 DOI: 10.1007/BF01542654]
- Gupta MK**, Arciaga R, Bocci L, Tubbs R, Bukowski R, Deodhar SD. Measurement of a monoclonal-antibody-defined antigen (CA19-9) in the sera of patients with malignant and nonmalignant diseases. Comparison with carcinoembryonic antigen. *Cancer* 1985; **56**: 277-283 [PMID: 2408729 DOI: 10.1002/1097-0142 (19850715)56:2 < 277: : AID-CNCR2820560213 > 3.0.CO; 2-M]
- Atkinson BF**, Ernst CS, Herlyn M, Steplewski Z, Sears HF, Koprowski H. Gastrointestinal cancer-associated antigen in immunoperoxidase assay. *Cancer Res* 1982; **42**: 4820-4823 [PMID: 6751528]
- Haglund C**, Roberts PJ, Kuusela P, Scheinin TM, Mäkelä O, Jalanko H. Evaluation of CA 19-9 as a serum tumour marker in pancreatic cancer. *Br J Cancer* 1986; **53**: 197-202 [PMID: 3456787 DOI: 10.1038/bjc.1986.35]
- Steinberg WM**, Gelfand R, Anderson KK, Glenn J, Kurtzman SH, Sindelar WF, Toskes PP. Comparison of the sensitivity and specificity of the CA19-9 and carcinoembryonic antigen assays in detecting cancer of the pancreas. *Gastroenterology* 1986; **90**: 343-349 [PMID: 2416628]
- Tian F**, Appert HE, Myles J, Howard JM. Prognostic value of serum CA 19-9 levels in pancreatic adenocarcinoma. *Ann Surg* 1992; **215**: 350-355 [PMID: 1348409 DOI: 10.1097/0000658-199204000-00008]
- Japanese Pancreas Society**. Report of Japanese pancreatic cancer register. Sendai: Japanese Pancreas Society, 1987

L- Editor: Filipodia E- Editor: Liu WX

## Analysis of amino acid constituents of gallstones

Ying Chen, Lian-Lian Wang, Yu-Xia Xiao, Jing-Hua Ni, Yan Yu

Ying Chen, Lian-Lian Wang, Department of Gastroenterology, Beijing Hospital, Beijing 100730, China

Yu-Xia Xiao, Jing-Hua Ni, Yan Yu, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100730, China

Dr. Ying Chen, female, born on 1942-12-14 in Shanghai, graduated from Nanjing Medical University in 1966, and having 33 papers published.

This project won The third-class award by the Beijing Hospital for research achievements.

Author contributions: All authors contributed equally to the work.

Correspondence to: Dr. Ying Chen, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100730, China  
Telephone: +86-10-65132266-3135

Received: October 26, 1996  
Revised: December 15, 1996  
Accepted: April 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To seek drugs that will efficaciously dissolve bilirubin, glycoprotein and black stones and that will represent improved lithotriptic agents to resolve cholesterol stones, and to study the amino acid constituents of gallstones.

**METHODS:** According to characteristics determined by infrared spectroscopy and to the contents of bilirubin determined by semi-quantitative chemical analysis, 30 of 148 cases of gallstones were selected and divided into 5 groups. Amino acids of the 30 cases were detected by high-speed chromatography.

**RESULTS:** The quantity of amino acids was highest in black stones (226.9 mg/g) and lowest in pure cholesterol stones (1.4 mg/g). In the 5 groups of gallstones, the quantity of amino acids followed the hierarchy of black stone > mixed bilirubin stone and glycoprotein stone > mixed cholesterol stone > pure cholesterol stone. The proportions were: 95.95:29.02 and 28.05:5.78:1. Aliphatic amino acids accounted for ~ 50% of the total amino acids in the gallstones, with glycine accounting for 15.3% of the total amount of the 17 kinds of amino acids.

**CONCLUSION:** For mixed stones, the higher level of bilirubin, the higher content of amino acids. Acidic amino acids were relatively higher in bilirubin stones than in cholesterol stones.

**Key words:** Gallstones; Amino acids/analysis; Bilirubin; Glycine

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All

rights reserved.

Chen Y, Wang LL, Xiao YX, Ni JH, Yu Y. Analysis of amino acid constituents of gallstones. *World J Gastroenterol* 1997; 3(4): 255-256 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/255.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.255>

### INTRODUCTION

Dissolution of cholesterol stones can be achieved by oral ursodeoxycholic acid or chenodehydrocholic acid, and by perfusion with methyl tertiary butyl ether as well. However, no effective means of dissolving bilirubin, glycoprotein and black stones has been reported. In order to identify better lithotriptic agents, the contents of amino acids in gallstones needs to be studied.

### MATERIALS AND METHODS

#### Sample selection

Gallstones were obtained from 148 patients who were treated by surgery in our hospital during the years of 1988 to 1992. The specimens were subjected to qualitative assessment by infrared spectroscopy, and 48 cases were also subjected to semi-quantitative chemical analysis. According to the characteristics corresponding to the infrared spectrum and the constituents of bilirubin, 30 cases were selected and divided into the following 5 groups: Pure cholesterol stones ( $n = 10$ ), mixed cholesterol stones ( $n = 7$ ), mixed bilirubin gallstones ( $n = 10$ ), glycoprotein stones ( $n = 2$ ), and black stone ( $n = 1$ ).

#### Sample treatment<sup>[1]</sup>

After pulverizing by agate mortar and drying, 20 mg of powder from each gallstone was added to 6 mL of HCl (6 mol), nitrogen sealed and baked at 110 °C for 24 h. The volume was brought to 25 mL with distilled water. After filtration, 4 mL was collected, dried in a rotary evaporator, and washed twice with distilled water. The remaining sample was dissolved in 2 mL distilled water, of which 50 µL or 100 µL was used to measure the 17 amino acids, and taurine and ammonia concentrations. A trace of tryptophane was detected in 2 cases.

#### Analytical methods

Amino acids were detected by high-speed chromatography (L-8500; Hitachi Corp, Japan). The column was 4.6 mm × 60 mm, and 5 buffer solutions were used for the stepwise wash-off, with resin of 2622 s.c. (Hitachi ion exchange resin was used). The standard amino acid samples were provided by Sodium Glutamate Corp. (Japan). The quantitative analysis was conducted with extensional calculation. The coefficient of variation (c.v.) was 1.5% in this experiment.

Table 1 Content of various amino acids in 5 groups (30 cases) of gallstones ( $\bar{x} \pm s$ , mg/g)

	Pure cholesterol		Mixed cholesterol		Mixed bilirubin		Glycoprotein		Black
	<i>n</i>	$\bar{x} \pm s$	<i>n</i>	$\bar{x} \pm s$	<i>n</i>	$\bar{x} \pm s$	<i>n</i>	$\bar{x} \pm s$	
Gly.	10	0.635 ± 0.498	7	2.333 ± 2.890	10	10.459 ± 4.451	2	19.185 ± 0.955	16.552
Ala.	8	0.109 ± 0.084	7	0.794 ± 0.622	10	4.048 ± 2.409	2	1.925 ± 2.298	13.551
Val.	9	0.133 ± 0.089	7	1.043 ± 0.674	10	4.202 ± 2.709	2	3.500 ± 0.948	15.383
Ile.	5	0.080 ± 0.044	7	0.496 ± 0.330	10	2.407 ± 1.374	2	1.590 ± 0.523	4.921
Leu.	9	0.150 ± 0.093	7	1.113 ± 0.671	10	5.914 ± 3.983	2	4.510 ± 0.410	16.834
Thr.	10	0.112 ± 0.077	7	0.743 ± 0.499	10	3.784 ± 2.874	2	3.080 ± 0.820	11.642
Ser.	10	0.117 ± 0.077	7	0.790 ± 0.565	10	2.836 ± 1.799	2	3.085 ± 0.870	11.643
Pro.	4	0.116 ± 0.054	5	0.621 ± 0.203	10	2.953 ± 2.167	2	2.560 ± 0.968	12.421
Sys.	2	0.060 ± 0.014	6	0.422 ± 0.211	10	2.018 ± 1.199	2	2.235 ± 0.813	9.943
Met.	4	0.038 ± 0.013	4	0.398 ± 0.479	10	1.080 ± 0.670	2	0.800 ± 0.070	3.309
Phe.	10	0.160 ± 0.089	7	0.797 ± 0.451	10	3.499 ± 2.397	2	3.060 ± 0.509	9.928
Thr.	8	0.108 ± 0.055	7	0.596 ± 0.351	10	2.483 ± 1.713	2	1.710 ± 0.453	6.907
Asp.	10	0.220 ± 0.162	7	1.374 ± 0.869	9	6.066 ± 4.039	2	5.250 ± 1.796	24.103
Glu.	10	0.293 ± 0.194	7	1.723 ± 1.088	10	8.595 ± 5.468	2	7.460 ± 1.950	34.358
Lys.	9	0.126 ± 0.076	7	0.680 ± 0.405	10	4.058 ± 3.195	2	2.405 ± 1.124	17.582
His.	9	0.071 ± 0.039	7	0.401 ± 0.258	10	2.041 ± 1.983	2	0.945 ± 0.247	4.316
Arg.	9	0.012 ± 0.078	7	0.716 ± 0.418	10	4.012 ± 2.492	2	3.010 ± 1.923	13.517
Tau.	5	0.052 ± 0.028	9	1.695 ± 1.697	9	1.695 ± 1.697	1	1.540	1.608
Ammon.	10	0.662 ± 0.232	10	5.332 ± 1.590	10	5.332 ± 1.590	2	6.130 ± 1.174	6.435

## RESULTS

The various contents of amino acids for the 30 gallstone cases in the 5 groups are presented in Table 1. All 30 had glycine, glutamic acid, threonine, phenylalanine and ammonia; among these, glycine content was the highest, accounting for 15.34% of the total amount, followed by glutamic acid, accounting for 13.01%. Asparagine, serine, valine, leucine, lysine, histidine and arginine were detected in 29 of the gallstone cases. There was a strong correlation ( $P < 0.01$ ) between the above-mentioned amino acids. In 29 gallstone cases, there were more acidic amino acids than alkaline amino acids (1.39-2.73:1), except for a single bilirubin mixed stone (1:1.77) which had the appearance of black mud and came from a patient with malignant changes in the gallbladder and liver metastasis detected in postoperative pathologic examinations. The content of amino acids in one sample of pure cholesterol stones was the lowest for the 30 cases of gallstones examined (1.37 mg/g). Six amino acids (glycine, glutamic acid, aspartic acid, serine, threonine, and phenylalanine), taurine and ammonia were detected in this case. In the 5 groups of gallstones, the constituents of amino acids of one case of black gallstone were the most complete and the total amount of amino acids also was the highest (226.93 mg/g), only the content of glycine was slightly lower than that of glycoprotein.

In 10 cases of pure cholesterol stones, the content of glycine was higher than that of glutamic acid and aspartic acid, and that of glutamic acid was higher than that of aspartic acid, with the differences being statistically significant ( $P < 0.005$ ).

In 7 cases of mixed cholesterol stone cases, the content of glutamic acid was significantly higher than that of aspartic acid ( $P < 0.01$ ), but the content of glycine was higher, but not significantly, than that of glutamic acid and aspartic acid ( $P > 0.05$ ).

In 10 cases of mixed bilirubin stones, although the content of glycine was higher than that of glutamic acid and aspartic acid, and that of glutamic acid was higher than that of aspartic acid, there was no significant differences ( $P > 0.05$ ).

## DISCUSSION

The amino acid is the fundamental unit of protein constitution. Nowadays, it is known that there are 20 kinds of amino acids<sup>[2]</sup>, which are controlled by genetic code in protein molecules. They are called living proteinic amino acids, and consist of glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, tyrosine, serine, threonine, cysteine, glutamic acid, aspartic acid,

histidine, lysine, arginine, asparagine and glutamine. Of the 20 living proteinic amino acids, 17 kinds were detected in this study; asparagine and glutamine were not detected because during this experiment<sup>[3]</sup> they would be completely hydrolyzed to free aspartic acid and glutamic acid, leading to artifactually higher amounts. Moreover, the proteins in this study were heated and hydrolyzed with 6 mol HCl, and tryptophan was damaged; additionally, cystine, methionine, threonine and serine were also influenced by the method, so the results for each were expected to be low. It has been reported that cysteine only exists in bilirubin gallstones, but in our experiment cysteine was not detected and only a small amount of the bisulfide compound of cysteine cystine and taurine produced during the conversion of cysteine in liver was detected.

Our results indicate that protein amino acids generally exist in gallstones. The components of amino acids detected in the gallstones were: Aliphatic amino acid > Acidic amino acid > Alkaline amino acid > Aromatic amino acid > Sulfur amino acid. In the 5 groups of gallstones of this study, the contents of amino acids were: Black gallstone > Mixed bilirubin stones and glycoprotein stone > Mixed cholesterol stone > Pure cholesterol stone. Their proportions were: 95.95:29.02 and 28.05:5.78:1. Glycine was the most abundant among the 8 kinds of aliphatic amino acids or the 17 kinds of amino acids in gallstones. For mixed stones, the higher the content of bilirubin, the higher the content of amino acids. With the exception of the black gallstones, all gallstones showed higher amount of glycine than that of aspartic acid and glutamic acid. However, in the pure cholesterol stones, glycine content was higher than aspartic acid and glutamic acid content ( $P < 0.005$ ); thus, it was clear that proportion of acidic amino acids in bilirubin stones was relatively higher than that in cholesterol gallstones. In 30 cases of gallstones, the components of acidic amino acids were higher than those of alkaline amino acids, except for the bilirubin mixed stones from a patient with malignant changes in the gallbladder and liver metastasis that was determined by postoperative pathologic examination. It is likely that the metabolic product of malignant tissue is not favorable for the stable existence of acidic amino acids. The mechanism underlying this observation, however, remains unknown.

## REFERENCES

- 1 Dal XW, Chen SZ, Yu Y, He KX, Zhang JQ. Analysis of amino acids of biliary pigment stones and indissoluble constituents. *Zhonghua Yixue Zazhi* 1989; **69**: 350-351
- 2 Liang ZY, Zhang HZ, Chen SS, Yuan HJ, Zhang YZ, Lin SY. *Physiologic chemistry*. Shanghai: The Publishing House of Science and Technology, 1985: 2-3
- 3 Shi F, Zhang JQ, Chen CC, XY, Wang LT, Sheng YZ. Analysis of amino acids of pigment stone. *Zhonghua Waikao Zazhi* 1987; **25**: 333-334

L- Editor: Filipodia E- Editor: Liu WX

## Comparative study of changing patterns of concanavalin A-binding proteins in early stage of cholesterol gallstone formation

Yu-Qiang Chen, Duan Cai, Yan-Lin Zhang, Tian-Fang Hua

Yu-Qiang Chen, Department of Surgery, Shanghai First People's Hospital, Shanghai 200080, China

Duan Cai, Yan-Lin Zhang, Tian-Fang Hua, Department of Surgery, Shanghai Hua Shan Hospital, Shanghai 200080, China

Yu-Qiang Chen, MD, attending surgeon, having 7 papers and 2 books published.

Author contributions: All authors contributed equally to the work.

Supported by The National Science Foundation of China, No. 39170718.

Correspondence to: Yu-Qiang Chen, MD, Department of Surgery, Shanghai Hua Shan Hospital, Shanghai 200080, China

Received: February 16, 1997  
Revised: March 29, 1997  
Accepted: October 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To elucidate the importance and the changing patterns of biliary concanavalin A-binding proteins (CPs) in the early stage of cholesterol gallstone formation.

**METHODS:** CP concentration and nucleation activity were measured by lectin affinity chromatography in bile samples of patients with cholesterol gallstones, pigment gallstones, gallbladder cholesterosis and non-biliary diseases.

**RESULTS:** The concentrations of CPs were much higher in patients with cholesterol gallstones ( $0.39 \pm 0.11$  g/L,  $n = 36$ ,  $P < 0.01$ ) or gallbladder cholesterosis ( $0.40 \pm 0.09$  g/L,  $n = 9$ ,  $P < 0.01$ ) than in those with pigment gallstones ( $0.2 \pm 0.12$  g/L,  $n = 7$ ) and/or non-biliary diseases ( $0.27 \pm 0.09$  g/L,  $n = 10$ ). Pronucleating activities were much stronger in patients with cholesterol gallstones (nucleation time ratio:  $0.57 \pm 0.21$ ,  $n = 5$ ,  $P < 0.01$  vs pigment gallstones and/or non biliary diseases) and gallbladder cholesterosis (nucleation time ratio:  $0.44 \pm 0.23$ ,  $n = 5$ ,  $P < 0.01$  vs pigment gallstones or non-biliary diseases). The binding percentages of CPs to model biliary vesicles were also higher for patients with cholesterol gallstones ( $n = 6$ ) than those with pigment gallstones ( $n = 6$ ) ( $2.4\% \pm 0.9\%$  vs  $0.9\% \pm 0.5\%$ ,  $P < 0.01$ ).

**CONCLUSION:** Hypersecretion of CPs, especially those in vesicular phase, may be an important change in the early stage of cholesterol

gallstone formation.

**Key words:** Bile; Concanavalin A binding proteins; Cholesterol gallstone; Chromatography

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen YQ, Cai D, Zhang YL, Hua TF. Comparative study of changing patterns of concanavalin A-binding proteins in early stage of cholesterol gallstone formation. *World J Gastroenterol* 1997; 3(4): 257-259 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/257.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.257>

### INTRODUCTION

Protein in bile has been considered for more than 3 decades as a contributing factor to the pathogenesis of gallstone formation<sup>[1]</sup>. But the essential progress in our understanding of this process was made just only 10 years ago, when Groen and colleagues<sup>[2]</sup> used lectin affinity chromatography to isolate and purify a potent pronucleating glycoprotein which has since been recognized as the bile-form aminopeptidase N. Since then, numerous nucleation promoting and inhibiting biliary proteins have been characterized<sup>[3-5]</sup>. But, as of yet, nearly nothing has been done in the research field of the most important or key nucleating proteins.

Concanavalin A-binding proteins (CPs), a group of biliary proteins containing almost all the well known pronucleating proteins, provided an opportunity to study the nucleation-effecting proteins systematically. Vesicular proteins are functionally location-specific pronucleating proteins, their concanavalin A-binding regions may exert important actions in gallstone formation. In this article, the authors determined the concentrations and nucleation activities of total biliary proteins, biliary CPs and vesicles related to CPs, so as to elucidate the relationship between changes in quantity and quality of biliary CPs and gallstones formation and study systematically the importance of CPs in pathogenesis of gallstone formation.

### MATERIALS AND METHODS

#### Patients

Sixty-six patients were enrolled in this study, including 43 with cholelithiasis (36 with cholesterol gallstones, 7 with pigment gallstones), 9 with gallbladder cholesterosis, 2 each with gallbladder adenomyomatosis and adenoma, and 10 with non-biliary diseases (5 with

**Table 1** Concentrations of proteins in human gallbladder bile (g/L,  $\bar{x} \pm s$ )

Patient group	n	Total protein	CP concentration
Cholesterol gallstones	36	4.1 ± 1.5	0.39 ± 0.11
Pigment stones	7	3.2 ± 1.8	0.26 ± 0.12
Gallbladder cholesterosis	9	4.4 ± 1.6	0.40 ± 0.09
Gallbladder adenomyomatosis	2	3.8 ± 1.2	0.29 ± 0.19
Gallbladder adenoma	2	3.9 ± 1.1	0.26 ± 0.14
Non-biliary diseases	10	4.5 ± 1.6	0.27 ± 0.09

<sup>a</sup>*P* < 0.05 vs other groups; <sup>b</sup>*P* < 0.01 vs pigment gallstones and non-biliary diseases. CP: Concanavalin A-binding protein

**Table 2** Nucleation time of human gallbladder bile ( $\bar{x} \pm s$ )

Patient group	n	Nucleation time (d)
Cholesterol gallstones	36	7.8 ± 4.2
Solitary	20	5.3 ± 2.7
Multiple	16	10.1 ± 5.6
Pigment gallstones	7	14.7 ± 6.0 <sup>1</sup>
Gallbladder cholesterosis	9	6.1 ± 3.7
Stone-free diseases	14	16.3 ± 3.2 <sup>2</sup>

Notes: Those (<sup>1</sup>3 cases, <sup>2</sup>8 cases) without nucleation after 21 d were recorded as 21 d.

colon cancer, 2 with gastric cancer, 1 each with peptic ulcer perforation and pancreatic pseudocyst). All the patients met the following criteria: No acute inflammation of the gallbladder, and no obstruction at the cystic duct or common bile duct; no abnormality in liver biochemistry, and negative bile culture; total lipid concentration in bile of > 50 g/L; serum protein concentration within normal range. Bile was sampled by aspiration according to the standard method<sup>[6]</sup> during operation and then stored at -20 °C. Observation of cholesterol monohydrate crystal in bile was carried out immediately after sample obtainment.

Stone classification was based on gross inspection and cholesterol content. Cholesterol gallstone was identified by cholesterol dry weight percentage > 70%; pigment gallstone was identified if the cholesterol dry weight percentage was < 30%.

#### Isolation of biliary vesicles and quantitation of vesicular protein

Biliary vesicles were isolated from 10 mL bile of each patient according to the method described by Amigo *et al.*<sup>[7]</sup>, and further purified according to the method described by Stone *et al.*<sup>[8]</sup>. Vesicular protein concentrations were determined using a pooled concentrated eluent that had undergone ultrafiltration.

#### Quantitative and nucleating activity analysis of CPs

CPs isolated from 1 mL bile were the bound fractions for concanavalin A affinity chromatography, and determined quantitatively using the Coomassie bright blue method. Nucleation activities were determined as follows: Lyophilized CPs prepared from 1 mL gallbladder bile as stated above were put into 1 mL artificial bile, and then incubated at 37 °C. Nucleation time (NT) was determined according to Holan's method<sup>[9]</sup>, and the NT ratio was calculated as the relative value to the blank control; final cholesterol crystal concentration (FCCC) was that after 3 wk at 37 °C, and its relative percentage to the blank control was the FCCC percentage.

#### Preliminary study of the distribution of CPs in vesicles

To determine the distribution of CPs in the vesicular phase of native bile, concanavalin A was first linked to horseradish peroxidase (HRP) (con A-HRP, 1:1 molar ratio) according to Guo's report<sup>[10]</sup>. One drop of the con A-HRP solution was added into one drop of bile on a 200-mesh zinc grid that was covered with carbon film, and stored overnight in moist saturation at 37 °C. The excess liquid was soaked out the next morning and dyed with 0.01% diaminobenzidine (DAB) solution (with dropwise addition of H<sub>2</sub>O to bring the volume up to 20 mL) for 15 min. The prepared zinc grids were then gently washed 3 times with buffer solution, dried and examined by a JEM-1200EX electronic microscope at a magnification of 30000 and using an accelerating voltage of 80 KV.

The binding characteristics of CPs to vesicles and micelles in model bile were determined using gallbladder bile samples from 6

**Table 3** Cholesterol crystals and biliary proteins ( $\bar{x} \pm s$ )

Patient group	n	Total protein (g/L)	CPs (g/L)
Gallstones	36		
With crystal	29	4.9 ± 2.7	0.43 ± 0.14
Without crystal	7	3.4 ± 2.1	0.29 ± 0.16
Gallstone-free	30		
With crystal	14	4.1 ± 1.9	0.40 ± 0.17
Without crystal	16	3.2 ± 2.1	0.31 ± 0.17

**Table 4** Influence of Concanavalin A-binding proteins on nucleating activities of model bile ( $\bar{x} \pm s$ )

Patient group	n	Nucleation time ratio	Final cholesterol crystal concentration percentage
Cholesterol gallstones	5	0.57 ± 0.21	143.4 ± 12.6
Pigment gallstones	5	0.71 ± 0.19	121.3 ± 27.4
Gallbladder cholesterosis	5	0.44 ± 0.23	152.7 ± 18.4
Non-biliary diseases	5	0.73 ± 0.11	130.4 ± 15.7

patients with cholesterol gallstones and 6 with pigment gallstones. The samples were processed to harvest CPs as described above; after 20 min of ultracentrifugation at 10000 rpm, the CPs were then added into model bile to a final concentration of 0.2 g/L. After incubation at 37 °C for 2 wk, the contents of vesicular and micellar protein in these solutions were analyzed.

#### Preparation of model bile

According to the method described by Kibe *et al.*<sup>[11]</sup>, this model bile has a CSI of 1.2, total lipid concentration of 100 g/L and cholesterol/lecithin molar ratio of 4. The concentration of biliary protein was determined following the method described by Gallinger *et al.*<sup>[12]</sup>. Dunnett's *t*-test was used to compare the mean values between test groups and control group, and the *U* test was used to compare the mean values between two groups. A probability of < 0.05 was considered significant.

## RESULTS

### Concentration of total biliary protein and nucleating activities in human gallbladder bile

Table 1 presents our observations of no significant differences in total biliary protein concentration for the patients with cholesterol gallstones and/or gallbladder cholesterosis as compared to other groups. But, a significantly higher concentration of biliary CPs was found in patients with cholesterol gallstones and/or gallbladder cholesterosis (*P* < 0.05). A very significant difference in biliary CPs was also found between patients with gallbladder cholesterosis and pigment gallstone as well as those with non-biliary diseases (*P* < 0.01). Gallbladder bile from patients with cholesterol gallstones and/or gallbladder cholesterosis nucleated more rapidly than those with pigment gallstone and/or other stone-free diseases (*P* < 0.001, Table 2).

### Relationship between cholesterol crystals and biliary protein

As shown in Table 3, both the gallstone and stone-free patients with crystals have a higher concentration of total biliary protein than those without crystals, but significant differences were only found between gallstone patients with crystals and stone-free patients without crystals (*P* < 0.05). Biliary CP concentrations, however, were significantly higher in all patient groups with crystals than in the crystal-free patients (*P* < 0.05).

### Nucleation activities of biliary CPs

The nucleation time induced by CPs from patients with cholesterol gallstones and gallbladder cholesterosis was significantly shorter than that from patients with non-biliary diseases and pigment gallstones (*P* < 0.05). An even more significant difference was found between patients with gallbladder cholesterosis and pigment gallstone as well as those with non-biliary diseases (*P* < 0.01). CPs from patients with cholesterol gallstones or gallbladder cholesterosis caused a significantly higher FCCC ratio than those with pigment

gallstones and non-biliary diseases ( $P < 0.01$ , Table 4).

#### Distribution of CPs in vesicle phase

Using a concanavalin A-affinity staining method, we observed many dark brown spherical vesicles of varied size in native bile. After incubation of artificial bile with CPs for 2 wk, the binding percentage of CPs to vesicles was  $2.4\% \pm 0.9\%$  in patients with cholesterol gallstones, which was significantly higher than that in patients with pigment gallstones ( $0.9\% \pm 0.5\%$ ,  $P < 0.01$ ).

## DISCUSSION

Both pro- and antinucleating proteins have been found in human bile, and dozens of pronucleating proteins and several antinucleating proteins have been discovered in recent years; however, which among them plays the most important or key role in cholesterol nucleation remains unknown. CPs are glycoproteins that contain almost all the nucleating proteins reported. Systematic studies of CPs may provide some approaches for identifying these key factors, but up to now few reports have dealt with this issue.

Theoretically, increase of nucleating proteins in bile would cause an elevation of total protein level<sup>[13]</sup>, but inconsistent results have been reported because too many factors may influence the results. Our current data showed that patients with stone-free diseases had a higher level of biliary total protein than those with cholesterol gallstone (but with no statistical significance; Table 1). Nucleation time, however, was arbitrarily much faster in patients with cholesterol gallstones than those with pigment gallstones (Table 2), indicating that the differences between them arose from their differences in quantity and quality of nucleating proteins. Our results, as shown in Table 1, demonstrated a higher concentration of biliary CPs in patients with cholesterol gallstones and gallbladder cholesterosis than those with pigment gallstones and stone-free diseases, and a stronger nucleating activity for CPs from the former than the latter, indicating that the differences in CPs determined nucleation of cholesterol in bile.

The presence of cholesterol crystal in bile often indicates a high nucleating and crystal growth activity. In the current study, the concentration of biliary CPs in patients with cholesterol crystals was found to be higher than in those without the crystals; moreover, the concentration of biliary CPs in stone-free patients with crystals was higher than that in gallstone patients without crystals (Table 3), which suggests that elevation of nucleating proteins was not secondary to gallstone formation.

Owing to their special location, vesicular proteins have been considered as a very important group of pronucleating proteins<sup>[14]</sup>, and

it was predicted that CPs, which include almost all the pronucleating proteins, also exist in the vesicular phase. We confirmed this prediction by an affinity staining method devised to display the presence of CPs in the vesicular phase. This investigation of CPs bound to artificial vesicles also demonstrated that only a small part of the CPs could bind to vesicles and the binding percentage was much higher for CPs from patients with cholesterol gallstones than that from patients with pigment gallstones. These results imply that vesicle-binding CPs may exert some special effects on cholesterol nucleation in human bile.

## REFERENCES

- 1 Bouchier IA. Biochemistry of gallstone formation. *Clin Gastroenterol* 1983; **12**: 25-48 [PMID: 6347457]
- 2 Groen AK, Noordam C, Drapers JA, Egbers P, Jansen PL, Tytgat GN. Isolation of a potent cholesterol nucleation-promoting activity from human gallbladder bile: role in the pathogenesis of gallstone disease. *Hepatology* 1990; **11**: 525-533 [PMID: 2328950 DOI: 10.1002/hep.1840110402]
- 3 Harvey PR, Upadhyaya GA, Strasberg SM. Immunoglobulins as nucleating proteins in the gallbladder bile of patients with cholesterol gallstones. *J Biol Chem* 1991; **266**: 13996-14003 [PMID: 1856228]
- 4 Lippset PA, DSouza MP, Kaufman HS, et al. Differences in biliary nonmucin glycoproteins with and without gallstones. *Gastroenterology* 1992; **102**: 319A
- 5 Lippset PA, Samantary DK, Falconer SD, et al. Pronucleating proteins localize preferentially in gallbladder biliary vesicles. *Hepatology* 1993; **18**: 96A
- 6 Strasberg SM, Harvey PR. Biliary cholesterol transport and precipitation: introduction and overview of conference. *Hepatology* 1990; **12**: 1S-5S [PMID: 2210636]
- 7 Amigo L, Covarrubias C, Nervi F. Rapid isolation of vesicular and micellar carriers of biliary lipids by ultracentrifugation. *J Lipid Res* 1990; **31**: 341-347 [PMID: 2324652]
- 8 Stone BG, Larsen LJ, Knoll DA, Bloomfield VA, Duane WC. Separation of bile vesicles and micelles by gel filtration chromatography: the importance of the intermicellar bile salt concentration. *J Lab Clin Med* 1992; **119**: 557-565 [PMID: 1583413]
- 9 Holan KR, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology* 1979; **77**: 611-617 [PMID: 467918]
- 10 Guo C, Guo Q. Introduction of a simple, rapid and highly effective antibody labeling method using peroxidase activated by sodium periodate. *Shanghai Mianyixue Zazhi* 1983; **3**: 97
- 11 Kibe A, Holzbach RT, LaRusso NF, Mao SJ. Inhibition of cholesterol crystal formation by apolipoproteins in supersaturated model bile. *Science* 1984; **225**: 514-516 [PMID: 6429856 DOI: 10.1126/science.6429856]
- 12 Gallinger S, Harvey PR, Petrunka CN, Ilson RG, Strasberg SM. Biliary proteins and the nucleation defect in cholesterol cholelithiasis. *Gastroenterology* 1987; **92**: 867-875 [PMID: 3556994]
- 13 Afdhal NH, Niu N, Gantz D, Small DM, Smith BF. Bovine gallbladder mucin accelerates cholesterol monohydrate crystal growth in model bile. *Gastroenterology* 1993; **104**: 1515-1523 [PMID: 8482463]
- 14 Miquel JF, Rigotti A, Rojas E, Brandan E, Nervi F. Isolation and purification of human biliary vesicles with potent cholesterol-nucleation-promoting activity. *Clin Sci (Lond)* 1992; **82**: 175-180 [PMID: 1311655 DOI: 10.1042/cs0820175]

L- Editor: Filipodia E- Editor: Liu WX

## A comparative study of biliary trace elements and clinical phenotypes in Wilson's disease

Ming-Shan Ren, Yu-Xin Fan, Ren-Min Yang, Yong-Zhu Han, Guo-Jun Wu, Yu-Rong Xin, Long Yu

Ming-Shan Ren, Ren-Min Yang, Yong-Zhu Han, Institute of Neurology, Teaching Hospital, Anhui College of T.C.M., Hefei 230031, Anhui Province, China

Yu-Xin Fan, Guo-Jun Wu, Yu-Rong Xin, Long Yu, National Laboratory of Genetic Engineering, Institute of Genetics, Fudan University, Shanghai 200433, China

Ming-Shan Ren, Associate Professor of Internal Medicine, MS in Neurology; Research Fellow of University of Rouen in Rouen, France, 1994-1995; having 18 papers and 1 book published.

Author contributions: All authors contributed equally to the work.

Supported by The Natural Science Foundation of Anhui Province, No. 97412001.

Correspondence to: Ming-Shan Ren, Associate Professor, Institute of Neurology, Teaching Hospital, Anhui College of T.C.M., Hefei 230031, Anhui Province, China  
Telephone: +86-551-2816764-2107

Received: April 11, 1997  
Revised: April 25, 1997  
Accepted: June 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To further explore the etiological mechanism of Wilson's disease (WD) by comparing the changes of biliary trace elements and its clinical phenotype.

**METHODS:** WD patients with different types and conditions ( $n = 20$ ), non-WD patients with chronic liver damage ( $n = 22$ ), and healthy volunteers ( $n = 10$ ; used as controls) were studied. Biliary samples were taken by duodenal drainage. Atom absorption spectrophotometer was used to assay the copper and zinc content of each sample.

**RESULTS:** In WD, the copper content and copper/zinc ratio of biliary juice were evidently lower than those of non-WD patients with chronic liver damage and of healthy controls ( $F = 14.76, 25.4; 14.92, 26.2$  respectively;  $P < 0.01$ ), while the biliary zinc level had no significant difference from the two non-WD control groups ( $P > 0.05$ ). There were significant differences in biliary copper excretion among patients with different types and conditions ( $F = 3.75, P < 0.05; F = 6.20, P < 0.01$ ).

**CONCLUSION:** Copper excretion by liver and the biliary system decreases obviously in WD, which plays a key role in the phenotypic copper retention, and the biliary copper retention is closely related

with the severity of hepatic injury and illness.

**Key words:** Wilson's disease; Copper; Zinc; Duodenal drainage; Bile

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Ren MS, Fan YX, Yang RM, Han YZ, Wu GJ, Xin YR, Yu L. A comparative study of biliary trace elements and clinical phenotypes in Wilson's disease. *World J Gastroenterol* 1997; 3(4): 260-262 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/260.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.260>

### INTRODUCTION

Wilson's disease (WD) is an autosomal recessive disorder of copper transport, first described by Kinnear Wilson in 1992 as hepatolenticular degeneration. The disorder has a worldwide frequency of between 1/35000 and 1/100000 and a corresponding carrier frequency of 1/90. Its symptoms develop from the toxic build up of copper primarily in the liver, and subsequently in the brain, kidney, cornea and other tissues. The resulting liver cirrhosis and/or neurological damage are fatal if not treated with copper chelating agents<sup>[1,2]</sup>.

The gene responsible for WD was independently identified by three research teams in 1993<sup>[3-5]</sup>, and is located on chromosome 13q14.3. The gene codes for a putative intracellular copper transport protein (ATP7B, a member of the cation-transporting P-type ATPase family), spans > 80 kb of genomic DNA and consists of 22 exons<sup>[6]</sup>. Because of a defective ATP7B, the patients with WD have two fundamental disturbances of copper metabolism: A reduction in the rate of incorporation of copper into ceruloplasmin and a reduction in biliary excretion of copper. Although the role of copper in its pathogenesis has been known for several decades, few studies have been carried out to investigate the exact etiological mechanism of copper retention. Our study was designed to further explore the etiological mechanism of WD by observing the biliary trace element content of WD patients and non-WD patients in combination with its clinical phenotype.

### MATERIALS AND METHODS

#### Subjects

Twenty in-patients with WD were chosen for this study, consisting of 13 men and 7 women with a mean age of 25-year-old. Their diagnoses all met the criteria proposed by Houwen *et al*<sup>[7]</sup>. Control subjects were chosen from among patients in the Department of Internal Medicine and healthy volunteers, all of who were carefully screened by clinical and copper metabolism examination for the

**Table 1** Values of biliary trace elements of the Wilson's disease group and control groups ( $\bar{x} \pm s$ ,  $\mu\text{mol/L}$ )

Group	n	Copper	Zinc	Copper/Zinc
Wilson's disease	20	4.42 $\pm$ 0.44	55.94 $\pm$ 3.14	0.13 $\pm$ 0.02
1 <sup>st</sup> control	22	41.70 $\pm$ 1.97 <sup>a</sup>	34.46 $\pm$ 1.85	1.54 $\pm$ 0.27 <sup>a</sup>
2 <sup>nd</sup> control	10	42.01 $\pm$ 2.63 <sup>a</sup>	34.30 $\pm$ 2.84	1.56 $\pm$ 0.24 <sup>a</sup>

<sup>a</sup> $P < 0.01$  vs Wilson's disease group.

**Table 2** Comparison of biliary trace elements for different types of Wilson's disease ( $\bar{x} \pm s$ ,  $\mu\text{mol/L}$ )

Type	n	Copper	Zinc	Copper/Zinc
Neurological	11	5.02 $\pm$ 0.61 <sup>b</sup>	62.58 $\pm$ 22.84	0.13 $\pm$ 0.06
Psychiatric	5	4.78 $\pm$ 0.80 <sup>b</sup>	57.41 $\pm$ 26.36	0.17 $\pm$ 0.14
Hepatic	4	2.31 $\pm$ 0.17	35.85 $\pm$ 11.51	0.10 $\pm$ 0.07

<sup>b</sup> $P < 0.05$  vs hepatic type.

**Table 3** Variations of biliary trace elements in different severity of Wilson's disease ( $\bar{x} \pm s$ ,  $\mu\text{mol/L}$ )

Grade	n	Copper	Zinc	Copper/Zinc
I	7	5.67 $\pm$ 0.86 <sup>c</sup>	82.31 $\pm$ 33.71	0.10 $\pm$ 0.05
II	7	4.72 $\pm$ 0.55 <sup>c</sup>	51.99 $\pm$ 19.57	0.17 $\pm$ 0.12
III-IV	6	2.61 $\pm$ 0.22	29.76 $\pm$ 8.26	0.12 $\pm$ 0.07

<sup>c</sup> $P < 0.01$  vs III-IV grades.

absence of clinical evidence of WD and then divided into two groups. The first control group consisted of 14 males and 8 females with mean age of 34-year-old, including 12 cases of chronic hepatitis, 6 of cirrhosis and 4 of primary biliary cirrhosis; the second control group of 10 healthy volunteers consisted of 5 men and 5 women with a mean age of 30-year-old.

### Laboratory studies

All subjects maintained their regular diet throughout the study and underwent duodenal drainage to obtain a biliary sample after admission to the hospital. Eight of 20 patients with WD had no medicinal treatment before hospitalization, and the remaining 12 had histories of treatment with penicillamine, zinc sulfate, dimercaprol (BAL) and sodium dimercaptosuccinate (DMS); all of the patients with treatment histories were asked to stop taking the above-mentioned medicines for 4 wk prior to the sampling. None of the control subjects had taken any agents that may affect the metabolism of internal trace elements during the 4 wk before sampling. The biliary samples were all collected at 09:00 am. The Hitachi-208 atom absorption spectrophotometer was used to assay the copper and zinc content of each sample. All values are presented as  $\bar{x} \pm s$ . The *F*-test for variation analysis was employed to determine the statistical significance of differences between the means. *P* values of  $< 0.05$  were considered significant.

### Typing and grading

As WD patients have genetic heterogeneity, they were classified according to the following clinical symptoms: Neurological type, 11 cases with predominantly neurological symptoms; psychiatric type, 5 cases with mental symptoms; and hepatic type, 4 cases with liver symptoms<sup>[8]</sup>. The severity of disease was graded using the modified Goldstein method: Grade I, 7 cases with mild extrapyramidal system symptoms, no liver symptoms or obstacles to daily life; Grade II, 7 cases with obvious extrapyramidal system symptoms, no liver symptom or mild hepatosplenomegaly with normal liver function, and no hypersplenism; Grade III, 4 cases with serious extrapyramidal system symptoms, obvious hepatosplenomegaly and/or liver function injury; and Grade IV, 2 cases with serious extrapyramidal system symptoms, bedridden, obvious hepatosplenomegaly or complicated with ascites and liver function injury. Typing and grading were accomplished independently by two experienced neurologists in our department, neither of who were aware of the results of biliary trace

elements.

## RESULTS

The biliary copper content and copper/zinc ratio of WD patients were notably lower than those of the first and second control groups and the differences were significant ( $F = 14.76, 25.4; 14.92, 26.2; P < 0.01$ ). The comparison of biliary zinc content among the three groups showed no statistical significance ( $F = 1.76, 1.98, P > 0.05$ ), indicating that the internal copper deposit of WD is directly related with the decrease of copper excretion by the liver and biliary system (Table 1).

The biliary trace elements of WD patients with different types are shown in Table 2. The biliary copper content in cases of neurological or psychiatric types was significantly higher than that in the hepatic type ( $F = 3.75, P < 0.05$ ). In contrast, the biliary zinc content and copper/zinc ratio were similar among the three types ( $F = 0.246, 0.855, P > 0.05$ ), showing that biliary copper excretion is consistent with the severity of hepatic injury of WD (Table 2).

The relationship between biliary trace elements and the severity of WD is shown in Table 3. The severity of the disease was classified into three groups (Grade I, Grade II and Grade III-IV). The biliary copper content of Grade III-IV WD patients was significantly lower than that of Grade I-II patients ( $F = 6.20, P < 0.01$ ), but the biliary zinc content and copper/zinc ratio showed no significant differences ( $F = 1.171, 1.081, P > 0.05$ ) among the 3 groups, illustrating that the severity of WD had a direct influence on biliary copper excretion but had no effect on biliary zinc excretion (Table 3).

## DISCUSSION

Wilson's disease has various clinical manifestations caused by large amounts of deposition of internal copper resulting from abnormal copper metabolism. Patients with untreated WD are in positive copper balance. At present, there are many hypotheses to explain the etiological mechanism of copper retention, and the biliary copper excretion disturbance is considered as one of the main intrinsic mechanisms leading to the copper retention of WD<sup>[9]</sup>; however, the evidence for this remains deficient and there are few published studies of biliary copper excretion for WD. Many assumptions have failed to further explain its relations with the observed clinical conditions of the WD patients. An actual demonstration of its involvement in abnormal copper metabolism of WD remains to be further explored.

The biliary copper excretion speed for normal individuals averages 500-1300  $\mu\text{g/d}$ , basically counteracting the copper absorption in gastrointestinal tract, while the excretion rate in urine and sweat is very small. Copper balance is maintained mainly through biliary copper excretion. In view of this, considerable emphases have been laid on the fact that biliary copper excretion abnormality may underlie the disorder. Indeed, many investigators have been trying to elucidate the exact point of breakdown in the copper metabolic chain of WD. Gibbs and Walshe<sup>[10]</sup> used <sup>64</sup>Cu to observe directly the excretion process of the biliary tracts of two WD patients with biliary tract fistula and non-WD patients, and found that the biliary copper content of the common bile duct and the copper excretion of the biliary tract for the former were much lower than those for the latter.

Courtoy *et al.*<sup>[11]</sup> found that intravenously administered polymeric IgA in rats was bound to a receptor in the liver parenchymal cells and then transported *via* the endosomes to the bile canaliculus where it was excreted. Lyengar *et al.*<sup>[12]</sup> further provided evidence that a high molecular weight copper-binding substance existing in the hepatic cells of normal subjects was absent in patients with WD by studying the cholecystokinin-stimulated biliary secretions. Afterwards, Hoof *et al.*<sup>[13]</sup> pointed out that there was a similar mechanism for the transport of a copper protein to the human bile and that the genetic defect of this metabolic pathway in WD will lead to insufficient copper excretion.

Our results demonstrate that the biliary copper content and copper/zinc ratio of the patients with WD are significantly lower than those of non-WD patients with chronic liver damage and of healthy volunteers ( $P < 0.01$ , respectively). Additionally, we observed that the severity of liver lesions and patient conditions correlate well

with the decrease of biliary copper excretion, while the biliary zinc excretion has nothing to do with WD ( $P > 0.05$ ). All these findings strongly suggest that liver and biliary pathways play important roles in the copper excretion dysfunction of WD and could, therefore, participate directly in the pathophysiology of copper retention. We think that due to a non-functional gene, the patients with WD lack a copper-transporting P-type ATPase (ATP7B) that would otherwise control endosome-mediated copper excretion to the bile canaliculus in the hepatic cells. Consequently, the copper will be routed to the lysosomes, where it will accumulate and fail to be discharged into the bile, leading to obvious decrease of biliary copper excretion and internal copper accumulation. The copper trapped in the hepatic cells further damages the endosomes, resulting in the vicious circle of disruption in copper transport and obvious hepatic damage<sup>[14]</sup>.

This vicious circle may be the reason why healthy individuals and non-WD patients with chronic liver damage and other forms of biliary retention, such as chronic hepatitis or primary biliary cirrhosis, do not have the same outcome, and why the severity of hepatic damage in WD correlates well with the decrease of biliary copper excretion. Therefore, our findings, on one hand, have provided the experimental basis for our final understanding of WD pathogenesis, and, on the other hand, have indicated that if the biliary copper excretion can be promoted therapeutically, satisfactory results will be yielded both in maintaining the negative balance of copper metabolism and alleviating pathological damage to organs.

## REFERENCES

- 1 **Thomas GR**, Bull PC, Roberts EA, Walshe JM, Cox DW. Haplotype studies in Wilson disease. *Am J Hum Genet* 1994; **54**: 71-78 [PMID: 8279472]
- 2 **Chelly J**, Monaco AP. Cloning the Wilson disease gene. *Nat Genet* 1993; **5**: 317-318 [PMID: 8298634 DOI: 10.1038/ng1293-317]
- 3 **Bull PC**, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993; **5**: 327-337 [PMID: 8298639]
- 4 **Yamaguchi Y**, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* 1993; **197**: 271-277 [PMID: 8250934 DOI: 10.1006/bbrc.1993.2471]
- 5 **Tanzi RE**, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Parano E, Pavone L, Brzustowicz LM. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; **5**: 344-350 [PMID: 8298641 DOI: 10.1038/ng1293-344]
- 6 **Thomas GR**, Roberts EA, Walshe JM, Cox DW. Haplotypes and mutations in Wilson disease. *Am J Hum Genet* 1995; **56**: 1315-1319 [PMID: 7762553]
- 7 **Houwen RH**, Roberts EA, Thomas GR, Cox DW. DNA markers for the diagnosis of Wilson disease. *J Hepatol* 1993; **17**: 269-276 [PMID: 8100247]
- 8 **Walshe JM**. Wilson's disease. In: Vinken PJ, Bruyn GW, Klawans HL, eds. *Handbook of clinical neurology*. Amsterdam: Elsevier 1986: 223-228
- 9 **Yang YF**. Normal copper metabolism and the etiological mechanism of Wilson disease. *Zhongguo Shenjing Jingshen Jibing Zazhi* 1984; **19**: 60-62
- 10 **Gibbs K**, Walshe JM. Biliary excretion of copper in Wilson's disease. *Lancet* 1980; **2**: 538-539 [PMID: 6105588 DOI: 10.1016/S0140-6736(80)91863-2]
- 11 **Courtroy PJ**, Limet J, Quintart J, Schneider YJ, Vaerman JP, Baudhuin P. Transport of IgA into rat bile: ultrastructural demonstration. *Ann n Y Acad Sci* 1983; **409**: 799-802 [DOI: 10.1111/j.1749-6632.1983.tb26927.x]
- 12 **Iyengar V**, Brewer GJ, Dick RD, Chung OY. Studies of cholecystokinin-stimulated biliary secretions reveal a high molecular weight copper-binding substance in normal subjects that is absent in patients with Wilson's disease. *J Lab Clin Med* 1988; **111**: 267-274 [PMID: 3125292]
- 13 **Van Hoof F**, Den Tandt W, Scharpe S. Wilson's disease: hypothesis of a deficiency of copper excretion via the endosome to the bile. *Arch Neurol* 1992; **49**: 800 [PMID: 1524511 DOI: 10.1001/archneur.1992.00530320020007]
- 14 **Thomas GR**, Forbes JR, Roberts EA, Walshe JM, Cox DW. The Wilson disease gene: spectrum of mutations and their consequences. *Nat Genet* 1995; **9**: 210-217 [PMID: 7626145 DOI: 10.1038/ng0295-210]

L- Editor: Filipodia E- Editor: Liu WX

## Altered expression of tumor suppressors p16 and Rb in gastric carcinogenesis

Qi Zhou, Jian-Xiang Zou, Yu-Long Chen, Hui-Zhen Yu, Li-Dong Wang, Yong-Xin Li, Hua-Qin Guo, Shan-Shan Gao, Song-Lian Qiu

Qi Zhou, Jian-Xiang Zou, Yu-Long Chen, Hui-Zhen Yu, Li-Dong Wang, Yong-Xin Li, Hua-Qin Guo, Shan-Shan Gao, Song-Lian Qiu, Laboratory for Cancer Research, Medical Experimental Center, Zhengzhou 450052, Henan Province, China

Jian-Xiang Zou, Yu-Long Chen, Department of Gastroenterology, First Affiliated Hospital, Henan Medical University, Zhengzhou 450052, Henan Province, China

Hui-Zhen Yu, Henan Red-Cross Blood Station Center, Zhengzhou 450053, Henan Province, China

Received: April 11, 1997

Revised: May 25, 1997

Accepted: June 28, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To determine whether expression of the tumor suppressors p16 and Rb is altered in gastric carcinoma.

**METHODS:** Mucosal biopsies were endoscopically obtained from patients with superficial gastritis ( $n = 12$ ), atrophic gastritis ( $n = 15$ ), atypical hyperplasia ( $n = 20$ ) and gastric cancer ( $n = 40$ ). Upon obtainment, all samples were immediately fixed with 10% buffered formalin, embedded in paraffin, and sectioned serially. Protein expression of p16 and Rb was detected by immunohistochemistry

(ABC method).

**RESULTS:** The gastric epithelium samples showed various degrees of nuclear immunostaining for p16 and Rb according to the different stage of lesion. Progressive pathology of the lesions was associated with a decreasing trend in positive immunostaining for p16 protein (83.3% > 73.3% > 30.0% > 27.5%) but an increasing trend for Rb protein (25.0% > 46.7% > 60.0% > 67.5%). A negative correlation was found between these two parameters and gastric cancer. Correlation analysis of the 40 cases of gastric cancer identified a negative correlation for 20 of the cases. When positive ( $n = 9$ ) and negative tissues ( $n = 11$ ) were compared, a statistically significant difference was found (50.0%, 22.5%, 27.5%) ( $P < 0.05$ ).

**CONCLUSION:** Abnormal expression of p16 and Rb may play an important role in gastric carcinogenesis.

**Key words:** Genes, suppressor, tumor; Gene expression; Retinoblastoma protein/metabolism; Stomach neoplasms/metabolism; Carcinoma/metabolism

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Zhou Q, Zou JX, Chen YL, Yu HZ, Wang LD, Li YX, Guo HQ, Gao SS, Qiu SL. Altered expression of tumor suppressors p16 and Rb in gastric carcinogenesis. *World J Gastroenterol* 1997; 3(4): 262 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/262.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.262>

L- Editor: Filipodia E- Editor: Liu WX

## Seropharmalogical effects of Fuzheng Huayu decoction on rat Ito cell morphology and function in culture

Cheng-Hai Liu, Cheng-Liu, Ping Liu, Lie-Ming Xu

Cheng-Hai Liu, Cheng-Liu, Ping Liu, Lie-Ming Xu, Institute of Liver Diseases, Shanghai Academy of Traditional Chinese Medicine (TCM), Shanghai 200032, China

Cheng-Hai Liu, MD and PhD, male, born on 1965-02-18 in Jingmeng City, Hubei Province, graduated from Hubei College of TCM in 1986, graduated from Shanghai University of TCM as PhD in 1996, specialized in hepatology, having 10 papers and 3 books published.

Author contributions: All authors contributed equally to the work.

Project supported by The National Natural Science Foundation of China, No. 39570889.

Correspondence to: Cheng-Hai Liu, MD and PhD, Institute of Liver Diseases, Shanghai Academy of Traditional Chinese Medicine (TCM), Shanghai 200032, China  
Telephone: +86-21-64036889

Received: October 6, 1996  
Revised: December 13, 1996  
Accepted: June 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To investigate the mechanisms of anti-liver fibrosis actions of Fuzheng Huayu (FZHY) decoction, which acts to strengthen the body's resistance and promote blood circulation.

**METHODS:** Ito cells were isolated from rats and cultured. Serum samples were collected from healthy (normal) rats after administration of FZHY decoction and added to the subcultured cells. The effects of FZHY decoction on the Ito cells were investigated by contrast microscopy (to observe cell morphology), [<sup>3</sup>H]Pro incorporation assay (cell viability), [<sup>3</sup>H]TdR incorporation and MTT colorimetric assay (cell proliferation), and [<sup>3</sup>H]Pro incorporation and collagenase digestion (collagen synthesis rate).

**RESULTS:** The rat sera samples from rats treated with FZHY decoction had no influence on Ito cell morphology, but improved cell viability and markedly inhibited cell proliferation and collagen synthesis. The magnitude of these effects showed dependence on treatment dosage and drug concentration in serum.

**CONCLUSION:** The seropharmalogical method can be efficiently used to investigate the pharmacological mechanism of anti-fibrotic traditional Chinese herbs and formulae. Inhibition of Ito cell proliferation and collagen synthesis may be two of the major mechanisms underlying the anti-fibrosis actions of the FZHY decoction.

**Key words:** Fuzheng Huayu; Liver fibrosis; Ito cell; Collagen synthesis

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Liu CH, Liu C, Liu P, Xu LM. Seropharmalogical effects of Fuzheng Huayu decoction on rat Ito cell morphology and functions in culture. *World J Gastroenterol* 1997; 3(4): 263-265 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/263.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.263>

### INTRODUCTION

Ito cells (also known as hepatic stellate cells, fat-storing cells or hepatic lipocytes) play a key role in liver fibrosis, the main hallmark of chronic liver diseases. In the chronic liver disease condition, Ito cells are activated, proliferative and synthesize large amounts of various components of the extracellular matrix, which may lead to liver fibrosis<sup>[1]</sup>. When cultured on uncoated plastic plates, Ito cells undergo activation and share the similar features of cell activation that are observed in the *in vivo* condition<sup>[2]</sup>. Our previous study<sup>[3]</sup> demonstrated that Fuzheng Huayu (FZHY) decoction, which acts to strengthen the body's resistance and promote blood circulation, exerts a protective effect on the liver in CCl<sub>4</sub>-induced fibrotic rats, suggesting its potential for improving liver status and function in patients with cirrhosis. In order to investigate the actions of FZHY decoction on liver cells, the seropharmalogical method was applied to an *in vitro* (culture) system with rat Ito cells to determine the effects on cell morphology and function (*i.e.* cell viability, proliferation and collagen synthesis).

### MATERIALS AND METHODS

#### Animals

Wistar male rats, weighing 350-500 g, were purchased from the Shanghai Science Academy.

#### Reagents

Minimum essential medium (MEM; developed by Eagle), 199 medium (M199) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco, USA. Pronase E, type IV collagenase, metrizimide and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Co., USA. L- [<sup>3</sup>H] proline ([<sup>3</sup>H]Pro) was purchased from Amersham Co., England.

#### Drug

The primary components of FZHY decoction are *Cerdecyps*, *semen Persiciae* and *Radix Salviae Miltiorrhizae*. The decoction fluid extract, containing 2.703 g of each of the above raw herbs per g of fluid, was supplied by the Shanghai Zhonghua Pharmaceutical Factory.

**Table 1** Effect of drug sera on Ito cell viability ( $\bar{x} \pm s$ )

Group	<i>n</i>	[ <sup>3</sup> H]Pro (cpm/well)
Controls	4	3610.18 ± 99.47
G	4	6578.13 ± 1690.95 <sup>a</sup>
G	4	9606.33 ± 950.85 <sup>a</sup>
13.8 g	4	4560.26 ± 2026.83

<sup>a</sup>*P* < 0.05 vs controls.**Table 2** Effect of drug sera on Ito cell proliferation ( $\bar{x} \pm s$ )

Group	<i>n</i>	[ <sup>3</sup> H]TdR (cpm/well)	MTT (OD <sub>570</sub> )
Controls	4	2864.50 ± 239.15	0.4206 ± 0.016
G	4	1979.65 ± 76.76 <sup>a</sup>	0.4625 ± 0.06
G	4	1882.05 ± 112.08 <sup>a</sup>	0.3475 ± 0.040 <sup>a</sup>
13.8g	4	1781.93 ± 49.69 <sup>a</sup>	0.3268 ± 0.081 <sup>a</sup>

<sup>a</sup>*P* < 0.05, vs controls. MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

### Preparation of drug serum

The FZHY fluid extract was diluted to concentrations of 2.3%, 4.6% and 13.8% (w/v) with distilled water. Healthy (normal) rats were administered one of the dilutions orally at 10 mL/kg wt, twice, at an interval of 2 h. Control rats were given normal saline. One hour after the last administration, blood was collected from the inferior vena cava of the rats under sterile conditions. The samples were centrifuged at 1700 × *g* for 30 min at 4 °C. The separated sera were then combined from rats that had received the same dosage of drug. The combined samples were mixed thoroughly, inactivated by incubating in a 56 °C water bath for 30 min, and stored in -70 °C freezer.

### Cell isolation and culture

Ito cells were isolated from livers of Wistar rats and cultured according to the modified Friedman method as previously described<sup>[4]</sup>. Briefly, the cells were identified by immunofluorescent staining with desmin antibody and according to typical appearance under light microscopy. The recovery rate was 2.5 × 10<sup>7</sup> per liver, purity > 95%, and viability > 98%, as determined by the trypan blue exclusion assay. The primary Ito cells were expanded in culture, with passaging using 0.25% trypsin 0.02% EDTA; upon reaching confluence, the cells were subcultured with M199 containing 10% fetal calf serum in a humid CO<sub>2</sub> incubator with 5% CO<sub>2</sub> and 95% air.

### Cell proliferation assay

The [<sup>3</sup>H]TdR incorporation method<sup>[5]</sup> was used to assess cell proliferation. Confluent subcultured Ito cells in 24-well plates were incubated with M199 containing 5%, 10% or 20% (v/v) drug sera respectively. After 48 h, [<sup>3</sup>H]TdR was pulsed in at 2.5 μCi/well and the cells were incubated for an additional 24 h, after which the cells were harvested for measurement of cpm by a Wallac 1410 Scintillator (Beckman, USA).

### Colorimetric MTT<sup>[6]</sup>

Confluent subcultured Ito cells in 96-well plates were incubated with 100 μL M199 containing drug serum. After 68 h, MTT solution (5 g/L in PBS) was added to each well at 10 μL per 100 μL medium and the cells were incubated for an additional 4 h. Next, acid-isopropanol (100 μL of 0.04 *n* HCl in isopropanol) was added, mixed thoroughly and allowed to incubate for a few minutes at room temperature to dissolve the dark blue crystals. The plates were read on an ELISA reader at wavelength of 570 nm.

### Cell viability assay

Cell viability was indicated by intracellular protein synthesis<sup>[7]</sup>. Specifically, the cells were processed as described above for the [<sup>3</sup>H]TdR incorporation method, but with use of the [<sup>3</sup>H]Pro (2.5 μCi/well) instead of [<sup>3</sup>H]TdR.

### Cell collagen synthesis rate assay

Greets' method<sup>[8]</sup> was used to assess the collagen synthesis rate. Specifically, confluent subcultured Ito cells in 6-well plates were

**Table 3** Effect of sera in different proportions to medium on Ito cell proliferation ( $\bar{x} \pm s$ )

Concentration	<i>n</i>	Controls	Drug sera	Inhibitive rate (%)
5%	4	1481.90 ± 168.03	703.03 ± 138.37 <sup>b</sup>	52.56 ± 9.34
10%	4	2013.88 ± 628.87	637.33 ± 214.53 <sup>b</sup>	68.35 ± 10.68
20%	4	2709.88 ± 788.19	480.20 ± 264.31 <sup>b</sup>	82.27 ± 9.75

<sup>b</sup>*P* < 0.01 vs controls.**Table 4** Influence of drug sera on Ito cell collagen synthesis (%), ( $\bar{x} \pm s$ )

Concentration	<i>n</i>	Intracellular		Extracellular	
		Controls	Drug sera	Controls	Drug sera
5%	6	0.42 ± 0.18	0.26 ± 0.10	4.02 ± 1.63	3.12 ± 30.33
10%	6	0.48 ± 0.16	0.25 ± 0.06 <sup>a</sup>	4.49 ± 1.28	2.71 ± 1.04 <sup>a</sup>
20%	6	0.58 ± 0.26	0.24 ± 0.08 <sup>a</sup>	4.80 ± 1.47	2.59 ± 1.06 <sup>a</sup>

<sup>a</sup>*P* < 0.05 vs controls.

incubated with M199 containing the drug sera. After 48 h, the culture medium was replaced with DMEM containing 5 μCi/mL [<sup>3</sup>H]Pro, 100 mg/L β-aminopropionitrile, and 50 mg/mL ascorbic acid. After 24 h, the new culture medium and cells were collected respectively. The cells were processed for whole cell extract. The samples were dialyzed thoroughly and reacted with collagenase. Measurement of the total radioactivity in the samples (cpm-t), and the radioactivity in the samples treated with collagenase (cpm-c) and without collagenase (cpm-b), was made by liquid scintillation spectrometry. The amount of collagen produced (*i.e.* the fraction of collagenous protein) was expressed as percentage of total radiolabeled protein and calculated using the formula:

$$\% \text{ collagen} = 100 / [5.4 \times (\text{cpm}_t - \text{cpm}_c) / (\text{cpm}_c - \text{cpm}_b) + 1]$$

## RESULTS

### Effects of drug serum on Ito cell morphology

Both normal rat and drug serum could sustain cell survival and growth, and there was no significant difference in cell shape. Compared with the fetal calf serum control treatment, normal rat serum and drug serum also showed no different influence on cell shape.

### Effects of drug serum on Ito cell viability

The drug serum from the rats treated with 2.3 g and 4.6 g doses improved cell [<sup>3</sup>H]Pro incorporation, while the latter producing a greater improvement (Table 1).

### Effects of drug serum on Ito cell proliferation

**Effects of sera from rats treated with different drug doses** All sera from the different dose groups were added to Ito cells in 10% (v/v) proportion of culture medium. The drug sera inhibited cell [<sup>3</sup>H]TdR incorporation and transformation of MTT to formazan, and this inhibitive effect was dose-dependent (Table 2).

**Effects of sera concentration on Ito cell proliferation** The drug sera derived from the 4.6% (w/v) group were prepared in 5%, 10% and 20% (v/v) proportion to M199 and added to the Ito cells, respectively. The results showed that the inhibitive effect on cell proliferation was enhanced as the drug concentration in M199 increased (Table 3).

### Effect of drug serum on Ito cell collagen synthesis

The drug sera derived from the 4.6% (w/v) group were added to cells in 5%, 10% and 20% (v/v) proportion to M199. The 10% and 20% proportions inhibited both intracellular and extracellular collagen synthesis, and showed a greater tendency of dose dependence (Table 4).

## DISCUSSION

It is well known that Ito cells play a central role in liver fibrogenesis, and inhibition of Ito cell activation is one of the main targets for

anti-fibrosis therapy<sup>[9]</sup>. Although the optimal anti-fibrosis drug has not been found yet, some herbal decoctions have been reported to prevent fibrogenesis effectively. Traditional Chinese Medicine (TCM) has shown a brilliant future in its application, in particular. FZHY decoction has been shown to prevent extracellular matrix production and deposits in CCl<sub>4</sub>- or DMN-induced fibrotic rats, with potential to improve fibrotic liver structure and function in patients with chronic hepatitis or cirrhosis<sup>[3]</sup>.

Chinese herbs have very complicated components, and their pharmacokinetics remain unclear. Therefore, it is difficult to investigate their pharmacological actions *in vitro* by direct addition of herbs or their rough extracts to cultured cells, because the direct addition method could change the *in vitro* environment (such as medium osmotic pressure and pH value, *etc.*) or cause loss of effective metabolites of the herbs or exert an action of unabsorbed substances. In clinical practice, most herbs are administered orally and absorbed into the blood circulation where they exert their functions. Therefore, the serum pharmacological method of indirect addition of a drug serum to culture systems *in vitro* could overcome the above shortcomings; in this way, when the drug is applied the herbal actions can be studied and their real pharmacological effects determined<sup>[5]</sup>.

We found that drug serum collected 1 h after two administrations of FZHY decoction in rats could be applied to perform convenient and efficient investigations<sup>[10]</sup>. In this study, the drug serum showed no obvious influence on Ito cell morphology, but could improve cell viability. Thus, the drug serum had no cytotoxicity, but produced an effect of "strengthening sufficiency". This also indicated that the seropharmacological method could reflect the practical effect of Chinese herbs.

Ito cell activation was characterized in this study by increased total cell number (proliferation) and enhanced per cell fibrogenesis. [<sup>3</sup>H]TdR incorporation represents the cellular DNA synthesis rate and the cleavage of MTT represents the activity of dehydrogenase enzymes in active mitochondria<sup>[6]</sup>, both of which reflect cell proliferation. Cell collagen synthesis consists of two processes: Intracellular translation and hydroxylation, followed by extracellular excretion

and processing. The [<sup>3</sup>H]Pro incorporation and collagenase digestion methods reflect both the intracellular and extracellular collagen production processes for individual cells. In this study, the drug serum decreased the Ito cells' [<sup>3</sup>H]TdR incorporation and MTT cleavage, and inhibited both intracellular and extracellular collagen synthesis per cell. These effects were related to the drug dose and drug serum concentration. Thus, FZHY decoction could inhibit Ito cell proliferation and collagen synthesis, and prevent cell activation. This may be one of the major mechanisms of the anti-fibrotic actions of FZHY decoction.

## REFERENCES

- 1 **Bissell DM**, Friedman SL, Maher JJ, Roll FJ. Connective tissue biology and hepatic fibrosis: report of a conference. *Hepatology* 1990; **11**: 488-498 [PMID: 2179098 DOI: 10.1002/hep.1840110322]
- 2 **Friedman SL**. Cellular sources of collagen and regulation of collagen production in liver. *Semin Liver Dis* 1990; **10**: 20-29 [PMID: 2186486 DOI: 10.1055/s-2008-1040454]
- 3 **Liu C**, Liu P, Hu YY, Xu LM, Zhu JL, Xie HM, et al. The study of anti-fibrosis actions of Fuzheng Huayu Decoction. *Zhongyi Zazhi* 1994; **35**: 602-604
- 4 **Xu LM**, Liu C, Liu P, Liu CH, Gu HT, Li FH, Chen LZI. A efficient and steady Ito cells isolation method. *J Cell Biol* 1995; **17**: 143-145
- 5 **Iwama H**, Amagaya S, Ogihara Y. Effect of shosaikoto, a Japanese and Chinese traditional herbal medicinal mixture, on the mitogenic activity of lipopolysaccharide: a new pharmacological testing method. *J Ethnopharmacol* 1987; **21**: 45-53 [PMID: 3695555 DOI: 10.1016/0378-8741(87)90093-6]
- 6 **Denizot F**, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods* 1986; **89**: 271-277 [PMID: 3486233]
- 7 **Mallat A**, Preaux AM, Blazejewski S, Rosenbaum J, Dhumeaux D, Mavier P. Interferon alfa and gamma inhibit proliferation and collagen synthesis of human Ito cells in culture. *Hepatology* 1995; **21**: 1003-1010 [PMID: 7705772 DOI: 10.1002/hep.1840210418]
- 8 **Geerts A**, Vrijnsen R, Rauterberg J, Burt A, Schellinck P, Wisse E. *In vitro* differentiation of fat-storing cells parallels marked increase of collagen synthesis and secretion. *J Hepatol* 1989; **9**: 59-68 [PMID: 2504809 DOI: 10.1016/0168-8278(89)90076-7]
- 9 **Brenner DA**, Alcorn JM. Therapy for hepatic fibrosis. *Semin Liver Dis* 1990; **10**: 75-83 [PMID: 2186490 DOI: 10.1055/s-2008-1040459]
- 10 **Liu CH**, Liu C, Liu P, Xu LM. Effects of Fuzheng Huayu decoction on Ito cells proliferation and collagen synthesis in rats. *Zhongguo Shiyang Fangjixue Zazhi* 1996; **2**: 16-19

L- Editor: Filipodia E- Editor: Liu WX

## Influence of fever on biliary elements of guinea pigs

Hou-Dong Lü, Ming-Guo Tian, Xiao-Peng Zhang, Huai-Lan Li

Hou-Dong Lü, Department of Microbiology, Jining Medical College, Jining 272113, Shandong Province, China

Ming-Guo Tian, Xiao-Peng Zhang, Department of Surgery, Jining Medical College, Jining 272113, Shandong Province, China

Huai-Lan Li, Linyi Municipal First People's Hospital, Linyi 276003, Shandong Province, China

Received: October 6, 1996  
Revised: December 13, 1996  
Accepted: June 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To study the influence of fever on biliary elements and gallstone formation in guinea pigs.

**METHODS:** Sixty guinea pigs were randomly divided and fed either a lithogenic diet (to induce gallstone formation) or a normal diet (for use as the non-gallstone controls), and each group was then subdivided into fever or non-fever subgroups. The fever condition was induced by subcutaneous injection of boiled non-fat milk (1

mL/kg, once a week for 4 wk). After 45 d, all the animals were euthanized for analysis; however, 36 h prior to euthanasia, the guinea pigs in the fever subgroups were injected subcutaneously with turpentine (1 mL/kg) to maintain the fever condition. Gallbladder lumens were examined and bile samples were analyzed.

**RESULTS:** Gallstone incidence was highest (40%, 6/15) in the group of animals that were fed the lithogenic diet and had fever. Compared to the non-fever subgroups, the fever subgroups had significantly higher total bile protein and bilirubin.

**CONCLUSION:** Fever influences biliary elements and may contribute to gallstone formation in guinea pigs.

**Key words:** Fever; Bile/metabolism; Bilirubin/metabolism; Cholelithiasis/etiology; Proteins/metabolism

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Lü HD, Tian MG, Zhang XP, Li HL. Influence of fever on biliary elements of guinea pigs. *World J Gastroenterol* 1997; 3(4): 265 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/265.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.265>

L- Editor: Filipodia E- Editor: Liu WX

## Clinical and experimental studies on stomach carcinoma treated with Yangwei Kangliu granules

Wen-Ping Lu, Gui-Zhi Sun, Bing-Kui Piao, Hai-Tao Dong, Zong-Yan Yang, Hong-Sheng Lin

Wen-Ping Lu, Gui-Zhi Sun, Bing-Kui Piao, Hai-Tao Dong, Zong-Yan Yang, Hong-Sheng Lin, Department of Tumor, Guanganmen Hospital, China Academy of TCM, Beijing 100053, China

Dr. Wen-Ping Lu, female, born on 1968-10-23 in Chengde City, Hebei Province, graduated from the China Academy of TCM as a postgraduate in 1995, currently Physician-in-Charge, having 3 papers published.

Author contributions: All authors contributed equally to the work.

Key project in The 8<sup>th</sup> five-year plan supported by the State Administrative Bureau (No. 85-919-01-02).

Correspondence to: Dr. Wen-Ping Lu, Department of Tumor, Guanganmen Hospital, China Academy of TCM, Beijing 100053, China  
Telephone: +86-10-63013311-303

Received: January 12, 1997

Revised: June 3, 1997

Accepted: June 28, 1997

Published online: December 15, 1997

### Abstract

**AIM:** To study the anti-cancer mechanism of Yangwei Kangliu (YWKL) granules from the view point of red blood cell (RBC) immunity and to investigate the relationship between RBC immunity and T lymphocyte immunity.

**METHODS:** Fifty patients with advanced gastric carcinoma were treated with a combination of YWKL granules and chemotherapy. Venous blood samples were obtained before treatment and after one course of treatment. The rosette rate of c-3b-receptor (RBC-C-3bRR), tumor and red cell (RRTR) and RBC immune complex (RBC-ICR) were measured under microscopy by counting the rosettes formed by sensitized or unsensitized yeast adherence. The T lymphocyte subset was observed by the method of APAAP. Control patients were treated with chemotherapy alone ( $n = 20$ ). In addition, mouse tumor studies were performed to investigate the dynamic changes of RBC-C-3bRR, RRTR and RBC-ICR in response to treatment with YWKL granules ( $n = 30$ ). Mice treated with chemotherapy alone ( $n = 30$ ) or water alone ( $n = 30$ ) were used as controls.

**RESULTS:** The clinical therapeutic effect of combination treatment with YWKL granules and chemotherapy (i.e. the treatment group) was markedly superior to that of chemotherapy alone (i.e. the control group) ( $P < 0.01$ ). In the treatment group, the rosette rates of RBC-C-3bRR and of RRTR were significantly increased ( $P < 0.01$ ) after treatment, the rate of RBC-ICR was markedly decreased ( $P < 0.01$ ), and the ratio of CD4 to CD8 was obviously elevated ( $P < 0.01$ ). Moreover, CD8 was much lower ( $P < 0.01$ ) and the ratio of CD4 to

CD8 was much higher ( $P < 0.01$ ) than that in the control group. The RRTR rate was positively correlated with the ratio of CD4 to CD8. In mice, on day 9 of bearing cancer, the tumor weight in the group treated with YWKL granules alone was much lower than that of the tumors in the control mice groups; in addition, the YWKL treated mice showed higher RBC immune function than the mice of the two control groups. On day 13 of bearing cancer, however, the differences in both tumor weight and RBC immune function had disappeared.

**CONCLUSION:** The anti-cancer mechanism of YWKL granules may involve enhancement of RBC immunity and of T lymphocyte immune function, which is supported by the finding of RBC immune function being correlated with T lymphocyte immune function.

**Key words:** Stomach neoplasms; Yangwei Kangliu granules; RBC immunity

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Lu WP, Sun GZ, Piao BK, Dong HT, Yang ZY, Lin HS. Clinical and experimental studies on stomach carcinoma treated with Yangwei Kangliu granules. *World J Gastroenterol* 1997; 3(4): 266-268 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/266.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.266>

### INTRODUCTION

Several studies have provided experimental and clinical data demonstrating that the immune system plays a key role in controlling the occurrence and development of tumors. As a result, red blood cell (RBC) immunity has emerged as a new subdiscipline in the modern field of immunology<sup>[1]</sup>. Since then, studies have identified a close correlated between RBC immunity and prognosis of tumors, and have characterized the functions as involving clearance of the immune complex in circulation, controlling and regulating other immune cells and effector-like actions.

The current study was designed to investigate the anti-cancer effects of Yangwei Kangliu (YWKL) granules and its function of strengthening body resistance by supporting a healthy qi and dissipating blood stasis and toxic material; specifically, its effects on RBC immune activity were studied in humans and a mouse model system.

### MATERIALS AND METHODS

#### Subjects

A total of 70 patients with pathology-confirmed gastric cancer who were admitted to our hospital or to the China-Japan Friendship Hospital for treatment were enrolled in our study and randomly divided

**Table 1** Change of red blood cell immune function in the Yangwei Kangliu treatment group (% ,  $\bar{x} \pm s$ )

	<i>n</i>	C-3b-RR	Immune complex	Tumor and red cell
Normal	30	17.3 ± 5.04	9.6 ± 1.72	40.6 ± 6.17
Pre-treatment	50	8.8 ± 2.37	13.2 ± 2.88	29.1 ± 6.32
Post-treatment	50	15.0 ± 2.71 <sup>b</sup>	8.9 ± 1.87 <sup>b</sup>	39.2 ± 1.96 <sup>b</sup>

<sup>b</sup>*P* < 0.01 vs pre-treatment.

into the YWKL treatment group (for receipt of a combination therapy with YWKL granules plus chemotherapy) and the standard treatment control group (for receipt of chemotherapy alone). The YWKL treatment group consisted of 50 patients, including 41 males and 9 females ranging in age from 35 to 70-year-old, with a median age of 55-year-old. The treatment control group consisted of 20 patients, including 18 males and 2 females ranging in age from 30 to 60-year-old, with a median age of 51 years-old.

The Karnofsky performance status (KPS) ranged from 60 to 70 for 13 cases in the YWKL treatment group and for 6 cases in the control group, from 71 to 80 for 33 cases in the YWKL treatment group and for 12 cases in the control group, and > 80 for 12 cases in the YWKL treatment group and for 4 cases in the control group. According to the TCM differentiation, 33 cases were classified as stomach and spleen deficiency type, 7 as functional incoordination of liver and stomach type, and 2 as preponderance of pathogenic heat.

#### Therapeutic method

The YWKL granules used in therapy were composed of *Rhizoma atractylodis*, *Radix astragali*, *Lignum sappan*, *Rhizoma paridis*, *Cordyceps*, etc. (manufactured by the Great Wall Pharmaceutical Factory, Beijing). The YWKL drug was administered orally, three times a day, 1 packet each time (equivalent to 30 g of raw herbs). Chemotherapy was administered simultaneous to the CMF regimen, and delivered as an intravenous infusion of carboplatin 400 mg on day 1, mitomycin 6 mg on days 7 and 14, and 5-fluorouracil 500 mg on days 7, 10, 14 and 17, for a total of 2-3 cycles (each cycle consisting of 4 wk, and one course consisting of 2-3 cycles).

The control group received the chemotherapy as described above, but without any YWKL.

#### Determination indices

The rate of c-3b-receptor (RBC-C-3bRR), tumor and red cell (RRTR) and RBC immune complex (RBC-ICR) were determined by the method described by Guo *et al.*<sup>[2]</sup>. The T lymphocyte subset was observed by the method of APAAP.

#### Criteria for assessment of efficacy

Efficacy of the treatments was assessed according to the short-term effectiveness criteria set forth by the World Health Organization. For complete response (CR), the foci completely disappear, and no new foci are present. For partial response (PR), the foci size decreases by 50% or more, and no new foci are present. For stable disease (SD), the foci size decreases by < 25% or increases by < 25%. For progressive disease (PD), a single focus size or the total size of multifocal lesions increases by 25% or more, or new foci are present.

#### Clinical outcome

For the purposes of this study, short-term effectiveness was summarized as follows. In the YWKL treatment group, 8 cases achieved PR, 40 achieved SD, and 2 achieved PD. In the control group, 0 cases achieved PR, 16 cases achieved SD, and 4 cases achieved PD. The differences for each outcome between the two groups were statistically significant (*P* < 0.05).

The changes of RBC immune function are presented in Table 1. Thirty healthy persons served as normal controls. Before treatment, the RBC-C<sub>3b</sub>RR and RRTR in patients with advanced gastric carcinoma were markedly lower than those in the normal controls (*P* < 0.01), while the RBC-ICR was much higher; after receipt of the combined treatment of YWKL and chemotherapy, the RBC-C<sub>3b</sub>RR

**Table 2** Change in T lymphocyte subset between the Yangwei Kangliu + chemotherapy (treatment) and chemotherapy only (control) group

Group	<i>n</i>	CD <sub>3</sub>	CD <sub>4</sub>	CD <sub>8</sub>	CD <sub>4</sub> /CD <sub>8</sub>
Treatment group					
Pre-treatment	50	66.00 ± 6.81	38.00 ± 4.52	32.36 ± 2.91	11.23 ± 0.166
Post-treatment	50	66.27 ± 5.55	39.00 ± 4.15	26.45 ± 2.84 <sup>b</sup>	1.48 ± 0.156 <sup>b</sup>
Control group					
Pre-treatment	20	63.59 ± 8.32	38.18 ± 6.7	31.56 ± 2.82	1.26 ± 0.22
Post-treatment	20	62.18 ± 9.03	36.37 ± 3.39	32.47 ± 4.89 <sup>d</sup>	1.21 ± 0.2 <sup>d</sup>

<sup>b</sup>Compared with the treatment group in pre-treatment *P* < 0.01; <sup>d</sup>Compared with the treatment group in post-treatment, *P* < 0.01.

and RRTR were both significantly enhanced, and the RBC-ICR was decreased as compared with those observed at the pretreatment stage (*P* < 0.01).

The changes in T lymphocyte immune function are presented in Table 2. After treatment, although the CD<sub>3</sub> and CD<sub>4</sub> levels were elevated, there were no difference from the pretreatment stage (*P* > 0.05). However, the level of CD<sub>8</sub> was significantly lower (*P* < 0.01) and the ratio of CD<sub>4</sub> to CD<sub>8</sub> was significantly greater (*P* < 0.01) than the pretreatment levels. The control group showed no differences of these indices from the pretreatment levels to the post-treatment levels. Comparison of the post-treatment indices of the treatment group and the control group showed no differences for the levels of CD<sub>3</sub> and CD<sub>4</sub> (*P* > 0.05). However, compared to the control group, the level of CD<sub>8</sub> in the treatment group was significantly lower (*P* < 0.01) and the ratio of CD<sub>4</sub> to CD<sub>8</sub> was also significantly higher (*P* < 0.01). The RRTR was positively correlated to the ratio of CD<sub>4</sub> to CD<sub>8</sub> (*Y* = 1.14 + 0.005*x*, *P* < 0.01).

## EXPERIMENTAL STUDIES

### Materials and methods

**Tumor model and transplantation** The fore-stomach carcinoma cell, which is a model of high pulmonary metastasis, were retrieved from storage in liquid nitrogen were thawed at 37-40 °C and inoculated subcutaneously into the right back of inbred 615-strain. When the resultant tumor had grown to 1.0-1.5 cm in diameter, the mice were sacrificed and the tumor tissues were resected and made into a single cell suspension. Then, this suspension was brought up to 0.2 mL with normal saline consisting of 1 × 10<sup>6</sup> tumor cells and was injected subcutaneously into the right back of a fresh mouse.

**Grouping** The transplanted mice were randomly divided into three groups of 30 for treatment with either TCM (administered 0.8 mL YWKL liquid, once daily, starting at 24 h after transplantation, with daily dose was equivalent to 1.4 g of raw drugs), chemotherapy (administered 25 mg/kg 5-fluorouracil, once every other day, intragastric), or control (administered 0.8 mL water, once daily, intragastric).

**Determination index** The inhibitory rates for tumor, RBC-C<sub>3b</sub>RR and RBC-ICR were determined as described above.

### Methods

A batch of mice were sacrificed respectively on post-inoculation day 3, 9 and 11; ten mice were sacrificed for each time point. The tumors were resected and weighed, and the inhibitory rate was calculated. Blood was collected by retro-orbital puncture and used to test the RBC-C<sub>3b</sub>RR and RBC-ICR.

## RESULT

Data for the dynamic changes of tumors in mice are shown in Table 3. After the latent stage (3-5 d of bearing tumors), the tumors grew rapidly; mice began to die on day 13 in all three groups. On day 3 post-inoculation, when the first batch of mice was sacrificed, no tumors were apparent. On day 9, the tumor weight was markedly lower in the TCM group than in the control or the chemotherapy group (*P* < 0.01 or *P* < 0.05). On day 13, there were no differences in tumor weights among the three groups (*P* > 0.05).

**Table 3** Dynamic changes in tumor weight between experimental groups

Group	n	Day 9 post-inoculation	Day 13 post-inoculation
Control	10	1.27 ± 0.47	2.07 ± 0.42
TCM	10	0.74 ± 0.43 <sup>ab</sup>	2.04 ± 0.21
Chemotherapy	10	1.196 ± 0.23	1.98 ± 0.35

<sup>b</sup>Compared with the control,  $P < 0.01$ ; <sup>a</sup>Compared with chemotherapy,  $P < 0.05$ .

Data for the dynamic changes of RBC immune function in mice are shown in Table 4. With the development of tumor, the rate of RBC-C<sub>3b</sub>RR decreased and that of RBC-ICR increased. On post-inoculation days 3 and 9, the RBC-C<sub>3b</sub>RR of the TCM group was higher and the RBC-ICR was lower than that of the chemotherapy and control groups ( $P < 0.01$ ); however, on day 13 there were no differences between the RBC-C<sub>3b</sub>RR and RBC-ICR for the three groups ( $P > 0.05$ ). At no time did the chemotherapy and control group show differences in RBC-C<sub>3b</sub>RR and RBC-ICR.

## DISCUSSION

Malignancy is closely correlated with RBC immunity. At present, RBC immune activity is often assessed by means of determination of RBC immune adhesion and the execution of RBC immune adhesion via the C<sub>3b</sub> receptor<sup>[2,3]</sup>.

Assuming that patients with advanced gastric carcinoma are always in a state of deficiency of healthy qi and stagnation of exogenous factor, YWKL granules would then function to support healthy qi, dissipating blood stasis and clearing away toxic materials. The prescription of YWKL used in this study included Rhizoma atractylodis and Radix scutellariae, both of which can strengthen body resistance, Lignum sappan, which can activate blood circulation to dissipate blood stasis in the stomach and spleen, and Rhizoma paridis, which can clear away heat and toxic material. Our clinical and experimental study of this prescription showed that, in both patients with gastric cancer and mice bearing tumors, the RBC -C<sub>3b</sub>RR and RRTR were low and the RBC-ICR was high before treatment. The experiment on mice further demonstrated that as the tumors grew, the red blood cell immunity gradually declined. On the ninth day of bearing tumor, the tumor weight in the YWKL treatment group was lower than that of the other groups; correspondingly, the RBC immunity was higher than that of the other two groups. On the thirteenth day of bearing tumor, there was no obvious difference of tumor weight, and no difference of RBC immunity existed among the three groups.

Clinical data had shown that the short-term effectiveness of

**Table 4** Dynamic changes in red blood cell-c-3b-receptor and red blood cell-immune complex

Group	Red blood cell-c-3b-receptor			Red blood cell-immune complex		
	D3	D9	D13	D3	D9	D13
Control	5.27 ± 2.96	3.57 ± 1.04	2.88 ± 1.10	7.14 ± 1.93	9.80 ± 2.55	12.29 ± 4.83
TCM	13.40 ± 5.70 <sup>av</sup>	7.20 ± 1.27 <sup>ac</sup>	4.37 ± 1.12	3.96 ± 1.51 <sup>ac</sup>	6.45 ± 1.03 <sup>ac</sup>	11.52 ± 2.81
Chemotherapy	3.53 ± 1.17	2.94 ± 1.18	3.15 ± 1.16	7.89 ± 2.76	9.05 ± 2.85	14.30 ± 5.09

<sup>a</sup>Compared with the control,  $P < 0.05$ ; <sup>c</sup>Compared with chemotherapy,  $P < 0.05$ ; D: Day.

YWKL combined with chemotherapy was superior to that of chemotherapy alone and that the post-treatment RBC immunity was higher than that of the pre-treatment samples from the patients who were treated with the combination of YWKL granules and chemotherapy. According to the amount concept<sup>[4]</sup>, the fewer the tumor cells the better the effect of the chemotherapy regimen, and of the immune therapy that is administered in conjunction or afterwards; moreover, when the number of tumor cells is below 10<sup>6</sup>, the immune system may be able to eliminate it. Therefore, we believe that the anti-cancer mechanism of YWKL granules involves its improvement of immune activity, especially of the RBC immune activity, and this can make the treatment level effective in cases with 10<sup>6</sup> and 10<sup>7-8</sup> tumor cells or even higher. This is also the theoretical basis that supports effectiveness of YWKL granules combined with chemotherapy is better than that of chemotherapy alone. Of course, whether YWKL granules can directly kill tumor cells as chemotherapy awaits further study. As to the cause underlying the observation that the inhibitory rate in the chemotherapy group was lower than that in the YWKL group, we speculate that 5-FU was not sensitive to Fc cells and it inhibited the immune function of the organism.

The anti-cancer effect of RBC immunity is due to its ability to not only clear the immune complex in circulation but also to control and regulate other immune cells, which is supported by the observed correlation between the RBC and T-lymphocyte subset.

## REFERENCES

- 1 Siegel I, Liu TL, Gleicher N. The red-cell immune system. *Lancet* 1981; **2**: 556-559 [PMID: 6116004]
- 2 Guo F, Yu Zi Q, Zhao Zhong P. Primary studies on red cell immunity. *Zhonghua Yixue Zazhi* 1982; **62**: 715
- 3 Shau H, Gupta RK, Golub SH. Identification of a natural killer enhancing factor (NKEF) from human erythroid cells. *Cell Immunol* 1993; **147**: 1-11 [PMID: 8462106 DOI: 10.1006/cimm.1993.1043]
- 4 Sun Y. Some important questions in internal treatment to tumor at present. *Pra Onc J* 1990; **5**: 129-134

L- Editor: Filipodia E- Editor: Liu WX

## Effect of esophageal cancer- and stomach cancer-preventing vinegar on N-nitrosoproline formation in the human body

Xian-Ke Guo, Ti-Jun Wang, Jing-Fan Gu

Xian-Ke Guo, 150<sup>th</sup> Central Hospital of PLA, Luoyang 471031, Henan Province, China

Ti-Jun Wang, Luoyang Anle Cancer-Prevention Vinegar Factory, Luoyang 471022, Henan Province, China

Jing-Fan Gu, Chinese Nutritional Society, Tianjin 300050, China

Author contributions: All authors contributed equally to the work.

Correspondence to: Dr. Xian-Ke Guo, 150<sup>th</sup> Central Hospital of PLA, Luoyang 471031, Henan Province, China

Received: April 8, 1997

Revised: June 2, 1997

Accepted: June 28, 1997

Published online: December 15, 1997

**Key words:** Esophageal neoplasms; Stomach neoplasms; Vinegar; N-nitrosoproline; Gas chromatography

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Guo XK, Wang TJ, Gu JF. Effect of esophageal cancer- and stomach cancer-preventing vinegar on N-nitrosoproline formation in the human body. *World J Gastroenterol* 1997; 3(4): 269-270 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/269.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.269>

### INTRODUCTION

N-nitroso-compound (NNC) exposure can induce formation of many kinds of tumors in the human body, and as such represents a potential environmental cause of some human cancers. Many of the precursors of NNC are abundant in the modern environment, such as nitrate, nitrite and amino acid compounds, all of which can synthesize into corresponding NNCs, and the occurrence of stomach cancer has been associated with the ingestion of these precursors. Indeed, the gastric juice of patients with stomach cancer have been shown to have higher concentrations of both nitrates and compounds that can form nitroso compounds, compared to healthy individuals<sup>[1]</sup>. Ohshima *et al*<sup>[2]</sup> measured the concentration of N-nitrosoproline (NPRO) and of nitroso-L-proline (L-Pro) in urine following ingestion of nitrates with the aim of monitoring NNC synthesis in the human body and of studying the various kinds of factors influencing synthesis. Moreover, it has been suggested that blocking the synthesis of NNC in the human body may have important theoretical and practical significance in preventing cancers.

Vitamin C is well known for its efficacy in blocking NNC forma-

tion in the human body. Therefore, in this study, we tested whether esophageal cancer- and stomach cancer-preventing vinegar (OSCPV, China-produced patent vinegar [Patent No. ZL90102404], which is rich in vitamins) exerts its protective effect by blocking the synthesis of NPRO in the human body.

### MATERIALS AND METHODS

#### Materials

Sodium nitrate AR was purchased from Xi'an Chemical Reagent Factory (Batch No. 83091). L-Pro chromatography depurant was purchased from Shanghai Biochemical Research Institute of the Chinese Academy of Sciences (Batch No. 8507169). NPRO and *N-nitrosopipecolic acid* (NPIC) (experimental quality) were purchased from IARC. Nitroso-methylurea, and OSCPV were also obtained.

#### Methods

All the subjects underwent gastroscopy. During the 2 d prior to the start of the experiment and during the experimental period, the subjects' food intake was restricted to rice and steamed buns. On the day when a urine sample was taken, the subjects were given a cooked wheat pastry for breakfast. The dietary rules strictly followed the requirements of the experiment. During the experiment, the subjects did not eat meat products or any other food that may contain NPRO, and they took no cigarettes, tea, beer, fruits, or medicines; only water was allowed for drinking.

The experiment course encompassed the following three stages. Stage A: On day 3 of the experiment, we tested the output of NPRO in the 24 h urine sample, which represented the background level. Stage B: At 7:00 a.m., the subjects drank 10 mL of water containing 300 mg NaNO<sub>3</sub> and another 10 mL of water containing 500 mg L-Pro as well as 15 mL of OSCPV, after which the counting time for urine collection was initiated. Twenty minutes later, the subjects ate breakfast. Stage C: At 48 h after stage B (this timing was used to ensure that the subjects had been able to completely discharge the NPRO gained in stage B), urine was collected. All urine samples were collected into individual polythene pails containing 10 mL of a solution of 3.6 *n* sulfosol in 20% ammonia sulfonate (AS solution). Each sample was tested respectively to measure output of NPRO corresponding to each stage of the experiment. To determine whether the urine collection was complete, we also tested the 24 h output of creatinine.

We used the methods of Ohshima *et al*<sup>[2]</sup> for extracting and testing NPRO in the urine. The main procedure is as follows: 25 mL urine is added to 400 mg NPIC (the internal standard) and 2 mL AS is added, after which the sample is extracted a total of 3 times using mixing with 30 mL, 20 mL and 20 mL ethyl acetate respectively. The final extract is dehydrated with anhydrous sulfate and placed into a rotary evaporator (inside temperature of 60 °C). After evaporation,

**Table 1** Effect of esophageal cancer- and stomach cancer-preventing vinegar in blocking synthesis of N-nitrosoproline in 76 patients

	Content of N-nitrosoproline (nmol/24 h urine)			P
	Stage A	Stage B	Stage C	
x	26.18	66.84	23.91	< 0.001
s	5.78	17.29	4.37	

**Table 2** Effect of esophageal cancer- and stomach cancer-preventing vinegar in blocking synthesis of N-nitrosoproline in 76 patients according to disease subgrouping

Group	Average age, years	Sex, male/female	Content of N-nitrosoproline			Blockage rate, %
			Stage A	Stage B	Stage C	
Esophagus	40	9/3	21.30	60.17	28.21	82.22
Esophageal + cardia	46	9/6	30.15	81.42	23.98	122.03
Esophageal + non-typical hyperplasia	48	6/6	26.22	46.60	20.86	126.30
Superficial gastritis	46	9/6	28.63	73.10	25.11	107.91
Atrophic gastritis	54	9/3	32.86	89.63	28.37	107.90
Intestinal metaplasia + non-typical hyperplasia	54	6/5	17.90	50.13	16.90	103.10

Blockage rate, % = [(Stage B-Stage C)/(Stage B-Stage A)] × 100%

the solute is mixed with 2 mL ether and dissolved in diazomethane methyl methyl ester for 5 min. The methods of preparing diazomethane methane and dissolving with methyl ester involved putting about 1 g nitroso urea (synthesized in-house) into a 250 mL flask and then adding 60 mL ether, 20 mL methanol and 10 mL 60% KOH solution, and shaking the flask. In order to dissolve with methyl ester, the ether solution (which is filled with diazomethane methane) was placed into a 2-mouth flask. The diazomethane methane was combined with mitogen into the solution of ether containing dissolved NPRO and NPIC. The methyl ester dissolution was terminated when the solution of ether turned yellow. The procedure was conducted in a hood. Next, the solution was condensed to 10 mL with nitrogen and 0.5 mL of GC-TEA was added to test the content of methyl ester of NPRO. The content of methyl ester of standard NPRO, which was taken through the same treatment, was used as a quantitative reference. The chromatographic column used in the next step was a 2 mm × 3 mm stainless steel column with filling material of 60-80 order chromosorb WAW-DMCS and 10% Carbowax 20 M spread. The carrier was argon and the speed of the flow was 20 mL/min. The temperature of the column was 185 °C, of the evaporator was 257 °C, of the TEA pyrolysis was 500 °C, and of the interface was 200 °C. Under these conditions, the NPRO and NPIC were able to be well dissociated, with no other interfering peak produced. The lowest detection limit was < 0.5 ppb, and the recovery rate was 85%.

## RESULTS

In the experiment, we took the output of NPRO—the level of endogenous nitrosification—as an index. After the 76 patients took 55 mg L-Pro, the output of NPRO in the 24 h urine sample rose from 26.18 nmol to 66.84 nmol, for an increase of 2.5 times. In contrast, after taking OSCPV and L-Pro, the NPRO in urine was reduced, to a concentration lower than the background level, which shows that OSCPV can block the synthesis of NPRO in the human body completely and suggests OSCPV as an ideal blockage agent for prevention of cancer (Tables 1, 2).

## CONCLUSION

OSCPV is an effective agent that can block the synthesis of NNC. It is effective in blocking the synthesis of NPRO, which can induce cancer in the human body, making this agent capable of preventing human cancers in an effective manner.

## REFERENCES

- 1 Reed PI, Smith PL, Haines K, House FR, Walters CL. Gastric juice N-nitrosamines in health and gastroduodenal disease. *Lancet* 1981; **2**: 550-552 [PMID: 6116002 DOI: 10.1016/S0140-6736(81)90939-9]
- 2 Ohshima H, Bartsch H. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. *Cancer Res* 1981; **41**: 3658-3662 [PMID: 7260920]

## Gastric emptying and plasma levels of gastrointestinal hormones in patients with peptic ulcer

Jian Chen, Jun-Man Li, Xue-Hui Li, Hong-Sheng Hao, Shu-Hua Fu

Jian Chen, Jun-Man Li, Xue-Hui Li, Hong-Sheng Hao, Shu-Hua Fu, Department of Gastroenterology, Affiliated Hospital of Shandong Medical University, Jinan 250012, Shandong Province, China

Received: January 12, 1997  
Revised: June 3, 1997  
Accepted: June 28, 1997  
Published online: December 15, 1997

### Abstract

**AIM:** To study the plasma level of gastrointestinal hormones and the time of gastric emptying in patients with peptic ulcer.

**METHODS:** Thirty patients with gastric ulcer (GU), 29 patients with duodenal ulcer (DU), and 12 healthy controls were studied. Plasma levels of somatostatin (SS), vasoactive intestinal peptide (VIP) and substance P (SP) were measured by radioimmunoassay. Gastric emptying half-time ( $GE_{T_{1/2}}$ ) was measured by the TC-99<sup>m</sup> resin/solid meal method.

**RESULTS:**  $GE_{T_{1/2}}$  was significantly longer in the GU patients than that in the healthy controls ( $65.9 \pm 14.8$  min vs  $53.3 \pm 4.3$  min,  $P < 0.01$ ) and plasma VIP levels were significantly higher ( $37.5 \pm 10.7$  ng/L vs  $18.4 \pm 5.9$  ng/L,  $P < 0.05$ ). There was a significant positive correlation between  $GE_{T_{1/2}}$  and plasma VIP levels ( $r = 0.55$ ,  $P < 0.01$ ).

No significant differences were found in SS and SP levels when GU patients were compared with healthy controls ( $P > 0.05$ ).  $GE_{T_{1/2}}$  was markedly shorter in the DU patients than in the healthy controls ( $41.7 \pm 10.2$  min vs  $53.3 \pm 4.3$  min,  $P < 0.01$ ) and plasma SS levels were significantly lower ( $6.4 \pm 2.5$  ng/L vs  $11.9 \pm 3.4$  ng/L,  $P < 0.01$ ). There was a significant positive correlation between  $GE_{T_{1/2}}$  and SS levels ( $r = 0.56$ ,  $P < 0.01$ ). Plasma SP levels in the DU patients were significantly higher than those in the healthy controls ( $54.4 \pm 12.7$  ng/L vs  $41.6 \pm 5.8$  ng/L,  $P < 0.01$ ). There was a significant negative correlation between  $GE_{T_{1/2}}$  and SP levels ( $r = -0.68$ ,  $P < 0.01$ ). No significant differences were found in the plasma VIP levels when DU patient were compared to healthy controls ( $P > 0.05$ ).

**CONCLUSION:** Elevation in VIP may contribute to occurrence of GU and its associated delay in  $GE_{T_{1/2}}$ . Increased SP and reduced SS may play important roles in  $GE_{T_{1/2}}$  acceleration and in the pathogenesis of DU.

**Key words:** Peptic ulcer/physiopathology; Gastric emptying; Gastrointestinal hormones/blood

© The Author(s) 1997. Published by Baishideng Publishing Group Inc. All rights reserved.

Chen J, Li JM, Li XH, Hao HS, Fu SH. Gastric emptying and plasma levels of gastrointestinal hormones in patients with peptic ulcer. *World J Gastroenterol* 1997; 3(4): 270 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v3/i4/270.htm> DOI: <http://dx.doi.org/10.3748/wjg.v3.i4.270>

L- Editor: Filipodia E- Editor: Liu WX



Published by **Baishideng Publishing Group Inc**  
8226 Regency Drive, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>  
<http://www.wjgnet.com>



ISSN 1007 - 9327

