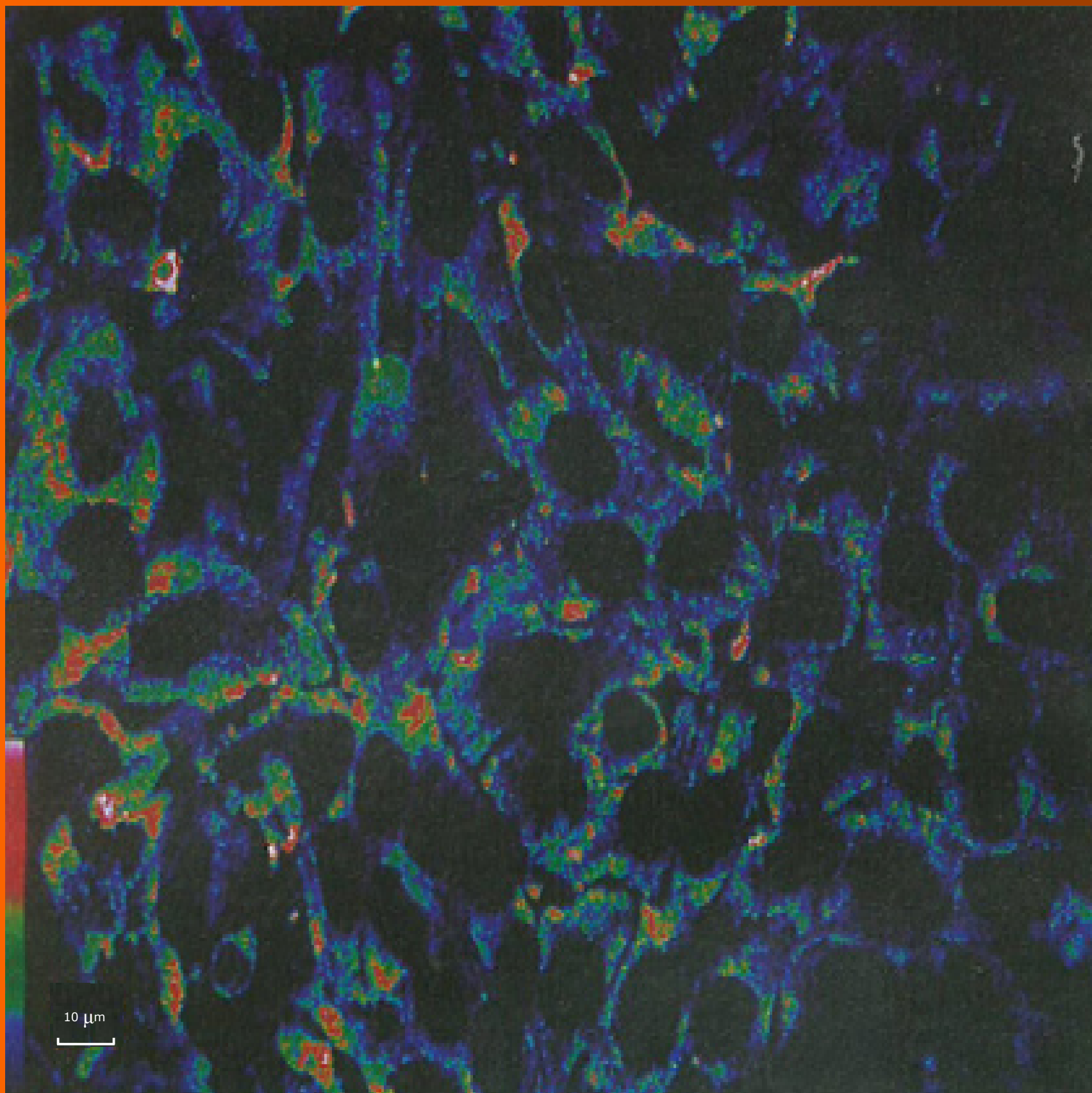


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Highlights in mechanisms and therapies for gastrointestinal and hepatic diseases: 1996 Shanghai International Gastroenterology Conference

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The 3rd 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).

β -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-carotenal 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- α , 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 HBcAg 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% HBcAg 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- γ , 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- β , 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).

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Uptake of bacterial lipopolysaccharide and expression of tumor necrosis factor- α -mRNA in isolated rat intrahepatic bile duct epithelial cells

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Abstract

AIM: To study the uptake of bacterial lipopolysaccharides (LPS) and expression of tumor necrosis factor α -mRNA (TNF- α -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- α -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- α -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- α -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- α -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- α -mRNA.

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

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INTRODUCTION

Endotoxins, mainly consisting of lipopolysaccharides (LPS) and proteins, can induce a number of reactions, both beneficial and harmful to the body^[1]. 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^[2]. 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^[3]. 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^[4]. 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^[5-6], 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- α -mRNA, the uptake of LPS and expression of TNF- α -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.

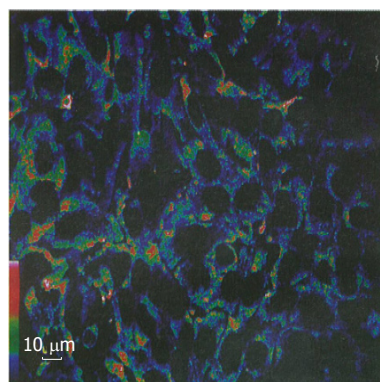


Figure 1 Fluorescent reactions to lipopolysaccharides in cultured intrahepatic bile duct epithelial cells. Positive reactions were obvious in the cytoplasm. The color bar in the bottom on the left side shows the fluorescent intensities (the highest is red and the lowest green).

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.*^[7]. 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 μ /mL, Ca^{2+} and Mg^{2+} 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 ($1-3 \times 10^5$ cell/mL). After incubation at 37 °C for 24 h in 35 mm plastic dishes in a 5% CO_2 and 40% O_2 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_2 and 40% O_2 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- α -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- α -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- α -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 ($121.45 \mu\text{FI}/\mu\text{m}^2 \pm 15.62 \mu\text{FI}/\mu\text{m}^2$ vs $32.12 \mu\text{FI}/\mu\text{m}^2 \pm 9.64 \mu\text{FI}/\mu\text{m}^2$, $P < 0.01$). No fluorescence was found in the negative controls using only the monoclonal antibody or FITC labelled secondary antibody.

Expression of TNF- α -mRNA

Positive FITC reactions to TNF- α -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^[1]. 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^[3-4]. The LPS injected through the portal vein could be detected in the intrahepatic bile duct epithelial cells in rats^[8]. 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

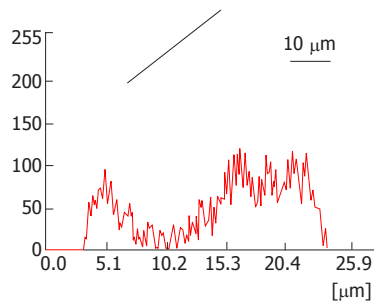


Figure 2 Fluorescent reactions to TNF- α -mRNA in cultured intrahepatic bile duct epithelial cells. Positive reactions were found in the cytoplasm and nuclei of the cells. The chart shows the fluorescent intensities along the line through the cells as indicated.

Table 1 Fluorescein isocyanate fluorescent intensities of TNF- α -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 ^b | 64.85 \pm 14.99 ^b |
| 6 h | 221.38 \pm 22.99 ^{bd} | 138.15 \pm 36.54 ^{bd} |
| 9 h | 170.00 \pm 15.25 ^b | 70.42 \pm 9.08 ^b |

^bCompared with the controls ($P < 0.01$); ^dCompared 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^[9]. 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^[10,11]. 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^[12,13]. 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^[14] and in cholestatic liver diseases they displayed aberrant expression of MHC class I and II antigens and of TNF- α receptors^[15]. The expression of TNF- α -mRNA was detected in the intrahepatic bile duct epithelial cells of LPS perfused livers^[16]. The results of the present study showed that strong expression of TNF- α -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- α -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^[5]. *In vitro*, the bile duct epithelial cells reacted to cytokines such as TGF, EGF and TNF to proliferate^[17]. 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- α -mRNA synthesis and distribution of tumor necrosis factor- α -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]

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Effects of γ -interferon on hepatic fibrosis of schistosoma japonicum-infected mice

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Abstract

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

METHODS: The amount and distribution of γ -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 γ -interferon were determined by immunohistochemical streptavidin biotin peroxidase complex method.

RESULTS: The amount of γ -IFN in liver peaked at the 16th week after infection (3 mice respectively reached 2+, 3+ and 4+ grade), which was higher than the levels of infected mice at the 8th-12th week ($P < 0.01$), and γ -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 8th 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 γ -interferon, however, the matrix proteins and collagens gradually increased, peaked respectively at the 20th and 24th 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 γ -IFN, only 3 infected mice had 2+ grade of fibronectin at the 20th week, and 2 mice had 3+ grade of type III collagen at the 24th 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: γ -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

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He YW, Liu W, Zen LL, Luo DD. Effects of γ -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^[1,2]. For example, studies in schistosomiasis mansoni^[3,4] and japonica^[5] showed that γ -interferon (γ -IFN) can affect the granuloma formation and inflammatory response. However, these studies just focused on the changes of γ -IFN in peripheral blood or induced *in vitro*. The amount and distribution of γ -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 γ -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 γ -IFN to infected mice to find out the relationship between γ -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 γ -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 8th, 12th, 16th, 20th, 24th and 28th week after infection, respectively; 30 healthy mice in control group were subdivided into 3 groups, and were killed at the 8th, 16th, and 24th week after infection respectively; and 20 infected mice were subdivided into 2 groups, each of which was treated with murine recombinant γ -IFN (Gibco BRL, United States) 50000 units daily I.M. for 20 d, starting at the 16th week after infection, and then were killed at the 20th and 24th week after infection, respectively. All fresh livers were stored in liquid nitrogen.

Table 1 Dynamics of γ -IFN and extracellular matrix in the liver of healthy control and infected mice

| Groups (wk) | Cases | γ -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 γ -IFN and extracellular matrix in the liver before and after γ -IFN treatment

| Groups (wk) | Cases | γ -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% H₂O₂/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 γ -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 μ L of solution A and 10 μ 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 γ -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

γ -IFN and extracellular matrix in the liver of healthy mice

In the liver of healthy mice, γ -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 γ -IFN and extracellular matrix at different stages (8th, 16th and 24th weeks) were insignificant (Table 1).

Distribution and dynamics of γ -IFN and extracellular matrix in the liver of infected mice

Table 1 shows the dynamics of γ -IFN and extracellular matrix in

livers of infected mice. γ -IFN in livers of 9 infected mice reached 1+ grade and 1 mouse 2+ grade at the 8th 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 γ -IFN in the liver of infected mice at the 12th week after infection was higher than that at the 8th week ($P < 0.01$), and peaked at the 16th week after infection (3 mice reached 2+, 3+ and 4+ grade respectively), which was significantly higher than that at the 12th week ($P < 0.01$). The more severe the inflammatory response was, the higher the amount of γ -IFN was. γ -IFN started to decrease at the 20th 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 8th and 12th 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 γ -IFN, at the 16th week after infection when severe inflammatory response appeared and γ -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 γ -IFN. FN and LN peaked at the 20th week after infection (7 and 8 mice with 3+ grade or over); type I and III collagens peaked at the 24th week after infection (7 and 8 mice with 3+ grade or over) (Figure 1A). Among them, the level of type I collagen at the 24th week was higher than that at the 16th week ($P < 0.05$). After 20th 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 γ -IFN treatment on extracellular matrix

γ -IFN treatment kept the hepatic γ -IFN at a high level in the 20th 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 16th 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 20th week, while 10 mice in the non-treated group ($P < 0.01$). At the 24th 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 20th week after infection before and after administration of γ -IFN ($\times 100$). A: Red and patched or band-like type I collagen in and around egg granuloma (3 + grade) before γ -IFN injection; B: Decreased type I collagen in and around egg granuloma (1+ grade) after γ -IFN injection.

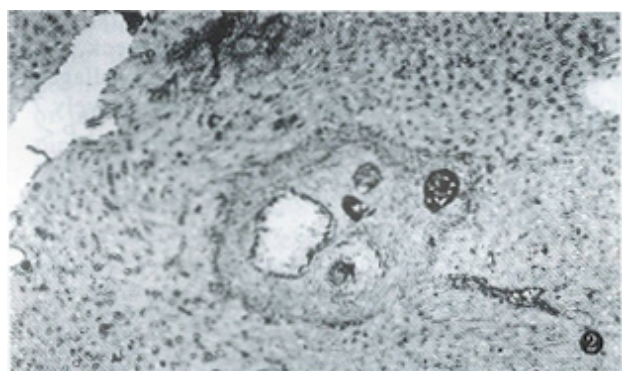


Figure 2 Type I collagen in the liver at 20th week after infection before and after administration of γ -IFN ($\times 100$). A: Red and patched or band-like type I collagen in and around egg granuloma (3 + grade) before γ -IFN injection; B: Decreased type I collagen in and around egg granuloma (1+ grade)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

of extracellular matrix at the 20th or 24th week after infection.

DISCUSSION

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

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

in and around the egg granuloma. After a repeated injection of recombinant γ -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 γ -IFN may inhibit the synthesis and excretion of extracellular matrix and the deposition of collagen^[6], which was mediated through the effect of γ -IFN on other cytokines. For example, the injection of monoclonal antibody to γ -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 γ -IFN can restrain the excretion of IL-2 and IL-4 and lighten the pulmonary granuloma formation. It is suggested that γ -IFN can abate the granuloma formation by inhibiting T cells from excreting IL-4 and IL-2^[4].

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^[7]. Our study revealed that the distribution of γ -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 γ -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 γ -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, Giambrone 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]

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

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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 α -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

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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^[1]. 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^[2,3]. Furthermore, the clinical trials have been approved in some countries for the treatment of brain tumor with *HSV-tk* gene/prodrug system^[4].

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

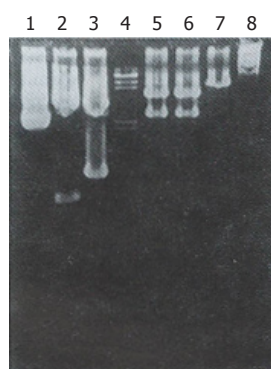


Figure 1 Identification of insertion of *pgk* + *HSV-tk* segment into the pMNM retroviral vector and plasmid pGEM 7Z-AFP. Lane 1: plasmid pGEM 7Z-AFP; Lane 2: pGEM 7Z-AFP/*Hind* III 3.3 kb, 406 bp; Lane 3: pGEM 7Z-AFP/*EcoR* I 3 kp, 727 bp; Lane 4: λ DNA *Hind* III marker; Lane 5: pMNP-tk/*Bam*H I 6.1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

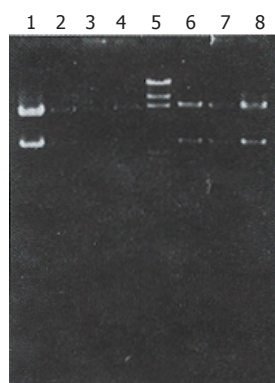


Figure 2 The orientation of human AFP enhancer core sequence was defined with *Hind* III and *Bam*H I digestion of the colonies of pMNPA-tk. Lane 1-4: right junction; Lane 5: λ DNA *Hind* III marker; lane 6-8: reverse junction.

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 α -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^[5]. 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 *Bam*HI 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).

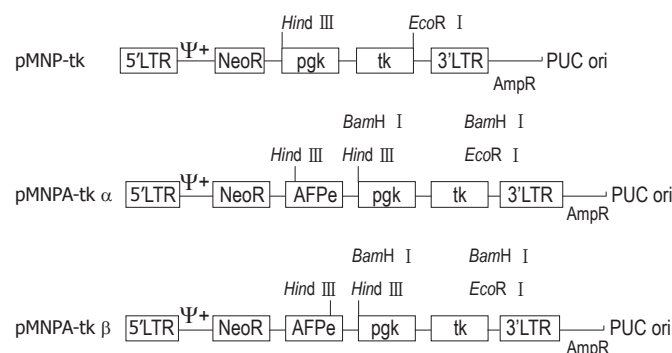


Figure 3 Structure of pMNP-tk, pMNPA-tk α , pMNPA-tk β retroviral vectors. LTR: Retroviral long terminal repeat sequence; NeoR: Neomycin phosphotransferase gene (for G418 selection); *pgk*: Human phosphoglycerokinase promoter; *tk*: Herpes simplex virus thymidine kinase gene; AFPe: Human α fetoprotein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

Construction of hepatoma specific expression retroviral shuttle vector^[5]

Plasmid pGEM 7Z-AFPe DNA was transformed into *E. coli* JM109. By α 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 pMNPA-tk α . Another colony exhibiting 321 bp, 2.8 kb and 6.5 kb fragments was reverse, named pMNPA-tk β (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 pMNPA-tk α and pMNPA-tk β

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^[6]. 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^[7]. 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^[8]. 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^[9] 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.*^[9] 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.*^[7] 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.

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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]

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

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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 μ 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

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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^[1]. 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^[2]. 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 (2nd 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^[3].

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^[4] and HC C2 isolate^[5].

Preparation of nucleic acid, cDNA synthesis and PCR

RNA from 50 μ 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 μ L of Tris HCl buffer (50 mmol/L, pH8.4) containing 8 mmol/L MgCl₂, 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 μ L containing 67 mmol/L Tris HCl, pH8.8, 1.5 mmol/L MgCl₂, 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 ddH₂O 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 NH₄Ac 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^[6].

Sequencing method^[7]

The primer labeling Two internal primers were respectively used as sequencing primers in both directions. Ten pmol of sequencing primer, 50 μ Ci (r-³²P) ATP (Ya Hui Company), 1 μ L of 10 \times kinase buffer (70 mmol/L Tris HCl, pH7.5, 10 mmol/L MgCl₂, 5 mmol/L dithiothreitol) and H₂O to a final reaction volume of 10 μ L were mixed, then 5 units of T4 polynucleotide kinase was added. The mixture was incubated at 37 °C for 30 min.

Sequencing Two μ 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'-³²P-labeled sequencing primer, 5 μ L of 5 \times sequencing buffer and 5 units of Taq polymerase were mixed in a total reaction volume of 16 μ L. Then 4 μ 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 μ 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 μ mol of each dNTP are used in PCR reaction, whereas the sequencing reactions are routinely performed in the presence of around 10 μ 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^[2,8]. 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^[6]. 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

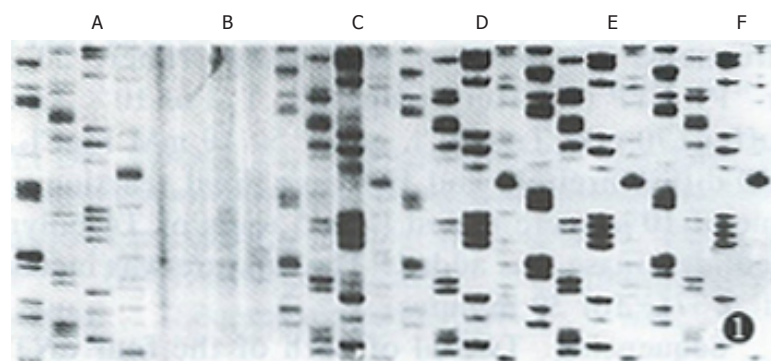


Figure 1 Effect of different methods recovering a template on sequencing. A: The first stage PCR products with lower concentration dNTPs and primers as a sequencing template; B: The second stage PCR products directly sequenced without purification; C: Sequencing of PCR amplified cDNA recovered by PEG; 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.

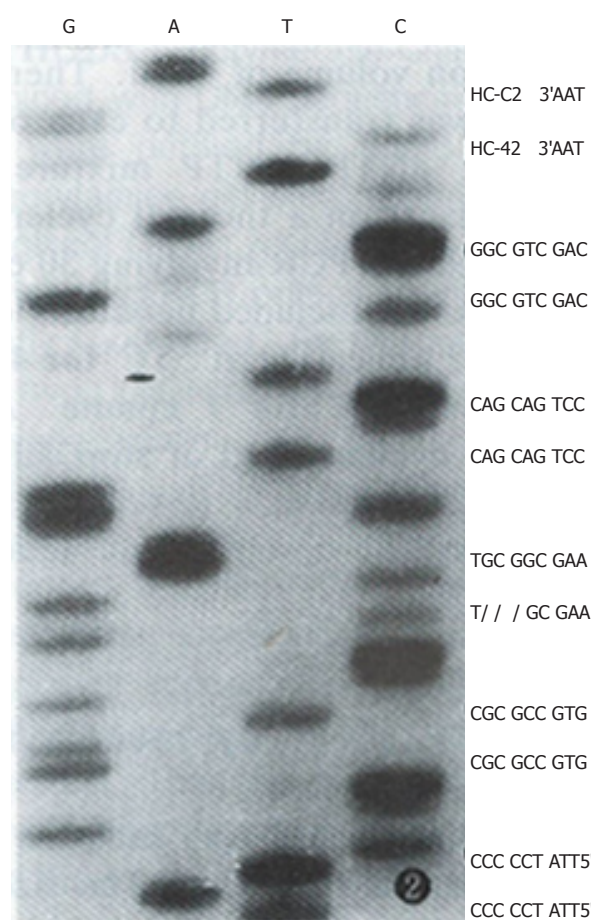


Figure 2 Deletion mutation in NS5B region of HC-42 and comparison with HC-C2. "—" denotes deletion.

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^[2]. In one strategy, lower concentrations of dNTPs (10-20 μ 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 μ 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^[9]. 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^[8]. 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^[10], 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^[11]. 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]

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

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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

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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^[1,2]. 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^[3].

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 (%) ¹ | 79.2 | 37.5 | 87.5 | 83.3 |
| No. of tumors ² | | | | |
| 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 |

¹ χ^2 test, **Experiment one:** $\chi^2 = 7.563$, $P < 0.01$; **Experiment two:** $\chi^2 = 1.003$, $P > 0.05$. ²Wilcoxon 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: $D = (d_1 + d_2)/2$, $V = (\pi/6) (d_1 + d_2)^3$. After 48 h fixation, the entire colon specimens were coiled up into "swiss rolls" and embedded in paraffin. The blocks were sectioned at 6 μ m and stained with hematoxylin and eosin (HE) for evaluation of histological changes.

Statistical analysis

Statistical analysis was made using the χ^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) ¹ | | | | |
| < 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 ³) ² | | | | |
| 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 |

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

²Wilcoxon 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^[1,2]. 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^[4]. 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^[5] 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, lipoxigenase and arachidonic acid uptake, which are known to play a role in inflammation and cell proliferation^[6,7]. 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]

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

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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

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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^[1-3]. 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^[4]. 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'-AACTGGTGGTGGTGGACCA-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

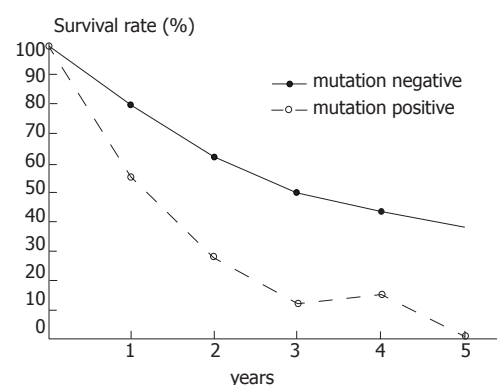


Figure 1 Five year survival rates of mutation negative and mutation positive after surgery for gastric cancer.

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^[5]. 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 MgCl₂, 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

| | <i>n</i> | 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) ^b |
| Lymph node metastases | | | |
| Without lymph node metastases | 35 | 33 | 2 (5.7) |
| With lymph node metastases | 53 | 39 | 14 (29.2) ^a |
| Staging | | | |
| Staging I and II | 43 | 42 | 1 (2.3) |
| Staging III and IV | 45 | 30 | 15 (33.3) ^c |

^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$

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^[6]. 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^[7]. 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^[5]. 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^[8,9]. However, Deng *et al.*^[6] 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^[10,11]. 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^[7,11], 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**, Wasylshyn 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]

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Cellular immune function and liver damage in post-hepatic cirrhosis

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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 ^3H -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 ± 13.0 vs 34.9 ± 21.7 , $P < 0.01$; 8.1 ± 6.0 vs 13.6 ± 5.8 , $P < 0.01$; 40.3 ± 21.7 vs 61.3 ± 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

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INTRODUCTION

Recent evidence has shown that deficiency of cell mediated immunity may play an important role in the pathogenesis of chronic HBV infection^[1-3]. 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 $1 \times 10^6/\text{mL}$. The cells were stimulated with PHA (final concentration 25 $\mu\text{g}/\text{mL}$). The assay was made by the ^3H -TdR incorporation technique and individual sample results were expressed as a stimulation index (SI):

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

IL-2 activity PBMC were stimulated with PHA (final concentration 150 $\mu\text{g}/\text{mL}$) 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.

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

NK cell activity The activity was measured with the ^3H -TdR post

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

| Assays | PHC (n) ^b | 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 ₃ (g/L) | 0.5 ± 0.2 (29) | 1 ± 0.6 (22) |

^b*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) ^a |
| IL-2 activity (SI) | 10.7 ± 7.9 (16) | 7.4 ± 3.4 (13) ^a | 5.2 ± 3.3 (12) ^b |
| NK activity (%) | 46.5 ± 19.9 (18) | 38.5 ± 15.4 (18) ^a | 19.5 ± 11.3 (13) ^b |

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

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

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^[3-6]. 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^[7]. 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^[8]. 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^[9].

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/yonj.1990.31.4.347]

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Surgical treatment of biliary ductal stricture complicating localized left hepatolithiasis

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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

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INTRODUCTION

Solitary left hepatolithiasis with left intrahepatic ductal calculi is a common and peculiar type of hepatolithiasis which is prevalent in East Asia^[1,2]. In order to treat left hepatolithiasis, it is necessary to deal with the intrahepatic biliary strictures^[3], 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^[4]. However, recently some problems have been noticed, such as a high incidence of residual stones after surgery^[5] and a high stone recurrence rate^[6] 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 (%) ^{a1} | Severe stricture (%) ^{a2} | 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) ^b |
| Left external hepatic duct | 75 | 29 (38.7) | 34 (45.3) | 63 (84.0) ^d |

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. ^{a1} $\chi^2 = 5.26$, $P > 0.05$; ^{a2} $\chi^2 = 4.90$, $P > 0.05$, among 3 groups. ^b $\chi^2 = 6.00$, $P < 0.01$; ^d $\chi^2 = 11.82$, $P < 0.01$, 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) ^f |
| 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) ^e |
| 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) |

^f $\chi^2 = 19.76$, $P < 0.01$; ^e $\chi^2 = 4.69$, $P < 0.05$, 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^[7], no special consideration has been given.

Biliary stricture is defined by Matsumoto *et al.*^[8] 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^[8,9], 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^[9]. 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^[7,10], bile stasis and liver function damage^[11].

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: 10.1097/00000658-198007000-00005]

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Study of the influence of hiatus hernia on gastroesophageal reflux

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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

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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^[1-4]. 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^[5-6]. 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^[7-10]. 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^[11-14]. 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 H₂ 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^[15]. 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^[16,17]. 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^[16,17]; 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*^[18] 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*^[19], 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^[20]. Kaul *et al*^[11] 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*^[21] found that impaired emptying in

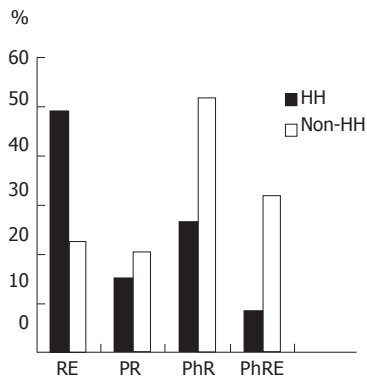


Figure 1 The incidences of Reflux esophagitis, Pathological reflux, Physiological reflux and Esophagitis with physiological reflux in patients with and without hiatus hernia. RE: reflux esophagitis, PR: Pathological reflux; PhR: Physiological reflux; PhRE: esophagitis with physiological reflux; HH: Hiatus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

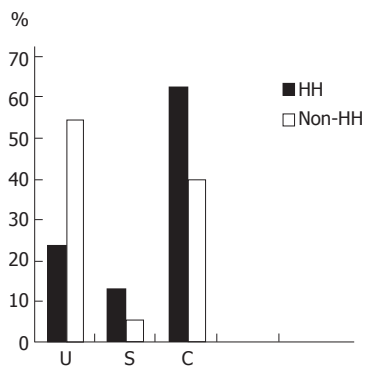


Figure 2 Percentages of upright, supine and combined reflux in patients with and without hiatus hernia. U: Upright reflux, S: Supine reflux; C: Combined daytime and nocturnal reflux, HH: Hiatus hernia, Non HH: Patients without HH.

patients with HH was attributable to an early retrograde flow, occurring immediately after lower esophageal sphincter relaxation. The results reported by Mittal *et al*^[22] 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^[23], 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^[24-27]. 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^[5,24,28-30]. Others have found evidence to support the opinion that daytime gastroesophageal reflux plays a more important role in the development of esophagitis^[31-34]. 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

- Pope CE. Pathophysiology and diagnosis of reflux esophagitis. *Gastroenterology* 1976; **70**: 445-454 [PMID: 765192]
- Dodds WJ, Hogan WJ, Miller WN. Reflux esophagitis. *Am J Dig Dis* 1976; **21**: 49-67 [PMID: 3966 DOI: 10.1007/BF01074140]
- 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]
- 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]
- 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]
- 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]
- 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]
- 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]
- Kramer P. Does a sliding hiatus hernia constitute a distinct clinical entity? *Gastroenterology* 1969; **57**: 442-448 [PMID: 4951149]
- Field P, Stalker MJ. Incompetence of the cardiac sphincter without radiologic demonstration of hiatus hernia. *Can J Surg* 1968; **11**: 412-419 [PMID: 5683598]
- 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]
- 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]
- 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]
- 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]
- Savary M, Miller G. The Esophagus. Handbook and Atlas of Endoscopy. Switzerland: Gassmann 1978: 125-132
- 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]
- 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]
- Clagett OT. Present concepts regarding the surgical treatment of oesophageal hiatal hernia. *Ann R Coll Surg Engl* 1966; **38**: 195-209 [PMID: 5931109]
- 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]
- DeMeester TR, Lafontaine E, Joelsson BE, Skinner DB, Ryan JW, O'Sullivan GC, Brunson 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]
- Sloan S, Kahrilas PJ. Impairment of esophageal emptying with hiatal hernia. *Gastroenterology* 1991; **100**: 596-605 [PMID: 1993483]
- 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]
- 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]

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

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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

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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^[1-3]. 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^[4-6]. The patients also have a decreased somatostatin level in gastric juice and antral mucosal tissue^[7]. Eradication of *H. pylori* may decrease hypergastrinemia, enhance antral somatostatin secretion, and then reduce gastric acid secretion^[8-10]. 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^[11,12].

Epidermal growth factor (EGF) is a polypeptide of 6000 daltons containing 53 amino acid residues^[13]. 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^[14-16]. Sialoadenectomy in rats damages the integrity of gastric mucosa^[16,17]. Mucosal ulceration can induce a novel EGF secreting cell lineage in human gastrointestinal stem cells^[18]. 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^[19], rapid urease test (commercial kits from Sanqing Biological Reagents Co, Fuzhou, China) and Warthin starry or Giemsa stains^[20]. 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^[21]: 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 $423.0 \text{ ng/L} \pm 104.0 \text{ ng/L}$, and $22.0 \text{ ng/L} \pm 11.1 \text{ ng/L}$, with no differences as compared with those in the control group ($405.6 \text{ ng/L} \pm 35.6 \text{ ng/L}$ and $22.0 \text{ ng/L} \pm 17.0 \text{ ng/L}$, 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 ($272.0 \text{ ng/L} \pm 96.3 \text{ ng/L}$ and $8.3 \text{ ng/L} \pm 2.4 \text{ ng/L}$, 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^[4,7-10].

A strong association between *H. pylori* and diseases of upper gastrointestinal tract has been reported^[1,2]. 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^[1]. The suggested mechanisms in antral organism cause a duodenal lesion including bacterial colonization of gastric metaplasia in the duodenum^[22], secondary changes in gastric acid or duodenal bicarbonate secretion^[23,24], or the changes caused by the infected organism and/or the inflammatory response to the host^[25,26]. 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^[27].

The possible hypotheses in explaining the relationship between *H. pylori* infection and duodenal ulcer have been described as "gastrin link"^[2,28,29] or "somatostatin link"^[10,30], 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^[4-6,27-30]. On the contrary, there was no consistent relationship between chronic *H. pylori* infection and acid secretion observed^[11,12,30,31]. Kang *et al*^[11] 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*^[31] 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"^[2,32], 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^[30], 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^[14,16]. Olsen *et al*^[15] 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^[16]. Chen *et al*^[33] 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 ^a | 423.0 ± 104.0 | 22.0 ± 11.1 |
| DU | 10 | 109.2 ± 24.5 ^b | 52.4 ± 13.8 ^b | 272.0 ± 96.3 ^b | 8.3 ± 2.4 ^a |

^a*P* < 0.05, ^b*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*^[34] 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 urogastrone 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 JJ, 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 **McColl 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 Shokakibyo Gakkai Zasshi* 1991; **88**: 1043-1050 [PMID: 1856997]

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Adherent properties of *Helicobacter pylori* to human epithelial cells

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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

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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^[1,2]. *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^[3,4]. 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% O₂, 10% CO₂ and 85% N₂ 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^[5].

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 3rd 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 |

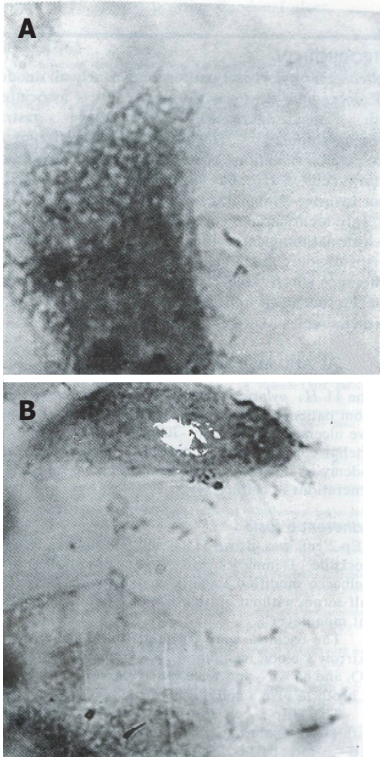


Figure 1 Adherence of *Helicobacter pylori* YC 11A to HEp2 cells (1000 ×). A: Incubation for 40 min; B: Incubation for 5 h.

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^[6] and M (microbial) selectins^[7]. Fibrillar hemagglutinin specifically binds sialyllactose^[6]. 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^[8]. 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*

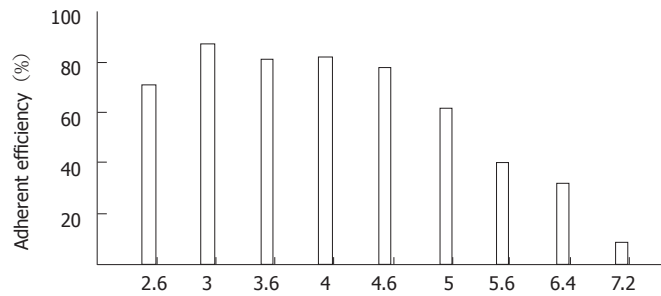


Figure 2 Effect of pH on adherence of *Helicobacter pylori* to HEp2 cells.

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^[9]. *H. pylori* infection stimulates immune response, leading to a much higher level of antibodies in sera^[4]. The monoclonal antibodies used were a cluster of antibodies specific to the predominant antigens of *H. pylori*^[5], 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]

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

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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

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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^[1,2]. 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. hemoccult) 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*^[3] 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^[4]. 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*^[5]. During analysis, subjects were divided into two groups: Neoplasm (cancer, adenoma

Table 1 Relationship between the 1st 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 2nd 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 3rd 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, χ^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^[6]. 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^[3] because the former had a higher sensitivity. In China, Zhou *et al*^[7] 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^[8].

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*^[9] 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^[10]. 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, Kawauchi 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 Strohlein JR, Fairbanks VF, McGill DB, Go VL. Hemoccult 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]

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

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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 LDH₅ doubled and the ratio of LDH₄ + LDH₅/LDH₁ + LDH₂ was 3.6 ± 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 LDH₅, 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

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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^[1,2]. 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^[3]. 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*^[4]. 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^[5], 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 ^d | 38.22 ± 19.77 ^b |
| Distal tissue | 16 | 44.81 ± 17.24 ^b | 39.92 ± 15.15 ^b |

^b*P* < 0.01, ^d*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 ₄ + LDH ₅ /LDH ₁ + LDH ₂ |
|------------------|----|--------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--|
| | | 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 ^a | 30.05 ± 4.04 | 37.03 ± 8.24 | 8.76 ± 6.04 ^e | 2.3 ± 1.2 ^a |
| Distal tissue | 16 | 4.28 ± 2.55 ^b | 22.17 ± 4.57 ^b | 34.38 ± 5.75 ^e | 30.76 ± 5.83 ^a | 8.11 ± 6.32 ^e | 1.7 ± 0.9 ^e |

^a*P* < 0.05, ^b*P* < 0.01, ^e*P* < 0.001 *vs* cancer tissue. LDH: lactate dehydrogenase.

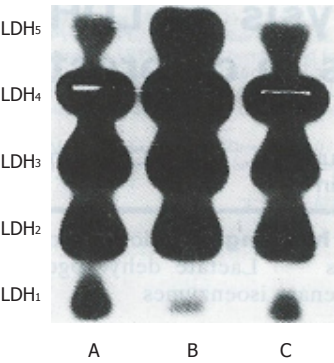


Figure 1 Lactate dehydrogenase isoenzyme patterns. A: proximal tissue; B: cancer tissue; C: distal tissue. LDH: lactate dehydrogenase.

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 LDH₁ and LDH₂ in tumors decreased significantly in comparison with proximal and distal noncancerous tissues; the percentage of LDH₃ decreased while that of LDH₄ increased in comparison with distal tissues; the percentage of LDH₅ was 2.2 and 2.4-fold higher than that in proximal and distal tissues, respectively. The ratio of LDH₄ + LDH₅/LDH₁ + LDH₂ 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.*^[6] and Hong *et al.*^[7].

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 LDH₅ contributes to the increase of total LDH activity in tumors; the ratio of LDH₄ + LDH₅/LDH₁ + LDH₂ 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^[8] 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

- 1 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]
- 2 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
- 3 Li QY, Xu MZ, Kong XY. Practices of Medical Laboratory Sciences. Wuhan: Hubei People's Publishing House, 1980: 341-343
- 4 Luo L, Yang ZH. A high sensitive method for determination of LDH isoenzymes. *Zhonghua Jianshan Yixue Zazhi* 1992; **15**: 6-7
- 5 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]
- 6 Carda-Abella P, Perez-Cuadrado S, Lara-Baruque S, Gil-Grande L, Nuñez-Puertas A. LDH isoenzyme patterns in tumors, polyps, and uninvolved 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]
- 7 Hong GY, Li JW, Xiao NQ. Systematic studies of human LDH isoenzymes. *Acta Sci Nat Univ Pekin* 1988; **24**: 195-201
- 8 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]

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

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Abstract

AIM: To observe the therapeutic effects and toxic side effects of ^{125}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}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}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}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}I within the tumor cells.

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

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INTRODUCTION

There have been many experimental and clinical reports about targeted treatment of hepatocellular carcinoma (HCC)^[1-5]. In 1980 the authors in 1980 started to use ^{131}I anti alpha fetoprotein (AFP) for the treatment of HCC, reported preliminary results in 1983^[5] and published the results of 72 cases in 1994, which showed rather good curative effects^[6]. However, ^{131}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}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}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}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}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 ¹²⁵I (supplied by the China Atomic Energy Research Institute) by the chloramine-T method. The product ¹²⁵I 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 ¹²⁵I 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 ¹²⁵I anti AFP intravenous drip and prednisone 30 mg was taken fractionally to prevent allergic reactions. The labeled drug was given once per month. The ¹²⁵I 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 ¹²⁵I 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 ¹³¹I anti AFP. The method of preparation followed that of Wu *et al.*^[6]. 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 T₃ and T₄ before treatment and during the monitoring of the blood, ¹²⁵I turned to zero. Detection of the biological bodily distribution of ¹²⁵I by FT-3120 isotope activity was performed in some cases to determine the blood ¹²⁵I concentration (% dose/mL) at different times after treatment and the urinary excretion rate (the percentage of 24 h urine ¹²⁵I administered dose amount). The organ surface cpm·min⁻¹ 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 14th and 19th 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, T₃ and T₄ 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 ¹³¹I or ¹²⁵I as warheads in targeted therapy for HCC. This therapeutic method was indicated for the treatment of unresectable HCC. Bretagne^[7], Baoul *et al.*^[8], Lu *et al.*^[4] and Wang *et al.*^[2] used ¹³¹I (or ¹²⁵I) 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^[9] used ¹³¹I 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^[1], using ¹³¹I 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 ¹³¹I 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 ¹²⁵I group and control (A, B, C) groups

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

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

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

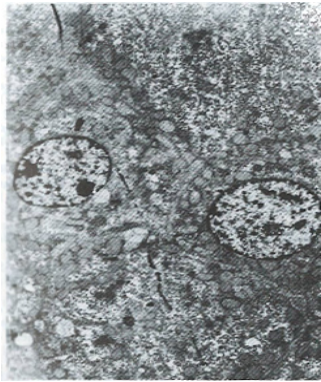


Figure 1 Electron microscope observation of resected specimen ¹²⁵I anti alpha fetoprotein treatment. (Li, male, 27 years old, HCC, admittance No.10206 tumor cross section, necrotic material, capsule intact electron microscope: Degeneration, necrosis of tumor tissue). Normal liver cell, from juxta tumor.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

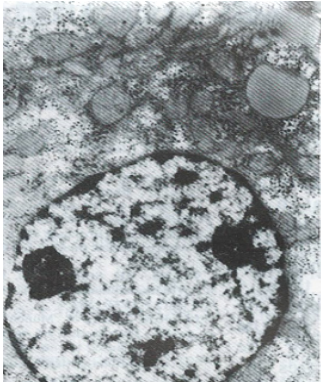


Figure 2 Electron microscope observation of resected specimen ¹²⁵I anti alpha fetoprotein treatment. (Li, male, 27 years old, HCC, admittance No.10206 tumor cross section, necrotic material, capsule intact electron microscope: Degeneration, necrosis of tumor tissue). Dissolution of nucleus. × 7700

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*^[10] reported that on the 7th day after the infusion of ¹²⁵I 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 ¹²⁵I 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 ¹²⁵I levels and the urinary excretion rates determined at different times in some of our treated patients, and as time passes, such blood ¹²⁵I levels and urinary excretion rates declined but rather slowly, showing that the radiation action of ¹²⁵I within the cancer cells might persist for quite a long time, thereby producing



Figure 3 Electron microscope observation of resected specimen ^{125}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. $\times 4000$



Figure 4 Electron microscope observation of resected specimen ^{125}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. $\times 7700$

comparatively good therapeutic results. This paper and the Koji experimental investigation suggest that ^{125}I anti AFP possesses a certain degree of specificity in the treatment of HCC. Of course, this awaits further clinical verification. Although ^{131}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, ^{125}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 ^{125}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 ^{131}I (or ^{125}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 ^{131}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 ^{131}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 ^{131}I -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 ^{131}I -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 ^{131}I 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]

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Drinking water and liver cancer

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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

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INTRODUCTION

AFB₁, 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^[1] 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^[2].

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 Cl₂ (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 ^b | + |
| 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 ₂ Cr ₂ O ₇ | 12 | 25.1 ± 5.10 ^b | + |

Each sample was compared with the distilled water (*t* test). ^b*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 ^b | + |
| Lingfan | 16.3 ± 4.73 ^a | + |
| Bayang | 15.3 ± 2.52 ^b | + |
| Liansui | 18.0 ± 2.00 ^a | + |
| Ruiling | 9.7 ± 0.58 ^a | + |
| Zhongyuan | 15.3 ± 2.50 ^b | + |
| Busha | 16.0 ± 1.60 ^a | + |
| Sairen | 20.0 ± 1.63 ^a | + |
| Lailu | 5.0 ± 1.00 | - |
| Sanhe | 8.7 ± 1.15 ^b | + |
| Dubang | 21.7 ± 4.16 ^b | + |
| Jutun | 19.3 ± 1.53 ^b | + |
| Tanlong | 18.0 ± 2.16 ^b | + |
| Distilled water | 4.4 ± 0.58 | - |
| K ₂ Cr ₂ O ₇ | 25.0 ± 3.24 ^b | + |

MCN of water from each spot was compared with distilled water (*t* test). ^b*P* < 0.01, ^a*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 ^a | + |
| Mumin | 3.0 ± 1.00 | - |
| Jucheng | 14.3 ± 3.05 ^a | + |
| destilled water | 4.1 ± 0.25 | - |
| K ₂ Cr ₂ O ₇ | 25.6 ± 5.20 ^b | + |

MCN of water from each spot was compared with distilled water (*t* test), ^b*P* < 0.01, ^a*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^[3,4]. 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 K₂Cr₂O₇ (3.4 × 10⁻⁶ 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 |
|---|--------------------------|-----------------|
| Ruiling | 5.00 ± 1.00 ^a | - |
| Sairen | 3.30 ± 0.47 ^a | - |
| Dubang | 4.67 ± 1.15 ^a | - |
| Tangan | 3.85 ± 0.57 ^a | - |
| Zhongyuan | 4.51 ± 0.51 ^a | - |
| Distilled water | 4.33 ± 0.52 | - |
| K ₂ Cr ₂ O ₇ | 24.9 ± 3.10 ^b | + |

MCN of water from each spot was compared with distilled water (*t* test), ^b*P* < 0.01, ^a*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 ₂ Cr ₂ O ₇ | 25.70 ± 2.30 ^b | + |

MCN of water from each spot was compared with distilled water (*t* test), ^b*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 ₂ Cr ₂ O ₇ | 25.71 ± 2.91 ^b | + |

^b*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 ^b | 2.34 |
| Well water by the ponds | 75.85 ^b | 2.17 |
| Well water | 63.12 ^a | 1.80 |
| River water | 45.69 ^a | 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, ^b*P* < 0.01, ^a*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^[5]. 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 AFB₁ and mycotoxins and so on^[6] 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 AFB₁ and drinking hygienic water^[7] 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.

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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

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

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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

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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^[1,2]. 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 IP₃, 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^[3]. Nishizuka pointed out that there were crosstalk between the cAMP/PKA and DAG/PKC pathways

and provided two models for their interaction^[4]. Tumor therapy by TCM has been reported^[5,6], 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

[³H] glycerol was purchased from Amersham, [³H] cAMP and [r-³²P] 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% CO₂ 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 ³H-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^[7]. cAMP level was expressed as pmol/10.6 cells.

DAG measurement

To label MGc80-3 cells with ³H-glycerol for 15 h according to Wolfman *et al.*^[7], 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.^[9] and Dabon *et al.*^[10] and the protein content in these extracts was determined according to Lowry's assay^[11].

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 4th 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^[12] 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 3rd or 4th day, indicating that the changes of messenger molecules appeared earlier than that of cell proliferation by several cell cycles^[13]. Regulation of

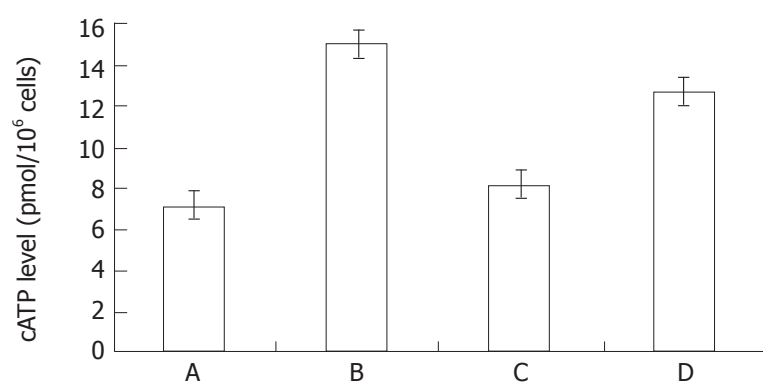


Figure 1 Changes of cAMP level in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ($n = 3$)

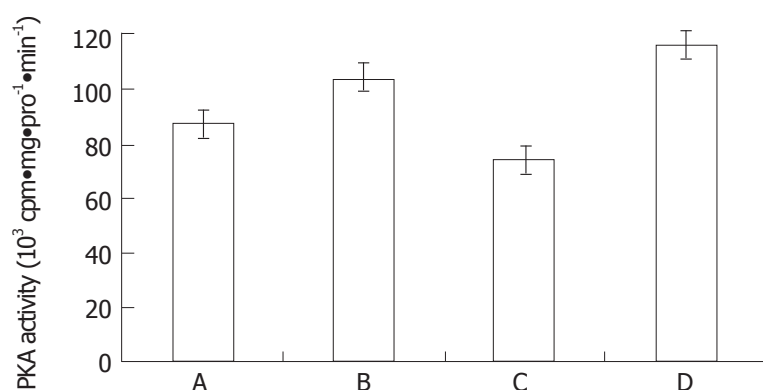


Figure 2 Changes of PKA activity in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ($n = 3$)

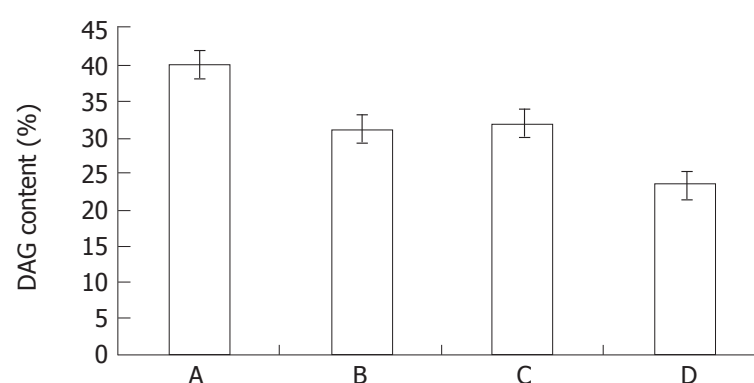


Figure 3 Changes of DAG activity in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ($n = 3$)

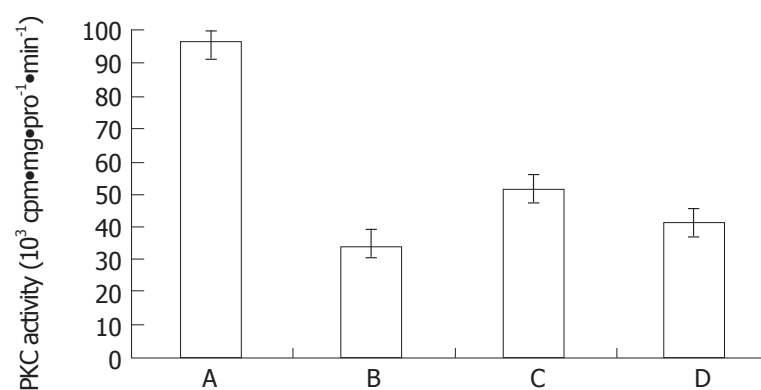


Figure 4 Changes of PKC activity in MGc80-3 cells treated in different conditions. A: control; B: 1.8 g/L bailong; C: 1.8 g/L bailong + 20 mg/L PKA inhibitor; D: 5 mmol/L PKC inhibitor ($n = 3$)

bailong was similar to that of HMBA in signal transduction of MGc80-3 cells^[14], 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^[15,16], 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^[4] model, in which the interaction of protein kinases and phosphatases were suited to the TCM theory of Yin Yang^[17]. 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 hexamethylenesacetamide-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, Rösel 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]

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

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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 ± 10.644 and 55.5173 ± 6.0500 , respectively; after such needling, the percentage was 43.7823 ± 10.1518 and 43.5147 ± 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 ± 0.3800 and 2.4020 ± 0.3536 , respectively, before needling and after needling, the frequency was 2.7090 ± 0.5865 and 2.9220 ± 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

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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 ± 8.0388 and frequency was 3.3426 ± 0.2523 C.P.M. After rotation, the percentage of disturbance wave was 51.6914 ± 5.9842 and frequency was 2.670 ± 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.

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

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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^[1]. 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^[2]. 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^[3].

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^[4]. *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^[5]. 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^[6]. 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^[7]. 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^[8]. 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^[9].

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^[10]. 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^[11]. Investigating the pathogenesis of *Hp* infection showed that the tissue TNF- α 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^[12]. 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^[13]. 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% ($P < 0.05$)^[14]. To assess the therapeutic efficiency of single, dual and triple therapy for eradication of *Hp* infection, ¹⁴C and ¹³C breath tests are presently used in large medical centers. A capsule-based modified microdose ¹⁴C 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^[15]. In a newly discovered test, ¹⁵N-urea excretion, which is devised here and can be used as a tracer for detection of clinical *Hp* infection, the ¹⁵N-excretion rate in urine ammonium was much higher in *Hp* positive than in *Hp* negative subjects. A single dose of ¹⁵N 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^[16].

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^[17]. Another advance was the study of the plasma and intracellular concentrations of vitamin A, C, E, β 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 β 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^[18] 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^[19]. 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 α , IL-1 β , IL-6 and TGF β . 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^[20]. 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^[21]. 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^[22]. 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^[23].

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^[24]. 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^[25]. 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^[26].

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^[27]. 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^[28]. In another study, the NS4 antigen of HCV in liver tissue from 9 patients treated with IFN- α 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- α . 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 INF- α than serum ALT and HCV RNA^[29]. 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^[30]. 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^[31]. Regarding a treatment regimen, a randomized control study was carried out comparing 3Mu IFN- α 2 α 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^[30]. 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^[32].

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 α -1 at a dosage of 40 μ g/d for three months has a similar effect as the Western product^[33]. 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^[34].

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 2nd 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^[35]. 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 β N-acetyl glucosamidase (β -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^[36]. On culture of lipocytes, IFN- γ and tocopherol were found to have an inhibitory action on ³H 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 α 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^[37]. 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^[38]. Tetrandrine had an inhibitory effect on ³H-proline incorporation at a concentration of 10 μ g/mL-50 μ g/mL in an experimental study of DNA and collagen synthesis of 3T3 cells and was considered antifibrotic^[39]. Another drug, cinnarizine was found to have the effect of blocking the G₁ phase cells of 3T3 fibroblasts from progressing to the S-phase and diminished the DNA content and mitosis, as demonstrated by flow cytometry^[40]. 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^[41]. 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 ¹⁵N-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^[42]. 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 reinstitution resulted in secondary patency. Hepatic failure and rebleeding were rare^[43,44]. 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^[45].

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^[46]. 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.^[47]. 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^[48]. 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^[49]. 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^[50]. 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^[51]. 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^[52]. 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^[53].

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^[54]. 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^[55]. 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^[56]. 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 α L-fucosidase, α -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_g 18.1 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^[57]. 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^[58]. 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 ¹⁴C 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 TXB₂, improving the ratio of TXB₂/PGF-1 α 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 ¹⁴C-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 ¹⁵N-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 antgastric cancer monoclonal antibody. *Changdao Bingxue* 1995; **1**: 20-22
- Ran ZH, Shan MJ, Xiao SD. Gastrointestinal bleeding of obscure origin aanalysis 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 stunt 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



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