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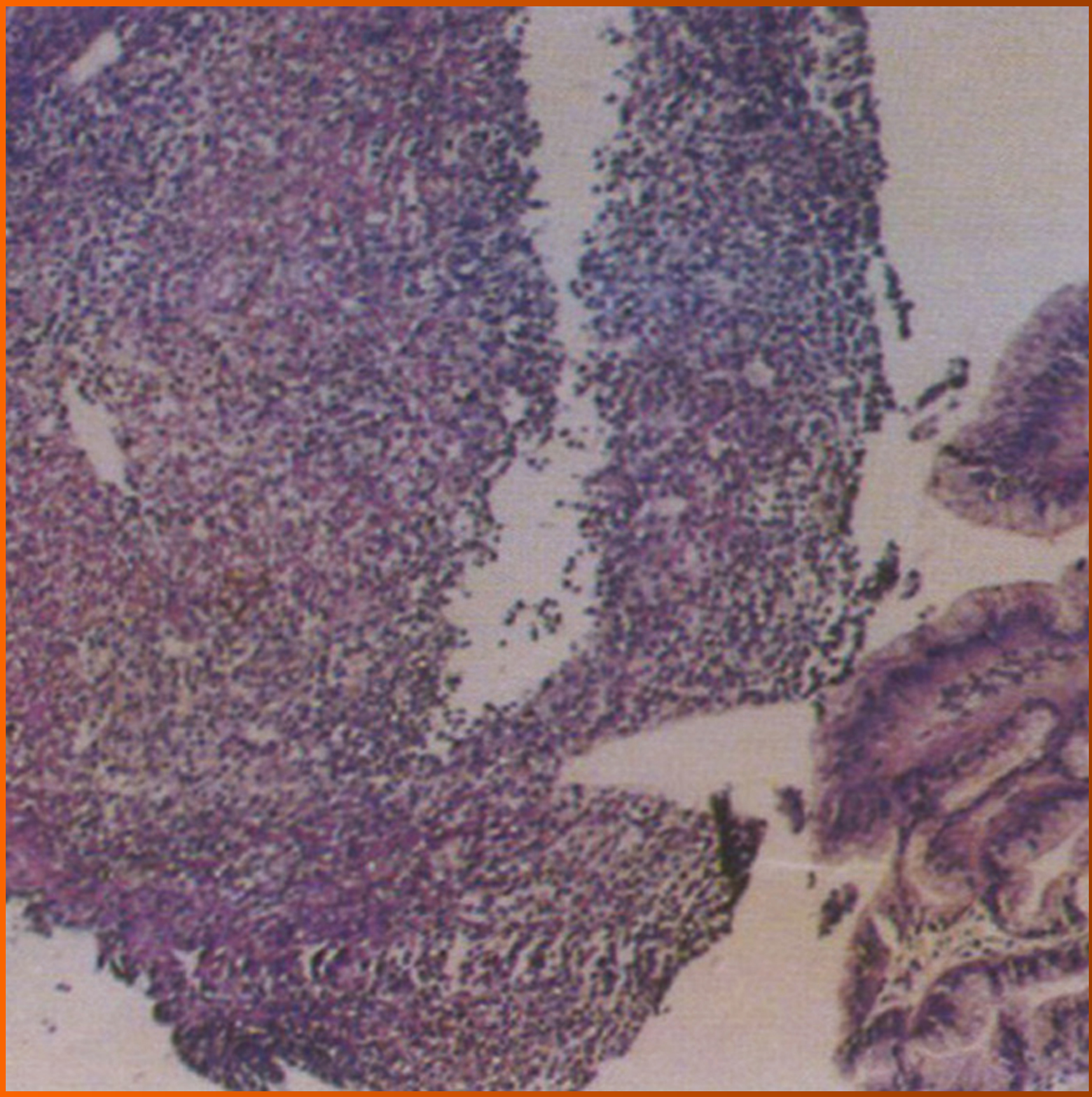

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Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893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

Developing a new gastroenterology with distinct Chinese features

Lian-Sheng Ma, Bo-Rong Pan

Lian-Sheng Ma, Member of the Committee of Gastroenterology, Chinese Association of the Combination of Traditional Chinese and Western Medicine. Director of Taiyuan Research and Treatment Centre for Digestive Diseases

Bo-Rong Pan, President of the China Specialty Council of Gastrology, Chinese Association of International Exchange and Promotion of Medicine and Hygiene, Professor of Internal Medicine, Rehabilitation Centre of the Fourth Military Medical University

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In the golden autumn, the season of harvest in China, the English Edition of the ***China National Journal of New Gastroenterology*** is coming out for expectant readers and authors at home and abroad. As a sister volume of the Chinese Edition of the ***Chinese Journal of New Gastroenterology***, which is monthly, the English Quarterly has been approved by the Science and Technology Commission of the People's Republic of China and the State Information and Publication Bureau, and registered at the Shanxi Provincial. This will be a unique academic journal in the field, distributed throughout the world for the purpose of an multi-angular interchange of views and multidisciplinary infiltration. The publication of the English edition will, of course, not only open up a new means of making academic contacts for gastroenterologists of all circles, but also play an active role in strengthening international cooperation and promoting our specialty.

What is meant by **new gastroenterology**? And how should we characterize it?

In recent years, with the wide application of gastrointestinal endoscopy and the extensive study of related subfields such as digestive immunology, oncology, microcirculation, rheology, *etc*., the diagnosis and treatment of digestive diseases have risen to a much higher level than ever before. This is one aspect of the matter. However, we still have much to learn. As far as traditional Chinese medicine is concerned, there has been a complete systematic theory on gastrointestinal disorders called "Treatise on the Spleen and Stomach", which combines all-round theoretical expounding and abundant clinical experience from "The Yellow Emperor's Internal Classic" until the famous physicians' works of the Ming and Qing dynasties. Therefore, as history advances, it is a necessary duty of the gastroenterologists of the country to merge modern medicine and traditional Chinese medicine into an organic whole so that a new gastroenterology with distinct Chinese features is gradually established. In this regard, Dr. Bei-Hai Wei made a thorough and accurate explanation when he put forward the characteristics of the new gastroenterology. In theoretical studies, there should be a number of glittering junctures to integrate Western and traditional Chinese medicine into one in which the new arguments and new findings should resemble neither the former nor the latter. In clinical diagnosis, a new integrated mode of diagnosis should be set up by uniting the identification of diseases with the overall analysis of illness, the patient's conditions, macro- and micro-observations, and the determination of cause, location, quality and quantity. In clinical treatment, an efficacy superior to that of Western or traditional Chinese medicine alone should stand repeated tests and open up the mechanisms of efficacy. An overall system of new gastroenterology is formed on the basis of a combination of medicine, physiotherapy, medication and nursing.

By keeping up with the rapid development of medical science, gastroenterology in our country has improved a great deal in the past few decades. This progress is reflected in an ever-growing number of specialists and several journals of our own, such as the ***Chinese Journal of New Gastroenterology, China National Journal of New Gastroenterology*** and ***Chinese Journal of Clinical Hepatology.*** However, language barriers are limiting our international exchanges. For this reason it is essential to increase mutual understanding among our colleagues throughout the world and to strive to raise our academic level. This is where the aim and purpose of the new journal lies. We are fortunate that our chief consultants include five academicians, two of whom are members of the Chinese Academy of Sciences: Prof. Ke-Ji Chen and Prof. Meng-Chao Wu. The other three academicians are members of the Chinese Academy of Engineering: the late Prof. Shao-Ji Jiang, Prof. Jian-Yu Tang and Prof. Jian-Hua Dong.

It is sincerely hoped that all of our colleagues in different countries who are concerned with and back up the English edition of the Journal will generously contribute articles and give us your advice regularly. The main sections of the Journal include: Prospects for the 21st Century, Comments on Basic Researches, Clinical Studies, Reviews, Case Reports, and Symposium Summaries. Contributions of an advanced international level and with national features of different countries worthy of interchanges across the world are warmly welcomed.

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Clinical research of hepatocellular carcinoma in the 21st Century

Zhao-You Tang

Zhao-You Tang, Professor of Surgery, Liver Cancer Institute, Shanghai Medical University, Shanghai 200032, China

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Correspondence to: Zhao-You Tang, MD, Professor of Surgery, Liver Cancer Institute, Shanghai Medical University, Shanghai 200032, China
Telephone: +86-21-4037181

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One century has gone by since the scientific basis was established. Much has been done and much remains to be done in the field of clinical research of hepatocellular carcinoma (HCC). In the first half of the 20th Century, no remarkable progress was made in the clinical aspect of HCC research; the average survival of patients with HCC was around 2-5 mo. In 1950s-1960s, based on the understanding of intrahepatic anatomy as well as the biochemical changes that occur after surgery, major resection was the only hope for a curative outcome of large HCC. In 1960s-1970s, with progress in immunology, liver transplantation became an option. In 1970s-1980s, due to alpha fetoprotein (AFP) serosurveys and ultrasonography monitoring in the high-risk population, small HCC resection opened a new era in the clinical research of HCC, resulting in a marked improvement in the survival rate. In 1980s-1990s, rapid progress in medical imaging and regional cancer therapies enabled substantial advances the in diagnosis and treatment of both small and large HCC, Along with multimodality combination treatment, cytoreduction and sequential resection for initially unresectable HCC has been a significant advance.

An analysis of three decades of materials in the Liver Cancer Institute of Shanghai Medical University indicated that HCC has been converted from "incurable" into "partly curable". During the period of 1960-1989, 212 HCC patients with ≥ 5-year survival were treated in the author's institute, whereas in 1905-1970, only 45 HCC patients with ≥ 5-year survival were reported in the worldwide literature (Curutchet, *et al*. 1971). The analysis of these 212 HCC patients with ≥ 5-year survival revealed that surgery remained the principal approach to long-term survival. The treatment modalities of the 212 patients were small HCC resection, 51.4%; large HCC resection, 34.9%; cytoreduction and sequential resection for unresectable HCC, 8.0%; and palliative surgery other than resection, 5.7%.

Resection remains the best option for a curative outcome, and resection for small HCC is more effective. In the author's institute, a comparison between small HCC (≤ 5 cm) resection (n = 549) and large HCC resection (n = 831) revealed that in the small HCC group, the resectability rate was higher (93.2% *vs* 49.9%), the curative resection rate was higher (95.1% *vs* 76.7%), the operative mortality rate was lower (1.3% *vs* 4.0%), and the 5-year survival rate was higher (62.9% *vs* 34.6%). By the end of 1994, 109 HCC patients survived more than 5 years in the small HCC resection group, whereas only 75 patients did so in the large HCC resection group. In the author's series, 86.1% of the HCC cases coexisted with cirrhosis. With the increasing proportion of limited resection (from 53.9% to 72.3%), the resectability of small HCC increased from 78.8% in the 1970s to 95.2% in the 1980s.

Re-resection for subclinical recurrence is important for further improving survival after the initial resection. In the author's institute, the 5-year recurrence rate was 61.5% in the entire series, and 43.5% in the small HCC resection group. Therefore, it is important to monitor with both AFP and ultrasonography every 2-3 mo for 5-10 year after the initial resection for an early detection of recurrence. Re-resection was the treatment of choice for subclinical recurrence in the liver or for solitary lung metastases. In author's institute, of the 97 patients with re-resection, the 5-year survival rate was 51.2% from the first resection and 38.7% from the re-resection.

Cytoreduction with sequential resection is a new approach to the treatment of localized unresectable HCC. With the progress of regional cancer therapies and multimodality treatment, some of the localized unresectable HCC cases could be converted into resectable ones. In the author's institute, 72 of the 663 cases with surgically verified unresectable HCC have been converted into resectable ones. Successful cytoreduction with a median diameter reduction from 10 cm to 5 cm was mainly due to triple or double combination treatment with hepatic artery ligation (HAL), hepatic artery cannulation with infusion (HAI), radioimmunotherapy and fractionated regional radiotherapy. The operative mortality rate was 1.4% for sequential resection and the 5-year survival rate was 61.2%. The analysis revealed that a single nodule, well-encapsulated, situated in the right lobe or hepatic hilum, associated with micronodular cirrhosis, had a higher sequential resection rate when treated with triple or double combination modalities. Patients with a solitary tumor confined to one lobe, without a tumor embolus or residual cancer in the specimen of sequential resection, had a longer survival rate. For cytoreduction, double or triple combination treatment was more effective than single treatment. Since 1985, clinical trials of targeting therapies has added weight to the combination of HAL and HAI. Regional fractionated radiotherapy was an alternative to radioimmunotherapy. The sequential resection rates were 14.3%-34.0% for triple combination treatment (HAL, HAI and radioimmunotherapy/radiotherapy), 10.1% for double combination treatment (HAL and HAI), and only 1.1% for single treatment. The

recent advance of transcatheter hepatic arterial chemoembolization (TACE) has provided a nonsurgical approach to cytoreduction of unresectable HCC, and sequential resection after TACE has also been reported in the author's institute.

Intraoperative regional cancer therapy is a recent trend for the treatment of unresectable HCC, namely: HAL, HAI, cryosurgery, and intralesional ethanol injection. In the author's institute, the 5-year survival rate for 107 patients treated by cryosurgery was 22.0%.

In short, the approaches that substantially improved survival were the early resection of small HCC, re-resection for subclinical recurrence, and cytoreduction and sequential resection for initially unresectable HCC. Progress in tumor markers, medical imaging, regional cancer therapies, and the concept of multimodality combination treatment have all contributed to the realization of these approaches.

In China, HCC was the second-most lethal cancer in rural areas and ranked third in cities. Approximately 110000 people were killed annually, which accounted for 40% of the HCC-related deaths worldwide. As the mortality rate of HCC was very close to that of gastric cancer in 1990, and because it is predicted that gastric cancer in China will gradually decrease, as it did in Japan, HCC will probably surpass gastric cancer as China's most lethal cancer in the 21st Century. Clinically, the major obstacle for further prolonging survival of HCC patients was recurrence and metastasis after resection. The recurrence rate of HCC after resection was as high as 80%-90%, and was 60%-70% after curative resection and 40%-50% after small HCC resection. Re-resection was an effective approach to prolonging further survival; however, it was limited by the multicentric origin of HCC. The current available modalities, such as TACE and percutaneous intralesional ethanol injection (PEI), might be useful for preventing and treating recurrence. Biotherapy might add weight to surgical therapy. Recently, several biological prognostic factors have been noted. In the author's institute, HCC invasiveness-related oncogenes and growth factors included p53, H-ras, c-erbB-2, TGF-α, and the epidermal growth factor (EGF) receptor; however, these factors did not correlate well to tumor size. Therefore, biological approaches, including gene therapy and tumor vaccines, will probably become important in the forthcoming years for further improving the prognosis after surgery. The establishment of an HCC metastatic model in nude mice is needed for research on HCC recurrence and metastasis, as well as for seeking new treatment modalities for metastasis. However, because gene therapy and other biotherapies are complex, the study of better combining old modalities will still be an useful approach. With progress on a humanized or bioengineered human-mouse chimeric antibody, immunotargeting therapy will probably be a routine treatment for HCC. Studies on multicentric origins was also important, particularly on the prevention of new lesion development. Liver transplantation will be significant in this particular aspect. However, the difficulty in getting donor organs, as well as the expenses, will make this study difficult, particularly in developing countries. The effective approach for noncompensated liver cirrhosis (Child C) was another challenge. In short, slow but substantial progress in the field of clinical research of HCC will be achieved in the forthcoming 21st Century.

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COMMENTARY

Acute liver failure: A progress report

Bo-Rong Pan, Si-Feng Yang, Lian-Sheng Ma

Bo-Rong Pan, Professor of Internal Medicine, gastroenterologist, member of EASE, IASL, CBE, DIA and STA; research fellow of Royal Postgraduate Medical School in London 1981-1982; President of China Speciality Council of Gastrology, China Research Network of Peptic Ulcer and China Association of Misdiagnostics: having 238 papers and 38 books published; enrolled in [Who's Who in Medicine] by International Biographical Centre, Cambridge, England.17 Changlaxilu, Xi'an 710033, Shaanxi Province, China

Si-Feng Yang, Professor of Internal Medicine, Director of Department of Gastroenterology Chinese PLA 222 Hospital, 47 Yueshanlu, Jilin 132011, Jilin Province, China

Lian-Sheng Ma, Physician-in-Charge, Director of Taiyuan Research and Treatment Centre for Digestive Diseases, 115 Qingnianlu, Taiyuan 030001, Shanxi Province, China

Author contributions: All authors contributed equally to the work.

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

Correspondence to: Dr. Bo-Rong Pan, Professor, A Fourth Military Medical University, Xi'an 710033, Shaanxi Province, China
Telephone: +86-29-3224890

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INTRODUCTION

Acute liver failure (ALF) includes many conditions in which severe injury of hepatocytes or massive necrosis occurs. Loss of hepatocyte function sets in motion a multiorgan response, and death may occur even when the liver has begun to recover. This article reviews recent advances in our understanding and treatment of ALF. Altered mental status (hepatic encephalopathy) and coagulopathy in the setting of an acute hepatic disease help define ALF. In 1946, Lucke *et al* paid attention to two fatal types of acute hepatitis: a fulminant form with an extremely rapid outcome and a subacute form with a slower course. ALF encompasses all these clinical presentations.

REDEFINING THE SYNDROMES

Existing definitions of clinical syndromes in ALF do not accurately reflect important differences in the clinical features and prognoses. Based on a large series of patients with ALF treated at King's College Hospital, London between 1972 and 1985, O'Grady *et al* proposed a new terminology. Hyperacute liver failure was suggested for cases in which encephalopathy occurs within 7 d of the onset of jaundice; this group includes the sizable cohort likely to survive with medical management despite a high incidence of cerebral edema. ALF was suggested for cases with an interval of 8-28 d from jaundice to encephalopathy; these patients also have a high incidence of cerebral edema, but have a poor prognosis without liver transplantation. Subacute liver failure was suggested for cases with encephalopathy that occurs 5-12 wk after the onset of jaundice; these patients are characterized by a low incidence of cerebral edema, but still have a poor prognosis. Adoption of this terminology should help in the management of these patients, in addition to standardizing the structure and interpretation of controlled trials of therapies (Figure 1, Table 1).

CAUSES OF ACUTE LIVER FAILURE

Viral hepatitis and drug-induced liver injury account for most cases of ALF, but there are great differences in incidence among countries. It is important to determine the cause as carefully as possible, since the prognosis and use of antidotes for certain forms of ALF depend on the identification of the causative agent (Table 2).

Viral hepatitis

The foremost cause of ALF is acute viral hepatitis, accounting for up to 72% of all cases. Viral hepatitis leads to hepatic failure in only a small proportion of cases (< 1%), and each of the five primary hepatotropic viruses (A, B, C, D, E) has been implicated in ALF. Hepatitis A rarely leads to ALF (0.35% of cases), but when it does, the patients have a good prognosis (> 60% survival) and seldom need liver transplantation. Acute hepatitis B may lead to ALF in 1% of patients, accounting for more than 70% of the patients with virus-induced disease in most countries. Hepatitis C virus has rarely been implicated in ALF, but may contribute to the massive necrosis that occurs in patients co-infected with hepatitis B virus. Hepatitis D virus in former carriers of hepatitis B virus accounts for fewer than 10% of all cases of acute hepatitis related to hepatitis B virus, and more than half the cases of ALF in patients positive for hepatitis B are due to hepatitis D virus rather than to hepatitis B alone. Hepatitis E occurs in epidemics exhibiting a high incidence of ALF, with the case fatality rate approaching 40% in pregnant women. Cytomegalovirus, Epstein-Barr virus, and Herpes viruses 1, 2, and 6 have occasionally been implicated as a cause of ALF. ALF related to herpes virus is usually responsive to immunosuppressive therapy, and can be treated with acyclovir.

Drugs and toxins

Drugs that cause liver injury can be divided into two categories: predictable and idiosyncratic. Acetaminophen is representative of the predictable toxic group; the toxicity of acetaminophen is dose-dependent and exaggerated by starvation or drugs (particularly

Figure 1 Professor Bo-Rong Pan.

Table 1 Characteristics of subgroups of patients with acute liver failure

Characteristics	Hyperacute liver failure	Acute liver failure	Subacute liver failure
Encephalopathy	Yes	Yes	Yes
Duration of jaundice	0-7 d	8-28 d	29-72 d
Cerebral edema	Common	Common	Seldom
Prothrombin time	Prolonged	Prolonged	Least prolonged
Bilirubin	Slightly elevated	Elevated	Elevated
Prognosis	Moderate	Poor	Poor

Table 2 Principal causes of acute liver failure

Causes	Agents responsible
Viral hepatitis	Hepatitis A, B, C, D, E, or F (?) virus
	Herpes simplex virus
Drug related liver injury	Epstein-Barr virus, Cytomegalovirus
Toxins	Adenoviruses, Paramyxovirus
Vascular events	Acetaminophen
Miscellaneous	Idiosyncratic reactions
	Drug-induced steatosis
	Carbon tetrachloride
	Amanita phalloides
	Phosphorus
	Ischemia or shock
	Veno-occlusive disease
	Heat stroke and Hypothermia
	Malignant infiltration
	Wilson's disease
	Acute fatty liver of pregnancy
	Reye's syndrome, Cryptogenic

Table 3 Drugs implicated in idiosyncratic acute liver failure

Infrequent causes	Rare causes	Synergistic causes
Isoniazid	Carbamazepime	Alcohol and acetaminophen
Valproate	Ofloxacin	Trimethoprim and sulfamethoxazole
Halothane	Ketoconazole	Rifampin and isoniazid
Sulfonamides	Niacin	Amoxicillin and clavulanic acid
Propylthiouracil	Labetalol	
Aiodarone	Etoposide (VP-16)	
Disulfiram	Imipramine	
Dapsone	Interferon alfa	
	Flutamide	

alcohol) that induce the cytochrome *P* 450 isoenzyme. Many other drugs produce rare catastrophic insults to hepatocytes that are considered to be idiosyncratic reactions (Table 3).

The fluorinated hydrocarbons trichoroethylene and tetrachloroethane produce hepatic injury in people exposed to industrial cleaning solvents. *Amanita phalloides,* the death-cap mushroom, causes numerous deaths among amateur mushroom gatherers in China's mountainous areas. ALF is preceded by muscarinic effects, such as profuse sweating, vomiting, and diarrhea. Early identification is helpful to use antidotes (penicillin and silybin). Successful liver transplantation has been reported.

Miscellaneous causes

ALF is one of the presentations of Wilson's disease. Cardiac-related

Table 4 Possible predisposing factors for acute liver failure

Etiologic agents	
Viruses	various hepatotropic viruses superinfection of hepatotropic viruses variants of a hepatotropic virus (mutants)
Chemicals	
Miscellaneous	
Host factors	
Hyperfunction of cellular immunity	
Hyperfunction of antibody production → immune complexes	
Endogenous endotoxemia	
Deficient phagocytosis of reticuloendothetial system	
Activation of macrophages	overproduction of TNF-α and IL-1 release of leukotrienes release of superoxides
Liver regeneration failure	overproduction of regeneration-suppressing factors disorders in cell receptors and signal transduction
Apoptosis	

hepatic ischemia produces centrilobular necrosis and ALF. Causes include myocardial infarction, cardiac arrest, cardiomyopathy, and pulmonary embolism. Hepatic sinusoidal obstruction with subsequent ischemia or interruption of sinusoidal flow has been reported in metastatic gastric carcinoma, carcinoid syndrome, breast cancer, oat-cell carcinoma, amyloidosis, and blastic infiltration with leukemic cells. Occlusion of hepatic venous outflow may occasionally produce a similar picture, either as Budd-Chiari syndrome or as veno-occlusive disease, in the setting of intensive cancer chemotherapy or bone marrow transplantation.

Acute fatty liver can occur in the third trimester of pregnancy and is characterized by the sudden onset of jaundice and altered mental status, accompanied by hypoglycemia and preeclampsia. ALT levels may be very high in late pregnancy and may be accompanied by hemolysis, thrombocytopenia, and preeclampsia in what is termed the HELLP syndrome (Hemolysis, Elevated Liver enzyme levels, Low Platelet counts). Other rare causes of ALF include amebic abscesses, disseminated tuberculosis, recrudescence of hepatitis B virus after the withdrawal of cancer chemotherapy, and bone marrow transplantation.

PATHOGENESIS AND PATHOPHYSIOLOGY

Although the causative agent is frequently known, a full understanding of the pathogenesis of ALF is elusive. A shock-like state and cerebral edema, shared by all forms of ALF, suggest a unified pathological mechanism. Endotoxemia is common and the levels of tumor necrosis factor α are increased. Prostaglandin metabolism is perturbed and may be important in producing or protecting against tissue hypoxia. Levels of prostaglandin E_2, thromboxane A_2, and prostacyclin are increased.

A group-specific component protein (Gc) is markedly diminished in ALF. The survival of patients with ALF after acetaminophen poisoning can be predicted by the level of Gc and the quantity of actin-Gc complexes. Depletion of Gc protein may contribute to the disease, since exhaustion of this actin scavenging mechanism would enhance the precipitation of actin filaments (and platelets) in the microcirculation. Plasma thrombomodulin levels are elevated in ALF. Endothelin-1 levels are elevated in patients with ALF and are highest in association with renal failure. It seems unlikely that a single pathological mechanism can explain all the abnormal events. Two aspects are involved – the host and the virus – as summarized schematically in Table 4 and Figure 2.

Encephalopathy and cerebral edema

The onset of encephalopathy is often abrupt. Agitation, delusional ideas, and hyperkinesis are common, and coma rapidly ensues. Benzodiazepine-like substances have been implicated in the pathogenesis of the encephalopathy. Elevated concentrations of 1,4-benzodiazepines have been detected in brain tissue.

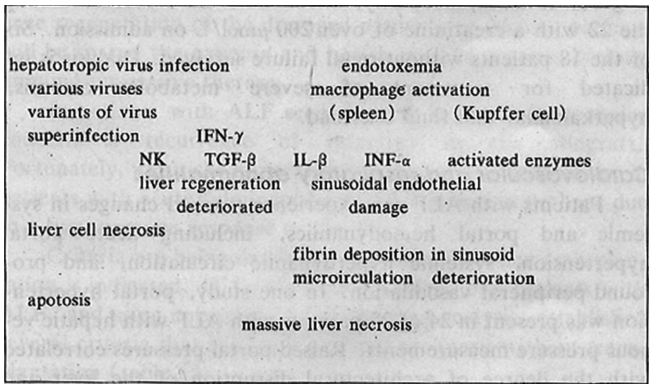

Figure 2 Hypothetical pathogenesis of acute liver failure.

Cerebral edema occurs in 75%-80% of ALF patients who progress to grade Ⅳ encephalopathy, and is the leading cause of death in these patients. Intracranial blood flow is markedly reduced in the patients. Cerebral edema in the confinement of the cranial vault raises intracranial pressure and decreases intracerebral perfusion. Transferring comatose patients to transplantation facilities by airplane or helicopter is hazardous, since the decreased pressure in flight exacerbates the condition. A CT scan to rule out an intracranial hemorrhage prior to surgery is necessary.

Intracranial pressure monitoring, which has shown that pressure changes evolve rapidly and erratically, is used in most transplantation centers to guide the treatment of preoperative and intraoperative pressure changes. Epidural monitors are less hazardous than subdural or parenchymal devices, albeit with a lower sensitivity. A persistent cerebral perfusion pressure of less than 5.33 kPa (40 mmHg) that is refractory to treatment with mannitol should preclude transplantation.

Coagulopathy

Coagulopathy and hemorrhage are common, and decreased levels of plasma fibrinogen and clotting factors (secondary to decreased synthesis by the damaged liver) are almost universal in ALF. Decreased levels of clotting factors Ⅱ, Ⅵ, Ⅶ, Ⅸ and Ⅹ account for the prolonged prothrombin time and partial thromboplastin time. Low-grade fibrinolysis and intravascular coagulation may occur. Antithrombin Ⅲ levels are decreased, and the level of thrombin-antithrombin complexes is increased.

Coagulation factor levels have been widely used to determine the prognosis in ALF. Pereira *et al* studied 22 patients with acetaminophen-induced ALF. The levels of factor Ⅴ on admission were significantly higher in patients who subsequently died of ALF or required liver transplantation than in patients who recovered. A factor Ⅷ/Ⅴ ratio of greater than 30 was predictive of an adverse outcome in 10 of 11 patients on admission.

Renal function

Renal failure occurs in more than 50% of patients with ALF and is a poor prognostic sign. Low urine output despite adequate cardiac output is commonly seen and may respond to low-dose dopamine loop diuretics. In 40 patients with ALF and stage Ⅲ-Ⅳ encephalopathy, there were no survivors among the 22 with a creatinine level of over 200 μmol/L on admission. Six of the 18 patients without renal failure survived. Dialysis is indicated for the treatment of severe metabolic acidosis, hyperkalemia, and fluid overload.

Cardiovascular and respiratory abnormalities

Patients with ALF can experience marked changes in systemic and portal hemodynamics, including acute portal hypertension, systemic hyperdynamic circulation, and profound peripheral vasodilation. In one study, portal hypertension was present in 24 of 25 patients with ALF. Elevated portal pressures correlated with the degree of architectural disruption of the liver and were most evident in patients with ascites and renal failure. Patients with ALF typically had a hyperdynamic circulation with a low systemic vascular resistance and a markedly increased cardiac output. The cause is unclear, and a variety of mediators have been proposed, including bile salts, prostaglandins, glucagon, endotoxin, serotonin, nitric oxide, and nonhumoral neural substances. The circulatory changes, which can be profound, are associated with renal vasoconstriction, a decrease in cerebral perfusion pressure, and hepatopulmonary syndrome, leading to renal failure.

Hypotension may result in impaired hepatic, renal, and cerebral function due to hypoperfusion. Pulmonary problems pose a special threat to patients with ALF. Aspiration with pneumonia may occur in comatose patients, who are at a higher risk of developing low pressure pulmonary edema and adult respiratory distress syndrome, and tissue hypoxia leads to anaerobic metabolism and ultimately to lactic acidosis. Prostacyclin, which has vasodilatory effects on the microcirculation, will increase peripheral oxygen uptake. Acetylcysteine, an acetaminophen antidote, enhances oxygen delivery and consumption, possibly by opening the microcirculation through the nitric oxide-induced control of vascular tone.

Infection and metabolic changes

Bacterial and fungal infections are common in ALF. In a study of 50 patients, 80% had culture-proven infection and 10% had a suspicion of infection. Most infections arise in the respiratory and urinary tract, and Gram-positive cocci – particularly Staphylococcus aureus – are most frequently implicated. Sepsis is common, with bacteremia documented in 20%-45% of patients. Susceptibility to infection may relate to a deficiency of complement or impaired serum opsonization and/or chemotaxis. Polymorphonuclear cell inhibition and Kupffer cell dysfunction may also increase the risk of sepsis. Patients with ALF are at risk for iatrogenic sepsis related to intravenous, arterial, or urinary catheters. Antifungal therapy should be considered prophylactically in patients with established renal failure who survived for five days post-admission. Spontaneous bacterial peritonitis is also common. Chu *et al* found that 32% of 82 patients developed bacterial peritonitis. These infected patients are likely to develop renal failure and gastrointestinal tract bleeding, with a significantly high mortality rate.

Hypoglycemia is common due to defective gluconeogenesis in the failing liver, as well as inadequate hepatic uptake of insulin, leading to hyperinsulinemia. Hypokalemia can occur due to central nervous system-induced respiratory alkalosis and the resultant renal excretion of potassium in exchange for hydrogen ions. Hypophosphatemia is also frequent and cardiac arrhythmias may occur.

TREATMENT

For each ALF patient, treatment should be complex and active based on the current understanding of the cause, pathogenesis and pathophysiology – not only with modern western medicine, but also with traditional Chinese medicine. Nevertheless, basic studies are particularly necessary in ALF, since intuitive treatment approaches have thus far been of limited value.

Systemic treatments such as corticosteroids, heparin, or insulin and glucagon have shown little efficacy, but recombinant human hepatic growth factor (rhHGF), HSS and hepatitis B immune globulin may have a beneficial effect. Antiviral agents have not been used to any extent for ALF, except Chinese herbs. Interferon is beneficial to block further liver destruction and the development of ALF from acute severe viral hepatitis. Levin *et al* reported favorable results in the use of human interferon γ in patients with ALF secondary to hepatitis A, B, or non-A, non-B. Blood or plasma exchange, hemodialysis, or other methods to detoxify the blood may improve the coma grade. Prostaglandins initially showed some promise; however, efficacy could not be demonstrated in controlled studies.

Since there are no specific treatments with proven efficacy (except for toxin antidotes), the guiding principle of therapy in ALF is to provide good intensive care to the comatose patients. Every effort must be made to elucidate the cause, as antidotes need to be given as early as possible for acetaminophen or mushroom poisoning. Initial management includes measuring glucose (giving 560 mmol/L (10%) dextrose if necessary), acetaminophen levels, ceruloplasmin (< 50 year of age), prothrombin time, hepatitis virus markers and

Table 5 Management of complications of acute liver failure

Hypoglycemia	(10%) dextrose continuous infusion Bolus (50%) dextrose solution	
Encephalopathy	Lactulose per NG enemas Neomycin/metronidazole/polymycin B GI therapy + branched chain amino acids	prostaglandins plasma exchange
	Rule out sepsis, GI bleeding	hypoxia, drug effects hypoglycemia, acid-base imbalance
Cerebral edema	Restrict fluids, Avoid patient stimulation Mannitol bolus Consider intracranial pressure	monitoring, thiopental infusion
Hypotension	Consider GI bleed/hypovolemia/septic shock Optimize cardiac filling pressure Dopamine ± norepinephrine infusion	
Hypoxia	Endotracheal intubation, Mechanical ventilation	
Sepsis	Broad-spectrum antibiotics, Consider fungal sepsis	

NG: Nanograms, GI: Glycemic Index.

Table 6 Criteria for predicting death and the need for liver transplantation at King's College Hospital, London[1]

Cause of ALF	Criteria
Acetaminophen poisoning	pH < 7.3 (irrespective of grade of encephalopathy) or Prothrombin time > 100 s and serum creatinine > 300 μmol/L (3.4 mg/dL) in patients with grade III or IV encephalopathy
All other causes	Prothrombin time > 100 s (irrespective of grade of encephalopathy) Any three of the following variables (irrespective of grade of encephalopathy): age < 10 yr or > 40 yr; liver failure caused by non-A, non-B hepatitis, halothane-induced hepatitis, or idiosyncratic drug reactions; duration of jaundice before onset of encephalopathy > 7 d; prothrombin time > 50 s; serum bilirubin > 300 mmol/L (17.5 mg/dL)

[1]Transplantation was considered if the likelihood of survival was less than 20%. ALF: Acute liver failure.

conducting toxicological screening. The patient's mental status, blood pressure, and urine output should be monitored carefully. H_2-receptor blockers should be routinely given to prevent stress ulcers and hemorrhage. Pulmonary artery monitoring is helpful for the management of intravascular volume and optimal oxygenation. Conventional pressor treatment for shock with dobutamine or dopamine is relatively ineffective, and may further impair peripheral oxygen delivery.

The appropriateness of liver transplantation should be soon assessed, since transfer to a specialized center is best accomplished when the patient has grade I or II encephalopathy. Evidence of cerebral edema, bleeding, infection, or changes in blood pressure or oxygenation should prompt aggressive treatment. For signs of cerebral edema, 100-200 mL of 200 mL/L mannitol (0.3-0.4 g/kg) should be given by rapid intravenous infusion and may be repeated at least once after several hours. Head-up tilting of up to 45 degrees is probably deleterious, since cerebral perfusion pressure diminishes with elevations above 20 degrees.

Crystalloid or colloid solutions should be infused to maintain blood pressure, but pulmonary capillary wedge pressures of over 1.60 kPa (12 mmHg) should be avoided to minimize the risk of precipitating cerebral edema. Endotracheal intubation and mechanical ventilation will provide protection of the airway from aspiration. Patients should be monitored with continuous pulse oximetry, and hypoxia should be treated with supplemental oxygen. Regular microbial surveillance of urine, sputum, blood and, if present, ascites and open wounds should be carried out. Sites of invasive catheter insertion should be examined regularly. Early broad-spectrum antibiotic therapy is indicated at the first suggestion of sepsis. In patients with a markedly elevated white blood cell count and pyrexia unresponsive to antibiotic therapy, antifungal therapy should be initiated with amphotericin B.

Patient survival is dependent on the rapid institution of comprehensive and aggressive medical care. If the multisystem failure associated with ALF can be successfully managed, liver regeneration and patient recovery may occur. The optimal management of patients with ALF may require the services of multiple specialties including surgery, intensive care, respiratory care, gastroenterology, hematology, nephrology, and psychiatry. ALF-related problems and their management are summarized in Table 5.

Management of acetaminophen overdose has focused on preventing glutathione depletion by administration of acetylcysteine as soon as possible. However, acetylcysteine given as late as 36 h after toxic ingestion can be beneficial. The lethal dose for acetaminophen is 10-20 g. Lesser amounts of acetaminophen may cause hepatotoxicity in patients taking medications known to induce the cytochrome P450 system, such as barbiturates, and in alcoholics. Thus, in these patients the plasma acetaminophen concentration threshold for acetylcysteine treatment should be lowered by 70%.

Liver transplantation

Several centers have published their results of orthotopic liver transplantation for patients with ALF. A total of 69 patients transplanted for ALF experienced a survival rate of 65%. In 42 patients who underwent orthotopic liver transplantation for ALF, stage IV coma, bleeding, and renal failure were defined as significant risk factors for mortality. Sepsis, uncorrectable metabolic acidosis, and hemodynamic instability requiring high-dose vasopressor therapy also appeared to be significant risk factors. Overall survival was 59.4% for 1a and 45.8% for 2a, 3a, and 4a. Contraindications for transplantation include ongoing sepsis, severe chronic cardiorespiratory disease, malignancy, active substance abuse, mental instability, portomesenteric venous thrombosis, and irreversible brain damage caused by cerebral edema.

Potential advantages of heterotopic liver transplantation over orthotopic liver transplantation include avoidance of decreased cardiac output and cerebral perfusion related to the anhepatic phase, as well as caval cross-clamping, reduced surgical trauma, and decreased operative time. Potential disadvantages of heterotopic transplantation include a lack of adequate room for the graft in the abdominal cavity, technical difficulties with graft vascularization and venous drainage, and functional competition with the native liver. Heterotopic liver transplantation may have a future role as a bridging measure in patients too unstable to undergo orthotopic liver transplantation; moreover, once regeneration of the damaged native liver occurs, the patients will be spared the expense and health risks of chronic life-long immunosuppressive therapy.

In patients with ALF secondary to hepatitis B, a major concern is the recurrence of infection in the allograft. Fortunately, the risks of recurrent infection are much lower in patients with acute fulminant hepatitis B infection, perhaps due to a hyperimmune response to the virus.

Criteria are being developed to determine when transplantation is indicated. O'Grady *et al* received 588 patients with ALF and, using univariate and multivariate analysis, established several criteria that indicated a dismal prognosis without transplantation (Table 6).

Serial recording of sensory-evoked potentials may be very helpful to identify: (1) a subgroup of patients selected for emergency liver transplantation by King's College criteria who may recover spontaneously without transplantation and (2) a subgroup of patients with severe, life-threatening brain dysfunction who should receive liver transplantation despite not fulfilling the King's College criteria.

New treatments

In theory, if liver function can be supported until hepatic regeneration occurs, liver transplantation or death can be avoided. Thus, an active area of investigation is the development of artificial hepatic support devices. A promising area currently being studied is the use of extracorporeal liver assist devices in which blood from patients with ALF is perfused through hollow fiber cartridges containing isolated hepatocytes. Alternate approaches, including

hemodiafiltration combined with plasma exchange, have shown favorable results. Hepatocyte transplantation in ALF has been reviewed. The potential problems with this procedure include the need to transplant an adequate number of hepatocytes to reverse ALF and the need for a system that will provide prompt engraftment and functioning of the hepatocytes. The implications of hepatocyte culture systems for artificial liver support, and a summary of previous studies using these techniques, have been reviewed. Extracorporeal hybrid designs containing hepatocyte culture systems capable of treating ALF may become a reality in the future.

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Expression of Span-21 and Ypan-21 in gastric cancer and subtypes of intestinal metaplasia

Dian-Chun Fang, Wei-Wen Liu

Dian-Chun Fang, Professor of Internal Medicine, having 75 papers published. Department of Gastroenterology Southwest Hospital, Chongqing 630038, China

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Telephone: +86-811-5318301

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Abstract

AIM: To analyze the relationship between intestinal metaplasia (IM) and gastric cancer (GC).

METHODS: The expression of the Span-1 and Ypan-1 antigens in GC (n = 110) and IM (n = 343) specimens was examined using the ABC immunohistochemical technique.

RESULTS: The expression rates of Span-1 and Ypan-1 in well and moderately differentiated adenocarcinoma (85.4% and 70.0%, respectively), signet-ring cell carcinoma (80.0%, 88.7%) and mucinous adenocarcinoma (88.6%, 76.5%) were significantly higher than the rates in poorly differentiated adenocarcinoma (48.6%, 45.9%), whereas the difference between early GC (59.2%, 65.4%) and advanced GC (73.8%, 65.5%) was insignificant. For IM, the expression of Span-1 was significantly higher in dysplasia, IM with GC, and chronic atrophic gastritis than in chronic superficial gastritis. In contrast, the expression of Ypan-1 was significantly higher only in IM with dysplasia (65.5%) than in chronic superficial gastritis (39.3%). When IM was classified into types I, II and III, the expression of both antigens in type III (79.0%, 75.2%) was higher than in type I (42.3%, 45.5%) and type II (51.2%, 50.0%), which themselves were similar.

CONCLUSION: Span-1 and Ypan-1 may be of value in detecting GC, even in the early stage, and type III IM should be considered precancerous.

Key words: Stomach neoplasms; Intestinal metaplasia; Antigens; Tumor-associated carbohydrate

Fang DC, Liu WW. Expression of Span-21 and Ypan-21 in gastric cancer and subtypes of intestinal metaplasia. *World J Gastroenterol* 1995; 1(1): 9-12 Available from: URL: http://www.wjgnet.com/1007-9327/full/v1/i1/9.htm DOI: http://dx.doi.org/10.3748/wjg.v1.i1.9

INTRODUCTION

The relationship between intestinal metaplasia (IM) and gastric cancer (GC) is still an open question. Some[1,2] but not all[3] authors consider type III IM to be precancerous, as the columnar cells contain sulfomucin. We have previously shown that both GC and type III IM could be significantly stained by MG7, a monoclonal antibody highly specific for GC, which might prove to be a useful tumor marker for GC and its precursor cells[4]. Recently, two new tumor markers for pancreatic cancer, Span-1 and Ypan-1, have been found to be expressed in GC as well[5,6]. It is worthwhile to assess the expression of both Span-1 and Ypan-1 in GC and different types of IM so as to evaluate the usefulness of these monoclonal antibodies in the early diagnosis of GC and to clarify the relationship between IM and GC.

MATERIALS AND METHODS

Histological specimens

Specimens of GC (n = 110, including 26 early and 84 advanced) were obtained from surgically resected specimens. The specimens contained well and moderately differentiated adenocarcinoma (WMDAC, n = 41, including papillary AC and tubular AC), poorly differentiated adenocarcinoma (PDAC, n = 37), signet-ring cell carcinoma (SRCC, n = 15) and mucinous adenocarcinoma (MAC, n = 17). Specimens of IM (n = 343) were obtained through endoscopical biopsy from the antrum (n = 292), body (n = 38) and fundus (n = 13) of the stomach. The specimens were divided into 5 groups according to their accompanying lesions: dysplasia (n = 32), GC (n = 79, within 2 cm from the margin of cancer), chronic atrophic gastritis (CAG, n = 173), gastric ulcer (GU, n = 31) and chronic superficial gastritis (CSG, n = 28). All the IM specimens were classified as mild (n = 107), moderate (n = 152) and severe (n = 84) according to the criteria defined by The National Cooperative Research Group for GC in 1980. Specimens of apparently normal gastric mucosa (n = 20, obtained through biopsy), intestinal mucosa (n = 15, obtained from resected specimens) and colonic mucosa (n = 20, obtained through biopsy) were included in the study. Specimens of fetal gastric (n = 10), intestinal (n = 10) and colonic (n = 10) mucosa obtained from 16-24-week-old fetuses after water bag absorption were also included.

Antibodies and reagents

Murine monoclonal antibodies specific for Span-1 and Ypan-1, obtained from Kim (GI research Lab, Veterans Administration

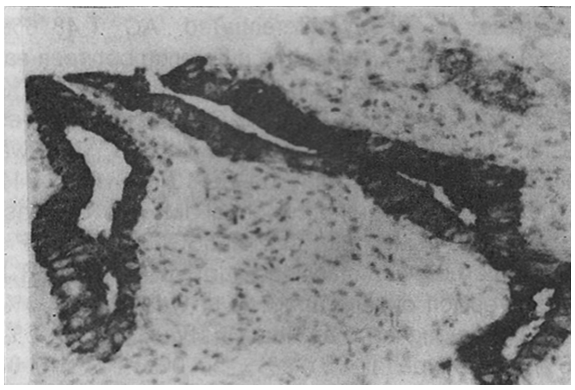

Figure 1 Span-1 immunohistochemical staining in gastric cancer. Magnification × 400.

Table 1 Expression of Span-1 and Ypan-1 in different types of gastric cancer

Histological type	*n*	Span-1 (%)	Ypan-1 (%)
WMDAC	41	35 (85.4)	29 (70.7)
PDAC	37	12 (48.6)[b]	17 (45.9)
SRCC	15	12 (80.0)	13 (86.7)
MAC	17	15 (88.2)	13 (76.5)

[b]$P < 0.01$, PDAC *vs* WMDAC, SRCC or MAC. WMDAC: Well and moderately differentiated adenocarcinoma; PDAC: Poorly differentiated adenocarcinoma; SRCC: Signet-ring cell carcinoma; MAC: Mucinous adenocarcinoma.

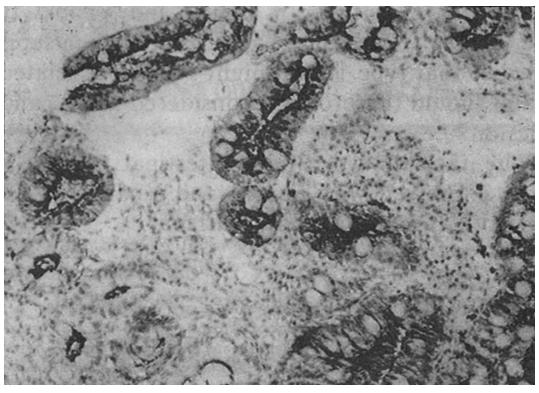

Figure 2 Span-1 immunohistochemical staining in intestinal metaplasia. Magnification × 200.

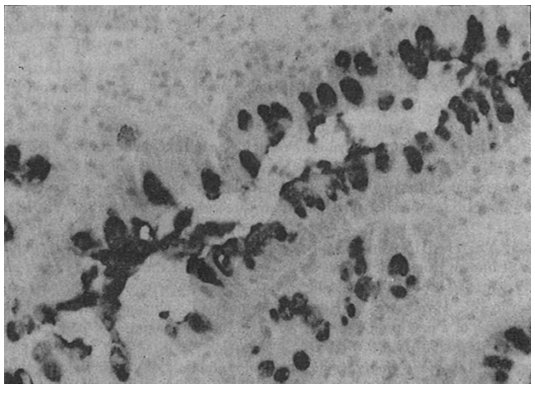

Figure 3 Ypan-1 immunohistochemical staining in intestinal metaplasia. Magnification × 400.

Medical Center, University of California), were produced against the human pancreatic cancer cell lines SW 1990 and Capan-2, respectively. The Vectastain ABC kit and biotinylated anti-mouse IgG were from Vector Laboratories, Inc. Reagents for histochemical mucin staining, including Alcian blue pH 2-5/PAS to visualize neutral and acid mucin, and high iron diamine to identify sulfomucin, were provided by The 3rd Shanghai Reagent Factory.

Histochemical mucin staining

Specimens were fixed in 10% formalin and embedded in paraffin. Six serial 5 μm-thick sections of each specimen were prepared: one for hematoxylin/eosin (HE) staining for histological diagnosis, two for mucin staining, and three for ABC immunohistochemical staining. IM specimens were stained with AB pH 2-5/PAS to visualize neutral mucin and sialomucin, and a high iron diamine/AB pH 2-5 stain was used to detect sulfomucin and sialomucin. IM specimens were classified into three types according to the results of the mucin and HE stains, as suggested by Filipe[7]: Type Ⅰ (complete): Characterized by mature absorptive and goblet cells, the latter secreting sialomucin; Paneth cells were often present. Type Ⅱ (incomplete): Absorptive cells were few or absent; Columnar "intermediate" cells were present in various stages of differentiation, secreting neutral and sialomucin; Goblet cells were secreting mainly sialomucin and occasionally sulfomucin; Paneth cells might not appear. Type Ⅲ (incomplete): Cell de-differentiation was more obvious than in type Ⅱ IM specimens; "intermediate cells" were secreting predominantly sulfomucin; Goblet cells were secreting sulfomucin or sialomucin; Paneth cells were hardly seen. There was a variable degree of disorganized glandular architecture frequently present in type Ⅱ and Ⅲ IM specimens.

Immunohistochemical staining

Span-1 and Ypan-1 mAbs were used at a dilution of 1:40, and the secondary antibody (biotinylated horse anti-mouse IgG) was used at a dilution of 1:100. A section of well-differentiated cancer known to be strongly positive for Span-1 and Ypan-1 was included in each assay as a positive control. As a negative control, the mAbs were replaced by phosphate buffer; none of the negative control sections showed staining.

Statistical analysis

Chi Square contingency tables were used for statistical analysis, and Fisher's exact test was used for small numbers. A P value < 0.05 was considered significant.

RESULTS

Expression of both antigens in GC

Eighty (72.7%) and 72 (65.5%) of 110 specimens of GC were positively stained for Span-1 and Ypan-1, respectively. The staining characteristics of both antigens in GC cells were essentially identical, appearing diffusely distributed in the cytoplasm. In some well-differentiated adenocarcinoma cells the antigenic material was found over the luminal surface of, or inside, the glandular cavities (Figure 1). The positive rates of Span-1 and Ypan-1 were 69.2% (18/26) and 65.4% (17/26), respectively, in the early GC specimens and 73.8% (62/84) and 65.5% (55/84) in the advanced GC specimens. The expression rates of both antigens among different types of GC are shown in Table 1.

Expression of both antigens in dysplasia and IM

The Span-1 antibody stained the apical aspect of the epithelial cells; the basal aspect was usually not stained (Figure 2). Ypan-1 staining was found mainly in the mucus of goblet cells (Figure 3). The staining rates are shown in Table 2. The expression rate of Span-1 in the IM with CSG specimens was significantly lower than the rate in the dysplasia specimens and the IM with CAG specimens. The differences in Span-1 staining among the dysplasia, IM with GC, IM with CAG and IM with GU specimens were not significant. The only significant difference in Ypan-1 staining was a higher rate in dysplasia than in IM with CSG.

Expression of both antigens in different subtypes of IM

When the IM specimens were classified into types Ⅰ (n = 156), Ⅱ (n = 82) and Ⅲ (n = 105), based on mucin and histological parameters, the expression rates of both antigens in type Ⅲ IM were significantly higher than the rates in types Ⅰ and Ⅱ. The expression rates of both Span-1 and Ypan-1 were similar between IM types Ⅰ and Ⅱ (Table 3).

Expression of both antigens in different grades of IM

The expression of both Span-1 and Ypan-1 in different grades of IM is shown in Table 4. There were significant differences in Span-1 expression between the mild, moderate and severe grades of IM. In addition, the Ypan-1 expression rates in severe and moderate IM were significantly higher than the rate in mild IM.

Table 2 Expression of Span-1 and Ypan-1 in dysplasia and intestinal metaplasia

Lesions		*n*	Span-1 (%)	Ypan-1 (%)
Ⅰ	Dysplasia	32	20 (62.5)	21 (65.6)
Ⅱ	IM with GC	79	44 (55.7)	41 (51.9)
Ⅲ	IM with CAG	173	103 (59.5)	101 (58.4)
Ⅳ	IM with GU	31	17 (54.8)	17 (54.8)
Ⅴ	IM with CSG	28	7 (26.5)[b]	11 (39.3)[a]

[b]$P < 0.01$, Span-1, Lesion Ⅴ *vs* Ⅰ, Ⅱ or Ⅲ; [a]$P < 0.05$, Ypan-1, Lesion Ⅴ *vs* Ⅰ. GC: Gastric cancer; IM: Intestinal metaplasia; CAG: Chronic atrophic gastritis; GU: Gastric ulcer; CSG: Chronic superficial gastritis.

Table 3 Expression of Span-1 and Ypan-1 in different types of intestinal metaplasia

Type	*n*	Span-1 (%)	Ypan-1 (%)
Type Ⅰ	156	66 (42.3)	71 (45.5)
Type Ⅱ	82	42 (51.2)	41 (50.0)
Type Ⅲ	105	83 (79.0)[b]	79 (75.2)[b]

[b]$P < 0.01$, Span-1 and Ypan-1, Type Ⅲ *vs* Type Ⅰ or Ⅱ.

Table 4 Expression of Span-1 and Ypan-1 in different grades of intestinal metaplasia

Grade	*n*	Span-1 (%)	Ypan-1 (%)
Mild	107	41 (38.3)	45 (42.1)[b]
Moderate	152	87 (57.2)[a]	88 (57.9)
Severe	84	63 (75.0)	58 (69.0)

[a]$P < 0.05$, Span-1, moderate *vs* mild or severe; [b]$P < 0.01$, Ypan-1, mild *vs* moderate or severe.

Table 5 Expression of Span-1 and Ypan-1 in normal adult and fetal Gastrointestinal mucosa

Mucosa	*n*	Span-1 (%)	Ypan-1 (%)
Normal adult			
Gastric	20	0	0
Intestinal	15	0	1 (6.7)
Colonic	20	0	4 (20.0)
Fetal			
Gastric	10	0	0
Intestinal	10	6 (60.0)	9 (90.0)
Colonic	10	0	2 (20.0)

Expression of both antigens in normal adult and fetal gastrointestinal mucosa

The expression of both Span-1 and Ypan-1 in normal adult and fetal gastric, intestinal and colonic mucosa was essentially negative or occasionally weak, except for fetal intestinal mucosa which was strongly stained (90.0%, 60.0%) for both antigens (Table 5).

DISCUSSION

Span-1 is a high molecular weight glycoprotein recognized by a mAb produced against the human pancreatic cell line SW 1990[8]. Preliminary studies revealed a high sensitivity (81.3%-90.0%) of the mAb for human pancreatic cancers[9]. Recent immunohistochemical studies using the Span-1 mAb showed 89%, 67% and 62% positive staining of cancer specimens from the pancreas, stomach and colon, respectively[5]. Similar to Span-1, Ypan-1 is recognized by a murine mAb produced against the human pancreatic cancer cell line Capan-2. The Ypan-1 mAb showed immunoreactivity with formalin-fixed specimens of pancreatic (89%), gastric (90%), and colonic (46%) cancers[6]. However, a comprehensive survey of the Span-1 and Ypan-1 antigens in GC and its precursors has not been performed.

Our data firmly showed that both Span-1 and Ypan-1 are highly expressed in the different types of GC, except for PDAC which had a significantly lower expression rate. Both SRCC and MAC expressed the antigens to the same extent as WMDAC.

It is interesting that early GC lesions expressed as much Span-1 and Ypan-1 as did the advanced lesions, and that a considerable percentage of premalignant lesions such as dysplasia and IM also strongly expressed the antigens. Normal adult gastric mucosa was entirely negative for the immunoreactivity of both antigens. These facts lead us to believe that both Span-1 and Ypan-1 might be useful for the detection of early lesions of GC and its precursors.

The relationship between IM and GC has not been fully explored. Our previous work has repeatedly shown that IM with abundant sulfomucin in the columnar cells (type Ⅲ) shares many biological characteristics with GC and thus should be treated as a precancerous lesion. In the present study, we found that, like dysplasia, those IMs which were adjacent to GC or accompanied with CAG were considerably immunoreactive for the Span-1 mAb. Moreover, type Ⅲ IM expressed significantly more Span-1 and Ypan-1 than did IM types Ⅰ and Ⅱ. These data further support the possibility that type Ⅲ IM is closely related to GC and should therefore be considered as a precancerous lesion.

The fact that fetal intestinal mucosa strongly expresses Span-1 and Ypan-1 indicates the oncofetal nature of both antigens.

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EXPERIMENTAL PAPERS

Immunohistochemical study of lactate dehydrogenase isoenzymes in gastric cancer

Yu-Lin Song, Guang-Lin Yang, Yu-Ming Dong

Yu-Lin Song, Department of Pathology, Anhui 21 Medical University, Meishanlu, Hefei 230032, Anhui Province, China

Yu-Lin Song, Ms in Pathology, having 4 papers published. Department of Pathology, Anhui 21 Medical University, Meishanlu, Hefei 230032, Anhui Province, China

Guang-Lin Yang, Yu-Ming Dong, Department of Pathology, Anhui Medical University

Author contributions: All authors contributed equally to the work.

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Correspondence to: Dr. Yu-Lin Song, Department of Pathology, Anhui 21 Medical University, Meishanlu, Hefei 230032, Anhui Province, China
Telephone: +86-551-3633411-2105

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Abstract

AIM: To study the metabolic features of gastric cancer cells, the relationship between intestinal metaplasia or dysplasia and gastric cancer, and the relationship between LDH isoenzymes and the biological behavior of gastric cancer.

METHODS: The content and distribution of LDH isoenzymes LDH-5 and LDH-H in 60 cases of gastric cancer were examined by immunohistochemistry and immunoelectron microscopy.

RESULTS: The content of LDH-5 was significantly higher in gastric cancer cells than in chief cells, surface epithelium and pyloric glandular epithelium, but was similar between gastric cancer cells and parietal cells. The content of LDH-H was significantly lower in gastric cancer cells than in parietal cells, but was similar between gastric cancer cells, chief cells and surface epithelium. The product of LDH-5 but not LDH-H was mainly distributed in the cytoplasmic matrix in the cancer cells. The content of LDH-5 in intestinal metaplasia and dysplasia was significantly higher than in the pyloric glandular epithelium, but was similar to the content in gastric cancer cells. The content of LDH-H was significantly higher in intestinal metaplasia and dysplasia than in both the pyloric glandular epithelium and gastric cancer cells.

CONCLUSION: Increased LDH in gastric cancer cells resulted mainly from increased levels of LDH-5. The LDH-5-mediated elevation in lactate levels could decrease the pH, thereby activating acid hydrolase and indirectly promoting the invasion and spread of gastric cancer cells. Intestinal metaplasia and dysplasia might be an intermediate phase between normal gastric mucosa and gastric cancer.

Key words: Gastric neoplasms; Oxidoreductase; Microscopy, electron; Immunohistochemistry

Song YL, Yang GL, Dong YM. Immunohistochemical study of lactate dehydrogenase isoenzymes in gastric cancer. *World J Gastroenterol* 1995; 1(1): 13-17 Available from: URL: http://www.wjgnet.com/1007-9327/full/v1/i1/13.htm DOI: http://dx.doi.org/10.3748/wjg.v1.i1.13

INTRODUCTION

Studies of lactate dehydrogenase (LDH) isoenzymes in gastric cancer tissues have generally been carried out using biochemical methods[1-3]. Immunohistochemical studies, which lack systematization, have scarcely been reported in the literature[4]. In the present study, the content and distribution of LDH isoenzymes LDH-5 and LDH-H in normal gastric mucosa, intestinal metaplasia, dysplasia and various kinds of gastric cancer were analyzed using immunohistochemistry (IHC) and immunoelectron microscopy. The metabolic features of gastric cancer cells and the relationship between LDH isoenzyme content and the biological behavior of gastric cancer are discussed.

MATERIALS AND METHODS

Light microscopy

Surgically resected specimens from 60 cases of gastric cancer (including 4 cases of early cancer), as well as normal mucosa > 2 cm from the tumor and transitional tissue between the tumor and normal mucosa, were fixed in 10% neutral-buffered formalin. After embedding the tissues in paraffin wax, 5 μm-thick serial sections were cut and stained by hematoxylin and eosin (H&E) or IHC. Antibodies specific for LDH-5 and LDH-H (both from Sigma, St. Louis, MO, United States) were diluted 1:100 and 1:25, respectively, and detected with an ABC kit from Vector Laboratories (Burlingame, CA, United States). Both negative and positive controls were used for all cases. Negative control slides were included by substituting the primary antibody with PBS, and skeletal muscle and myocardium were used as positive control tissues for LDH-5 and LDH-H, respectively.

Table 1 Staining intensity of LDH-5 and LDH-H in normal body mucosa and gastric body carcinoma

Grade	Surface epithelium		Parietal cells		Chief cells		Cancer cells	
	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]
-	16	10			7	2		
±							3	7
+	10	6	7	1	20	14	7	4
++	1		15	6			10	5
+++			5	9			7	
Total	27	16	27	16	27	16	27	16

[a]$P < 0.005$, [b]$P < 0.005$. LDH: Lactate dehydrogenase.

Table 2 Staining intensity of LDH-5 and LDH-H in normal antral mucosa and gastric antral carcinoma

Grade	Surface epithelium		Pyloric glandular epithelium		Cancer cells	
	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]
-	7	8	6	8		2
±					2	10
+	21	7	25	7	13	3
++	5		2		10	
+++					8	
Total	33	15	33	15	33	15

[a]$P < 0.005$, [b]$P > 0.75$. LDH: Lactate dehydrogenase.

Table 3 Staining intensity of LDH-5 and LDH-H in normal antral mucosa, intestinal metaplasia and antral carcinoma

Grade	Glandular epithelium		Intestinal metaplastic epithelium		Cancer cells	
	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]
-	2	5				1
±				1	1	6
+	10	4	7	4	6	2
++			4	4	4	
+++			1		1	
Total	12	9	12	9	12	9

[a]$P < 0.025$, [b]$P < 0.01$. LDH: Lactate dehydrogenase.

Table 4 Staining intensity of LDH-5 and LDH-H in normal antral mucosa, dysplasia and antral carcinoma

Grade	Glandular epithelium		Dysplastic epithelium		Antral cancer cells	
	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]	LDH-5[a]	LDH-H[b]
-	2	5			1	2
±				1	2	7
+	13	5	9	5	6	1
++			5	5	3	
+++			1		4	
Total	15	10	15	10	15	10

[a]$P < 0.025$, [b]$P < 0.025$. LDH: Lactate dehydrogenase.

Electron microscopy

Specimens from nine of the 60 cases of gastric cancer were cut in ultrathin sections using a pre-embedding immunoelectron microscopic technique in our laboratory[5].

Grading of staining patterns

Referring to Berry's grading[6], the IHC staining patterns were scored according to the relative intensity and extent of immunoperoxidase staining. The intensity of staining was scored as negative (0), weak yellow (1), yellow-brown (2) and dark brown (3). The extent of staining was scored as negative (0), less than one-third of the cells stained (1), one-third to two-thirds of the cells stained (2), and more than two-thirds of the cells stained (3). Then the scores were added and the cases were divided into five grades: no points/not stained at all (-), 2-3 points (±), 4 points (+), 5 points (+ +) and 6 points (+++). Grades (-) and (±) were regarded as negative staining, and grades (+) to (+++) as positive staining.

Statistical analysis

The IHC grades from the tissues were compared by Rank sum test, and the rates of staining were compared by Chi square test. $P < 0.05$ was considered significant.

RESULTS

The content and distribution of LDH-5 and LDH-H in normal gastric mucosa, precancerous lesions and gastric cancer cells are shown in Tables 1-4.

Normal gastric epithelium

The content and distribution of LDH-5 and LDH-H in the normal gastric body and antral epithelium (including surface epithelium, parietal cells, chief cells and pyloric glandular epithelium) were mainly observed in the present study. Staining in parietal cells was diffusely distributed in the cytoplasm, whereas staining in chief cells was confined to the supranuclear cytoplasm. The staining intensity and patterns of LDH-5 and LDH-H varied among the cell types. Parietal cells and chief cells were dominated by LDH-H, whereas surface epithelium and pyloric glandular epithelium were dominated by LDH-5. Parietal cells showed the strongest staining for LDH-H and LDH-5 in normal gastric epithelium. The staining intensity of LDH-H and LDH-5 in the surface epithelium and pyloric glandular epithelium were not significantly different. The positive product of LDH-5 and LDH-H in parietal cells, being mostly granular, was located in the cytoplasmic matrix around the mitochondria and on the microvilli of

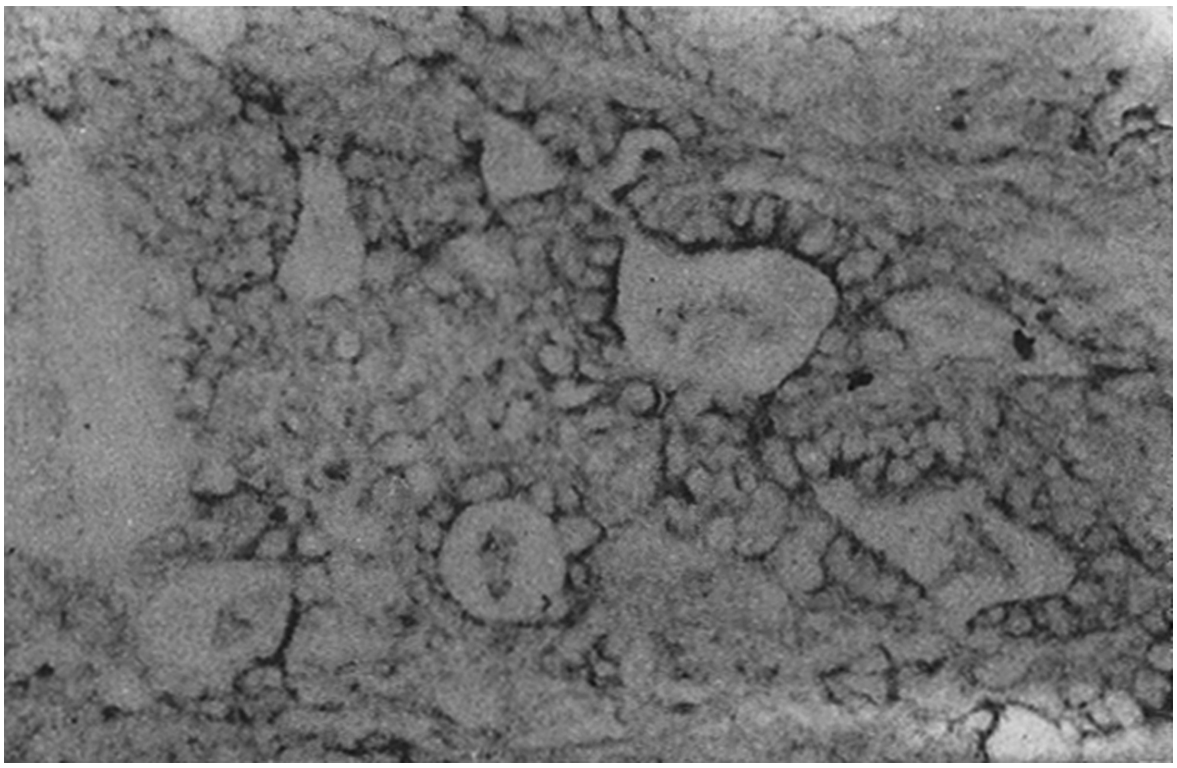

Figure 1 Tubular adenocarcinoma. LDH-5 staining is located primarily at the luminal side of the cancer cells and partly in the cytoplasm (magnification × 200). LDH: Lactate dehydrogenase.

intracellular canaliculi. In contrast, the positive product of LDH-5 and LDH-H in chief cells was located in the cytoplasmic matrix around the zymogen granules and endoplasmic reticulum, and sometimes on the membrane of endoplasmic reticulum.

Intestinal metaplastic epithelium

Intestinal metaplastic epithelium showed moderately strong staining for LDH-5 and LDH-H, whereas pyloric glandular epithelium showed weaker staining for both isoenzymes. The intestinal metaplastic epithelium was dominated by LDH-5. The staining product was located in the supranuclear cytoplasm of absorptive cells and in the cytoplasm around mucin of goblet cells.

Dysplastic epithelium

Dysplastic epithelium also showed moderately strong staining for LDH-H and LDH-5, and pyloric glandular epithelium also showed weaker staining for both isoenzymes. The dysplastic epithelium was dominated by LDH-5. The staining product was primarily located in the supranuclear cytoplasm of dysplastic epithelium.

Gastric cancer cells

The staining intensity of LDH-5 was stronger in gastric cancer cells than in surface epithelium, pyloric glandular epithelium and chief cells. There was no significant difference in LDH-5 staining between gastric cancer cells and parietal cells, intestinal metaplastic epithelium and dysplastic epithelium. The staining intensity of LDH-H was weaker in gastric cancer cells than in intestinal metaplastic epithelium, dysplastic epithelium and parietal cells. There was no significant difference in LDH-H staining between gastric cancer cells and surface epithelium, pyloric glandular epithelium and chief cells. Staining in cancer cells was dominated by LDH-5. The rate of LDH-5 and LDH-H staining in 27 cases of gastric body cancer was 88.9% (24/27) and 56.3% (9/16), respectively, whereas the rate of LDH-5 and LDH-H staining in 33 cases of gastric antral cancer was 93.9% (31/33) and 20.0% (3/15), respectively . No significant difference existed between the rates of staining in body cancer cells and antral cancer cells. Although the relative intensities of the two antibodies varied, LDH-5 and LDH-H generally exhibited the same staining patterns. The mAbs stained well-differentiated gastric adenocarcinoma primarily in the luminal aspect or in the apical cytoplasm of cancerous glands, and in the cytoplasm of some cancer cells (Figure 1). In poorly differentiated adenocarcinoma, the staining was mostly distributed in the cytoplasm (Figure 2). In signet-ring cell carcinoma the staining was distributed in a ring round the cell membrane. In addition, the heterogeneity of LDH-5 and LDH-H in gastric cancer cells was apparent both in the relative intensity of staining and the distribution of staining, *i.e.*, different intensities of staining and different types of distribution (polar and diffuse) were seen in different areas of the same section. By electron microscopy, the positive product of LDH-5 in cancer cells of tubular adenocarcinoma appeared as coarse, large granules distributed in the cytoplasmic matrix primarily around mitochondria and the endoplasmic reticulum (Figure 3) and partly near the cell membrane and on the microvilli. In contrast, the positive product of LDH-5 in poorly differentiated adenocarcinoma appeared as fine, small granules diffusely distributed in the cytoplasmic matrix. The positive product of LDH-H in cancer cells was distributed less on the membrane of the endoplasmic reticulum and in the cytoplasmic matrix around it. The positive product of LDH-5 in cancer cells showed stronger electron intensity than did the positive product of LDH-H.

Relationship of LDH staining to the biological behavior of gastric cancer

There was no significant correlation between the positive rate of LDH-5 and LDH-H staining in gastric cancer and the cancer's histological types, degrees of differentiation, size, clinical stages, manner of growth and lymph node metastases.

DISCUSSION

LDH is an important oxidoreductase in glycolysis, catalyzing the transition between pyruvate and lactate. LDH is actually a group of enzymes that are tetramers composed of two types of subunits (H and M). Differences in the proportions of M and H subunits in LDH can yield five types of isoenzymes (LDH-1 through LDH-5). Since the LDH isoenzymes possess different molecule constitutions, functional differences exist among them to some extent. LDH-5 easily converts pyruvate into lactate, which benefits anaerobic metabolism, whereas LDH-1 converts lactate into pyruvate, which can be oxidized in the citric acid cycle and thus benefits aerobic metabolism. In the present study, antibodies against LDH-5 and the H subunit (present in LDH-1 through LDH-4) were used in an IHC survey. We found that the LDH isoenzyme patterns varied with different kinds of cells in normal gastric epithelium, consistent with a previous study[4]. LDH-H dominated LDH-5 in parietal cells and chief cells, but LDH-5 dominated LDH-H in surface epithelium and pyloric glandular epithelium. Parietal cells showed the strongest staining

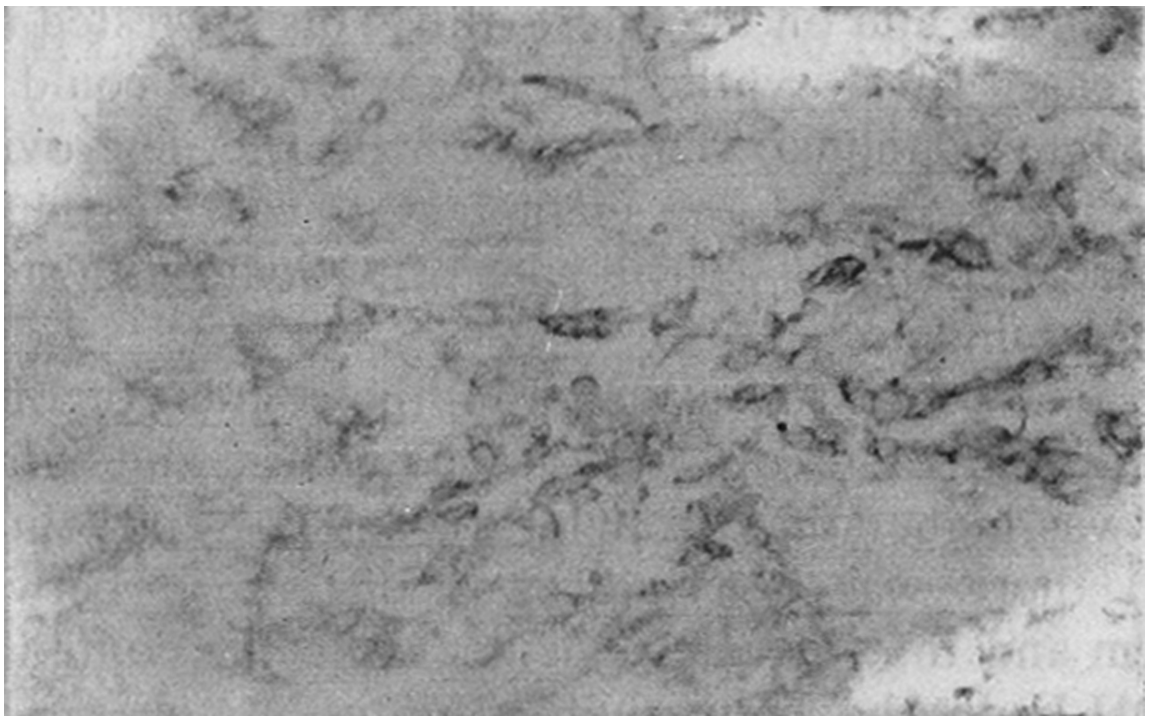

Figure 2 Poorly differentiated adenocarcinoma. LDH-5 staining is distributed in the cytoplasm (magnification × 200). LDH: Lactate dehydrogenase.

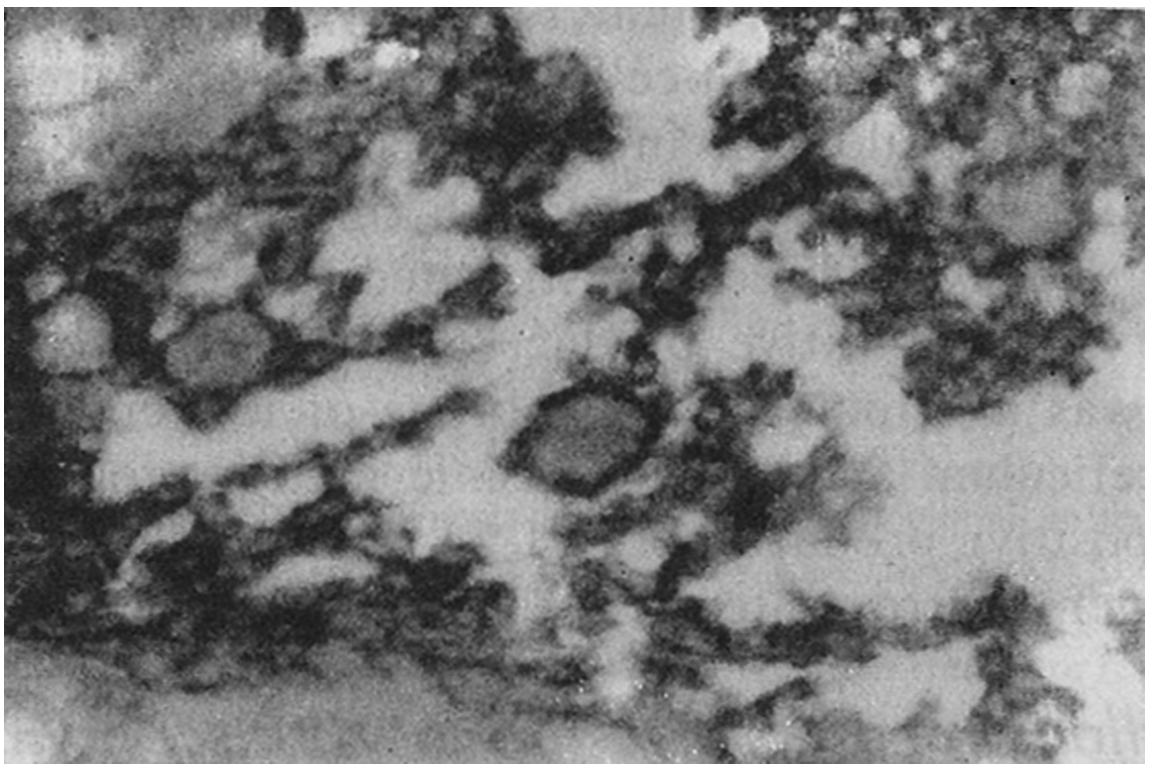

Figure 3 Tubular adenocarcinoma. LDH-5 staining is distributed in the cytoplasmic matrix around mitochondria and the endoplasmic reticulum (magnification × 3000). LDH: Lactate dehydrogenase.

for both LDH-5 and LDH-H, which is not surprising given the cells' active acid secreting activity[7]. The mechanisms of different LDH isoenzymes in various kinds of cells warrant further investigation.

Normal gastric epithelium is composed of many kinds of cells with varying structure and functions, and the histological origin of gastric cancer varies by cell type. It would be inappropriate if normal gastric mucosa was used as control without indicating its corresponding site in the stomach. In this study, we compared gastric cancer cells from different sites with the corresponding normal gastric epithelium in order to elucidate the metabolic features of the gastric cancer cells. We found that the content of LDH-5 was higher in gastric cancer cells than in chief cells, surface epithelium and pyloric glandular epithelium. The content of LDH-H in gastric cancer cells was lower than that in parietal cells and similar to the content in chief cells, surface epithelium and pyloric glandular epithelium. In the cancer cells, LDH-H was dominated by LDH-5. By electron microscopy, the positive product of LDH-5 was found intensely in coarse granules that were distributed in the cytoplasmic matrix around the mitochondria and endoplasmic reticulum. In contrast, the positive product of LDH-H could only be seen diffusely in the cytoplasmic matrix. Therefore, at both the cellular and ultrastructural level, our data indicate that the increased LDH in gastric cancer cells[2,3] is mainly due to a selective increase in LDH-5. This change in the gastric cancer cells would increase glycolysis and thereby rapidly generate energy for proliferation. The positive product of LDH-5 in the cancer cells was also seen in the cytoplasmic matrix near the cell membrane and on the microvilli. This location suggests that the newly-synthesized LDH-5 could enter into the glandular lumen through the microvilli, consistent with the increased levels of LDH-5 found in the gastric juice of patients with stomach carcinoma.

In intestinal metaplasia and dysplasia, the content of LDH-5 was higher in the surface epithelium than in the pyloric glandular epithelium, whereas the content of LDH-H was higher in the epithelium than in both the pyloric glandular epithelium and the cancer cells. LDH-H was dominated by LDH-5. Thus, LDH isoenzymes in intestinal metaplastic epithelium and dysplastic epithelium are similar to, though not the same as, those in cancer

cells. Intestinal metaplasia and dysplasia could be a borderline lesion between gastric cancer and normal gastric mucosa; though increased LDH-5 content might not be tumor-specific, it could be closely related to the proliferative activity of the cancer cells[4]. Our LDH-H results are not consistent with Carda-Abella's biochemical result[2], perhaps due to factors influencing the biochemical result[8] and the use of different counting methods.

Few studies have probed the relationship between gastric cancer cell LDH isoenzyme content and biological behavior[2,3,9]. Our study showed there was no significant correlation between the expression of LDH-5 and LDH-H in gastric cancer cells and their biological behavior. This result might be due to many factors, including the degrees of cellular carcinogenesis, invasion or metastasis. Increased levels of LDH, especially LDH-5, might increase glycolysis in cancer cells, raising lactate concentrations in the tumor and adjacent tissue. The subsequent decrease in pH by acid hydrolase might then promote invasion and metastasis of gastric cancer cells[9].

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Biological effects of types I and III collagens in human hepatocellular carcinoma tissue

Yao-Jun Wang, Zi-Qin Sun, Jun-Ji Yu, Xing-Zhao Xu, Xia Zhang, Qi-Zhen Quan

Yao-Jun Wang, Zi-Qin Sun, Jun-Ji Yu, Xing-Zhao Xu, Xia Zhang, Qi-Zhen Quan, Department of Gastroenterology, General Hospital of Chinese PLA, Jinan Command area 250031, Shandong Province, China

Yao-Jun Wang, Physician in charge, Master of Medicine, having 26 papers published Department of Gastroenterology, General Hospital of Chinese PLA Jinan Command area 25 Shifan Road, Jinan 250031, Shandong Province, China

Author contributions: All authors contributed equally to the work.

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Correspondence to: Dr. Yao-Jun Wang, Department of Gastroenterology, General Hospital of Chinese PLA, Jinan Command area 250031, Shandong Province, China

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Abstract

AIM: To explore the biological effects of type I and III collagens in human hepatocellular carcinoma (HCC) and the relationship between the collagens and tumor behavior.

METHODS: The distribution of types I and III collagens was determined by immunohistochemistry in 25 specimens of human HCC and surrounding liver tissue, as well as six normal liver specimens. In addition, the expression of types I and III collagens were studied by *in situ* hybridization in nine HCC and two normal liver specimens. Collagen content in the tissues was calculated according to the theory of sterology.

RESULTS: The content of types I and III collagens was significantly lower in HCC than in the surrounding liver tissue. In addition, the collagen content was significantly lower in invasive/metastatic HCC tissue than in non-invasive/metastatic HCC tissue. However, collagen gene expression and protein synthesis were increased in HCC tissue.

CONCLUSION: The decrease in collagen content in HCC tissue likely resulted from collagen degradation. Collagens may be inhibitors of tumor invasion and metastasis.

Key words: Liver neoplasms; Immunohistochemistry; Collagen; Sterology; *In situ* hybridization

Wang YJ, Sun ZQ, Yu JJ, Xu XZ, Zhang X, Quan QZ. Biological effects of types I and III collagens in human hepatocellular carcinoma tissue. *World J Gastroenterol* 1995; 1(1): 18-20 Available from: URL: http://www.wjgnet.com/1007-9327/full/v1/i1/18.htm DOI: http://dx.doi.org/10.3748/wjg.v1.i1.18

INTRODUCTION

Types I and III collagens regulate cell proliferation, migration, polarity and differentiation. In recent years, close attention has been paid to the relationships between collagens and tumor behavior[1]. The distribution, cellular origin and gene expression of interstitial collagen in hepatocellular cancer (HCC) tissues have been reported[2,3]. However, a quantitative study of collagen in HCC has not been performed, and the biological function of collagen in HCC is not clear. To explore the relationship between collagens and HCC behavior, a quantitative study of collagen was made according to the theory of sterology, and functions of collagen in HCC are discussed.

MATERIALS AND METHODS

Materials

Specimens of liver tissue were obtained from 25 HCC patients by surgery. In each case, tissue was obtained from three places: the tumor, the surrounding liver tissue (SLT), and the juncture of the cancerous and non-cancerous tissue. In addition, normal liver tissue specimens were obtained from six persons who were killed in accidents. The samples were fixed in 10% buffered formalin and 5 μm-thick paraffin sections were cut and used for H&E staining and immunohistochemistry. Nine of the HCC specimens and 2 of the normal liver specimens were also frozen for *in situ* hybridization. A specific antibody against type I collagen was obtained as a gift from Yamamoto. A rabbit antibody against bovine procollagen III peptide was obtained from the Second Military Medical University. The ABC staining kit was purchased from Vector Laboratories. Recombinant DNA clones for *in situ* hybridization were a gift from Vuorio: PHCAL1 for proα-1 (I) collagen mRNA and PHFS3 for proα-1 (III) collagen mRNA. In the 25 cases of HCC (21 male, 4 female), the average age was 44.3 year (range 28-63 year). The serum AFP level was ≥ 400 μg/L in 10 of the HCC cases, and the serum was positive for HBsAg in 13 of the HCC cases.

Methods

Collagen quantitative study: Localization of type I and III collagens in the tissues was determined by immunohistochemistry[2]. Qa, the number of intersections of collagen and the test line (average of 5 fields per section and 3 sections per specimen), was calculated with the M168 multifunctional coherent test system recommended by Weibel using 400 × magnification. Length density was calculated as $Jv = 2Qa/(Pc \cdot K_2 \cdot d^2)$ and analyzed by Student's *t* test.

In situ hybridization: cDNA probe preparation and *in situ* hybridization procedures in frozen sections were performed as

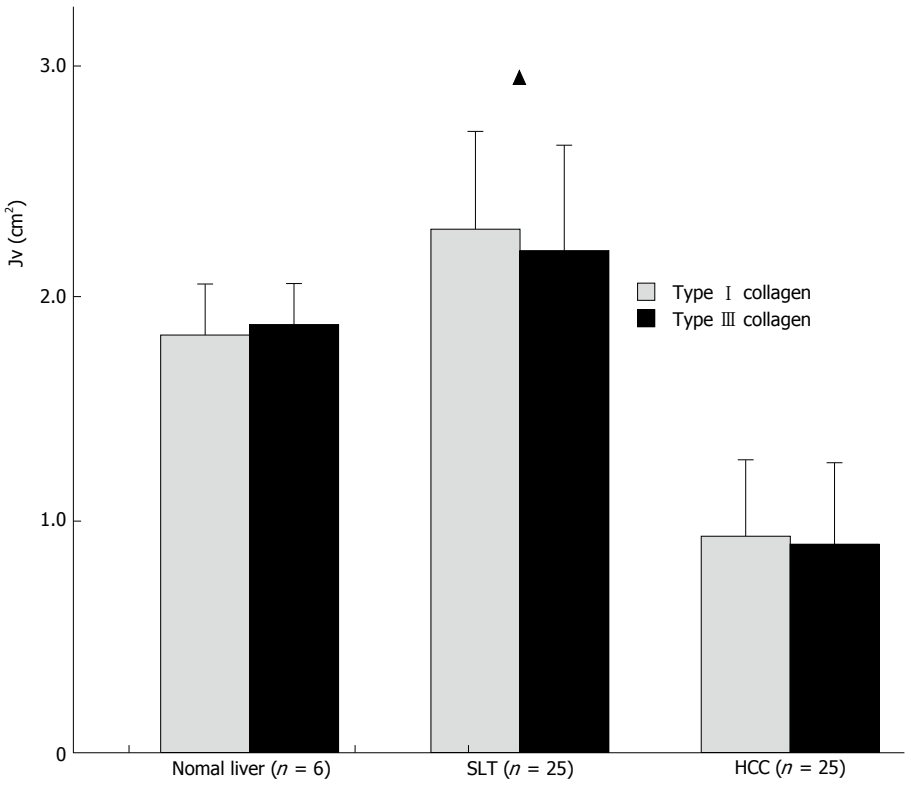

Figure 1 Content of types Ⅰ and Ⅲ collagen in HCC. HCC *vs* normal liver and *vs* SLT, $P < 0.01$; SLT *vs* normal liver, $P < 0.05$. HCC: Hepatocellular carcinoma; SLT: Surrounding liver tissue.

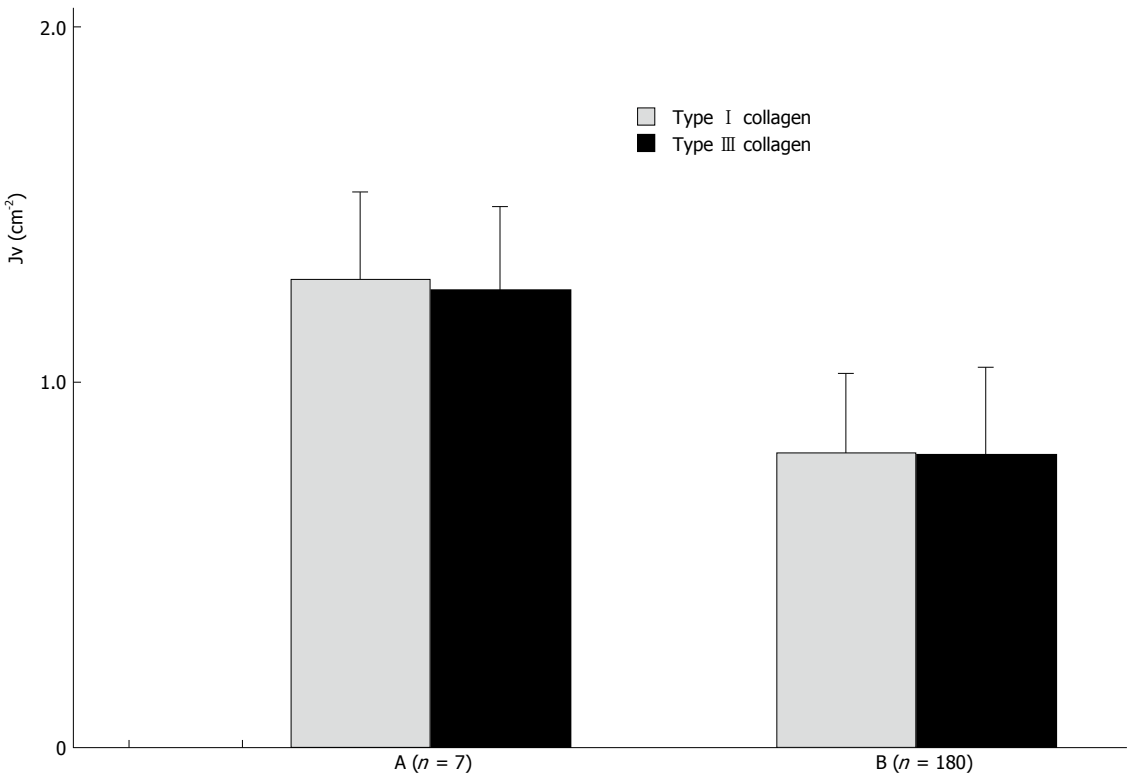

Figure 2 Content of types Ⅰ and Ⅲ collagen in HCC with invasion and/or metastasis (B) and in HCC without invasion and/or metastasis (A). A *vs* B, $P < 0.01$. HCC: Hepatocellular carcinoma.

previously described[3].

RESULTS

Pathological study

Histopathological observation was combined with gross specimen assessment and clinical data. According to Edmonson's grading criteria, of the 25 cases of HCC, one was grade Ⅰ, 11 were grade Ⅱ, 12 were grade Ⅲ and one was grade Ⅳ. Fifteen cases were accompanied by cirrhosis and eight cases were accompanied by chronic hepatitis. Tumors with invasion or/and metastasis were found in 18 cases. An entire envelope was formed in three cases.

Distribution of interstitial collagen

In normal liver tissue, type Ⅰ collagen was distributed in the portal connective tissue, the wall of central veins, and (non-continuously) in the sinusoidal wall. The distribution of type Ⅲ collagen was identical to that of type Ⅰ. In the SLT with chronic liver disease, deposition of types Ⅰ and Ⅲ collagen was found in connective tissue and in areas of inflammation and focal necrosis. Collagen was increased in the sinusoidal walls, which appeared wide and deep on immunohistochemical staining. The two types of collagen also had an identical distribution. However, within the tumor, the collagen content decreased significantly, surrounding the cancer nest and sometimes inside the nest. The tumors with a surrounding envelope had a significantly lower rate of invasion or/and metastasis.

Collagen content in tissues

Collagen content was scaled by the length density of collagen (Jv). Collagen content in the tissues is shown in Figures 1 and 2. There was no significant difference in content between types Ⅰ and Ⅲ collagens.

Collagen gene expression

In normal liver tissue, transcripts for types I and III collagens were not detected by *in situ* hybridization. In four cases of SLT (three with cirrhosis and one with persistent hepatitis), there were some weak signals in rare scattered mesenchymal cells. Interestingly, very intensive signals were found in many tumor cells in six of the nine cases of HCC.

DISCUSSION

According to the theory of sterology, we used the length density of collagen as a parameter for collagen content in HCC. We found that the content of types I and III collagen were much lower in HCC than in normal liver tissue and SLT (Figure 1). However, *in situ* hybridization showed that collagen gene expression is greater in HCC than in non-cancerous tissue. The rate of collagen protein synthesis in HCC has been shown to be increased, as well[4]. Therefore, we believe that the decrease in collagen content in HCC is due to excessive collagen degradation . Figure 2 shows that collagen content is significantly lower in cancer tissue with invasion or/and metastasis. It is possible that collagen degradation is closely linked to the tumor's malignancy. Based on a series of studies, Liotta suggested that tumor cells can produce collagenase and thereby degrade the collagen matrix in HCC and its SLT. The local matrix degradation results in the formation of spatial pathway for invasion and metastasis. Additionally, stimulated by cell adhesive molecules and scatter factors, the tumor cells could scatter. The ability of hepatoma cells to produce collagenases may be an important factor affecting the invasion and metastasis of the tumor. Based on the present study, we believe that collagen degradation might promote tumor invasion and metastasis and that types I and III collagens might prohibit malignant behavior. This viewpoint is supported by the fact that a significantly lower invasive and metastatic tendency was present in tumors with an entire envelope. Collagens may act as a "screen" or perhaps regulate gene expression in the tumor cells[5]. As for the difference in collagen content between SLT and normal liver tissue, we believe the difference is related to the chronic liver disease present in the SLT.

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Loss of heterozygosity at adenomatous polyposis coli, mutation in colorectal cancer and deleted in colorectal cancer genetic loci in colorectal cancers

Dian-Chun Fang, Yuan-Hui Luo, Rong Lu, Wei-Wen Liu, Feng-Xuan Liu, Zhi-Yong Liang

Dian-Chun Fang, Professor of Internal Medicine, Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 630038, China

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

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Abstract

AIM: To evaluate the role and analyze the loss of heterozygosity (LOH) of adenomatous polyposis coli (APC), mutation in colorectal cancer (MCC) and deleted in colorectal cancer (DCC) genes in the development and progression of colorectal cancers.

METHODS: LOH at *APC*, *MCC* and *DCC* genes was examined in 41 surgically resected specimens of colorectal carcinomas by polymerase chain reaction and restriction fragment length polymorphism analysis technique.

RESULTS: LOH of APC and MCC were observed in 7 of 25 (28.0%) and 8 of 22 (36.4%) of informative cases, respectively. When considered as one locus, the LOH frequency for APC/MCC was 14 of 36 (38.9%). LOH at *DCC* gene locus was detected in 21 of 38 (55.3%) of informative cases. No correlation was found between the LOH at *APC* or *MCC* gene and tumor histological types, size, invasion, lymph node metastasis and Dukes' stages ($P > 0.05$). However, LOH rates at DCC locus in the group with lymph-node metastasis (80.0%) and in Dukes' stages III and IV (71.4%) were significantly higher than those without lymph node metastasis (39.1%) and in Dukes' stages I and II (35.3%) ($P < 0.05$).

CONCLUSION: LOH at APC and/or MCC may occur more frequently in the early stages and plays a role in the initiation of colorectal cancer while LOH at DCC is frequent at late event and associated with the progression and metastasis of colorectal cancer.

Key words: Heterozygote detection; *APC* gene; *MCC* gene; *DCC* gene; Colorectal neoplasms

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INTRODUCTION

Inactivation of tumor suppressor genes has been shown to play an important role in the development of a variety of human cancers[1,2]. The mechanisms of inactivation include allelic deletion, chromosome rearrangement, point mutation, and binding of suppressor gene products with viral or cellular inactivating proteins[1-3]. To date, several tumor suppressor genes have been discovered which include, but are not limited, the retinoblastoma susceptibility, p53, Wilm's tumor, neurofibromatosis type I, adenomatous polyposis coli (APC), mutation in colorectal cancer (MCC), and deleted in colorectal cancer (DCC) genes. In this study, the loss of heterozygosity (LOH) at APC, MCC, and DCC genetic loci was further examined and analyzed.

MATERIALS AND METHODS

Tissues and DNA extraction

Matching normal and tumor tissues were obtained at the time of surgery from 41 patients with colorectal carcinoma (11 with colonic carcinoma and 30 with rectal carcinoma). Each specimen was frozen immediately and stored at 80°C until the analysis. A 5-μm section was cut from each tissue and stained with hematoxylin/cosin to ascertain whether the cancer cells in the tissues were predominant or not. Samples containing no cancer cells were considered normal, and those containing > 70% cancer cells were characterized as cancer-cell rich. Genomic DNA extraction was performed as previously described[4].

PCR Amplification

Polymerase chain reaction (PCR) was carried out as described previously[5]: 50 ng to 500 ng of genomic DNA were incubated at 95°C for 5 min in 20 μL buffer containing 10 mmol/L Tris HCl, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 25 pmol/L of each primer, 200 μmol/L concentration of each deoxynucleotide triphosphate, and 2 units of Taq DNA polymerase. Multiple primer sets were used for each loci (Table 1). The priming regions were located within specific tumor suppressor genes of a polymorphic sequence[6]. Either a restriction fragment length polymorphism analysis technique (RFLP)[7] or a variable number of tandem repeats type polymorphism[8] was utilized. PCR was performed under the

Table 1 Primer sets used in polymerase chain reaction-loss of heterozygosity assays

Primer set[1]	Priming region	Amplification size (base pairs)	Polymorphism type	Primer sequence
1	APC exon 11	133	RsaI RFLP	5'-GGACTACAGGCCATTGCAGAA-3' 5'-GGCTACATCTCCAAAAGTCAA-3'
2	MCC exon 10	79 or 93	Insertion	5'-TACGAATCCAATGCCACA-3' 5'-CTGAAGTAGCTCCAAACA-3'
3	DCC	396	MspI RFLP	5'-TTGCACCATGCTGAAGATTGT-3' 5'-ACCCTCCCCCTGATGACTTA-3'
4	DCC	240	MspI RFLP	5'-CGACTCGATCCTACAAAATC-3' 5'-TCTACCCAGGTCTCAGAG-3'
5	DCC	200	VNTR	5'-GATGACATTTTCCCTCTAG-3' 5'-GTGGTTATTGCCTTGAAAAG-3'

[1]Primer sets 1 and 2, Tumura *et al*[6]; Primer sets 3, 4, and 5, Gao *et al*[5]. VNTR: Variable number of tandem repeats; APC: Adenomatous polyposis coli; MCC: Mutation in colorectal cancer; DCC: Deleted in colorectal cancer; RFLP: Restriction fragment length polymorphism analysis technique; RFLP: Restriction fragment length polymorphism.

Table 2 Results of loss of heterozygosity assay at the adenomatous polyposis coli, mutation in colorectal cancer and deleted in colorectal cancer genetic loci in 41 colorectal carcinomas

Number	APC exon 11	MCC exon 10	DCC
1	HET	NI	NI
2, 6, 12, 23	HET	NI	HET
3, 5, 7, 30, 32	HET	NI	LON
4	LOH	HET	NI
8, 19, 24, 25, 31	NI	HET	LOH
9, 11, 21	HET	LOH	LOH
10, 28, 37	HET	HET	HET
13	LOH	LOH	HET
14, 33, 40	LOH	NI	HET
15, 22, 34, 38	NI	NI	LOH
16, 20, 39	NI	HET	HET
17	NI	LOH	NI
18, 29	NI	LOH	HET
26	HET	LOH	HET
27	HET	HET	LOH
35	LOH	NI	LOH
36	LOH	HET	LOH
41	NI	NI	HET

APC: Adenomatous polyposis coli; MCC: Mutation in colorectal cancer; DCC: Deleted in colorectal cancer; HET: Retained heterozygosity; NI: Not informative; LOH: Loss of heterozygosity.

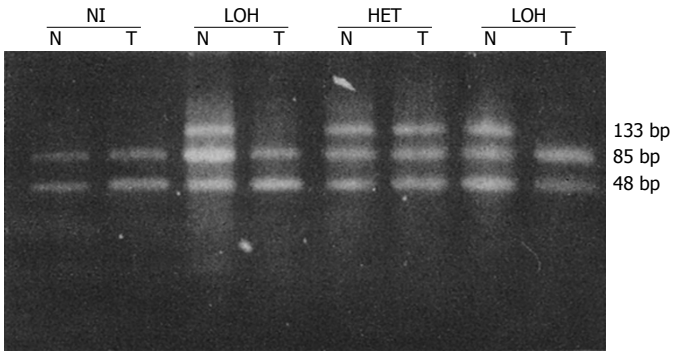

Figure 1 LOH assay of *APC* gene exon 11 (primer set 1). T: Tumor DNA; N: Normal DNA; NI: Not informative; LOH: Loss of heterozygosity; APC: Adenomatous polyposis coli; HET: Retained heterozygosity.

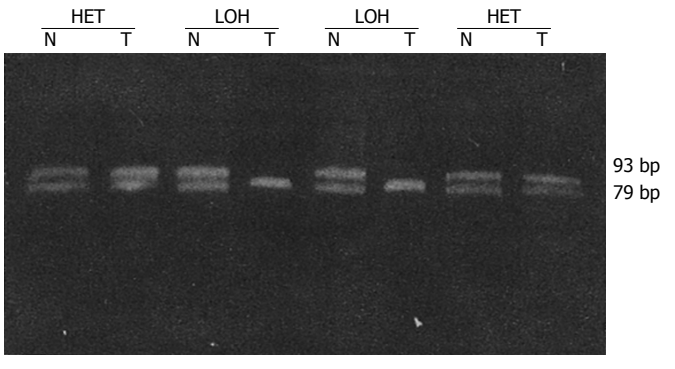

Figure 2 LOH assay of *MCC* gene exon 10 (primer set 2). T: Tumor DNA; N: Normal DNA; LOH: Loss of heterozygosity; HET: Retained heterozygosity; MCC: Mutation in colorectal cancer.

conditions described by Boynton *et al*[4] with a thermal cycler (Perkin Elmer Cetus, TOWN, COUNTRY). Annealing temperature, extension time and the number of amplification cycles were optimized for each primer set. PCR products were either digested with appropriate restriction enzymes (for RFLPs) or left intact (for a variable number of tandem repeats) and electrophoresed on either a 3% agarose gels or 8% polyacrylamide gel which were stained with ethidium bromide and photographed under UV light.

Data analysis

LOH was defined as a visible change in allele: allele ratio in DNA relative to the ratio in corresponding normal DNA.

RESULTS

LOH results obtained for each locus are in Table 2. Tissues from 41 patients were studied for LOH. When multiple polymorphic loci within each gene were used, a constitutional heterozygosity (informativity) was found at APC in 25 (60.9%), at MCC in 22 (53.7%) and DCC in 38 cases (92.7%). LOH of APC and MCC were observed in 7 of 25 (28.0%) and 8 of 22 (36.4%) of informative cases, respectively (Figures 1 and 2). When considered as a single locus, the LOH frequency for APC/MCC (number positive for LOH of one or both genes/number informative for one or both genes) was 38.9% (14/36). LOH at DCC genetic locus was detected in 55.3% (21/38) informative cases (Figures 3-5). LOH of at least one of these three genes was detected in 68.3% (28/41) of tumor informative at all loci.

Correlations between LOH at various loci and clinical pathological data of colorectal cancer are illustrated in Table 3. No significant correlation was found between the LOH at APC or MCC and tumor histological type, size, serosal invasion, lymph node metastases and Dukes' stages ($P > 0.05$). However, the LOH rates at DCC locus in the groups with lymph node metastases and in the Dukes' stages Ⅲ and Ⅳ were significantly higher than in groups without lymph node metastases and in Dukes' stages Ⅰ and Ⅱ ($P < 0005$).

Table 3 Correlation between loss of heterozygosity at the adenomatous polyposis coli, mutation in colorectal cancer and deleted in colorectal cancer genes and clinicopathological parameters of colorectal cancers

Group	LOH/ Informative (%)		
	APC	MCC	DCC
Grade (differentiation)			
Well/Moderate	4/14 (28.6)	4/12 (33.3)	8/20 (40.0)
Low differentiated	2/8 (25.0)	3/6 (50.0)	10/14 (71.4)
Mucoid	1/3 (33.3)	1/4 (25.0)	3/4 (75.0)
Size			
≤ 3 cm	3/8 (37.5)	4/12 (33.3)	7/15 (46.6)
> 3 cm	4/17 (23.5)	4/10 (40.0)	14/23 (60.9)
Serosal invasion			
Negative	5/16 (37.5)	4/14 (28.5)	10/23 (43.4)
Positive	2/9 (22.2)	4/9 (50.0)	11/15 (73.3)
Lymph-node metastasis			
Negative	4/16 (25.0)	5/14 (35.7)	9/23 (39.1)
Positive	3/9 (33.3)	3/8 (37.5)	12/15 (80.0)[a]
Dukes' stages			
Stages I and II	4/14 (28.5)	4/10 (40.0)	6/17 (35.3)
Stages III and IV	3/11 (27.3)	4/12 (33.3)	15/21 (71.4)

[a]$P < 0.05$. LOH: Loss of heterozygosity; APC: Adenomatous polyposis coli; MCC: Mutation in colorectal cancer; DCC: Deleted in colorectal cancer

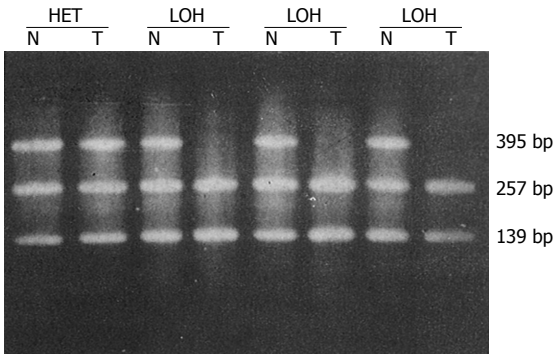

Figure 3 LOH of *DCC* gene (primer set 3). T: Tumor DNA; N: Normal DNA; LOH: Loss of heterozygosity; HET: Retained heterozygosity; DCC: Deleted in colorectal cancer.

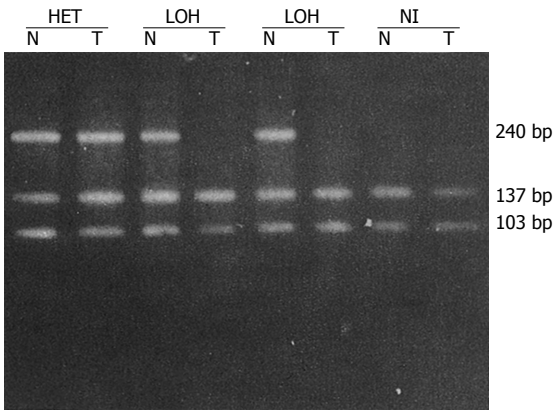

Figure 4 LOH of *DCC* gene (primer set 4). T: Tumor DNA; N: Normal DNA; NI: Not informative; LOH: Loss of heterozygosity; HET: Retained heterozygosity; DCC: Deleted in colorectal cancer.

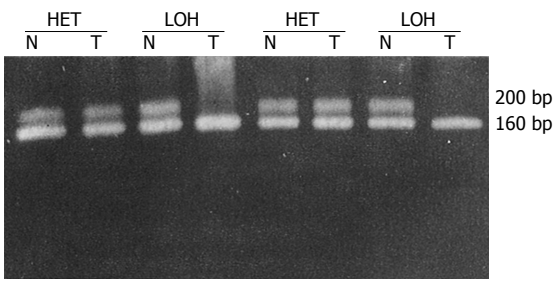

Figure 5 LOH of *DCC* gene (primer set 5). T: Tumor DNA; N: Normal DNA; LOH: Loss of heterozygosity; HET: Retained heterozygosity; DCC: Deleted in colorectal cancer.

DISCUSSION

Growing evidence suggests that the accumulation of multiple genetic events is responsible for the pathogenesis and/or progression of tumors. Multiple chromosomal deletions have been identified in colorectal cancer[9]. Recent studies on *APC*, *MCC*, and *DCC* gene aberrations have suggested that these genes may be involved in the carcinogenesis of human colorectal carcinoma[9-11]. LOH on chromosome 5q, where the *APC* and *MCC* genes are located, has been detected in 40.0% of sporadic colorectal carcinomas[9] and 33.0% of cancerous ulcerative colitis[11]. LOH on chromosome 18q, where the *DCC* gene is located, has been detected in 45.5% of sporadic colorectal carcinoma[9]. In the present study, LOH at APC and /or MCC was detected in 38.9% of colorectal carcinomas (APC, 28.0%; MCC, 36.4%), and at DCC in 55.3% of cases. These data suggest that deviations in *APC*, *MCC* and *DCC* genes may play a crucial role in the development and progression of colorectal carcinoma.

Genetic alterations such as ras mutation, 5q, 18q, and 17p deletions are believed to contribute to multistage carcinogenesis through colorectal adenoma to carcinoma[12]. LOH on 5q was observed most frequently in the intramucosal carcinoma[10,13]. With respect to the LOH on 18q, the frequency was very low in moderate and severe adenomas and intramucosal carcinomas, but it was high in invasive carcinomas[14]. In the present study, we did not find any correlation between LOH at APC and/or MCC and tumor histological type, size, serosal invasion, lymph node metastasis or the Dukes' stages. However, the LOH rates at DCC locus in groups with lymph node metastasis and the Dukes' stages Ⅲ and Ⅳ were significantly higher than in groups without lymph node metastasis and the Dukes' stages Ⅰ and Ⅱ. These data suggest that LOH at APC and/or MCC may occur more frequently in the early stages and play a role in the initiation of colorectal cancer. LOH at DCC is frequently a late event and is associated with the progression and metastasis of colorectal carcinoma.

Unexpectedly, there was no significant correlation between LOH of APC and MCC, even though these are closely linked loci on chromosome 5q. This finding suggests that LOH of APC occurs independently of LOH involving MCC. Similar discrepancies between these two genes have been previously reported in lung cancer[15] and esophageal cancer[4], as well as in colorectal cancer[16].

Some tumors did not lose heterozygosity at any of the tumor suppressor gene loci examined. One explanation is that these genes may be altered by another mechanism, such as point mutation, gene rearrangement, or microdeletion. Point mutations in APC, MCC, and DCC have been found in tumors without LOH[16]. Another explanation is that LOH at chromosomal regions, such as chromosomal 17q, is also important in pathogenesis. Furthermore, current assays may have limited sensitivity due to normal cell contamination of the specimens and/or lack of informativity at the RFLPs tested. And lastly, a subset of colorectal tumors may arise through the inactivation of other, as yet unknown, tumor suppressor genes and/or in combination with other genetic and epigenetic events. Further studies are required to explore these possibilities.

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The changes of antral endocrine cells in *Helicobacter pylori* infection

Ji-Yao Yu, Jia-Ling Wang, Li Yao, Ji-Yi Zheng, Ming Hu

Ji-Yao Yu, Professor of Pathology, interesting in gastrointestinal endocrine pathology. Director of the Department of Pathology, Chinese PLA Naval General Hospital, 6 Fuchenglu, Beijing 100037, China

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Correspondence to: Dr. Ji-Yao Yu, Professor of Pathology, interesting in gastrointestinal endocrine pathology. Director of the Department of Pathology, Chinese PLA Naval General Hospital, 6 Fuchenglu, Beijing 100037, China
Telephone: +86-10-6854229-58176

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Abstract

AIM: To study the changes of antral endocrine cells in *Helicobacter pylori* (Hp) infection, and to observe the relation between Hp infection and the number of gastrin (G) cells and somatostatin (D) cells.

METHODS: Sixty-five cases, 18 with Hp infection and 47 without Hp, were analyzed by endoscopy and immunohistochemical staining of the antral mucosal biopsies using antibodies against chromogranin A, gastrin, somatostatin, and bombesin. The positive cells were quantitatively studied by an image analyzer.

RESULTS: In the Hp infection group, the results were following: 71.28 G cells/mm^2, 5.32 D cells/mm^2, 8.68 bombesin positive cells/mm^2, and the G/D cell ratio was 13.40. By contrast, in the group without Hp infection, the number of G cells was 67.75/mm^2 while the number of D cells and bombesin positive cells were 13.65/mm^2 and 5.31/mm^2, respectively, with the ratio of G/D cells of 5.05. The difference in the number of D cells and the G/D cell ratio was statistically significant between the two groups ($P < 0.05$).

CONCLUSION: The gastrin increase in patients with Hp infection may be due to the decrease in D cells and somatostatin secretion.

Key words: *Helicobacter pylori*; Immunocytochemistry; Antral mucosa; Gastrin cells

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INTRODUCTION

It has been suggested that *Helicobacter pylori* (Hp) infection induces changes in the antral endocrine cells (EC). The relationship between EC and stomach diseases has been reported. The present study aimed to examine the influence of Hp infection on the antral EC using immunohistochemical staining.

MATERIALS AND METHODS

Sixty-five gastroscopic antral biopsy specimens including the entire height of the antral mucosa from the superficial epithelium to the muscularis mucosa were collected. The blocks were fixed in 10% formalin and embedded in paraffin. Serial sections (10) were cut from each block (5 μm). Hematoxylin and eosin staining was carried out. Hp was confirmed with the Warthin Starry silver staining.

The cellular localizations of endocrine substances were detected by biotin avidin (BA kit, Academy of Military Medical Sciences). The primary antibodies included anti-Chromogranin A (CGA, M869 DAKO, working dilution 1:400), anti-gastrin (GAT, A568, 1:300), anti-somatostatin (SS, A566, 1:400), and anti-bombesin (BOM, CA-08-210, Cambridge Research Biochemical Limited England, 1:20000). All antibodies were polyclonal. Peroxidase was revealed using diaminobenzidine tetrahydrochloride. Specificity of the immunoreaction was controlled by incubating consecutive sections with nonimmune serum, instead of the primary antiserum, or with the specific antiserum pre-absorbed with an excess of the respective antigens.

The EC in gastric antral mucosa was quantitatively analyzed with GmbHg image analyzer using 20 × objective. Care was taken to examine the same area in consecutive sections stained with anti-Chromogranin A and hormonal antibodies immunostaining. The number of the different types of EC in measured mucosa was counted and shown by the number of EC per square millimeter. Statistical analysis: The results were shown as $\bar{x} \pm s$ of each EC. Measurements were compared with the unpaired two-tailed Student's *t* test.

RESULTS

Table 1 shows the clinicopathological characteristics of 65 cases. The number of peptic ulcers in the Hp positive group was 8/19 (42.1%) and in the Hp negative group was 3/46 (6.5%) ($P < 0.05$). In the Hp positive group, 15.8% (3/19) of cases had chronic atrophic gastritis; the Hp negative group had 10.9% (5/46) of chronic atrophic gastritis ($P > 0.05$).

The mean numbers of G cells in the Hp positive and Hp negative groups are shown in Figure 1. The difference was not statistically significant ($P > 0.05$). Figure 2 shows the number of somatostatin positive cell (D cell) in the Hp positive group (5.32 ± 1.42/mm^2) and Hp negative group 1 (3.65 ± 3.56/mm^2) ($P < 0.05$). Figure 3 shows the number of bombesin positive cells in the two groups (Hp positive, 12.7 ± 2.6/mm^2; Hp negative, 5.31 ± 2.31/mm^2) ($P < 0.05$).

Table 1 Clinicopathological characteristics of the 65 cases

Characteristics	Hp positive	Hp negative
Cases (*n*)	19	46
Age (Yr)		
Median (age range in Yr)	51 (32-67)	48 (34-65)
Sex (F/M)	14/4	36/11
Pathological diagnosis		
CSG	8	38
CAG	3	5
PU	8	3

Hp: *Helicobater pylori*; CSG: Chronic superficial gastritis; CAG: Chronic atrophic gastritis; PU: Peptic ulcer.

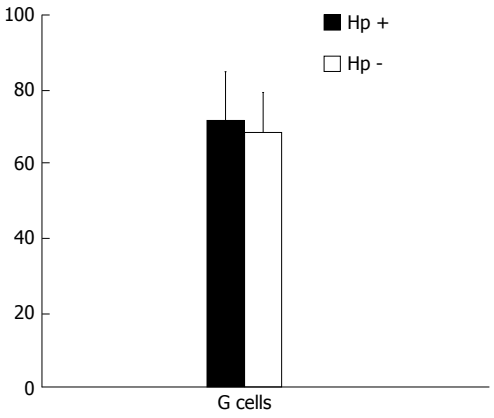

Figure 1 The mean number of G cell in *Helicobacter pylori* (Hp) positive group (71.28 ± 12.5/mm^2) and Hp negative group (67.65 ± 10.40/mm^2) (*P* > 0.05). G cell: Gastrin cells.

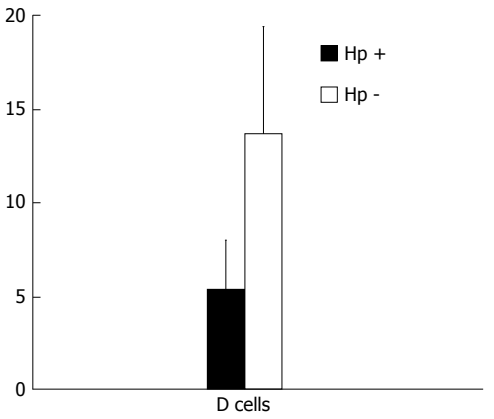

Figure 2 Number of D cells were 5.32 ± 1.42/mm^2 (Hp positive group) and 13.65 ± 3.56/mm^2 (Hp negative group) (*P* < 0.05). Hp: *Helicobacter pylori*; D cell: somatostatin cells.

DISCUSSION

Hp infection is a cause of gastritis and peptic ulcer[1]. The antral content of progastrin and its products are increased in Hp infected patients[2]. Our study showed a more intense immunostaining of G cells, using quantitative image analysis, in the presence of Hp

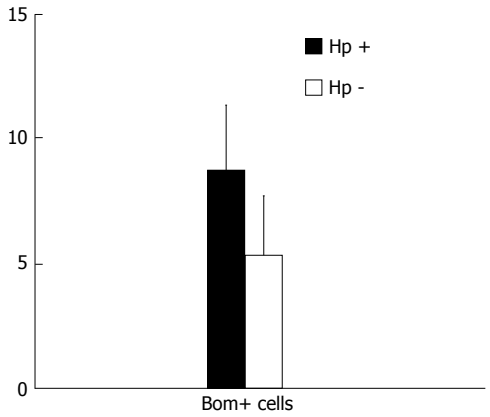

Figure 3 BOM + cell numbers were 12.7 ± 2.6/mm^2 (Hp positive group) and 5.31 ± 2.31/mm^2 (Hp negative group) in the two groups (*P* < 0.05). Hp: *Helicobacter pylori*; BOM: Bombesin.

than in uninfected patients ($P < 0.05$). The ratio of G/D cell was significantly different ($P < 0.05$). Gastrin release was normally inhibited by somatostatin. The decrease of D cells led to decreased inhibition in the G cells by somatostatin. Our results are in agreement with some reports[3,4] which showed a significant rise in somatostatin mRNA and somatostatin immunoreactive cell density after the eradication of Hp.

The stomach is rich in endocrine cells of various kinds. When it is infected with Hp, the changes in endocrine cells, including number, structure, and function are complex. This study showed an increase in the bombesin positive cells of antrum during Hp infection. Exaggeration of gastrin release followed bombesin stimulation[5]. Gastrin release can also occur as a result of γ interferon and interleukin 2 stimulation in a dog antrum[6]. Tumor necrosis factor, found in high concentration in the inflamed antrum, has been shown to increase gastrin gene transcription.

In conclusion, the increase of gastrin in patients with Hp infection may be due to the decrease in D cell and somatostatin secretion. Further investigation is required to elucidate the relations between EC in gastric mucosa and Hp infection.

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Substance P, vasoactive intestinal peptide, and leu-enkephalin in plasma and gastric juice of patients with precancerous lesions and gastric cancer

Chang-Tai Xu, Bo-Rong Pan, Yu-Min Wang, Rui-Ying Zhang

Chang-Tai Xu, Director of the Department of Gastroenterology & Hematology, Chinese PLA Air Force 473 Hospital, 216 Anningtonglu, Lanzhou 730070, Gansu Province, China

Yu-Min Wang, Rui-Ying Zhang, Chinese PLA Air Force 473 Hospital

Bo-Rong Pan, Professor of Internal Medicine, Room 12 Building 621, Fourth Military Medical University, 17 Chanlexilu, Xi'an 710033, Shaanxi Province, China

Author contributions: All authors contributed equally to the work.

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Correspondence to: Dr. Chang-Tai Xu, Director of the Department of Gastroenterology & Hematology, Chinese PLA Air Force 473 Hospital, 216 Anningtonglu, Lanzhou 730070, Gansu Province, China
Telephone: +86-931-7662362

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Abstract

AIM: To investigate the changes of the brain-gut-peptide concentrations in the plasma and gastric juice and their relations to gastric diseases.

METHODS: A total of 83 subjects were part of the study. Of those, 28 had chronic atrophic gastritis with precancerous lesions, 22 had gastric cancer in an advanced stage, and 33 were healthy subjects for a control group. Samples of fasting blood and gastric juice were collected. Levels of substance P (SP), vasoactive intestinal peptide (VIP) and leu-enkephalin (LEK) in plasma and gastric juice were measured with radioimmunoassay kits expressed as ng/L.

RESULTS: In patients with gastric cancer, the SP levels (83.7 ± 11.0 *vs* 39.6 ± 4.5, $P < 0.01$; 24.0 ± 1.6 *vs* 17.8 ± 1.5, $P < 0.05$) and LEK in plasma and gastric juice (226.2 ± 15.4 *vs* 180.3 ± 13.1, $P < 0.01$; 55.0 ± 3.4 *vs* 30.7 ± 2.4, $P < 0.05$), and VIP of gastric juice (80.5 ± 2.9 *vs* 64.3 ± 4.1, $P < 0.05$) were higher than those in the controls. The SP and LEK levels of plasma correlated with those of gastric juice ($r = 0.432$ and 0.516, $P < 0.05$). In the post-surgical gastric cancer, plasma levels of SP and gastric juice LEK were lower than the pre-surgical levels ($P < 0.05$). In the precancerous lesions, plasma and gastric juice LEK levels and gastric juice VIP levels were increased ($P < 0.05$), and the plasma LEK level correlated with the gastric juice LEK level ($r = 0.398$, $P < 0.05$).

CONCLUSION: Measurement of concentrations of SP, VIP, and LEK in plasma and gastric juice is of clinical significance in detecting certain stomach diseases.

Key words: Stomach neoplasms; Precancerous conditions; Substance P; Vasoactive intestinal peptide; Enkephalin, leucine

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INTRODUCTION

Multifocal chronic atrophic gastritis with intestinal metaplasia (IM) of the gastric mucosa is considered a precancerous lesion of gastric cancer[1,2]. The increased prevalence of this lesion in high-risk population is of little use in identifying individuals at the highest risk. It has been proposed that some types of IM represent a more advanced stage in the precancerous process, as represented by the colonic type of morphology and the secretion of sulfated mucins[3]. The poor prognosis of advanced gastric cancer has not changed substantially over the years[4]. This dismal outcome is in sharp contrast to the excellent five-year survival rate of surgically treated early gastric cancer, reportedly to be 90%-95%[5]. Since most patients with early gastric cancer are probably asymptomatic or have only vague and/or nonspecific complaints, they are usually seen in the clinic at a rather advanced stage of the disease.

Various gut regulatory peptides, including substance P (SP), vasoactive intestinal peptide (VIP) and leu-enkephalin (LEK), may act as endocrine hormones influencing gastrointestinal functions. The alterations of levels of beta-endorphin, motilin,, and gastrin in plasma and gastric juice have been reported to occur in gastric cancer and precancerous lesions[6]. Abnormal plasma levels of SP, VIP and LEK have been reported to occur in stomach diseases[7,8]. The present study was undertaken to determine if the production of these peptides is altered in gastric cancer and precancerous lesions, and if the measurement of the levels of the peptides in gastric juice and plasma could assist in unraveling the pathogenesis and early diagnosis.

MATERIALS AND METHODS

Subjects

Studies were performed on 22 patients with advanced gastric cancer (5 women and 17 men) with a mean age of 48.2 ± 2.6 years. A total of 28 patients had chronic atrophic gastritis with precancerous

Table 1 Comparison of plasma and gastric juice levels of substance P, vasoactive intestinal peptide and leu-enkephalin between patient and control groups ($\bar{x} \pm s$, ng/L)

Diagnosis	*n*	Plasma			Gastric juice		
		SP	VIP	LEK	SP	VIP	LEK
Gastric cancer	22	83.9 ± 11.0[b]	6.0 ± 1.0[a]	226.2 ± 15.4[a]	24.1 ± 1.6[a]	80.5 ± 2.9[a]	55.0 ± 3.4[b]
Precancerous lesion	28	42.0 ± 2.3[a]	7.0 ± 0.4[a]	213.9 ± 19.1[a]	20.0 ± 2.8[a]	78.4 ± 8.7[a]	41.7 ± 5.7[a]
Healthy control	33	39.6 ± 4.5	6.9 ± 1.0	180.3 ± 13.1	17.8 ± 1.5	64.3 ± 4.1	30.7 ± 2.4

[a]$P < 0.05$, [b]$P < 0.01$, *vs* healthy controls. SP: Substance P; VIP: Vasoactive intestinal peptide; LEK: Leu-enkephalin.

lesions (8 women and 20 men) with a mean age of 47.5 ± 3.1 years. A total of 33 healthy subjects (7 women and 26 men) with a mean age of 45.8 ± 2.5 years were used as controls. The diagnoses were based on the endoscopic, histologic or cytological data and the clinical and laboratory findings before therapy. Intestinal metaplasia of the gastric mucosa was considered precancerous lesions. Carcinoma was found in the antrum (n = 15), body (n = 3), and fundus (n = 4) of the stomach. Pathohistological diagnosis revealed adenocarcinoma (n = 16), squamous cancer (n = 1), and undetermined (n = 5). Patients with other diseases were not included in the study.

Methods

Patients under fasting conditions had 2 mL of a blood sample collected in prechilled heparinized tubes containing 1000 kIU aprotinin. The plasma was separated immediately by centrifugation, frozen and stored at -20 ℃ until analysis. The gastric juice samples (4 mL) were collected in tubes during gastroscopy at fasting and were immediately mixed with $NaHCO_3$ (0.1 N) to adjust the pH to 5-7, and stored at 20 ℃ until analysis. The concentrations of SP, VIP, and LEK were measured with radioimmunoassay kits (The General Hospital of Chinese PLA, Beijing, China)[9,10]. The coefficient variations were controlled to less than 9%. The smallest amount of peptides detectable with 95% confidence was 2 ng/L for SP, 0.5 ng/L for VIP, and 10 ng/L for LEK.

Statistical analysis

All values were given as $\bar{x} \pm s$. The unpaired Student's t test was used to compare the control group and patient groups. The paired Student's t test was used to compare post- and pre-surgical gastric cancer patients. Correlations were calculated for the peptide values in both plasma and gastric juice with linear regression analysis. $P < 0.05$ was considered significant.

RESULTS

The levels of SP, VIP and LEK peptides in the control and patient groups are summarized in Table 1. In patients with gastric cancer, a statistically significant correlation was found between the levels of SP and LEK in plasma and gastric juice: $r = 0.432$, $P < 0.05$ for SP; and $r = 0.516$, $P < 0.05$ for LEK. In patients with precancerous lesions, $r = 0.398$, $P < 0.05$ for LEK.

In gastric cancer, the differences in the levels of plasma SP and LEK, and in the gastric juice peptides between adenocarcinoma and other cancers did not reach statistical significance ($P > 0.05$). In gastric cancer patients, post-surgery plasma levels of SP (47.2 ± 11.6 ng/L, $P < 0.01$) and gastric juice LEK (35.9 ± 3.8 ng/L, $P < 0.01$) were lower than pre-surgery, but changes in the other peptides did not reach statistical significance.

To test if the differences in peptide levels were due to the difference in sex distribution between the patient and control groups, comparisons were made for each sex. No statistically significant difference was found.

DISCUSSION

Neuropeptides form a part of the brain-gut-axis which may regulate gastrointestinal functions, including immune regulation and development of gastrointestinal cancer[7,8]. Our results showed that gastric juice peptide levels changed in the precancerous lesion, as well as in established gastric cancer. The levels of SP did not change significantly in the precancerous lesion. The plasma levels of SP and LEK changed in gastric cancer as compared with controls, as well as the level of LEK in the precancerous states.

Hormonal influences on IM and malignant cells remain an important area of research in the study of stomach neoplasms and precancerous conditions. SP[11], VIP[12] and LEK[13] have each been localized to mammalian gastric enteric nerves using immunohistochemical and radioimmunological techniques. SP-containing nerve fibers are present in the myenteric plexus and circular smooth muscle of gastric corpus of several species. VIP has been localized to enteric neurons of the same gastric smooth muscle layers as the other neuropeptides[14]. LEK has important clinical implications between opiates and human immune system.

SP, an 11-amino acid peptide, is a natural polypeptide produced by the cells of the intestinal mucosa and brain. The primary action of this hormone is, by endogenous means, to stimulate the smooth muscle of stomach to contract. SP has been shown to play a secondary role in the pathogenesis of gastric cancer[15]. In the present study, we have demonstrated that SP levels in patients with gastric cancer were significantly increased. This may suggest either a destruction of the function of gastrointestinal cells by the malignant process or a primary failure which facilitates carcinogenesis.

VIP, a 28-amino acid peptide, is found extensively throughout the central nervous system and the gastrointestinal tract and is considered a neurotransmitter. Like SP, VIP has been shown to induce the release of histamine from mast cells. The release of histamine is inhibited by SP antagonist, suggesting that SP and VIP act on a common site or *via* a common receptor[16]. There is evidence to suggest that VIP modulates the neurogenic inflammatory response induced by SP, an effect probably related to its vasodilator activity[17]. VIP has also been shown to induce pepsinogen secretion in gastric glands, probably by increasing cAMP levels[18]. In several studies, VIP has been found to decrease gastric acid secretion by releasing somatostatin[19], whereas, in others, VIP had an inhibitory or no effect on gastric somatosatin[14,20]. Increased levels of VIP in gastric juice have been reported in the gastric cancer patients[15]. Karmeli *et al*[14] have provided evidence that VIP is involved in the pathogenesis of acute ethanol-induced gastric mucosal damage. In patients with gastric cancer or precancerous lesions, the changes in levels of VIP in gastric juice suggest that VIP may stimulate contraction of the smooth muscle of the stomach. The VIP mechanism may be enhanced by gastroduodenal mucosa since gastric emptying is influenced in gastric cancer or intestinal metaplasia involving gastric mucosa.

LEK is a 5-amino acid peptide. The fluctuations in LEK in various other gastrointestinal disorders are unclear. It is possible that gastrointestinal dysmotility may play a role in the development of gastric cancer and precancerous lesions. The regulation of gastrointestinal motility is a complex process involving the inherent properties of the mural musculature, both intrinsic and extrinsic neural control mechanisms, hormonal influences, and other factors. A wide range of gastrointestinal peptides, such as SP, VIP and LEK may influence the motor function of the gut. LEK levels have been previously reported to increase in patients with gastric cancer[15]. In our study, we have demonstrated that LEK levels in patients with gastric cancer and precancerous lesions were significantly elevated.

A good correlation was found between the plasma and gastric juice concentrations of SP and LEK in gastric cancer patients, and of LEK in precancerous lesions, although in individual patients there was a clear difference. Many gut hormones, including SP, VIP and

LEK, will change their levels in plasma or gastric juice as a result of the disease of the stomach[15,16,21]. Our results show that the difference in levels of SP, VIP, and LEK between the patient and control groups are significant. Therefore, a diagnostic laboratory should choose one peptide for routine screens. In practice the values of SP, VIP, and LEK are not likely to be useful in the diagnosis and the follow-up of patients with gastric cancer and precancerous lesions. However, given the established interactions between stress and opiates and the immune system, our findings might justify further consideration of these factors in relation to causation of gastric cancer.

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Gastroendoscopic biopsy diagnosis of mucosa-associated lymphoid tissue lymphoma

Xiao-Long Ji, You-Quan Cheng, Shu-Qing Wang

Xiao-Long Ji, You-Quan Cheng, Shu-Qing Wang, Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China

Author contributions: All authors contributed equally to the work.

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Correspondence to: Dr. Xiao-Long Ji, Professor, Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China

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Abstract

AIM: To differentially diagnose gastric lymphoma by gastroendoscopic biopsy and clinicopathology.

METHODS: A retrospective review of 38 lymphoma cases diagnosed by gastroendoscopic biopsy in the period between 1984 and 1994 from gastroendoscopy files in the Department of Gastroenterology. The histology slides were examined retrospectively. Diagnostic criteria were discussed according to the new classification of non-Hodgkin's lymphoma.

RESULTS: Of 53400 gastroendoscopy, 1672 were malignant neoplasms of which 38 were cases of the primary gastric lymphoma as diagnosed by both endoscopic findings and histological examination. A total of 22 men and 16 women, age 16 to 82 year, with a median of 47.7 year, were included in the study. The endoscopic evaluation found 12 cases of ulcerative, 11 cases of diffusely infiltrating, six cases of massive, four cases of large mucosal fold, and five cases of mixed type. The histological evaluation resulted in 34 cases of mucosa-associated lymphoid tissue lymphoma (89.5%), two cases with lymphoblastic type and two cases unclassified due to the crushed neoplastic cells.

CONCLUSION: These findings are present in about 90% of endoscopic biopsy specimens of low-grade gastric lymphoma. The majority of the cases of the primary low-grade gastric lymphoma have morphologic and clinical features that justify their inclusion in the category of low-grade lymphoma of mucosa-associated lymphoid tissue.

Key words: Stomach neoplasms; Gastroendoscopy; Lymphoma; Mucosa-associated lymphoid tissue

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INTRODUCTION

Primary gastric lymphoma is the most common extranodal lymphoma and accounts for 24% of all such tumors. In addition, non-neoplastic lymphoid hyperplasia is not uncommon in the stomach. The distinction between reactive lymphocytic proliferations and gastric lymphomas remains troublesome. This is reflected in numerous publications devoted to this problem[1]. It has been postulated that lymphomas involving mucosal surface arise from mucosa-associated lymphoid tissue and characterize a low-grade lymphoma called mucosa-associated lymphoid tissue lymphoma (MALToma), often with numerous reactive-appearing lymphoid follicles[2-4]. Therefore, the differential diagnosis of MALToma from reactive lymphoid hyperplasia remains difficult, especially by endoscopic biopsy with small pieces of tissue.

MATERIALS AND METHODS

All gastric lymphomas diagnosed at the Department of Gastroenterology by gastroendoscopic biopsy during the period 1984-1994 were reviewed. Based on the new classification of lymphoid neoplasms[5], MALToma was confirmed if the following criteria were met: (1) invasion of epithelial structures resulting in lymphoepithelial lesions; (2) small lymphocytes, marginal zone cells and/or monocytoid B cells; and (3) infiltration of diffuse, perifollicular, interfollicular, or even follicular type due to colonization of reactive follicles. Tissue for light microscopy analysis was prepared in paraffin sections from 10% formalin and stained with hematoxylin and eosin.

RESULTS

Clinical features

From 1984 to 1994, a total of 53400 gastroendoscopy were performed, and 1672 cases of malignant gastric neoplasms were detected, including 38 cases of gastric lymphoma (2.3% of malignant tumors of the stomach). The patients (16 women and 22 men) ranged in age from 16 to 82 year, with a median of 47.7 year.

Gastroendoscopic features

The endoscopic evaluation showed gastric lymphoma in various locations which included 10 cases in gastric antrum, six in gastric body, one in gastric fundus, 14 in gastric body and antrum, one in gastric body and fundus, four in gastric fundus, body and antrum,

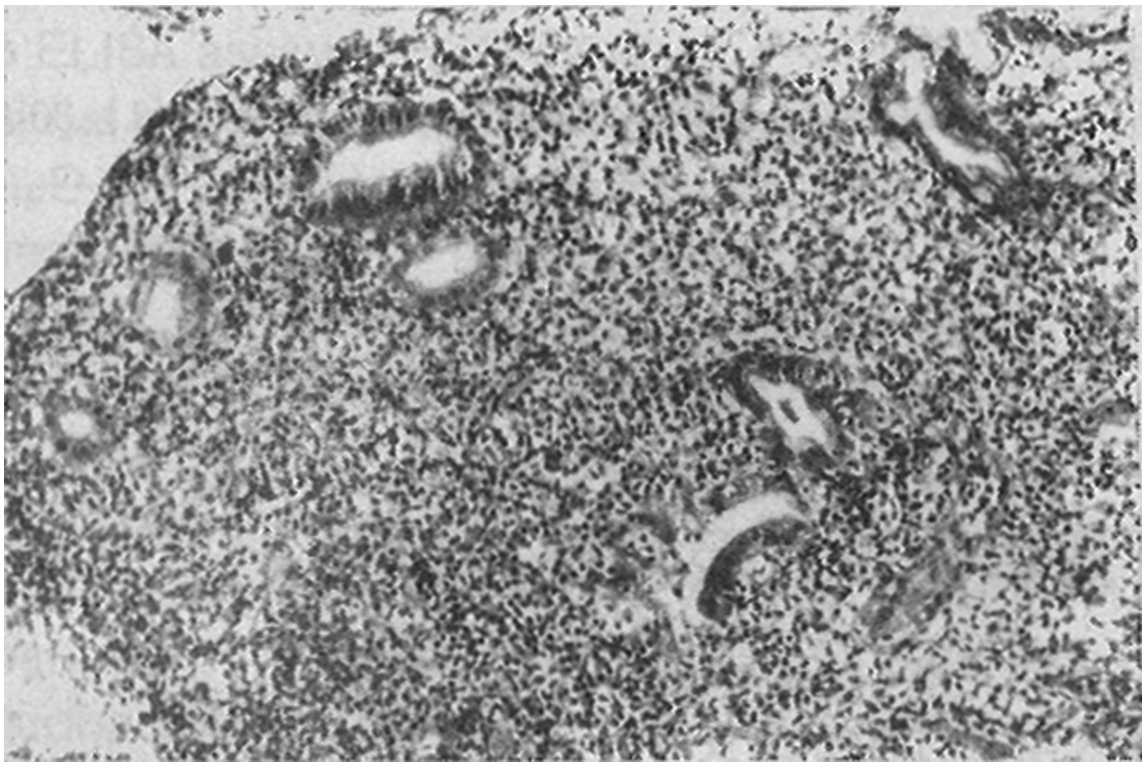

Figure 1 Monocytoid B cells lymphoma infiltrating diffusely gastric mucosa. Hematoxylin and eosin stain, × 200

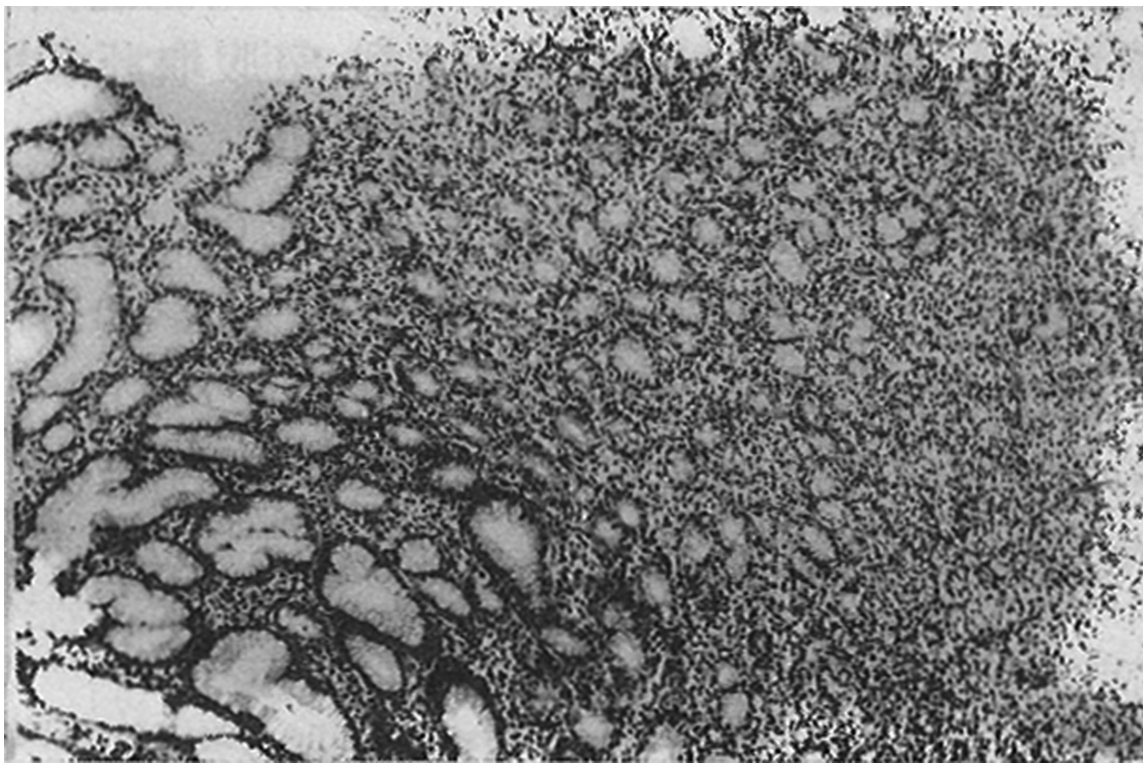

Figure 2 Neoplastic lymphocytes partially infiltrating gastric mucosa. Hematoxylin and eosin stain, × 200

and two in gastric fundus and antrum. The endoscopic diagnosis showed five types of gastric lymphoma: ulcerative (12 cases), diffuse infiltrating (11 cases), massive (6 cases), large mucosal fold (4 cases), and mixed type (5 cases).

Histopathological features

Based on the appearance of biopsy specimens, all 38 cases were gastric lymphomas of which 34 were MALToma (low-grade), two were lymphoblastic lymphoma (high grade) and two unclassified because of the crushed neoplastic cells.

Initially, the low-grade MALToma was diagnosed as either suspected lymphoma (12/34, 35.3%) or as lymphoid hyperplasia (4/34, 11.8%). Of the 34 MALToma cases, 18 were seen with the diffuse infiltrating pattern of neoplastic lymphocytes (Figure 1), 12 with the interfollicular pattern (Figure 2), and four with the follicular pattern. Both of the latter two patterns were originally misdiagnosed as non-neoplastic lesions.

For the neoplastic lymphocytes, there were 21 cases with marginal zone cells, 12 with small lymphocytes, and five with monocytoid B-cells. All of them showed obvious lymphoepithelial lesions.

DISCUSSION

Several previous studies have reported the morphologic features of the primary gastric MALToma in the endoscopic biopsy specimens. The histopathological criteria for the diagnosis of gastric MALToma have largely been based on the analysis of partial gastrectomy specimens[4]. From a routine biopsy diagnosis by gastroendoscopy, it is very difficult to distinguish MALToma from reactive lymphoid hyperplasia on small pieces of tissue. In our series, 47.1% (16/34) of cases were misdiagnosed as non-neoplastic lesions. Nearly half of the cases had failed to have the right diagnosis of MALToma of the stomach.

Histologically, our results showed that the prominent

lymphoepithelial lesions in the endoscopic biopsy specimens were one of the most important features for diagnosing MALToma of the stomach. The lymphoepithelial lesions were seen in 100% of the biopsied specimens. The diffuse infiltrating pattern was more appropriate than either interfollicular or follicular pattern for diagnosing MALToma of the stomach.

From our studies, we reflect that the key point for the diagnosis of MALToma is for the infiltrating lymphocytes to show homogeneity in the marginal zone cells, small lymphocytes, or monocytoid B cells. All of the three types of cells comprise low-grade lymphomas composed of a dense infiltrate with a superficial and peripheral plasma cell component.

Cytologically, neoplastic lymphocytes are characterized by cellular heterogeneity, including centrocyte-like cells (small, atypical cells with more abundant cytoplasm), monocytoid B cells, small lymphocytes, and plasma cells. Occasionally large cells (centroblast- or immunoblast-like cells) are present. If reactive follicles are present, the neoplastic cells will occupy the marginal zone and/or the interfollicular region. When the neoplastic lymphocytes take on a follicular pattern, it is called follicular colonization. Therefore, the diagnosis of MALToma of the stomach is not based on the infiltrating pattern, but on the cellular morphology.

In conclusion, we have demonstrated that nearly half of the MALToma cases of the stomach are misdiagnosed using the biopsy specimens. The diagnosis of gastric MALToma should be based on (1) dense lymphoid infiltrate with marginal zone cells, small lymphocytes or monocytoid B cells; or (2) prominent lymphoepithelial lesions. If a case is highly suspected for MALToma of the stomach, repeat biopsy should be performed. The presence of germinal centers, acute inflammation, crypt abscesses, and even associated *Helicobacter pylori* infection do not exclude lymphoma.

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Evaluation of the serum alpha-fetoprotein-reactive-lentil-lectin in the diagnosis of hepatocellular carcinoma

Bai-He Zhang, Meng-Chao Wu

Bai-He Zhang, Institute of Hepatobiliary Surgery, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

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Correspondence to: Dr. Bai-He Zhang, Institute of Hepatobiliary Surgery, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
Telephone: +86-21-5347018-728

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Abstract

AIM: To determine the serum concentration of alpha-fetoprotein (AFP) with anti-human AFP variant monoclonal antibody (AFP-R-LCA mAb) in detection of hepatocellular carcinoma (HCC) and to ascertain the value of this tumor marker in the diagnosis of HCC.

METHODS: Cell fusion was used for the preparation of anti-human AFP-R-LCA mAb which was assayed with a two-site sandwich enzyme linked immunosorbent assay (ELISA) method. Using this method, the serum concentration of AFP-R-LCA was tested in 99 patients with HCC, 67 patients with benign liver diseases (BLD), 30 pregnant women, and 30 normal controls.

RESULTS: The threshold value of the serum AFP-R-LCA was set to 10 μg/L, in reference to normal controls. The concentration of AFP-R-LCA in serum was 1.27 ± 0.6 μg/L and 0 μg/L in pregnant women and controls, respectively. As shown by the rocket immune-electrophoresis, the serum AFP-R-LCA increased from 62.6% to 86.8% in HCC patients ($P < 0.05$) while the positive rates were decreased from 19.4% to 1.45% in BLD patients ($P < 0.05$) and from 26.7% to 0% in pregnant women ($P < 0.05$). The false positive rate was 1.5%; the two-site sandwich ELISA assay had a specificity of 99.0% in the diagnosis of HCC.

CONCLUSION: Our AFP-R-LCA variant mAb had a higher affinity and specificity to AFP-R-LCA than the routinely used anti-AFP polyclonal antibody. Anti-human AFP-R-LCA variant mAb two-site sandwich ELISA assay had a low false positive rate so that it could be used for both early diagnosis of HCC and the differential diagnosis of HCC and BLD. The method is simple, accurate, and reproducible.

Key words: Liver neoplasms; Alpha-fetoproteins; Enzyme linked immunosorbent assay; Monoclonal, antibody

Zhang BH, Wu MC. Evaluation of the serum alpha-fetoprotein-reactive-lentil-lectin in the diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 1995; 1(1): 33-36 Available from: URL: http://www.wjgnet.com/1007-9327/full/v1/i1/33.htm DOI: http://dx.doi.org/10.3748/wjg.v1.i1.33

INTRODUCTION

Alpha-fetoprotein (AFP) is one of the most useful carcinoembryonic proteins. The structure of the protein moiety and the biological activities of the AFP in fetal liver and hepatocellular carcinoma (HCC) are almost identical, with only slight differences in the carbohydrate composition. Recently, Aoyagi *et al*[1] reported a difference in the binding pattern of AFP with lens culinaris agglutinin (LCA) between HCC and benign liver disease (BLD), using the crossed immunoaffinoelectrophoresis (CIAE). An increase of the LCA reactive species of AFP in patients with HCC indicated that fucosylation of the sugar chain was the molecular basis for this AFP variation. However, the CIAE method requires great skill and is, therefore, not appropriate for routine clinical work. The present study describes a clinical application of the two-site sandwich enzyme linked immunosorbent assay (ELISA) assay with anti-human AFP-R-LCA variant monoclonal antibodies (mAb) to detect serum AFP-R-LCA. The results showed that these mAb are clinically useful for the differential diagnosis of HCC and BLD and for increasing the early detection rate of HCC.

MATERIALS AND METHODS

Samples

A total of 226 cases were included in the study, of which 99 had a histopathological diagnosis of HCC. Thirty patients had tumors < 5 cm in diameter while the rest had tumors > 5 cm in diameter. The serum AFP levels, determined by the rocket immune-electrophoresis (RIE), were < 100 μg/L in 38 (38.4%) patients, < 400 μg/L in 46 (46.4%) patients, and > 400 μg/L in the rest of the patients (15.2%). The serum levels of AFP-R-LCA determined by the CIAE were higher than 25% in 79 patients. AIAT, γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), LDH, PHI, and CEA were also detected in HCC patients with tumors < 5 cm in diameter. A total of 67 cases of BLD were included of which 30 had post-hepatitis cirrhosis, 10 acute hepatitis, and 27 chronic active hepatitis. All of these patients received a histopathological diagnosis. A total of 30 pregnant women (3-4 mo in pregnancy) and 30 healthy adult blood donors were taken as controls. AFP levels were determined in all the patients and controls by RIE.

Preparation of anti-human AFP-R-LCA mAb

BALB/C mice were immunized with purified AFP-R-LCA (provided

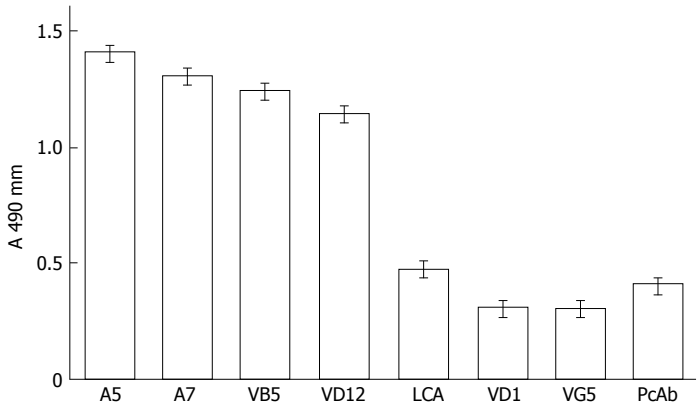

Figure 1 Solid state antigen competitive inhibition assay A5, A7, anti-AFP mAb; VB5, VD12, VD1, VG5, anti-AFP-R-LCA mAb; PcAb: Anti-AFP polyclonal antibodies; LCA: Lens culinaris agglutinin; mAb: Monoclonal antibody.

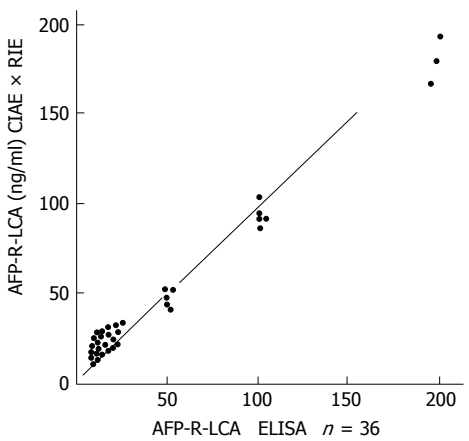

Figure 2 The correlation analysis of the two-site sandwich ELISA assay and RIE × CIAE (*n* = 36). RIE: Rocket immune-electrophoresis; CIAE: Crossed immuno-affinoelectrophoresis; AFP-R-LCA: Alfa-fetoprotein reactive lens culinaris agglutinin; ELISA: Enzyme linked immunosorbent assay

by the Hepatobiliary Surgery Institute, Changhai Hospital, the Second Military Medical University). The spleen cells were removed and flushed with FO myeloma cells (Balb/c origin) by 50% PEG methods. After ELISA monitoring and five limit diluting clonozation, five cell lines secreting anti-human AFP-R-LCA mAb were obtained. The titers of the specific mAb, as determined by ELISA, were 2 × 10 in a culture supernatant and 1 × 10 in ascites induced in mice. The hybridoma cell lines were cultured for 5 mo *in vitro* and passed on for 60 generations. After being frozen in liquid nitrogen and thawed, these cell lines had the ability to secret mAb steadily. Chromosome analysis demonstrated that these cell lines had the characteristics of hybridoma cells. The mAb belonged to IgG class.

Purification and enzyme labeling of mAb

The mAb was purified from ascites by diethylaminoethyl cellulose 52 chromatography. The purified mAb and horseradish peroxidase (HRP) were first treated with periodic acid and then precipitated twice with 500 g/L $(NH_4)_2SO_4$. After an exhaustive dialysis against 0.01 mol/L PBS (pH 7.4), the mixture was then passed through Sephadex G25 column. The HRP-labelled mAb were obtained with a molecular ratio 1.4. The working concentration was 1:2000.

Solid state antigen competitive inhibition assay

Polystyrene plate was coated overnight with 0.2 mL/well AFP-R-LCA (5 μg/mL). The plate was washed four times with 0.01 mol/L-PBS, followed by addition of 0.1 mL/well of HRP-mAb and 0.2 mL/well of four different ascites (1:10 diluted). The reaction mixtures were incubated for 2 h at 37 ℃. Substrate colorization was stopped after 30 min, and O.D. value at 490 nm was determined.

Two-site sandwich ELISA assay

The mAb used for coating did not have cross-reaction with HRP-mAb. Standard curve was drawn for each assay. The accuracy of the ELISA assay had intra-batch coefficients of variability (CV) values ($CV = s/\bar{x} \times 100$; s, standard deviation; $\bar{x}$, mean) of 4%, 3.8%, 5.3%, 6.4% and 6.8%, respectively and intra-batch CV values 8.5%, 10.2%, 9.3%, 8.6% and 12.8%, respectively. The obtained accuracy rate was 96.2% ± 5.4%.

RESULTS

Epitope specificity of mAb directed antigen

The results of the competitive inhibition assay demonstrated that antigen binding sites of anti-AFP mAb A5 and A7 and anti-AFP-R-LCA mAb VB5 and VD12 were different from those of VD1 and VG5 (Figure 1). VD1 and VG5 may bind the same antigenic sites, or the antigenic sites they bound were very close to each other. Polyclonal horse anti-human AFP antibodies and LCA also inhibited the binding of HRP-mAb VG5 to AFP-R-LCA. The presence of two different mAb with no cross-reactivity in the prepared anti-AFP-R-LCA formed the basis of the two-site sandwich ELISA assay.

The relationship of two-site sandwich ELISA with RIE and CIAE

Serum AFP-R-LCA was determined by the two-site sandwich ELISA assay. Serum AFP and the ratio of AFP/AFP-R-LCA were determined by RIE and CIAE. The correlation of the two-site sandwich ELISA with RIE × CIAE was analyzed in the curve of ELISA against RIE × CIAE. There was a significant positive correlation between the results of ELISA and those of RIE × CIAE as demonstrated in Figure 2 ($\gamma = 0.8401$, $P < 0.01$).

Serum test results

AFP-R-LCA value for pregnant women and normal controls were tested, and the concentration of AFP-R-LCA in serum was 1.27 ± 0.6 μg/L and 0 μg/L, respectively. Concerning the previously published paper, we set the threshold value of serum AFP-R-LCA to 10 μg/L. No false negatives were observed, and the false positive was only 1.45%.

The results of the eight parameters obtained from 30 patients with small HCC are shown in Table 1. The range of serum AFP concentration was 0 μg/L–2000 μg/L. When > 400 μg/L of AFP was taken as a positive threshold, 10 patients were positive (33.3%) for AFP. The concentration range for AFP-R-LCA was 0 μg/L-300 μg/L in these patients. When AFP-R-LCA threshold was set at > 10 μg/L, 26 patients were positive. None of the other six parameters had a positive rate larger than 30%. In four patients, no AFP-R-LCA could be detected, so these patients were regarded as AFP-negative primary liver cancer.

The results showed that serum AFP positive rate was increased from 62.6% (RIE) to 86.8% (two-site sandwich ELISA assay) in HCC patients while the positive rates were decreased from 19.4% to 1.45% in BLD ($P < 0.05$) and from 26.7% to 0% in pregnant women. The two-site sandwich ELISA assay had a specificity of 99.0% in the diagnosis of HCC (Table 2).

In 46 HCC patients with AFP 400 μg/L and 38 HCC patients with AFP 100 μg/L, the AFP-R-LCA concentrations were 36.8 ± 10.2 μg/L and 19.1 ± 10.4 μg/L, respectively. In 54 BLD patients with AFP 400 μg/L and 51 BLD patients with AFP 100 μg/L, the AFP-R-LCA concentrations were 4.40 ± 2.3 μg/L and 1.91 ± 0.9 μg/L, respectively ($P < 0.01$). In 30 pregnant women, the AFP-R-LCA concentration was 1.27 ± 0.6 μg/L ($P < 0.01$). The above results indicate that although HCC patients may have AFP levels lower than the positive threshold, the AFP-R-LCA concentrations were higher than the positive threshold and also much higher than that in BLD patients.

DISCUSSION

As of recently, more attention has been paid to carbohydrates as "functional codes". Since lectins can specifically bind to carbohydrates, they have been widely used in the study of the heterogeneity of AFP carbohydrate chains. Consequently, much progress has been made in the field. In the current study, Du *et al*[2] found that the reactivity of serum AFP from 20 patients with HCC with immobilized lentil-lectin was significantly greater (39% ± 18%) than that of the same protein from patients with chronic liver disease (11.2% ± 3.3%), fulminant hepatic failure (10% ± 8.4%),

Table 1 Eight parameters found in hepatocellular carcinoma patients

Item	Number	Concentration	Positive rate %[1]
AFP	30	0-2000 μg/L	33.3
AFP-R-LCA	30	0-300 μg/L	86.7
AIAT	28	250-560 μg/L	14.3
γ-GT	30	20-420 U	25.0
ALP	30	6-28 U	13.3
ADH	25	120-860 U	28.0
PHI	25	23-216 U	20.0
CEA	14	6.8-62.7 U	21.4

[1]Positive rate: AFP 400 μg/L, AFP-R-LCA 10 μg/L, AIAT 420 μg/L, γ-GT 100 U, ALP 8 U, LDH 500 U, PHI 300 U, and CEA 15 U. AFP: Alpha-fetoprotein; AFP-R-LCA: Alfa-fetoprotein reactive lens culinaris agglutinin; γ-GT: γ-glutamyl transpeptidase; ALP: Alkaline phosphatase.

Table 2 Comparison of alfa-fetoprotein and alfa-fetoprotein reactive lens culinaris agglutinin in the diagnosis

Patients	*n*	Positive rate of AFP (%)	Positive rate of AFP-R-LCA (%)
HCC	99	62.6	86.8
BLD	67	19.4	1.45
Pregnant women	30	26.7	0
Normal controls	30	0	0

HCC: Hepatocellular carcinoma; BLD: Benign liver diseases; AFP: Alfa-fetoprotein; AFP-R-LCA: Alfa-fetoprotein reactive lens culinaris agglutinin.

and normal pregnant women (4.1% ± 2.7%). However, in these experiments the lack of correlation between the ConA and lentil-lectin reactivities of AFP in HCC suggests that the implied structure changes involving D-mannose/D-glucose and L-fucose respectively, resulting from quite distinct cellular lesions.

Aoyagi *et al*[3] has purified LCA reactive and non-reactive heterogeneous AFP variants from HCC ascites and analyzed their carbohydrate chain structure. Their results showed that LCA-reactive AFP has N-acetyl 1-glucose residue bound to Asn, and this residue has a bound fucose residue. The group tested the sera from the patients with HCC and found that the ratio of LCA-R-AFP heterogeneous variant significantly increased. This may be a result of decreased fucosidase activity. Since the fucosidase activity decreases, the fucose residue bound to G1cNAc can not be removed during the process of AFP carbohydrate chain modification, and so forms the characteristic structure that can be recognized by LCA.

The degree of AFP fucosylation has been revealed as a good marker for discrimination of HCC from nonmalignant liver diseases[4]. The fucosylated and non-fucosylated molecular species of AFP have been identified by CIAE in the presence of LCA, by taking advantage of the affinity of the fucosylated species with this lectin. Similar methods have been used for diagnosis of neural tube defects and liver disease with lectins such as concanavalin A and LCA[5]. However, the methods using CIAE, immunoblotting techniques, and affinity chromatography are complicated and require a high level of technical skill.

Several investigators have described the application of mAb assay systems as quantitative measurement of serum markers in patients with certain disease[6]. In recent years, many studies have demonstrated that mAb can immunologically distinguish among AFP from different organs. At least 10 mAb against AFP have been reported, and their introduction increased the detection sensitivity and specificity. However, these antibodies still could not distinguish BLD from HCC for these two AFPs belong to the same fetal liver type. Thus, it was necessary to develop mAb that can specifically recognize the relatively specific reverse differential protein AFP-R-LCA induced by transformed cells in HCC for the early diagnosis of HCC and the differential diagnosis of BLD and HCC. Suzuki *et al*[7] described a new enzyme immunoassay (EIA) that distinguished between purified fucosylated and non-fucosylated AFP using monoclonal anti-AFP antibody in the presence of LCA at AFP concentration from 200 μg/L to 1000 μg/L. They suggested that their new EIA with mAb18H4 would be useful for detection of an early stage of HCC. However, a great quantity of LCA is consumed in EIA, and economic load on patients would increase consequently. On the other hand, our anti-AFP-R-LCA mAbs have high specificity and affinity, especially mAbs VG5 and VD1, which recognize new antigenic sites (different from the already known AFP a and b antigenic sites). The two-site sandwich ELISA assay set up using these two mAbs can quantitatively analyze the AFP-R-LCA concentration and increase the detection rate of HCC. This ELISA assay is superior to the CIAE, which is currently widely used in clinical studies. The results of our study show that 10 μg/L of AFP-R-LCA is a reasonable threshold. Based on this threshold, the detection rate was 86.8% for HCC, 86.7% for small HCC, and 100% for AFP positive HCC. No false negatives were encountered. Only one BLD patient had serum AFP-R-LCA higher than 10 μg/L (false positive rate, 1.45%). No one in pregnant women and control groups had AFP-R-LCA higher than 10 μg/L. In HCC patients with low AFP levels, 46 cases (46.6%) had AFP levels less than the positive threshold of 400 μg/L. However, the mean AFP-R-LCA concentration was 36.8 ± 10.2 μg/L in these patients, much higher than the positive threshold. When AFP levels were determined by RIE, the positive rate was 62.9%, and the false positive rate was 19.4% for HCC. Since six BLD patients and 53 HCC patients had AFP of 400 μg/L, the coincidence of diagnosis was 89.9% (53/59). The detection rate for HCC was 79.7% (79/99) by CIAE. CIAE and RIE had lower sensitivity and in accuracy than ELISA assay.

We suggest from this study that the quantitative analysis of serum AFP-R-LCA is useful not only in increasing the early diagnosis rate and accuracy of HCC with low AFP but also for the early diagnosis of small HCC. At present, it is difficult to distinguish HCC from AFP-positive BLD in a clinical setting. If the two-site sandwich ELISA assay is used to analyze quantitatively AFP-R-LCA concentration, the accuracy of differential diagnosis of HCC and BLD will increase and enable patients to gain the opportunity of an early treatment. The mAbs are easy to prepare and have higher specificity than polyclonal antibodies. Combining mAb with ELISA provides a simple, fast, and sensitive method which can be used widely in clinical practice. It is possible that this mAb two-site sandwich ELISA assay will replace the routine methods for AFP analysis.

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Genesis and distribution of motilin in the human fetus

Yu-Xin Huang, Cai-Fu Xu, Hong Liao, Qing-Li Wang

Yu-Xin Huang, Cai-Fu Xu, Qing-Li Wang, Department of Gastroenterology, Tangdu Hospital, the Fourth Military Medical University, Xi'an 710038, Shaanxi Province, China

Hong Liao, Department of Gastroenterology, Air Force Guangzhou Hospital, TOWN 510001, China

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Correspondence to: Dr. Yu-Xin Huang, professor, Department of Gastroenterology, Tangdu Hospital, the Fourth Military Medical University, Xi'an 710038, Shaanxi Province, China

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Abstract

AIM: To investigate the pattern of distribution and ontogeny of motilin (MTL) in the human fetus.

METHODS: MTL concentrations were determined systematically, using radioimmunoassay, in the tissues of the central nervous system (CNS, six regions) and the digestive system (20 regions) in human fetuses.

RESULTS: The results showed a wide distribution of MTL in the tissues but at different concentrations. The MTL concentrations in the digestive system tended to be higher than those in the CNS, and the concentrations increased with the fetal age, especially in the digestive system. High concentrations of MTL were found in hypophysis (the earliest MTL generation region in the CNS), spinal cord and cerebellum; however, there were no significant differences among them. In the digestive system, MTL was detectable as early as 16th week in jejunum, duodenum, ileum, stomach, transverse colon, sigmoid colon, and rectum, while in other regions MTL was not detectable until after the 23rd week. By the 24th week, MTL showed an adult distribution pattern in all of the tissues. In the digestive system tissue of different fetal ages, the highest MTL concentration was found in jejunum, followed by duodenum and ileum, and MTL was positively correlated with the fetal age. The MTL concentration in ileum was about 6-7 times higher than in the CNS. In addition, we detected MTL at a considerable concentration in the appendix for the first time.

CONCLUSION: The findings might help reveal distribution and ontogeny of MTL in fetuses.

Key words: Motilin; Central nervous system; Digestive; System; Fetus

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INTRODUCTION

Motilin (MTL) is a gastrointestinal hormone which stimulates gastrointestinal tract motility. Despite rapid progress in the MTL research, especially regarding molecular biology and receptor theory, the studies of embryogenesis of MTL have been limited to intestinal organs such as duodenum, jejunum, ileum, and colon. Furthermore, there has been no report on the MTL genesis in the central nervous system (CNS) in human fetus[1]. In our present study, MTL concentrations were determined in the tissues of both CNS and digestive system of human fetuses age 16 wk to 40 wk.

MATERIALS AND METHODS

Collection of gross specimens

Twelve normal fetuses (5 males and 7 females) in water bag were obtained from healthy pregnant women by labor induction and were stored in a freezer (-32 ℃) for no more than one month. Full ethical permission was obtained for this study. Deduced from the time of the last menstrual period of the pregnant women, the fetuses were divided into three groups, 16–23 wk, 24–31 wk, and 32–40 wk.

Dissection of the fetuses

Fetuses were defrosted, and the esophagus, stomach, duodenum, jejunum, ileum, colon, appendix and gall bladder were dissected under direct vision at 10 ℃. The organ lengths were measured, and the tissue specimens were taken from each organ. The blood was removed, and the specimen put in vials which were immediately placed on crushed ice. The samplings from the medullary bulb, cerebral cortex, hypothalamus, cerebellum, hypophysis and spinal cord were taken out as previously described. After all the tissue specimens (20 regions in the gastrointestinal tract and six regions in the CNS) had been taken, they were stored in a freezer (-32 ℃).

Extraction of MTL from tissue

Tissues weighing 20–100 mg were taken from the cerebrospinal cord and each organ in the gastrointestinal tract. The tissues were defrosted, and 1 mL of 2 mol/L iced acetic acid was added. After been plunged into vigorously boiling water for 10 min, the tissues were cooled down to 4 ℃ and then homogenized for 10 min. After centrifugation at 3500 rpm for 20 min at 4 ℃, the supernatant was collected. Just before the radioimmunoassay (RIA) assay,

Table 1 The distribution of motilin in cerebrospinal tissues of different fetal ages (pmol/g, n = 4, $\bar{x} \pm s$)

Region	Fetal age (wk)		
	16-23	24-31	32-40
Cerebral cortex	UD	0.222 ± 0.052	0.967 ± 0.738
Hypothalamus	UD	0.347 ± 0.152	1.015 ± 0.634
Hypophysis	0.884 ± 0.417	0.599 ± 0.408	1.678 ± 1.404
Cerebellum	UD	0.276 ± 0.116	1.272 ± 1.101
Medullary bulb	UD	0.357 ± 0.068	1.086 ± 0.749
Spinal cord	UD	0.270 ± 1.131	1.611 ± 1.019

UD: Undetectable.

Table 2 The distribution of motilin in digestive tissues of different fetal ages (pmol/g, n = 4, $\bar{x} \pm s$)

Region	Fetal age (week)		
	16-23	24-31	32-40
Esophagus	UD	0.801 ± 0.483	1.441 ± 0.896
Cardia	UD	0.404 ± 0.239	1.775 ± 0.968
Stomach	0.125 ± 0.071	0.618 ± 0.537	1.966 ± 1.441
Pylorus	UD	0.745 ± 0.724	1.591 ± 0.753
Duodenum	1.562 ± 0.009	3.764 ± 2.471	4.656 ± 2.480
Jejunum	2.302 ± 0.127	3.947 ± 2.729	8.488 ± 4.927
Ileum	0.880 ± 0.173	1.066 ± 0.641	2.420 ± 1.769
Cecum	UD	1.119 ± 0.379	1.726 ± 0.636
Appendix	UD	0.688 ± 0.096	1.632 ± 0.787
Colon	0.250 ± 0.083	1.054 ± 0.363	2.286 ± 1.745
Rectum	0.112 ± 0.037	0.719 ± 0.461	2.318 ± 1.033
Gallbladder	UD	0.457 ± 0.077	1.164 ± 0.397

UD: Undetectable.

the supernatant was centrifuged for another 10 min for further purification.

Radioimmunoassay (RIA)

MTL was determined using a non-equilibrium RIA with motilin antibody. The kit was provided by the General Hospital of the People's Liberation Army. The specimens were assayed in duplicate.

Statistical analysis

The data were analyzed using the statistical analysis of variance and correlation.

RESULTS

Genesis and distribution of MTL in CNS

The MTL concentrations in cerebrospinal tissue of different fetal ages are given in Table 1. Hypophysis was the first region to secrete MTL as early as the 16th week while in other regions MTL was not detectable until after a 23rd week. MTL concentrations in human fetal cerebrospinal tissues, hypophysis, cerebellum, and spinal cord were high. However, there was no significant difference in the MTL concentration between the cerebrospinal tissues assayed ($P > 0.05$).

Genesis and distribution in digestive system

The MTL concentrations in digestive tissues of different fetal ages are shown in Table 2. In the digestive system, MTL was detectable before the 16th week in jejunum, duodenum, ileum, stomach, colon, and rectum, whereas in other regions it was detected no earlier than the 23rd week. It was not until the 24th week that the fetuses displayed a similar distribution pattern of MTL to that of adults. MTL levels in both the digestive system and the CNS increased with the age of the fetuses, and the MTL level in the former tended to be elevated more significantly than that of the latter.

In all the digestive system tissues of different fetal ages, the highest MTL concentration was found in jejunum, followed by duodenum, ileum, and colon. The concentrations in the descending part of the duodenum, jejunum, and colon were positively correlated with the age of the fetuses ($P < 0.01$ and $P < 0.05$, respectively) (Figure 1). In addition, for the first time, MTL was found in the appendix.

The length of the small intestine (including jejunum and ileum) was closely correlated with the age of the fetuses ($r = 0.0056$, $P < 0.01$, Figure 2).

DISCUSSION

Genesis, distribution and significance of MTL in cerebrospinal tissues

In the cerebrospinal tissues, hypophysis was the first to secrete MTL (16th week), whereas in other regions it was not until 24th week. The MTL secreting cells in the adenohypophysis might be responsible for the early secretion of MTL in hypophysis[2]. The later secretion of MTL in cerebellum and other central nervous tissue suggests that the cells in adenohypophysis might secrete MTL earlier than the Purkinje cells.

MTL in fetuses could be seen as a type of a cerebral enteropeptide, for it is widely distributed in the CNS tissue, especially in hypophysis, cerebellum, and spinal cord, and it exists at various levels in the hypothalamus, medullary bulb, and the cerebral cortex. Although there was no significant difference between these regions, the MTL concentration in hypophysis was twice that of cerebral cortex which might be related to the density distribution of the MTL secreting cells. However, further confirmation is necessary. The MTL concentration in the CNS increased with the fetal age and reached the peak level by the 40th week. This might be due to the gradual maturation of the fetal CNS as the fetus developed, the MTL secreting cells in the CNS increase in numbers and improve in function.

MTL in CNS was different from cholecystokinin and vasoactive intestinal peptide in the subcellular distribution. Rather than located in a vesicle, MTL was located in the nucleus[3]. In the early CNS developmental period, MTL might act either as a chemical signal or as a nutrient for cell migration and linkage between specific neurons and their target cells.

Genesis, distribution and significance of MTL in gastrointestinal system

The regions in which MTL was detectable before the 16th week were jejunum, duodenum, ileum, stomach, transverse colon, rectum and sigmoid colon while in other regions it was not detectable until after the 16th week. However, there was an adult distribution pattern in all regions by the 24th week which is in agreement with the 20-24 wk adult distribution pattern of most other gastrointestinal hormones[4]. The genesis of MTL in the majority of gastrointestinal system regions was earlier than that in the CNS.

MTL was widely distributed in the gastrointestinal system. In our

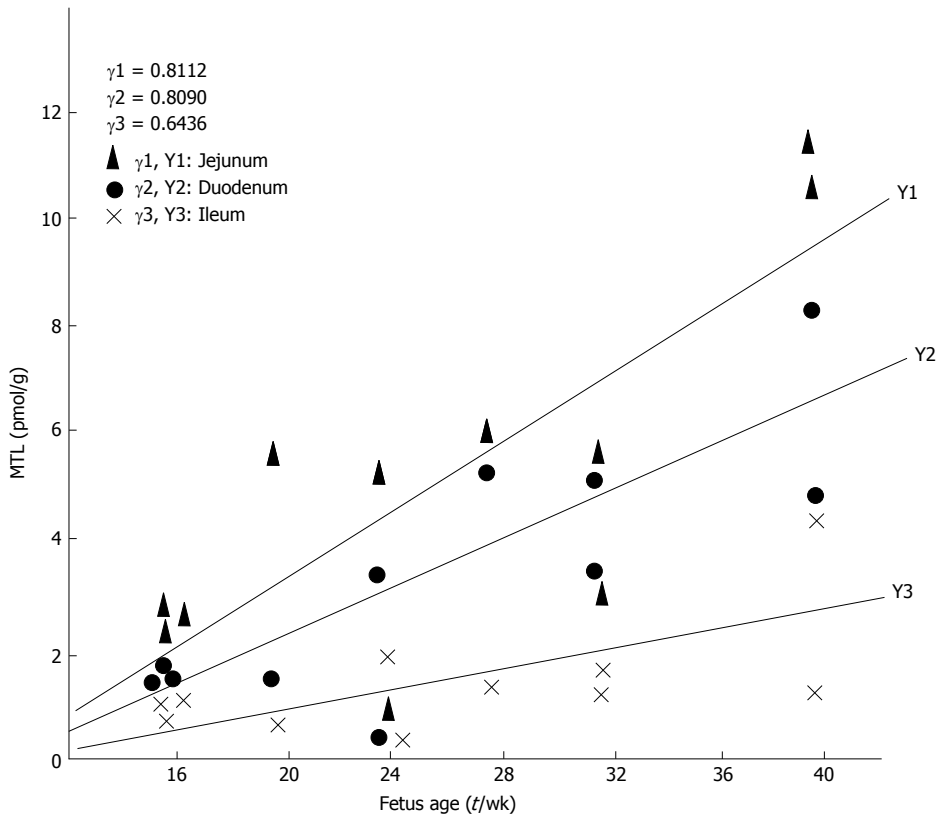

Figure 1 The relationships between fetus age and the Motilin levels in jejunum, duodenum, and ileum. $Y_1 = -2.8120 + 0.2986X$, $Y_2 = -2.1830 + 0.2124X$, $Y_3 = -0.6704 + 0.0777X$. MTL: Motilin.

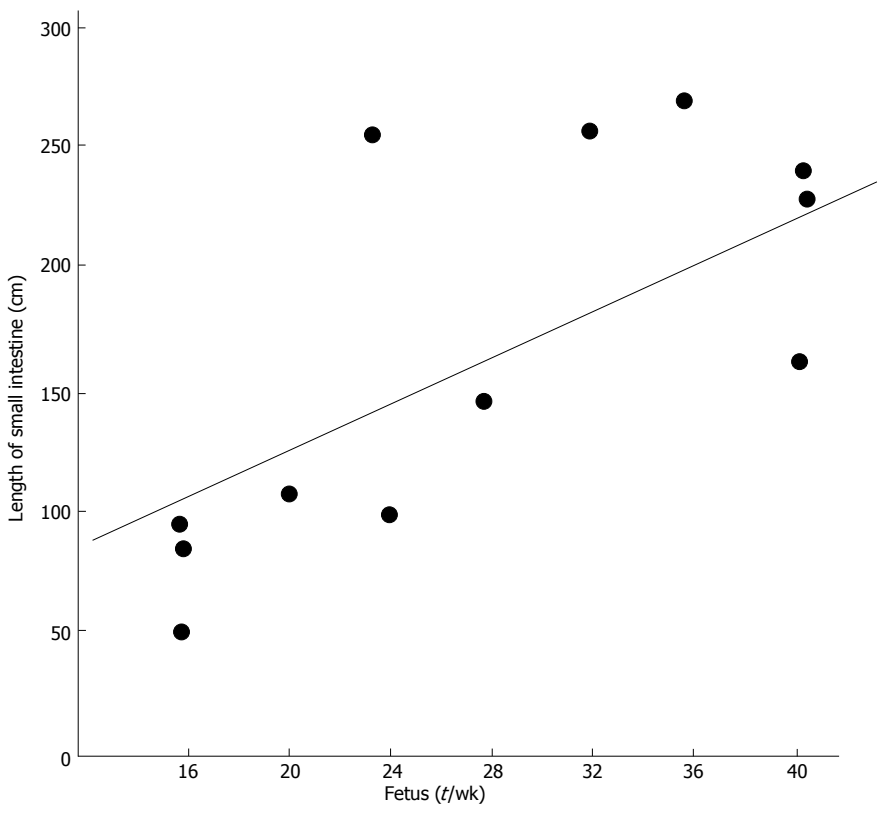

Figure 2 The relationship between fetus age and the length of the small intestine.

present study, it was detected in all of the 20 regions, though at different levels. In all age groups, the highest MTL concentration was noted in jejunum tissues which was twice that in the duodenum, three times that in ileum and colon, six times that in the esophagus and eight times that of a gall bladder. The result confirmed the immunohistochemical report that the most densely distributed region of the motilin-immunopositive cells was the upper segment of the small intestine. MTL concentration in the gastrointestinal system had a tendency to increase with the fetal age and reached the peak level by the 40th week. Having detected MTL of considerable concentration in the appendix for the first time, we deduced that motilin-immunopositive cells might exist in the mucosa of the appendix where appendix potentially acts as a secretory organ. MTL was present in the gall bladder tissues, but was hardly detectable in bile, suggesting a lack of secretion of MTL by the gall bladder tissue into bile, and if any, it was decomposed by an enzyme in bile soon after MTL was secreted. MTL concentrations in the fetal duodenum, jejunum, and ileum were positively correlated with the fetal age. Thus, the assay of MTL concentrations in these regions was of great value in assessing the development of fetal small intestine.

In short, MTL has a wide distribution in both the gastrointestinal and cerebrospinal tissue, with the MTL concentrations in the former were higher than those in the latter. The most remarkable discovery was the MTL concentration in jejunum was about 6-7 times that in the CNS[5].

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Influence of *Helicobacter pylori* on the gastric mucosal barrier

Cai-Pu Xu, Xian-Yong Gui, Wei-Wen Liu, Zhen-Hua Wang, Shao-Wu Pan

Cai-Pu Xu, Department of Gastroenterology, Southwest Hospital, 3rd Military Medical University, Chongqing 630038, China

Author contributions: All authors contributed equally to the work.

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Correspondence to: Dr. Cai-Pu Xu, professor, Department of Gastroenterology, Southwest Hospital, 3rd Military Medical University, Chongqing 630038, China
Telephone: +86-811-5318301-73374

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Abstract

AIM: To study the influence of *Helicobacter pylori* (Hp) on the gastric mucosal barrier (GMB) by the measurement of the potential difference (PD).

METHODS: Fifty seven chronic gastritis cases were diagnosed endoscopically and confirmed by forceps mucosal biopsy. PD was measured by the Takeuchi method, and Hp was detected by both culture (modified Skirrow method) and press printing method with the Giemsa stain. Patients were divided randomly into three groups (De-Nol, WeiTong-Ling, and Placebo) for a course of 6 wk therapy.

RESULTS: PD across the mucosa of antrum was significantly lower in Hp (+) patients than in Hp (-) patients (16.44 ± 2.36 *vs* 19.58 ± 2.44, $P < 0.0001$). In Hp (+) patients, PD in the antrum increased markedly (16.88 ± 2.56 *vs* 20.03 ± 2.21, $P < 0.0001$) after Hp was cleared up by the De-Nol treatment.

CONCLUSION: Our data strongly indicated that Hp infection might cause a gastric mucosal barrier to be impaired markedly while the clearance of Hp by De-Nol recovered the integrity of the gastric mucosal barrier significantly.

Key words: *Helicobacter pylori*; Gastric transmucosal potential difference; Gastric mucosal barrier

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INTRODUCTION

Helicobacter pylori (Hp) infection may play an important role in the etiology of chronic gastritis (CG) and peptic ulcer, but its mechanism remains unclear. Obvious damages to the gastric mucosal barrier (GMB) were found in various CG, but there were few reports about the influence of Hp on GMB. The purpose of this study was to explore the influence of Hp on GMB by the measurement of the potential difference (PD).

MATERIALS AND METHODS

Fifty seven CG patients were diagnosed endoscopically and confirmed by the forceps mucosal biopsies. Of the 58 CG patients, 14 had chronic superficial gastritis (CSG), and 43 had chronic atrophic gastritis (CAG). A total of 38 men and 19 women with mean age of 37.8 year were included in the study. PD was measured by the Takeuchi method[1,2] at eight sites in the gastric antrum and body. Hp was detected by both culture (modified Skirrow method) and the Giemsa stain. Fifty seven CG patients were randomly divided randomly into three groups. In the group Ⅰ, 120 mg of De-Nol (9 cases) was administrated three times a day for six weeks. The group Ⅱ, the WeiTong-Ling group, patients were administered 1.7 three times a day for six weeks. The group Ⅲ was the control group (Placebo).

RESULTS

Hp was detected in 28 of 57 patients (49.12%; CSG, 35.75%; CAG, 53.5%; $P < 0.05$). The Hp clearance rate was 77.8% in the De-Nol group and 33.3% in the WeiTong-Ling group ($P < 0.01$). In the control group, Hp was not cleared in any of the patients.

As is shown in Table 1, PD in the antrum was lower in Hp (+) patients than in Hp (-) patients ($P < 0.0001$), but no significant difference was found in PD in the gastric body. In Hp (+) patients, PD in the antrum increased markedly ($P < 0.0001$) after Hp has been cleared by the De-Nol treatment (Table 2). No significant change in PD was found in Hp (-) patients after the therapy (Table 3).

DISCUSSION

PD is a good indicator of the integrity of the gastric mucosa, and it runs parallel with the degree of mucosal damage or recovery[2]. Present data showed that Hp infection lowered the PD significantly in the antrum and exacerbated the damage of GMB. Once Hp is cleared by the medical therapies (the De-Nol treatment), the GMB improves, and the inflammatory infiltration reduces. The mechanism of damage to GMB caused by Hp is poorly understood. Goodwin *et al*[3] found that the content of neutral mucus on the Hp-infected gastric mucosa decreased markedly. Tasman-Jones *et al*[4] reported the similar results. Bode *et al*[5] found that the viscosity of mucus infected by Hp was lowered. The production of mucus in the damaged epithelial cell may be decreased[6]. Other causes weakening the GMB included the

Table 1 Potential difference in *Helicobacter pylori* positive or negative patients

Group	Antrum				Corpus			
	n	Hp (+)	*n*	Hp (-)	*n*	Hp (+)	*n*	Hp (-)
CSG	5	17.96 ± 2.70	9	20.43 ± 2.08[e]	5	32.13 ± 3.79	9	32.93 ± 5.47
CAG	23	16.44 ± 2.36	20	19.58 ± 2.44[e]	25	31.74 ± 3.63	20	31.94 ± 4.84

[e]$P < 0.001$, *vs* Hp (-). CSG: Chronic superficial gastritis; CAG: Chronic atrophic gastritis ; Hp: *Helicobacter pylori*.

Table 2 Potential difference in *Helicobacter pylori* (+) patients before and after treatment

Group	*n*	Antrum		Corpus		YPD
		before	after	before	after	
I	9	16.88 ± 2.53	20.03 ± 2.21[b]	29.58 ± 2.50	32.41 ± 2.33	3.15 ± 1.9
II	6	16.50 ± 3.12	17.80 ± 3.63	31.84 ± 5.61	33.18 ± 4.22	1.30 ± 0.79
III	6	17.93 ± 2.58	17.53 ± 3.95	31.35 ± 1.57	31.33 ± 2.70	-0.39 ± 1.7

[b]$P < 0.01$, YPD = elevated PD in antrum after treatment. Group I: The De-Nol treatment; Group II: The WeiTong-Ling treatment; Group III: Placebo; PD: Potential difference.

Table 3 Potential difference in *Helicobacter pylori* (-) patients before and after treatment

Group	*n*	Antrum		Corpus		YPD
		before	after	before	after	
I	9	18.78 ± 2.50	20.11 ± 2.46	32.73 ± 3.61	33.30 ± 3.09	1.34 ± 2.37
II	9	19.69 ± 2.71	20.86 ± 1.99	34.27 ± 5.26	34.54 ± 4.97	1.17 ± 1.34
III	4	19.46 ± 2.99	19.67 ± 2.53	37.13 ± 3.32	36.96 ± 3.42	0.21 ± 0.39

Group I: The De-Nol treatment; Group II: The WeiTong-Ling treatment; Group III: Placebo; YPD: Elevated potential difference in antrum after treatment.

loosing of the connection of epithelial cells by the Hp infection and local increment of ammonia products.

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Prognostic value of silver-stained nucleolar organizer regions in colorectal carcinoma

Zheng-Fen Zhou, Shi-Zhen Yuan

Zheng-Fen Zhou, Associate Professor of Medicine, having 30 papers and books published. Director of the Department of Gastroenterology, the First Affiliated Hospital of Kunming Medical College, and member and Secretary of Yunnan Provincial Gastroenterology Society, won an Advanced Technology & Science Prize, 153 Xichanglu. Kunming 650031, Yunnan Province, China

Shi-Zhen Yuan, Gastroenterology Research Laboratory, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University of Medical Sciences, Guangzhou 510120, Gangdong Province, China

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Abstract

AIM: Recently, silver-stained nucleolar organizing regions (AgNOR) analysis has been used as a criterion for tumor diagnosis and research. The purpose of this study was to investigate the prognostic value of AgNOR analysis in colorectal carcinomas.

METHODS: The silver staining technique was applied to paraffin embedded tumor tissue sections from 114 patients with colorectal carcinoma. The number, morphology, size, and distribution of AgNOR were counted and analyzed.

RESULTS: (1) The number of AgNOR in patients who died within 5 year of carcinoma diagnosis ($\bar{x} \pm s$: 8.8 ± 2.3 per nucleus, n = 27) was significantly higher than that in those who survived beyond 5 year (6.3 ± 1.8, n = 30, $p < 0.001$). The number of large sized (> 2 μm) and small sized (< 1 μm) AgNOR was significantly higher in patients who died ($\bar{x} \pm s$: 85.9 ± 20.7, 661.7 ± 250.5 in 100 nuclei) than in those who survived (71.7 ± 27.0, 398.3 ± 225.4, p = 0.04, 0.00 respectively). The concentrated type of distribution was significantly fewer in those who died (10.2%) than those who survived (31.4%, p = 0.00), whereas the mixed type of distribution was significantly greater in those who died (25.7%) than in those who survived (7.1%, p = 0.00). And (2) The number of AgNOR was also related to other factors that affected prognosis of colorectal carcinoma, such as age, histological type, depth of invasions, and metastasis to lymph nodes.

CONCLUSION: The AgNOR analysis is a novel and useful parameter for assessing the prognosis of colorectal carcinoma.

Key words: Nucleolus organizer region; Colonic neoplasms; Prognosis

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INTRODUCTION

Nucleolar organizer regions (NOR) are markers of ribosomal DNA (rDNA) and rDNA transcription. It can provide information regarding gene regulation and the control of cell proliferation and differentiation[1] and can reflect the biological behavior of cancer cells. Recently, silver-stained nucleolar organizing regions (AgNOR) analysis has been applied to predict cancer prognosis; but the results have been controversial and no study to date has been performed in Chinese patients. In this study, we examined whether AgNOR quantitative analysis is predictive for colonic cancer prognosis and explored its relationship with other prognostic factors.

MATERIALS AND METHODS

Selection of specimens

One hundred fourteen patients with colonic cancers were examined, of which 60 were male and 54 were female. Among them, 11 were < 29 year old (9.7%); 70 were 30-59 year old (61.4%); and 33 were over 60 (29.0%). In 44 cases, the cancer was located at the sigmoid colon; 18 at the ascending colon; 14 at both the descending colon and ileocecum; nine at the transverse colon; eight at the splenic flexure, and seven at the hepatic flexure of colon. In 57 cases, carcinomas invaded the serosa; 27 were beyond the serosa; 25 invaded deep smooth muscles; and 5 invaded the mucosa, submucosa, and shallow smooth muscles. Histological classification was graded in accordance with the diagnosis standards[2], as follows: 86 tubular adenomas (including 29 high, 36 moderate, and 21 low differentiations), six undifferentiated adenomas, five signet-ring cell adenomas, 12 mucoid adenomas, two papillary adenomas, and three polypoid adenomas. The tumors were classified according to malignant degree[3], as follows: 31 low malignant adenomas (including papillate and highly differentiated adenomas), 51 moderately malignant adenomas (including polypoid, moderately differentiated, and mucoid adenomas), 32 highly malignant adenomas (including low differentiated, undifferentiated, and signet ring adenomas). According to operation findings and

Table 1 The number of silver-stained nucleolar organizing regions in the died and survived groups with different histologic types of colonic cancer $\bar{x} \pm s$ (range)

Histologic types	AgNOR numbers/nucleus			
	n	Survived group	*n*	Died group
Tubular adenoma				
highly differentiated	7	4.64 ± 0.23	8	6.26 ± 0.72
		4.17-4.85		5.60-7.38
moderately differentiated	11	5.94 ± 0.92	7	8.44 ± 0.84
		4.33-7.10		7.47-9.55
low differentiated	4	7.65 ± 0.74	6	11.23 ± 0.74
		6.72-8.37		10.89-12.71
Mucous adenoma	5	6.33 ± 0.66	3	7.75 ± 0.39
		5.70-7.29		7.42-7.94
Other types[1]	3	9.64 ± 3.51	3	12.20 ± 0.39
		6.02-11.02		11.88-12.63
Total[2]	30	6.29 ± 1.83	27	8.76 ± 2.29
		4.17-11.02		5.60-12.63

[1]Survived group: One undifferentiated adenoma, one polypous adenoma, and one signet-ring cell adenoma. Died group: Two undifferentiated adenomas and one signet-ring cell adenoma. [2]t = 4.5115, P = 0.00, between two groups. AgNOR: Silver-stained nucleolar organizing regions.

pathohistological data from resected specimens, 56 cases had no metastasis, 47 had metastasis, and 11 cases were unclear in metastasis of lymphonodi. Among the cases of lymphonodi metastasis we studied, there were 39 cases of primary and metastatic cancers based on the AgNOR technique in identical slides. Fifty-seven cases were followed up for over 5 year , among whom 27 died and 30 survived. These preserved specimens of biopsy from patients with colonic cancer were obtained between 1984-1991 from the Department of Pathology, Sun Yat-sen Memorial Hospital, Sun Yat-Sen Medical University.

Methods

Preparation of AgNOR specimens. Tissues were fixed in 10% formalin solution and processed in paraffin wax. Each paraffin embedded block was cut into two 3 μm thick sections, with one routinely processed with hematoxylin and eosin stains and the other submitted to AgNOR staining according to Ploton's modification one step method[4].

Quantitative analysis. Sections were examined in 20 ×, 40 ×, and 100 × immersion lenses and 100 × oil immersion lens. Fields were selected at random for analysis, and 100 cells were examined continuously. Each nucleus was examined in four respects, as follows: (1) Number of nucleoli: The number of nucleoli and the AgNOR number besides nucleoli were recorded. A nucleolus was defined as an AgNOR dot, and the mean number of AgNOR dots was calculated. (2) Shape: In each specimen, the number of regular type and abnormal type of AgNOR dots in 100 nuclei was counted in accordance with the following standards: 1. Regular type: the shape of the AgNOR dots was circular, and the rim was more or less smooth, 2. Abnormal type: the shape of the AgNOR dots was a bar shape rhombus or otherwise strange, with a diameter longer than 3 μm. (3) Size: The size of the AgNOR dot of each nucleolus was measured and classified into one of three groups: Large (2 μm), medium (1 μm), and small (0-1 μm), as measured with a C2 net type objective ruler (Shanghai Third Optical Instrument Factory, Shanghai, China). And (4) Distribution: In each specimen, the distribution of AgNOR dots in 100 nuclei was classified, as follows: 1. Gathered type: Regular dots were gathered at the center of nuclei. Generally, there were less than four dots per nucleus. 2. Scattered type: Irregular dots were scattered in the nuclei like satellites, and there were more than five dots per nucleus. 3. Mixed type: Nuclei had the characteristics of both gathered and scattered types.

Statistical analysis. Analysis of variance (ANOVA) or Kruskal and Wallis rank sum test (H test) were employed to detect the differences among groups. Meanwhile, Student's t test or Wilcoxon rank sum test was performed to compare the differences between two groups. For paired designed data, either paired t test or Wilcoxon's signed rank sum test was used.

RESULTS

AgNOR was clearly recognized as black or brown dots in the nuclei or nucleoli after staining.

AgNOR and sex

The mean number of AgNOR dots per nucleus was 7.1 ± 2.3 in the male colonic cancer group and 6.9 ± 2.1 dots in the female colonic cancer group ($p > 0.05$).

AgNOR and age

In the age groups < 29, 30-59, and over 60, the mean number of AgNOR was 8.5 ± 2.3, 7.1 ± 2.2, and 6.4 ± 1.8 dots per nucleus, respectively. The number of AgNOR in the < 29 age group was significantly higher than that in the 30-59 and the over 60 group ($p < 0.05$), and there was no difference between the latter two age groups ($p > 0.05$).

AgNOR and location of cancers

The mean number of AgNOR per nucleus was 6.6 ± 1.8 at the sigmoid colon, 7.6 ± 2.2 at the descending colon, 7.3 ± 3.0 at the splenic flexure of the colon, 7.0 ± 2.4 at the transverse of the colon, 6.7 ± 1.3 at the hepatic flexure of colon, 6.7 ± 2.1 at the ascending colon, and 8.5 ± 2.9 at the ileocecum. There were no significant differences among these groups.

AgNOR and infiltrative degree

The mean number of AgNOR of the colonic cancers that had invaded different layers was as follows: mucosa 5.6 ± 0.6, submucosa and shallow smooth muscle, 5.3 ± 0.9, deep smooth muscle, 7.4 ± 1.8, serosa, and 8.2 ± 2.9 in outer serosa. The number of AgNOR in cancers that had invaded the mucosa and submucosa and shallow smooth muscles was less than that in cancers that had invaded the serosa and outer serosa ($p < 0.05$). The mean number of AgNOR in the deep smooth muscle group was significantly different from that in the serosa and outer serosa groups ($p = 0.00$ for both), but there was no difference among other groups ($p > 0.05$).

AgNOR and histological types

Different types of colonic cancers: The mean number of AgNOR per nucleus was 6.6 ± 1.9 in tubular adenomas, 6.5 ± 0.9 in mucous adenomas, 11.9 ± 1.3 in undifferentiated adenomas, 10.5 ± 1.1 in signet ring cell adenomas, 5.7 ± 0.5 in polypous adenomas, and 6.3 ± 0.2 in papillate adenomas. The AgNOR number per nucleus in tubular adenomas was significantly different from that in signet ring cell adenomas and undifferentiated adenomas (both $p < 0.01$). In addition, the AgNOR number for mucosa adenomas was significantly different from both signet-ring cell and undifferentiated adenomas. No differences were detected among the other groups. Regarding different degrees of malignancy of colonic cancer, the mean number of AgNOR per nucleus was 5.2 ± 0.9 in the low malignancy group, 6.5 ± 1.2 in the moderate malignancy group, and 9.3 ± 2.5 in the high malignancy group ($p < 0.01$ for all).

AgNOR and metastasis of lymphonodi

In the metastasis of lymphonodi positive group, the number of AgNOR per nucleus was more than that in the lymphonodi metastasis negative group (7.6 ± 2.1 *vs* 6.4 ± 2.0 $p = 0.00$), and the AgNOR of the lymphonodi metastatic cancers was more than those of cancers *in situ* (8.1 ± 2.1 *vs* 7.3 ± 1.9, $p = 0.00$).

AgNOR and clinical prognosis

The mean number of AgNOR in those who died (8.8 ± 2.3) was significantly higher than that in those who survived ($p = 0.00$), and there was no overlap between the range of AgNOR number in these two groups, although the histological type was similar. The size and distribution of AgNOR, but not the shape, were significantly different between the two groups (Tables 1-3, Figures 1-4).

DISCUSSION

In recent years, there have been a number of substantial studies on the relationship between AgNOR and the prognosis of various cancers. Several studies on cancers of the digestive system have

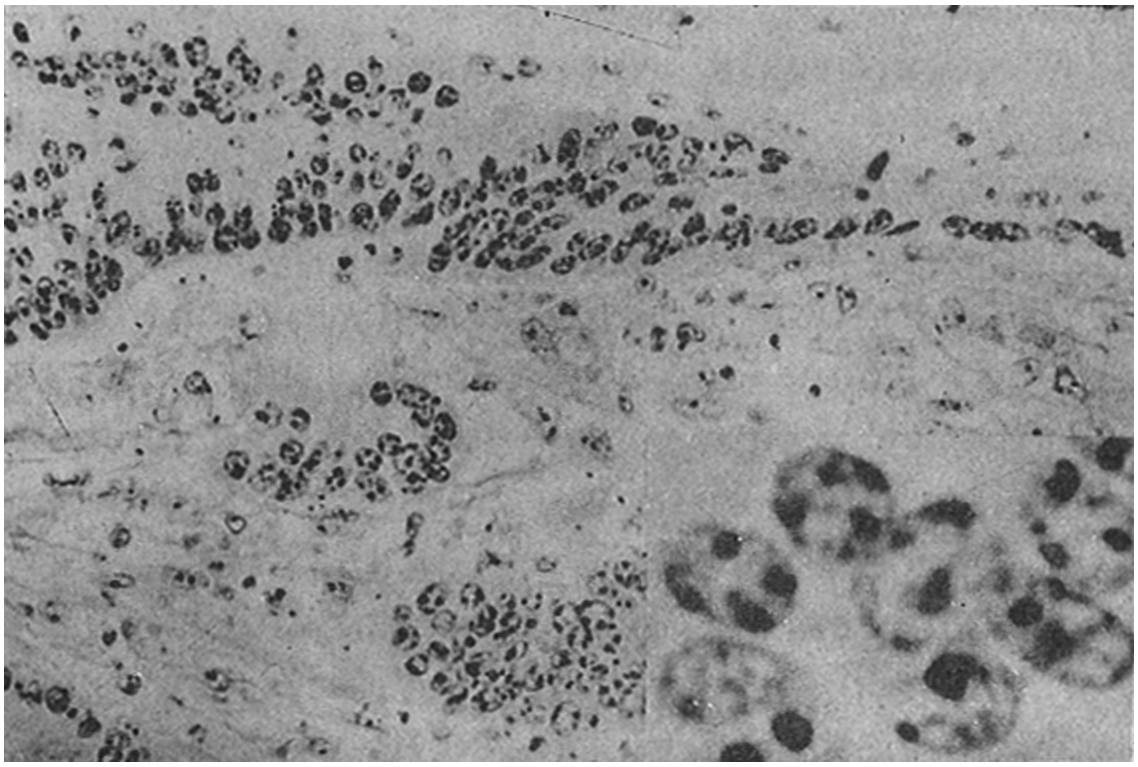

Figure 1 Moderately differentiated tubular adenomas (survived case) 10 × 20, 10 × 100).

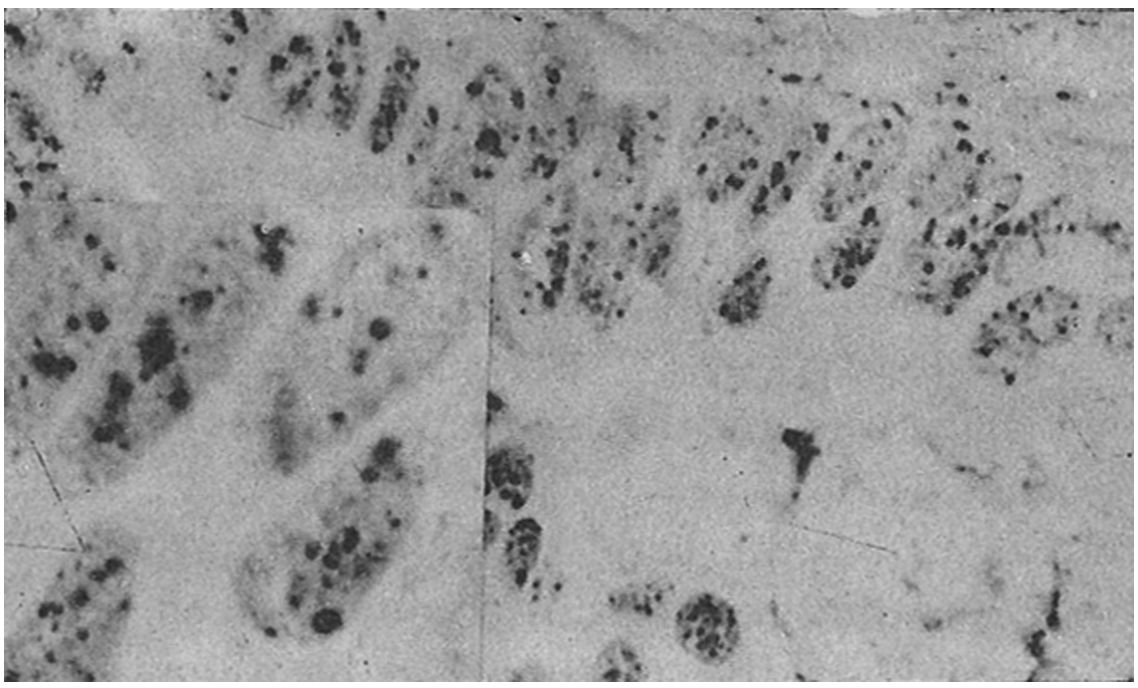

Figure 2 Moderately differentiated tubular adenomas (died case). The AgNOR numbers, abnormal dots, large and small dots, and scattered and mixed types are significantly increased compared with survived cases (10 × 40, 10 × 100). AgNOR: Silver-stained nucleolar organizing regions.

Table 2 The number of abnormal shape and giant silver-stained nucleolar organizing regions in died and survived groups ($\bar{x} \pm s$/100 nuclei)

Group	*n*	Abnormal shape AgNOR	Giant AgNOR
Survived	30	56.57 ± 19.67[1]	23.77 ± 14.89[2]
Died	27	67.00 ± 24.12	31.37 ± 16.15

[1]t = 1.7968, P = 0.08; [2]t = 1.8494, P = 0.0698 *vs* died group. AgNOR: Silver-stained nucleolar organizing regions.

Table 3 The size of silver-stained nucleolar organizing regions in died and survived groups ($\bar{x} \pm s$/100 nuclei)

Group	*n*	0 μm	1 μm	2 μm
Survived	30	398.30 ± 225.36	158.87 ± 75.74	71.67 ± 27.01
Died	27	661.70 ± 250.52[3]	128.00 ± 72.65[1]	85.89 ± 20.70[2]

[1]t = 1.6347, P = 0.1078; [2]t = 2.0958, P = 0.0407; [3]t = 4.1973, P = 0.0001 *vs* survived group.

suggested that the number of AgNOR in patients who died was significantly higher than those who survive[5], and that the number of AgNOR was increased in the cases of invasion and metastasis of cancer cells[6]. Griffiths *et al*[7] evaluated 100 cases of rectum adenomas and found no relationship between AgNOR number and prognosis, cell proliferation, and cell DNA ploidy. Liu *et al*[8] found that the number of AgNOR was significantly different between patients who died and those who survived non-Hodgkin's lymphoma, but there was some overlap between the two groups in a few cases. Eusebi *et al*[9] found that the mean area of AgNOR from short term (< 34 mo) breast cancer survivors was larger than that of long term (> 3 year) survivors, with no overlap observed between the two groups. Therefore, the utility of AgNOR in predicting cancer prognosis is controversial.

In this study we used AgNOR quantitative analysis to assess the prognosis of colonic cancer. We found that the relationship between AgNOR and clinical prognosis is of value in clinical practice, as the number of AgNOR in the group of patients who died was

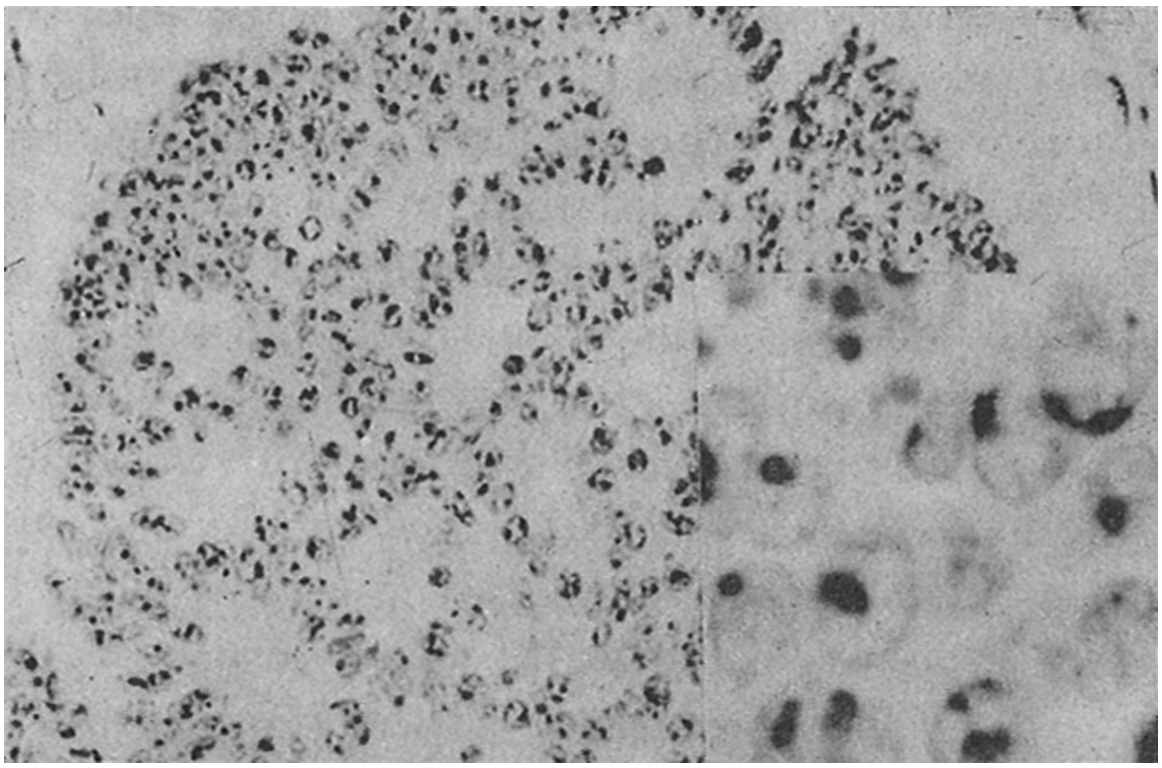

Figure 3 Moderately differentiated tubular adenomas (primary cancer of colon): 10 × 20, 10 × 100).

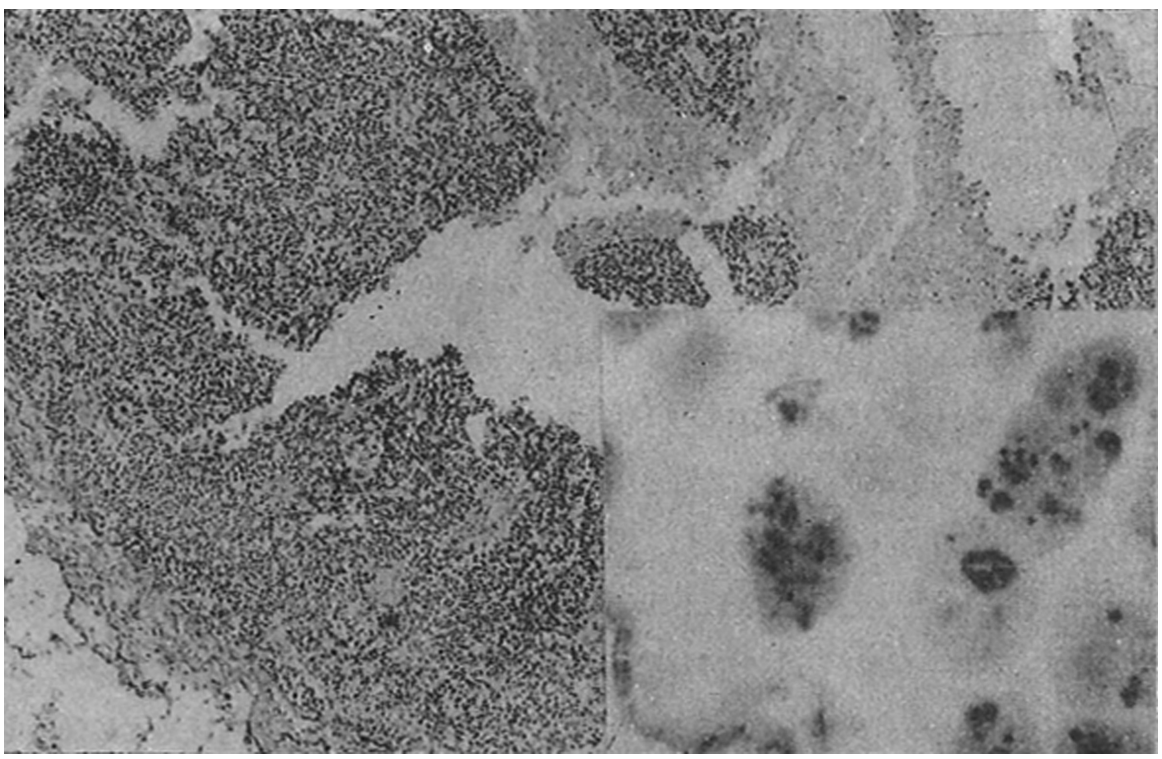

Figure 4 Moderately differentiated tubular adenomas (lymphonodic metastasis). Compared with the primary cancer, the AgNOR number, large and small dots, and scattered type are increased significantly (10 × 10, 10 × 100); AgNOR: Silver-stained nucleolar organizing regions.

significantly higher than that in the group that survived. In addition, the number of large and small dots increased; the distance between two polarities was increased (with the increase of small dots being most significant); and the distribution of gathered type decreased while the distribution of mixed type increased in the died group compared to the survived group. These results suggested that not only were the number of AgNOR different between the two groups but also their size and distribution. In addition, there was no overlap in the range of AgNOR numbers between the died and the survived groups, although the histological type was identical. These results were consistent with Eusebi's report. Moreover, we identified a relationship between AgNOR and correlative factors of colonic cancer prognosis. Some studies have suggested that the histological type of colonic cancer played a role in determining survival rate. Our data showed that the number of AgNOR was significantly different in different histological types of colonic cancers and that the number of AgNOR increased with the degree of malignancy. These results indicated that quantitative analysis of AgNOR could reflect cell proliferation, differentiation, and degree of malignancy and that clinical course process correlated well with survival.

Depth of invasion is one of the most important factors in colonic cancer prognosis, and prognosis was significantly different between the groups with shallow invasion and those with deep muscle, serosal, or beyond serosal invasion. Our study showed that the number of AgNOR per nucleus was significantly higher in the latter than in the former. These results also suggested that the number of AgNOR is correlated with the prognosis.

Lymph node metastasis is an important factor in the prognosis of colonic cancers. Bockmuhl *et al*[10] found that the number of AgNOR in breast cancer with lymph node metastasis was higher than that in breast cancer without lymph node metastasis. Kakeji *et al*[11] also found that the number of AgNOR was increased in gastric cancer when lymph node metastasis occurred. Our study showed that the number of AgNOR in the lymph node metastasis positive

group was higher than that in the lymph node metastasis negative group. The increase of AgNOR number in infiltrative and metastatic cancer cells suggested that those cells were more biologically active and that rRNA gene duplication and transcription activity was much greater. Therefore, AgNOR number contributed to the prediction of cancer cell invasion, metastasis, and relapse after surgery. We also found that AgNOR number of lymph node metastatic cancer was higher than that of primary colonic cancer. The results were consistent with those previously reported by Ohno *et al*[12] in lung cancer with metastasis of cartilaginous sarcoma. The phenomenon might correlate with the aberrance of cancer. More efforts are needed to explore the mechanism in colonic cancers. The prognosis of cancers located at the rectum was poorer than that located at the colon, and the prognosis of cancers in the ileocecum and ascending colon was better than that in other parts of the colon. No difference among other sites of the colon was found[3]. However, our study showed that the number of AgNOR was not different in any location of colonic cancers, suggesting that the number of AgNOR was not correlated with the site of colonic cancer. Regarding age, the prognosis in the group below 30 year old was poorer than that in the 30-59 year and over 60 year groups[3]. Our study showed that in the below 30 year group, the number of AgNOR was higher than that in 30-59 year and over 60 year groups, suggesting that the number of AgNOR was increased in young people who had poorer prognosis.

AgNOR is a marker of cell proliferation and rRNA transcription. Therefore, cancers with a higher number of AgNOR have more active cell proliferation. These cancers are exuberant, with more biological activity. The patient's condition advanced faster, and their prognosis was worse. In contrast, in those with cancer with a lower AgNOR number, the proliferation of cells was relatively slow with good biological activity. The patients' condition was stable, and the prognosis of patients was good. Moran *et al*[5]studied the prognosis of advanced colonic cancers and found that the traditional clinical and pathological indices were too difficult to use in the prediction of colon cancer prognosis. However, AgNOR were considered a reliable prognostic index. Ofner *et al*[13]studied AgNOR and several other prognostic indices of colonic cancer in post-operative patients and found that the value of AgNOR for predicting the prognosis of colonic cancers was more reliable and more accurate than that of the World Health Organization (WHO) classification system (UIC dividing terms, Jass classification and Duke's classification). Our study showed that AgNOR correlated well with clinical prognosis, age, histological types, the degrees of invasion, and lymphonodi metastasis. Between the died and survived groups with identical histological types, no overlap was observed. As shown in the present study, AgNOR quantitative analysis is a new, useful, and reliable index for predicting the prognosis of colonic cancers. In particular, it can be used for pathologic diagnoses before and after operation of colonic cancers. This analysis can also be used to direct treatment and to predict the metastasis and relapse of cancers. Taken together, our data suggest that AgNOR quantitative analysis has broad prospects for use in clinical practice.

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Domperidone improves gallbladder emptying function in patients with irritable bowel syndrome

Shi-Zhu Chen, Xu-Chung Chen, Wan-Xi Liu, Zhang-Shan Yang, Xiao-Ling Guo

Shi-Zhu Chen, associate professor, Director of Fifth Department of Internal Medicine, member of International Gastro-Surgical Club, having 53 papers published, 11 of them in UK, USA and Greece. Chinese PLA 451 Hospital, 39 Youyidonglu, Xi'an 710054, Shaanxi Province, China

Xu-Chung Chen, Zhang-Shan Yang, Department of Ultrasonics, Chinese PLA 451 Hospital

Wan-Xi Liu, Vice President, Chinese PLA 451 Hospital

Xiao-Ling Guo, engineer, Department of Information, Chinese PLA 451 Hospital

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Correspondence to: Dr. Shi-Zhu Chen, Associate professor, Director of Fifth Department of Internal Medicine, member of International Gastro-Surgical Club, having 53 papers published, 11 of them in UK, USA and Greece. Chinese PLA 451 Hospital, 39 Youyidonglu, Xi'an 710054, Shaanxi Province, China
Telephone: +86-29-5252350-372

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Abstract

AIM: To investigate the pathogenesis of abnormal gallbladder (GB) emptying and the effect of domperidone (Dom) on GB emptying in patients with irritable bowel syndrome (IBS).

METHODS: The effects of DOM on GB emptying were studied in 20 IBS patients and 18 healthy controls by real time ultrasonography, using randomized, double-blind, and controlled methods.

RESULTS: Fasting GB volume was significantly higher in IBS patients than in controls (24.136 ± 1.38 cm^3 *vs* 19.793 ± 1.487 cm^3, $\bar{x} \pm s_{\bar{x}}$, $p < 0.01$). In controls, 30 min after 10 mg Dom orally, the GB ejection fraction (GBEF) was decreased significantly ($p < 0.005$), and the magnitude of this decrease was greater after 20 mg Dom. The difference between these two doses was not significant ($p > 0.05$). In IBS patients, GBEF was significantly increased 15 min after 10 mg Dom orally ($p < 0.01$), and the magnitude of this increase was greater with 20 mg Dom ($p < 0.001$). This difference was even more marked with prolongation of time after oral Dom. The GBEF in IBS patients with segmental contraction was significantly less than that with hypermotility ($p < 0.01$), and the increase of GBEF was more marked after oral Dom in IBS patients with segmental contraction than those with hypermotility ($p < 0.01$).

CONCLUSION: GB emptying function is abnormal in patients with IBS. The feeble contractility of the GB and/or the incomplete relaxation of Oddi sphincter may be factors that directly affect GB emptying in IBS patients. Dom can significantly improve GB emptying function and may decrease the risk of forming GB stones in these patients.

Key words: Colonic diseases, functional; Gallbladder; Domperidone

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INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic disease with smooth muscle dysfunction in many organs[1-4]. In IBS patients, abnormal gallbladder (GB) emptying function, increased fasting GB volume (GBV), and incomplete postprandial GB emptying have been found[5,6]. Nevertheless, the pathogenesis of abnormal GB emptying remains unknown, and there is no reasonable treatment strategy for improving GB emptying in IBS patients. Domperidone (Dom), a dopamine receptor antagonist, can promote stomach emptying[7], but it is not clear whether it can improve GB emptying in IBS patients. There are adrenergic and cholinergic receptors in the smooth muscle of the GB and the Oddi sphincter (OS)[8], and it is assumed that there may be dopamine receptors in these sites, thereby explaining the effect of Dom on GB emptying. Because decreased GB emptying is a predisposing factor for GB stone formation, it is necessary to investigate the pathogenesis of abnormal GB emptying in patients with IBS and to observe the effect of Dom on GB emptying. Here, we performed a randomized, double-blind, controlled study using ultrasonography to measure GBV and to investigate the effect of Dom on GB emptying.

MATERIALS AND METHODS

Subjects

Twenty patients with IBS (IBS group) and 18 healthy controls (control group) were included in this study, and these two groups were comparable for age, sex, height, and body weight (Table 1).

All patients were randomly selected inpatients, and their disease courses were between 2 to 28 (mean 8.7) yr without any operation history. Clinical, laboratory, and other examinations revealed no organic pathologic process and no gastrointestinal disorders induced by diabetes mellitus, *etc*., and IBS was diagnosed in accordance with the criteria[9]. According to criteria[10] based on main

Table 1 Characteristics of patients and controls studied

Characteristics	Patients (n = 20)	Controls (n = 18)
Mean age (a)	33.15 ± 2.16	32.85 ± 2.25
(Range)	(18-56)	(22-54)
Males/Females	11/9	9/9
Mean weight (kg)	59.45 ± 1.47	57.77 ± 1.26
(Range)	(46.5-70.4)	(47.0-67.5)
Mean height (cm)	165.50 ± 1.13	166.80 ± 1.21
(Range)	(155.5 ± 178.2)	(154.6 ± 179.0)
Mean body index (kg/m^2)	22.24 ± 1.21	22.07 ± 1.26
mass (Range)	(16.24 ± 26.21)	(17.69 ± 24.82)

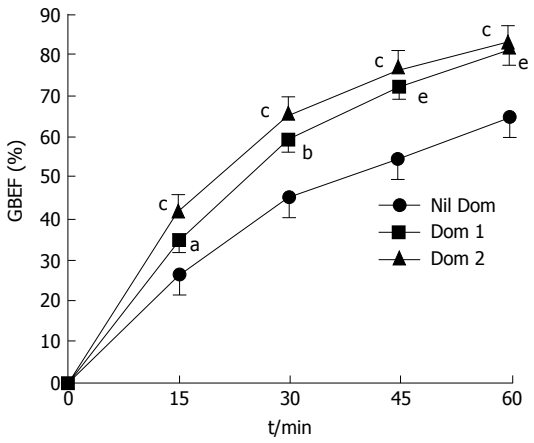

Figure 1 Gallbladder (GB) emptying after oral domperidone in 20 irritable bowel syndrome (IBS) patients after a meal stimulus. ● no drug, or ■ 10 mg Dom and ▲ 20 mg Dom. $^aP < 0.05$, $^bP < 0.01$, $^eP < 0.001$, *vs* no Dom. GBEF: Gallbladder ejection fraction; Dom: Domperidone.

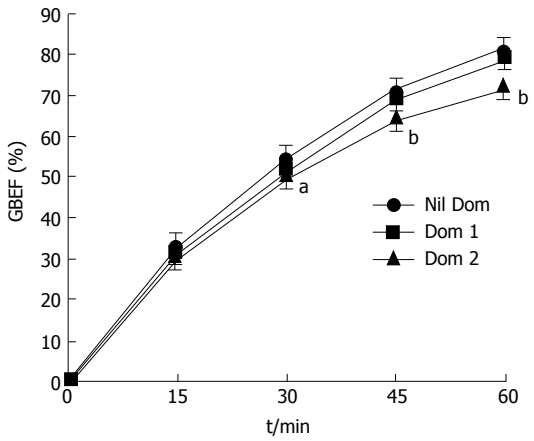

Figure 2 Effect of oral Domperidone on meal induced Gallbladder emptying in 18 controls. ● no Dom or ■ 10 mg Dom and ▲ 20 mg Dom. $^aP < 0.05$, $^bP < 0.01$, *vs* no Dom. GBEF: Gallbladder ejection fraction; Dom: Domperidone.

manifestations of the digestive tract, nine patients were classified with hypermotility type (in which the main manifestations were diarrhea or accompanied by abdominal pain), seven patients were classified as segmental contraction of intestine type (in which the main symptoms were abdominal pain and dry feces, the frequency of bowel movement might not decrease), and the remaining four cases could not be classified into the other two categories and were included in a separate group. All drugs were stopped 3 d before ultrasonographic examination.

Healthy subjects (Table 1) were randomly selected healthy volunteers who had no disease in the livers, GB, pancreas, or gastrointestinal tract, and all had normal tests for blood sugar and lipids. Alcohol was avoided 3 d before and during the study.

GB ultrasonography

All subjects fasted for 12 h and were examined using the same real time ultrasonographic unit (SSD-630, Hitachi Aloka Medical Ltd., Tokyo, Japan). GBV was determined according to the method of Jonderko *et al*[11] by the same investigator who did not know the aims and contents of the study and the diagnosis of the patients. The examination was carried out in the same position, same place (skin guide marks were used in each case), and same phase of respiration each time for each subject. GBV was calculated by the formula.

$V = 1/6\pi \cdot L \cdot W \cdot H$

Where: V is GBV, L is GB length (sagittally), W is width, and H is GB height. The GB ejection fraction (GBEF) was calculated by the formula for each considered interval after the meal stimulus:

$GBEFt = (Vf - Vt)/Vf \times 100\%$

where: GBEFt is GB ejection fraction at time t (t = 15, 30, 45, 60 min), Vf is before the meal GBV, Vt is GBV at time t.

Study design

Drug preparation: The powder of 10 mg (Dom_1) and 20 mg (Dom_2) (product of Janssen Pharmaceutical Ltd, Xi'an, China) was filled in a separate capsule of the same color and size by a pharmacist, and bottled and labeled as "three" and "four".

GBV measurement: All subjects received ultrasonography separately at 8:30 AM in the recumbent position, and the fasting GBV was determined three times and the mean values were taken to be a reference value. Then, a fatty meal (260 mL cream and egg containing 35 g fat) was given to the subject to take within 2 min. The GBV was determined at 15 min intervals for 60 min. On a separate day, the fasting GBV was determined in the same way as above. Afterwards, an investigator, who did not know the name of the drug in the capsule, randomly took a capsule (the number and order were recorded) and gave it to the subject to take. Ten minutes later, the fatty meal was given as previously. A small drink of water (50 mL) was allowed to facilitate the passage of the capsule along the esophagus. The subjects lay on a couch while the GBV measurement was being taken, and they were allowed to move freely between measurements.

Statistical analysis

GBV (cm^3) and GBEF (%) are expressed as $\bar{x} \pm s$. Paired Student's t test was used to compare the difference between two groups, and linear regression was used to analyze the relationship between variables. $p < 0.05$ (two sides) was considered to be statistically significant.

RESULTS

All subjects completed the study. The fasting GBV in the IBS group (24.136 ± 1.138 cm^3) was significantly higher than that in controls (19.793 ± 1.487 cm^3) ($p < 0.01$). GB emptying function and the effect of Dom on GB emptying in IBS patients and controls are shown in Figures 1 and 2, Table 2.

Comparison of GBEF in patients with IBS of different subtypes (Table 3)

DISCUSSION

The present study found that there was a significant abnormality in GB emptying function and an increased fasting and postprandial GBV in IBS patients compared to healthy controls. These measures were also remarkably different among the different subtypes of IBS. Oral Dom treatment improved GB emptying and increased GBEF, and these effects were more remarkable for Dom_2 than for Dom_1. In healthy controls, oral Dom_1, and especially Dom_2, decreased GB emptying Dom_2; and the decrease at 60 min after the meal reached the level of IBS patients who did not take the drug. This suggests

Table 2 Comparison of Gallbladder ejection fraction after oral domperidone in irritable bowel syndrome patients and controls

Drug	Subjects	15 min	30 min	45 min	60 min
No drug	patients	25.93 ± 2.06[b]	44.54 ± 2.49[b]	54.16 ± 1.87[b]	65.11 ± 2.10[b]
	controls	32.63 ± 2.34	53.79 ± 2.32	70.32 ± 1.85	80.13 ± 1.81
Dom 1	patients	34.26 ± 1.42[a]	58.37 ± 1.77[b]	71.22 ± 1.50[a]	79.66 ± 1.09[e]
	controls	30.63 ± 2.40	50.68 ± 3.05	67.72 ± 1.95	77.74 ± 1.09
Dom 2	patients	41.10 ± 1.55[e]	64.98 ± 1.03[b]	74.81 ± 1.45[a]	82.74 ± 1.32[b]
	controls	29.63 ± 1.38	49.34 ± 2.24	63.26 ± 1.69	70.95 ± 1.45

[e]$P > 0.05$, [a]$P < 0.05$, [b]$P < 0.01$, *vs* controls. Dom: Domperidone.

Table 3 Comparison of Gallbladder ejection fraction after oral domperidone in irritable bowel syndrome patients with different subtypes

Drug Type	15 min	30 min	45 min	60 min
Nil Hypermotility	26.68 ± 2.83	47.61 ± 2.62	58.18 ± 2.31	69.80 ± 1.87
Type Segmental contraction	29.20 ± 2.68[a]	40.98 ± 2.39[b]	49.49 ± 2.51[c]	60.96 ± 1.92[c]
Others	24.42 ± 1.82[b]	24.42 ± 1.82[b]	48.43 ± 1.64	55.43 ± 1.72[b]
Dom 1 Hypermotility	33.34 ± 2.15	62.04 ± 2.11	73.99 ± 1.76	82.27 ± 1.86
Segmental contraction	35.11 ± 1.66	57.90 ± 1.63	69.34 ± 1.87	77.45 ± 1.59[b]
Others	31.34 ± 2.63[b]	55.89 ± 2.51	68.29 ± 2.03	77.64 ± 1.83
Dom 2 Hypermotility	40.09 ± 2.22	66.02 ± 1.91	79.69 ± 1.70	84.78 ± 1.70
Segmental contraction	38.87 ± 1.81[a]	62.93 ± 1.65[b]	74.62 ± 1.64[b]	81.38 ± 1.78[a]
Others	43.91 ± 2.06	69.71 ± 1.81[b]	77.91 ± 1.55[b]	83.01 ± 1.58

[a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$. Dom: Domperidone.

that dysfunction of GB smooth muscle or OS and intestinal smooth muscle is similar in IBS; and that the factors that are inhibited by Dom in healthy patients may be the same as those affected in IBS patients. Sood *et al*[5] observed that there was no significant difference in GB emptying between patients with predominant diarrhea and those with constipation. This is in contrast to our results, and this difference may result from either (1) variance with sampling or (2) classification of our IBS patients by not only symptoms but also the mechanism of inducing symptoms[10] because the underlying mechanisms may be different in different subtypes[12,13]. In addition, our patients were randomly collected, and the number was limited. Not all subtypes were included, and the number of cases of some subtypes was relatively small. Therefore, the GB emptying function in patients with IBS of different subtypes and the exact effects of Dom on GB emptying in them remain to be determined.

GB emptying is a complex process involving many factors with many components, being controlled by nerves, regulated by hormones[14,15], and influenced directly by the functional conditions of GB and OS. Of these, the most important is the GB contraction, OS relaxation, and coordination of the two. There are α and β cholinergic receptors, cholecystokinin (CCK) receptors, and others[16]in the wall of GB and OS. Immunohistochemical studies have documented the presence of dense concentrations of neuropeptide containing myenteric nerves in the OS[17]. Physiologic studies have confirmed that these peptides exert significant effects on biliary motility. In healthy people, the entry of a fatty meal into the duodenum results in release of CCK, which causes the GB to contract, the OS to relax, and the GB to empty; GBEF can reach 80% or more 60 min after the meal. The mechanism by which GBEF decreases after oral Dom may be *via* Dom-induced OS contraction or inhibition of relaxation of OS, making OS relaxation weak. Dom antagonism of the β or dopamine receptors in OS blocks the excretion of bile from the biliary duct. In the present study, we did not determine the concentrations of CCK and other neuropeptides in blood and the pressure of OS before and after oral Dom. Also, we do not know if there were changes of CCK release from intestinal mucosa or differences in the number and sensitivity of the receptors, such as β, CCK, *etc*., in GB and OS[18] because there were abnormal changes in these aspects in IBS patients. In addition, the disorder of gastrointestinal motility was frequent in IBS patients. Since it is known that Dom promotes stomach emptying[7], it is possible that changes in GB emptying occur after oral Dom in our patients. According to reports by Duan *et al*[19] and Guelrud *et al*[20] and the results gained in the present study, healthy persons and IBS patients respond differently to Dom, so the effect of stomach emptying on GB emptying function can be excluded. Our main finding is that Dom can strengthen the contraction of weakened GB smooth muscle in IBS patients and at the same time may cause the OS to relax through stimulating dopamine receptors.

The present study demonstrates that IBS is a chronic systematic dysfunctional disease involving all smooth muscles[1] and that its pathogenesis may differ within the subtypes. Oral Dom can significantly improve GB emptying in IBS patients. Our study has revealed a role for abnormal GB emptying function in the pathogenesis of IBS, providing support for the use of Dom clinically in the treatment of IBS.

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REVIEW

Clinical application of 5-HTa-3R antagonist tropisetron in chemotherapy patients

Chang-Tai Xu, Bo-Rong Pan

Chang-Tai Xu, Physician in Charge, Director of the Department of Gastroenterology and Haematology, Chinese PLA 473 Air Force Hospital, 216 Anning donglu, Lanzhou 730070, Gansu Province, China

Bo-Rong Pan, Professor of Internal Medicine, Room 12 Building 621, Fourth Military Medical University, 17 Changlexilu, Xi'an 710033, Shaanxi Province, China.

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Correspondence to: Dr. Chang-Tai Xu, Physician in Charge, Director of the Department of Gastroenterology & Haematology, Chinese PLA 473 Air Force Hospital, 216 Anning donglu, Lanzhou 730070, Gansu Province, China

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INTRODUCTION

The prevention and control of vomiting and nausea is of major clinical importance, particularly in the realm of oncology, where intractable vomiting and nausea may severely limit the use of cytotoxic therapy. For the patient, the most distressing aspect of chemotherapy is its associated nausea and vomiting, and this may cause refusal of further therapy, even when potentially curative[1]. This is particularly true of cisplatin, which is used in the management of solid tumors and is regarded as the most emetogenic of the cytotoxics (Table 1).

PHARMACOLOGICAL PROPERTIES

Chemistry and composition

Navoban, also known as (1H-indol-3 carboxylic acid 8-methy1 - 8-azabicyclo[1-3] oct 3α-Y1-eater), has the molecular formula $C_{17}H_{20}N_2O_2HCl$. Navoban was derived from a modification to 5-HT_3, the indole moiety being used as the nucleus. Like other 5-HT_3 receptor antagonists, navoban has a 6.5 aromatic nucleus connected to a basic nitrogen atom *via* a carbonyl group and a 4-atom unit, and it is thought that the aromatic nucleus may be responsible for the blockade of the 5-HT_3 receptor. Navoban is a white, crystalline powder with good stability, and it has a shelf-life of 3 year. No special packaging or protective condition is required for storage of navoban.

Both navoban capsules and 5 mL ampules, for intravenous administration, contain 5.64 mg of navoban hydrochloride, which is equivalent to 5 mg of the base.

Activity at 5-HT3 receptors

The 5-HT_3 receptors are involved in nausea and vomiting, gastrointestinal motility, anxiety, drug dependency, schizophrenia, the intradermal flare response, the blister-base pain response, and mediation of the von Bezold Jarisch reflex.

In their study comparing zacopride (a metoclopramide analogue) with navoban and ondansetron, Conhen *et al* (1989) used the inhibition of serotonin-induced bradycardia in rats as a measure of duration of action. Oral navoban maintained its inhibition of serotonin-induced bradycardia for 3 h, with heart rate reverting to "normal" (control) levels by 6 h. In contrast, inhibition by oral ondansetron persisted for less than 3 h.

Studies of post-mortem human brain revealed that the nucleus of the solitary tract, the vagus nerve, and the spinal trigeminal nucleus were rich in 5-HT_3 receptors. However, it is the finding that the area postrema possesses the highest density of these receptor sites that is of interest, for this area is known to be important in the vomiting reflex, supporting a central site of action for the 5-HT_3 antagonists.

Antiemetic effects

Gamse (1990) reviewed the early work on acute cisplatin-induced vomiting in humans and concluded that "total inhibition of emesis after a single 5 mg dose…occurred in 80% of [those] receiving 50-100 mg/m^2 cisplatin and in 50% of those treated with > 100 mg/m^2." Two or less than two episodes of vomiting occurred in 92% and 90% of patients, respectively.

The mechanism by which cytotoxic drugs induce vomiting is thought to involve the release of serotonin by damaged intestinal enterochromaffin cells, the subsequent activation of vagal afferents, and the initiation of the vomiting reflex. This theory is supported by studies in which the concentrations of plasma

Table 1 Emetogenic potential of chemotherapeutic agents; 5 = highest, 1 = lowest[1]

Grade 5		Grade 4		Grade 3		Grade 2		Grade 1
Drug	Dose (mg/m^2)	Drug	Dose (mg/m^2)	Drug	Dose (mg/m^2)	Drug	Dose (mg/m^2)	Drug
Cisplatin	> 100	Cisplatin	50-100	Dactinomycin	> 0.3	Dactinomycin	< 0.3	Amsacrine
				Carboplatine	> 150	Altreamine		Asparaginase
				Carmustine	> 75	Carboplatin	≤ 150	Bleomycin
				Chlormethine	> 6	Carmustine	≤ 75	Etoposide
				CyClo		Chlormethine	≤ 6	Fluorouracil
				Phosphamide	> 50	Cisplatin	≤ 50	Mercaptopurine
				Cytarabine	> 1000	Cyclo		Methotrexate
				Dacarbazine	> 100	Phosphamide	≤ 50	Mitomycin
				Daunorubicin	> 45	Cytarabine	≤ 1000	Mitoxantrone
				Doxorubicin	> 45	Dacarbazine	≤ 100	Procarbazine
				Epirubicin	> 75	Daunorubicin	≤ 45	Teniposide
				Ifosfamide	> 1000	Doxorubicin	≤ 45	Thioguanine
						Epirubicin	≤ 75	Vinorelbine
						Fotemustine		Vinblastine
						Ifosfamide	≤ 1000	Vincristine
								Vindesine

Abbreviations: A agents are classified as Grade 1 irrespective of dose.

Table 2 Pharmacokinetic parameters of navoban in poor and extensive metabolizers, normalized for a 10 mg dose

Parameter	Extensive metabolisers		Poor metabolisers	
	IV (n = 18)	Oral (n = 43)	IV (n = 36)	Oral (n = 12)
T_{max} (h)		2		3.6
C_{max} (μg/L)	84	21.7	82	29.9
AUC (μg/L·h)	239	230	1192	1579
$t_{1/2\beta}$ (h)	7.3	8.6	30.3	41.9
V_β (L)	554		463	
F (%)	100	60-100[a]	100	60-100[a]

[a]: Dose dependent; T_{max}: Time to maximum plasma concentration; C_{max}: Maximum plasma concentration; AUC: Area under plasma concentration-time curve; $t_{1/2\beta}$: Terminal phase elimination half life; V_β: Volume of distribution during termination phase; F: Bioavailability.

serotonin and urinary 5 hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, were increased in patients experiencing cisplatin induced vomiting. Selective inhibition of 5-HT$_3$ receptors by navoban could interrupt the vomiting reflex at one or more of the following sites[4]: (1) gastrointestinal receptors; (2) afferent vagal fibers; (3) brain-stem receptors, either in the chemoreceptor trigger zone (CTZ) or the vomiting center; and (4) afferent vagal fibers. Navoban is likely to exert its influence on the peripheral, afferent, and central components of the reflex[4].

PHARMACOKINETIC PROPERTIES

Absorption, plasma concentration and distribution

After oral administration, the absorption of navoban is rapid, with a mean absorption half-life of approximately 20 min. More than 95% of a 100 mg dose is absorbed within 2.2 h[4], and peak plasma concentrations are reached within 3 h. As a result of a saturable metabolic capacity, bioavailability is dose dependent: a dose under 15 mg is 60% bioavailable (under fasting conditions, 75%-80% is bioavailable when taken with food), and doses of ≥ 45 mg are 100% bioavailable. Despite individual variability, the bioavailability is similar for capsules and oral solutions.

Metabolism

Navoban undergoes little hepatic first-pass metabolism. The drug is oxidized mainly at positions 5, 6, or 7 of the indole ring, with hydroxy metabolites being further metabolized to glucuronides and sulfates. N-demethyl and N-oxide navoban are detected in trace amounts only. The metabolism of navoban in humans is linked to the polymorphically expressed cytochrome P-450 IID6 enzyme system, which also determines the metabolism of sparteine, debrisoquine, and other drugs, such as neuroleptics, β blockers, tricyclic antidepressants, and antiarrhythmics. The ratio of "extensive" to "poor" metabolizers in Western populations is approximately 12:1. In extensive metabolizers, between 8% and 9% of a 20 mg dose of navoban is excreted unchanged in the urine, 70% as metabolites, and 15% in the feces, almost entirely as metabolites. The metabolites of navoban are not pharmacologically active[4].

Poor metabolizers excrete a greater proportion of unchanged navoban in the urine than their extensive metabolizer counterparts. While this suggests a potentially greater risk of side effects related to drug accumulation, this risk is negligible when navoban is administered at the recommended dosage (5 mg per day for 6 d)[5], and the side effect profiles of extensive and poor metabolizers are comparable. Therefore, similar doses may be given to both groups and, in the same way that patients need not be screened before initiation of β blocker therapy, screening for poor metabolizers before administration of navoban is not a requirement. The pharmacokinetic parameters for extensive and poor metabolizers are detailed in Table 2[4].

Elimination

The elimination half-life of navoban (oral and intravenous) in extensive metabolizers is about 8 h (range from 7.3-8.6 h). By comparison, the elimination half-life for oral and intravenous ondansetron ranges from 3.2-3.7 h and for intravenous granisetron ranges from 3-4 h (in healthy volunteers) or 10-12 h (in cancer patients)[6]. The relatively long half-life of navoban allows for effective once daily dosing; while the oral forms of granisetron and ondansetron, by comparison, require a bid and tid (or qid) dose, respectively.

Drug interactions

Protein binding is moderate (59%-71%), implying that drug-drug interaction due to displacement of the drug from plasma binding sites is unlikely[4]. Navoban does not induce or inhibit

cytochrome P-450 dependent enzymes that are not induced or linked to the IID6 polymorphism, but P-450 enzyme-inducing drugs, such as rifampicin, phenobarbital, and phenylbutazone, increase the elimination and shorten the half-life of navoban. Extensive metabolizers who are on concomitant therapy with such drugs may, therefore, need a higher dosage of navoban to achieve effective plasma levels[4]. In contrast, liver enzyme inhibitors, *e.g.*, cimetidine, have a negligible effect on navoban plasma concentrations, and the customary 5 mg/d dosage of navoban need not be altered.

The pharmacokinetics of navoban in elderly patients are similar to those of younger patients. Ondansetron, by contrast, has been reported to undergo slower clearance in the elderly[7]. No dosage adjustment is required in patients receiving navoban.

Metabolic clearance of navoban in patients with hepatitis and fatty liver disease is similar to that in healthy extensive metabolizers but is lower (by 50%) in cirrhotic patients[4]. Nonrenal clearance of avoban is reduced, again by half, in those with moderate or severe renal dysfunction. However, when the recommended 6-day course of 5 mg daily is administered, even a 25% loss of hepatic function or risk associated with accumulation of the drug, no dosage adjustment is required in patients with hepatic or renal impairment[4].

Recent studies indicated that navoban (maximum 5 mg/d) is well tolerated by pediatric patients and that the efficacy and tolerability results in children are similar to those in adults[8,9].

Relation between plasma concentration and clinical efficacy

A clear dose response relationship has been demonstrated with the 5-HT_3 antagonists. A navoban dose finding study that compared navoban doses of 5, 10, 20, and 40 mg failed to demonstrate significant differences between the doses for total control of acute nausea and vomiting, with all doses achieving complete control in more than 50% of patients. It has, however, been estimated that a plasma concentration of more than 3 μg/L of navoban is necessary for inhibition of ≥ 90% of 5-HT_3 receptors.

NAVOBAN IN CLINICAL PRACTICE

The development of the 5-HT_3 receptor antagonists has irrevocably changed the treatment of chemotherapy induced nausea and vomiting. Nausea and vomiting are now clearly differentiated, and, with the successful treatment of vomiting, nausea has become the primary therapeutic target in this field of research[10]. Alleviation of nausea and vomiting in patients treated with cytotoxic drugs is of major importance, since this side effect of anticancer therapy may cause the patients considerable distress and may even cause the patient to refuse or delay further courses of such therapy[1].

Some of the general guidelines for effective antiemetic therapy in chemotherapy patients can be summarized as follows: (1) prevention of nausea and vomiting is easier than treatment of established nausea and vomiting; (2) complete abolition of symptoms is required if anticipatory vomiting is to be prevented; and (3) the choice of antiemetic depends on the relative emetogenic potential of the cytotoxic agent used.

Dose finding studies

In multicenter, dose finding studies, navoban (2 and 5 mg and 5, 10, 20, or 40 mg) was administered to patients receiving cisplatin chemotherapy. Total control of nausea and vomiting in the first 24 h was achieved in up to 71% of patients. The 5 mg dose of navoban was more effective than the 10, 20, or 40 mg doses and also more effective than the 2 mg dose, which was apparently subtherapeutic in some patients. Furthermore, on day 1 of chemotherapy course 1, the 5 mg dose was able to achieve total or major control of vomiting and nausea in a greater percentage of patients than the 2 mg dose (86% *vs* 68%, $p = 0.055$ and 92% *vs* 86%, respectively, NS). Comparison of the efficacy of the 5 mg and 2 mg doses in successive courses of this study is questionable, since strict treatment failure criteria caused a reduction (by about 50%) in the efficacy population in courses 2 and 3 (Stamatakis *et al* 1990).

The dosing of navoban is simple. The optimal daily dose for the prevention of nausea and vomiting associated with chemotherapy is 5 mg[4], a single intravenous dose of navoban being sufficient to protect most patients for at least 24 h after chemotherapy.

Navoban in acute nausea and vomiting

Acute nausea and vomiting occur within the first 24 h after the onset of chemotherapy. They usually begin within 1.5 to 3.0 h and last 2-6 h. The effects of navoban on acute nausea and vomiting in cancer patients receiving chemotherapy have been assessed in dose-finding, non-controlled studies.

The antiemetic effects of the optimal dose of navoban (5 mg), as defined in dose finding studies, have already been discussed. Stamatakis *et al* (1990) and ven Belle *et al* (1990) reported that in the first 24 h after chemotherapy (course 1), complete control of cisplatin induced vomiting in 70% and 71% of patients and complete control of nausea in 65% and 71 %, respectively, was achieved with a single intravenous dose of navoban. Stamatakis *et al* (1990) found that with 5 mg navoban, 86% and 92% of patients achieved total or partial control of acute vomiting (≤ two episodes) and nausea (≤ 2 episodes). In a small subgroup of patients on high dose cisplatin (> 90 mg/m^2), navoban (5 mg) achieved 100% total plus partial control of vomiting in the first 24 h.

In their open label study of 476 patients who were refractory to standard antiemetic therapy and who were receiving chemotherapy of varying emetogenic potential, Bleiberg *et al*[1] reported that 62% of patients on day 1 of course 1 had a complete response (no nausea and no vomiting) to pretreatment with navoban (5 mg or 10 mg) by intravenous injection. The number of patients with a complete or partial response (1-4 vomiting episodes and/or episodes of nausea) was as high as 91%. These results are in line with those of a similar study involving patients on cisplatin and non-cisplatin regimens, 67% of whom were completely protected from nausea and vomiting on day 1 of course 1. An additional 27% had a partial response, raising the complete plus partial response result to 94%[11].

When comparing the results of chemotherapy naïve patients with those of patients with prior experience of chemotherapy, Sorbe *et al*[11] found that the former had a higher rate of control (73% *vs* 61%, $p < 0.02$) of acute nausea and vomiting on day 1, and fewer of the naïve group required rescue therapy as a result of treatment failure than did their non-naïve counterparts (3% *vs* 33%, $p < 0.0001$). Bleibery *et al* reported a complete response (no vomiting and no nausea) in 73% and 63% ($p = 0.05$) of chemotherapy-naïve and non-naïve patients, respectively.

Sorbe *et al*[11]demonstrated that navoban was able to achieve complete control of acute nausea and vomiting in 51% of cisplatin-treated patients, compared with 78% of non-cisplatin treated patients ($p < 0.001$). In contrast, however, Bleiberg *et al*[1] found that the emetogenic grade of chemotherapy did not significantly affect the antiemetic response.

Dogliotti and colleagues[12] also administered navoban to patients receiving cisplatin and, although they chose severity of nausea rather than duration, their results for complete control of nausea and vomiting can be compared with those from other studies. They reported that a single intravenous dose of 5 mg navoban afforded

Table 3 Intensity of delayed vomiting on days 1 and 2 after cisplatin therapy with navoban (20 courses) or metoclopramide plus lorazepam (20 courses)

Vomiting	Navoban (% of courses)	Metoclopramide (% of courses)
Day 1	$n = 18$	$n = 19$
No vomiting episodes	75	30
1 to 2 vomiting episodes	10	5
> 2 vomiting episodes	15	65
Day 2	$n = 2$	$n = 5$
No vomiting episodes	90	75
1 to 2 vomiting episodes	10	10
> 2 vomiting episodes	0	15

complete protection from acute nausea and vomiting in 44% and 53%, respectively, of 104 courses.

Comparative efficacy studies have usually compared navoban with metoclopramide monotherapy or metoclopramide-based cocktails. In a study comparing navoban with a metoclopramide-based regimen in chemotherapy naïve patients placed on a non-cisplatin course, 5 mg navoban provided complete protection from acute vomiting for significantly more patients than the comparative regimen (46% *vs* 22%, $p = 0.013$). Complete protection from acute nausea, too, was greater with navoban, although the difference between the two treatments was not statistically significant (25% *vs* 12%). Total or partial control of vomiting occurred in 67% of navoban treated patients compared to only 46% of metoclopramide-treated patients, and this difference was statistically significant ($p = 0.044$). The difference between treatments for complete or partial control of nausea was not significant (63% *vs* 57%).

Dogliotti *et al*[12] studied the acute and delayed antiemetic effects of navoban compared with metoclopramide plus lorazepam in patients receiving cisplatin and reported that for both acute nausea and acute vomiting, navoban was significantly more effective than metoclopramide plus lorazepam ($p < 0.001$). Complete control of vomiting and nausea was achieved in 75% and 40% of navoban patients, respectively, compared with 30% and 30% of metoclopramide patients.

In another study involving 172 patients, McVie *et al*[13] compared navoban with a metoclopramide based antiemetic regimen in the control of vomiting in the first 24 h after cisplatin or cisplatin based chemotherapy. Before chemotherapy, patients treated with the antiemetic cocktail received 2 mg/kg metoclopramide and 20 mg dexamethasone by intravenous infusion plus either oral 50 mg diphenhydramine or 1 mg lorazepam; 4 h later a second, identical dose of metoclopramide with diphenhydramine or lorazepam was administered. Metoclopramide 10 mg tid was given either orally or by suppository on days 2 to 7. Navoban and the control of acute vomiting in 50% and 60% of patients, respectively. Compared with navoban, the metoclopramide-based cocktail achieved significantly superior control of acute nausea (rate of total control 54% *vs* 31%).

In their multicenter, randomized study, Bruntsch *et al*[14] compared the acute antiemetic efficacy of navoban and conventional antiemetic regimens, including dopamine antagonists, antihistamines, tranquillizers, and steroid in 231 patients who had responded poorly to previous antiemetic therapy and who were treated with a variety of chemotherapeutic agents. Navoban was significantly more effective in providing complete protection from acute vomiting (53% *vs* 29%, $p < 0.001$); it was also more effective than the conventional antiemetic therapy for acute nausea, reducing the duration of nausea by 2.5 h ($p < 0.001$) and totally controlling nausea in a significantly greater percentage of patients than the comparative regimens (32% *vs* 19%, $p < 0.05$)[15]. These results confirm those of an earlier interim analysis of the study population[13].

Navoban has also been compared with alizapride for its antiemetic effects in high-dose alkylating agent chemotherapy (cyclophosphamide or melphalan) and was shown to be more effective in providing complete control of acute vomiting (13% *vs* 6%), while the addition of the antidopaminergic haloperidol to navoban further enhanced its antiemetic efficacy[16].

Navoban in delayed nausea and vomiting

Delayed nausea and vomiting are associated with the highly emetogenic chemotherapeutics and occur more than 24 h after the start of chemotherapy. In general, the effects are less severe than the acute form, but they may persist for up to 7 d. For this reason, the use of navoban may be of particular importance in the outpatient setting.

Other studies have shown a somewhat different pattern. McVie *et al*[13]found that control of delayed vomiting was comparable for navoban and a metoclopramide-based cocktail, although metoclopramide was superior in its protection from delayed nausea ($p = 0.003$). Dogliotti *et al*[12] showed that navoban was significantly more effective in controlling nausea and vomiting the day after cisplatin, although by the following day, both nausea and vomiting were less pronounced and the between-treatment difference had diminished (Table 3).

Both patients and investigators rated navoban efficacy highly: 71% of patients and 72% of investigators scored it "very good" or "good", compared with 32% of patients and 31% of investigators in the optimal standard antiemetic group. Thus, even in a population selected for previous treatment failures, navoban was highly effective[15].

A recent study evaluated the antiemetic efficacy of navoban in combination with dexamethasone, both given for 6 d, in patients who achieved only partial control with navoban monotherapy[17]. Chemotherapy-naïve patients who received two identical courses of chemotherapy were assessed according to stringent efficacy criteria. The results of the study showed that patients who were completely controlled by navoban in course 1 were also well controlled in course 2. Those who were incompletely controlled in course 1, however, benefited from the addition of dexamethasone in course 2.

Navoban in multiple courses of chemotherapy

The question of whether or not an antiemetic can maintain its efficacy over several courses of chemotherapy arises, since most patients receive more than one course of chemotherapy. Furthermore, patients inadequately protected from nausea and vomiting in one course are more likely to respond poorly to antiemetic agents in subsequent courses of chemotherapy. A certain reduction in efficacy over multiple courses of chemotherapy may, therefore, be expected. Sorbe *et al*[11]confirmed the ability of navoban to maintain efficacy over multiple courses in patients who received up to 10 courses of chemotherapy, of which only half were chemotherapy naïve. Complete plus partial control of nausea and vomiting was achieved in 90%-100% of patients on day 1 of each

course, with a slightly reduced response on days 2 to 4.

Some factors may be important in predicting patient response to navoban: age, gender, previous chemotherapy, alcohol abuse, and emetogenic potential of the chemotherapeutic agent have all been previously cited[18]. Bleiberg *et al*[1]. analyzed the impact of various factors on antiemetic response in their study of patients who had previously been refractory to standard antiemetic therapy. The emetic grade of chemotherapy had a statistically non-significant effect on the response to navoban, and while non-naïve patients responded well to navoban, their chemotherapy naïve counterparts achieved significantly better response rates.

One thousand seventy two patients who were scheduled to receive at least two identical cycles of emetogenic chemotherapy were treated with 5 mg navoban once daily in their first chemotherapy course. Complete response rates (no nausea and no vomiting) were 72% for day 1 and 48% for days 1 through 6 of course 1. During course 2, more complete responders were observed when dexamethasone was added, both for day 1 (76% *vs* 66%, $p = 0.020$) and for days 1 through 6 (50% *vs* 34%, $p = 0.0004$). A moderate increase in the complete response rate was seen with the addition of conventional dose alizapride (day 1, 75% *vs* 68%, $p = 0.14$; day 1 through 6: 47% *vs* 37%, $p = 0.041$). Doubling the dose of navoban did not change the complete response rate. These data show that the addition of dexamethasone significantly increased the complete response rate of both acute and delayed emesis in patients who have incomplete disease control with navoban alone[19].

Dosage and administration

The recommended dosage of navoban is 5 mg daily for 6 d. The first dose, on day 1, should be administered intravenously shortly before chemotherapy, either as an infusion (1 ampule diluted in 100 mL of a common infusion fluid, such as normal saline, Ringer's solution, 50 g/L glucose, or 50 g/L levulose) or as a slow injection. Thereafter, on days 2 to 6, a single 5 mg oral capsule should be taken with water immediately on rising and at least 1 h before eating. Patients known to be poor metabolizers do not need to alter the recommended 6 d course of 5 mg navoban[5]. Similarly, as indicated in the basic prescribing text, the recommended dose need not be reduced in the elderly or in patients with impaired hepatic or renal function. The dosage regimen for navoban is currently the most simple and convenient of any for the 5-HT_3 antagonists.

SAFETY AND TOLERABILITY

The integrated safety results of two navoban dose finding and five comparative treatment studies have been summarized by de Bruijn *et al*[4]. In total, 417 patients received 5 mg navoban daily, 51 patients received metoclopramide, and 222 received an antiemetic cocktail, in which the dose of metoclopramide was approximately twice that of metoclopramide monotherapy.

Navoban was generally well tolerated at the recommended dose of 5 mg daily for 6 d. As with the other 5-HT_3 antagonists, headache, constipation, diarrhea, and fatigue were the most frequently reported adverse effects, but their relation to antiemetic therapy was not easily assessed, since aggressive chemotherapy or the cancer itself could have accounted for some symptoms. Only headache and constipation with abdominal pain recurred in the same patients with repeated courses of navoban, which suggests that these symptoms were in fact related to the administration of the drug.

Adverse effects (headache, constipation) were mild, seldom requiring symptomatic treatment, and withdrawal of navoban because of adverse effects was rare (0.2%); reports of "extrapyramidal symptoms" (*e.g.*, as ataxia, tremor, and cramps) were not only extremely uncommon (0.3%) but were not clearly attributable to navoban. The side effect profile of navoban did not alter with repeated administration over several courses[11]. There was no evidence of: (1) laboratory of electrocardiogram abnormalities at the recommended dose; (2) induction of liver enzymes; and (3) exacerbation of cisplatin nephrotoxicity, neurotoxicity, or bone marrow suppression.

The concomitant administration of navoban with liver enzyme inducers, such as rifampicin and phenobarbital, may result in a lower plasma concentration of navoban. While extensive metabolizers may require an increase in the dosage of navoban, this is not the case for poor metabolizers. In contrast, liver enzyme inhibitors, such as cimetidine, have a negligible effect on navoban plasma concentrations; the customary 5 mg/d dosage of navoban need not, therefore, be altered.

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CASE REPORTS

Caliber persistent artery of the stomach: An unrecognized cause of massive gastric hemorrhage

Tu-Xing Chen

Tu-Xing Chen, Associate Professor of Internal Medicine, having 72 papers and three books published. Department of Gastroenterology, the First Affiliated Hospital, Nanjing Medical University. 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, China

Author contributions: Chen TX solely contributed to this work.

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Correspondence to: Dr. Tu-Xing Chen, Associate Professor of Internal Medicine, having 72 papers and three books published. Department of Gastroenterology, the First Affiliated Hospital, Nanjing Medical University. 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, China
Telephone: +86-25-3428186

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INTRODUCTION

The caliber persistent artery of the stomach, also known as aneurysm, Dieulafoy's exulceration simplex, submucous arterial malformation, gastric arteriosclerosis, and peptic ulcer of peculiar location, was once considered to be a rare cause of massive gastric hemorrhage. Recently, however, the number of reports on caliber persistent artery of the stomach have been increasing, suggesting that this lesion is probably underdiagnosed and not a rare cause of massive gastric hemorrhage[1-3]. Two patients with caliber persistent artery of the stomach were admitted to our department 2 years ago, and diagnosis was confirmed subsequently by surgical operation and endoscopy and angiography.

CASE REPORT

Case 1

A 64-year-old man with a past medical history of about 1 wk use of self prescribed acetylsalicylic acid (4.5 g daily) for relieving pain from cholecystitis and cholelithiasis presented to our department with hematemesis consisting of 600 mL of bright red blood accompanied by melena. Emergency endoscopy showed a large amount of blood clots in the gastric cavity, but no active hemorrhage was noted. On admission, physical examination revealed pallor of skin, a heart rate of 100 beats/min, and orthostatic hypotension. The results of laboratory tests showed anemia (hemoglobin 78 g/L). Over the following 7 d, he continued to bleed despite the administration of omeprazole (Losec) and thrombin. Endoscopic examination was repeated, revealing a solitary superficial erosion in normal appearing gastric mucosa near the esophagogastric junction on the posterior wall, and a 0.2 cm × 0.2 cm fresh red tissue was seen at the base, with no active hemorrhage. The patient received altogether about 7000 mL blood transfusion but still had persistent liquid dark stools with recurrent loss of consciousness. Thus, a surgical intervention was given. During the operation, a protruding artery was seen at the posterior wall of the body of the stomach near the cardia. There was a small blood clot on it, and a large amount of blood spurted out as the blood clot was removed. The patient underwent oversewing and ligation of the ruptured vessel and Bancroff operation (Billroth Ⅱ). Histological examination showed submucous arterial malformation. The patient tolerated the operation well and was discharged 2 wk after the operation.

Case 2

A 49-year-old man experienced his first episode of hematemesis, consisting of 500-1000 mL of bright red blood followed by melena (about 300 g) two times. No history of complaints regarding the gastrointestinal tract was present. He was not taking medications before admission and denied intake of nonsteroidal anti-inflammatory drugs (NSAIDs) and ethanol. On admission, his blood pressure was 12/6.5 kPa, heart rate was 90 beats/min, and hemoglobin was 81 g/L. Emergency endoscopy performed on admission disclosed a large amount of fresh and coagulated blood in the esophagus and gastric and duodenal cavities, but the blood source was not obvious. Conservative management, including blood transfusion, histamine H_2 blockers, and thrombin was instituted. The patient still had recurrent massive hematemesis at times associated with melena. His blood pressure fell to 8/4 kPa. A selective angiography performed on day 2 of hospitalization showed the leakage of contrast from a branch of the gastroduodenal artery. The patient underwent surgical, during which a small, round mucosal defect with a protruding tissue at its base was observed in the small curvature of the body of the stomach. Spurting bleeding from the protruding artery was also be seen. In the gastric cavity, 200-300 mL of fresh blood and about 500 g of blood clots were found. The vessel was ligated and oversewn. Postoperative recovery was excellent, and the patient was discharged from the hospital.

DISCUSSION

Caliber persistent artery of the stomach was first described by

Gallord in 1884 and was later characterized by Dieulafoy in 1896 as the exculceration simplex. This lesion is characterized as follows: a negative past medical history, independence from peptic ulcer disease, sudden onset, increasing bouts of hematemesis, subcardial location, a tiny mucosal lesion, an open submucous artery of seemingly large caliber, failure of conservatism in treatment, and 60.5% overall lethality[1]. Although it used to be considered a rare cause of massive gastric hemorrhage, more recently this lesion has been identified more frequently. Therefore, it is likely to be an under-recognized and undiagnosed cause than a rare cause of massive gastric hemorrhage[1-4]. Its incidence as a source of upper gastrointestinal bleeding was shown to range from about 0.3%-6.7%[5]. Pointner *et al*[6] found 22 of 1432 patients and Baettig *et al*[7] found 28 of 480 patients (5.8%) with severe gastrointestinal hemorrhage who underwent emergency endoscopy. Here, we present two cases that we have seen in the recent two years. Thus, physicians' awareness of the existence of this lesion should be heightened to improve the likelihood of uncovering this "unknown cause" of massive gastric hemorrhage.

Caliber persistent artery of the stomach has been reported in a wide age range of patients, from 16 to 93 years[3,8], with a moderate male predominance[1,9]. In Pointner's 22 cases, eight cases were female, and 14 were male, ranging in age from 29 to 80 years. Nine of these patients were older than 60 year[6]. Our two patients were males, 64 and 49 year old.

The bleeding site for this lesion is often within 6 cm of the gastroesophageal junction in the cardia or fundus of the stomach[10]. In Reilly's review, approximately 98% of the lesions located in the stomach were in its upper portion. Sixty-seven percent were located high in the body and 25% in the gastric fundus[5]. The recent reports have identified similar lesions in the duodenal bulb[11], the jejunum[12,13], and in the right colon[14]. Endoscopically, the gross appearance of the lesion is that of a solitary lentil sized, round mucosal defect with a protruding artery at the base[6,15]. Juler *et al*[4] used multiple tissue staining techniques and showed the following histopathological characteristics: (1) a gastric mucosal defect with fibrinoid necrosis at the base; (2) large thick walled artery loops in the base of the defect; (3) a tortuous dysplastic artery below the muscularis mucosa; (4) large thick walled veins adjacent to the artery; and (5) lymphoid aggregates in the lamina propria.

The typical clinical presentation of this lesion is recurrent and massive hematemesis at times associated with melena, hematochezia, and hypotension. Of the 177 cases described in the literature, 28% presented with hematemesis alone, 51% had hematemesis accompanied by melena, and 18% had melena alone[5]. There have been inferences in the literature regarding associations with the use of alcohol and NSAIDS. In our case 1, the patient had a past medical history of 1 wk use of acetylsalicylic acid before gastric hemorrhage. It is assumed that the drug injured the mucosa of the stomach, which then triggered a rupture of the arterial branch in the gastric submucosa.

The caliber persistent artery of the stomach is the most dangerous forms of gastrorrhagia. It was observed that 100% of patients who were treated conservatively (*i.e.*, nonsurgically, nonendoscopically) died as a result of hemorrhagic shock[15]. The mortality rate of the cases published since 1980 ranged from 40.0%-60.5%[1]. Since this lesion has an extremely high mortality rate, it is necessary for the physicians to consider the existence of the lesion when conservative therapy fails in a patient with a sudden onset massive gastric hemorrhage, and a careful examination should be given as soon as possible. Recently, some authors[6,16,17] have pointed out that emergency endocopy is the most effective method of diagnosing the lesion. About 82% of the patients with caliber persistent artery of the stomach were identified with endoscopy. Forty-nine percent of lesions were identified during the initial endoscopic examination, while 33% required a second endoscopy to confidently identify the lesion as the source of bleeding[5]. However, the source of the bleeding was not identified with endoscopy in 18% of patients evaluated[5]. An alternative diagnostic method to assist in the diagnosis of this lesion is angiography. According to the literature, this technique was used in 14 patients, and it was helpful in the localization of the bleeding site in 11 patients[2,11,12,14,18-20]. Seven of the patients had lesions in the stomach, while the remaining four had one in either the duodenal bulb, jejunum, or ascending colon[11,12,14]. The source of bleeding in our case 2 was not identified initially by the endoscopy but verified subsequently by angiography, showing that angiography is also an effective diagnostic tool for localizing the lesion.

Regarding treatment, surgical intervention is the treatment of choice[21]. The most widespread surgical measures used are oversewing and wedge resection[9,15]. These are safe and often successful procedures[9]. Endoscopic therapy included epinephrine injection[7,9,15], polidocanol injection[6], bipolar electrocoagulation[6,9], heater probe coagulation[9], and YAG laser photocoagulation[9]. According to a review by Reilly *et al*[5], endoscopic therapy was utilized in 79 patients, and permanent hemostasis was accomplished in 85% of these patients following the first therapeutic endoscopy session. Twelve patients (15%) re-bled. Repeat endoscopic therapy was successful in eight patients (10%), and surgical intervention was needed in four patients (5%). Stark *et al*[9] reported that endoscopic therapy was successful in 18 of 19 patients (95%), and there were neither deaths due to bleeding nor endoscopic complications. Baetting *et al*[7] have followed up 21 patients treated by endoscopy for a mean of 28.3 months, and, of these, 20 patients had no recurrence of hemorrhage. Angiography with gel-foam embolization is another therapeutic option available for the treatment of caliber persistent artery of the stomach. With this technique, successful results were achieved in three out of four patients[18,19].

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Esophageal Crohn's disease: A case report

Guo-Hang Yu, Zheng-Gao Su, Jiang Zou, Yuan-Ben Wang

Guo-Hang Yu, Zheng-Gao Su, Jiang Zou, Yuan-Ben Wang, Department of Gastroenterology, Chinese PLA 271 Hospital, Tianjin 300191, China

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INTRODUCTION

Crohn's disease is relatively rare and may occur in any part of the digestive tract. However, according to domestic reports, less than 10% of the disease is found in the upper digestive tract and even less in the esophagus. Here, we report the case of an 81-year-old man who was originally diagnosed with esophageal cancer because of progressive dysphagia. After he was admitted to our hospital, we carried out a series of examinations and diagnosed him with esophageal Crohn's disease. We discuss the differential diagnosis of esophageal cancer and esophageal Crohn's disease.

CASE REPORT

An 80-year-old man began to have progressive dysphagia in Feb 1992. In June, he was able to take only a small amount of liquid food without any other symptoms. The evident esophageal stenosis, located 30 cm from incisor teeth, was found by gastroscopy, and the gastroscope could not be passed through it. There was about 5 cm esophageal mucosa stiffness, and the area above the stenosis appeared dirty. Biopsy revealed that the mucosa had congestion and edema, and granulation tissue was found in the submucosa. A clinical diagnosis of esophageal cancer was made. Since then, his dysphagic symptoms gradually subsided without any treatment, and the patient was asymptomatic for the next 26 mo. At this time, his condition began to rapidly deteriorate until 1994. He had difficulty drinking water and vomited mucous secretion frequently. Weight loss amounted to 10 kg. The results from a second gastroscopy were similar to those of the first. Clinical diagnosis was still esophageal cancer. General treatment was of no use, and he was admitted to our hospital for endoscopic treatment on June 20, 1994. During history taking, he said: "I am healthy" and denied having any suffering or other symptoms. Physical examination revealed that his temperature, pulse, and blood pressure were normal; and no obvious positive findings were found on systemic examination, except for some weight loss and a narrowing of his chest. Laboratory examination of blood, urine, and stool was routine; and plasma albumin and globulin, alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatine, blood sugar, serum potassium, and sodium chloride were all normal. Erythrocyte sedimentation rate (ESR) was slightly elevated, being 22 mm/h. Stool occult blood was negative. There was fungus present in the esophageal mucosa. X-ray chest film, electrocardiogram, and ultrasonic electrocardiogram were normal. B type ultrasonic examination showed one 3.5 cm × 3.5 cm stone in his gallbladder. The third gastroscopy done on June 29 revealed evident esophageal stenosis 32 cm distal to the incisor teeth, with a luminal diameter of approximately 1 mm. Findings proximal to the stenosis were the same as that on first examination. Biopsy reported an abundance of inflammatory necrotic tissue that contained fibrotic cells and granulation tissues, with blood capillaries and inflammatory cells. Normal esophageal mucosa was not found (Figure 1). In order to understand the condition of the stenosis in the lower esophagus, we used 38% biligrafin to examine the esophagus both by mouth and through gastroscope pouring. X-ray film revealed an almost complete obstruction. Its margin was smooth and complete, and there was no mucosal destruction (Figure 2). There was a 4 cm string like stenosis below the obstruction; but its margin was complete, and the cardia opened normally (Figure 3). Initial differential diagnosis: 1. esophageal Crohn's disease; 2. esophageal cancer; or 3. gallbladder stone (inactive stage). On July 7, a trial treatment was carried out to target esophageal Crohn's disease under careful observation by intramuscular injection of dexamethasone (10 mg twice daily). The patient's dysphagic symptoms gradually improved after 3 d. He could take solid food after 6 d. However, on day 6 of treatment, he felt epigastric pain and suddenly vomited blood. Emergency gastroscopy revealed that the original esophageal stenosis disappeared. The mucosa of the middle and lower part of the esophagus appeared whitish, thickened, and coarse. There were superficial erosions and an ulcer. A ring like stenosis, approximately 5 cm above the cardia, was found; but the gastroscope could not get to the stomach smoothly, and there was a lot of retained bloody fluid. After the fluid was cleared away, many small erosions and blood oozing sites were found spread over the stomach fundus and body, with atrophic changes over the whole stomach, including the fundus, body, and antrum. The pylorus was slightly deformed, and there was evident congestion at the duodenal bulb. Blood oozing stopped after spraying with mansa's solution and thrombin. Endoscopic differential diagnosis: 1. esophageal Crohn's disease; 2. acute gastric mucosal disease; and 3. chronic atrophic gastroduodenitis. After emergency gastroscopy, dexamethasone was reduced to 5 mg twice daily by intramuscular injection. Then, the patient instead received prednisone 50 mg/d and a proton pump

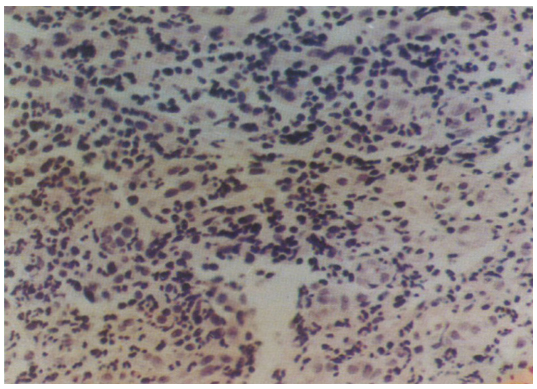

Figure 1 Inflammation granular tissue (HE × 200).

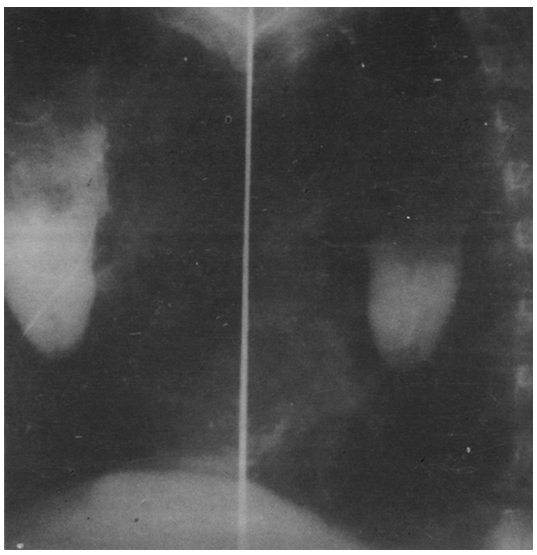

Figure 2 Esophageal obstruction piace is blind and no mucosa destroyed.

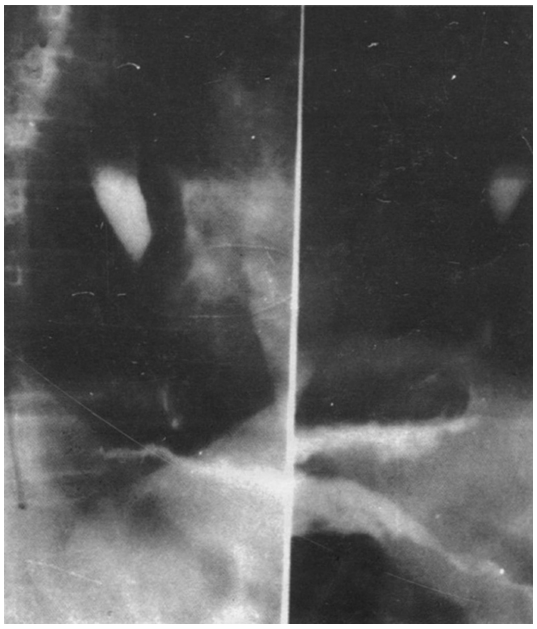

Figure 3 Esophageal intubation injection of contrast medium. There is a line-like stenosis blow the obstruction.

inhibitor and De Nal. The patient had no symptoms afterwards. Barium meal examination was performed, and no evident diseases were found below the esophagus 30 d after treatment. The fifth gastroscopy revealed that the ring-like stenosis still existed 32 cm distal to the incisor teeth, but the gastroscope could not pass through. There were a few superficial ulcers on the esophagus, but erosions of the stomach fundus and body disappeared, leaving some atrophic change. Biopsies from the antrum, fundus, and body

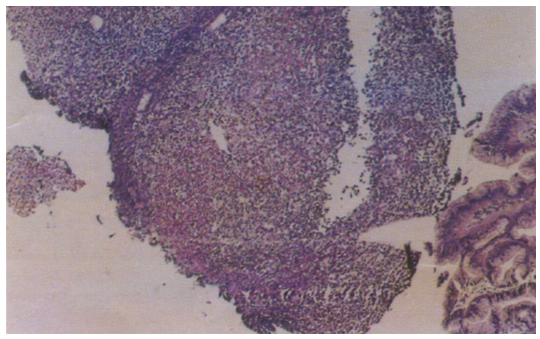

Figure 4 Local part seems to be a crack-like ulcer (HE × 45).

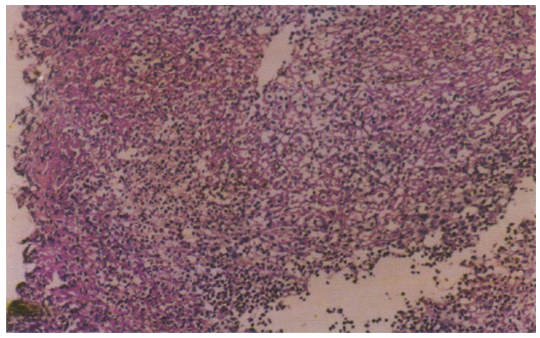

Figure 5 Local part seems to be a crack-like ulcer (HE × 75).

of the stomach and esophagus found: (1) chronic atrophic gastritis; (2) presence of inflammation and granulation tissue on the mucosa of the lower esophagus. Ulceration was considered, and some parts seemed to crack like ulcers. These findings were consistent with esophageal Crohn's disease (see Figures 4 and 5). The patient took prednisone continuously and left the hospital without any symptoms. The total hospital stay was 52 d. After discharge from the hospital, he continued to take prednisone, and his condition remained very good. The symptoms only reappeared when he stopped taking medicine, and they disappeared when the patient continued to take medicine. Follow-up has been done for one year, and the patient is still healthy.

DISCUSSION

Pathological diagnosis is considered the gold standard for detection of Crohn's disease. During their research on 41 children suffering from Crohn's disease and 47 children from ulcerative colitis with pathological changes in the upper digestive tract, Runska *et al* found that only 12 children developed Crohn's disease granuloma (one case in the esophagus, eight in the stomach, and three in the duodenum), all of which were confirmed by pathological diagnosis. Therefore, symptoms only cannot rule out Crohn's disease and ulcerative colitis, and pathological diagnosis is crucial. Based on medical records of 24 children of the same age with Crohn's disease, Schmidt-Sommerfeld *et al* pointed out that if a patient is suspected of suffering from Crohn's disease, biopsy and pathological examination were required even though the mucosa looked normal during gastroscopy. Otherwise, the histological changes of the disease might be neglected, and a definite diagnosis would be delayed. In our case, the reason why the patient was misdiagnosed as esophageal cancer three times was that changes of the stenosis of the esophageal mucosa initially could not be detected because of the severe degree of stenosis of the esophageal lumen. It was only after trial treatment when the esophageal obstruction disappeared was a biopsy of the stenosis taken, allowing for the final diagnosis of Crohn's disease to be established. It is only by pathological diagnosis that Crohn's disease can be definitely differentiated from esophageal cancer, especially in elderly patients.

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Rectal Bleeding caused by Haemadipsa japonica: First case report in China

Ji-Tuan Xu

Ji-Tuan Xu, Physician in Charge, having 4 papers published. President of Nanzhnng Hospital of Nanhai City, Guangdong 528210, China

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CASE REPORT

A 56-year-old male was admitted to the hospital because of lower gastrointestinal tract bleeding. He was previously diagnosed with internal hemorrhoids on May 27, 1989. Two days prior to admission to the hospital, he had a sense of a foreign body, with itching and bleeding in his right eye, upon returning home after cutting firewood in the mountains. The following day he had 10 episodes of bloody stools, with approximately 30 mL of fresh blood every time, but without bowel movement, mucus, tenesmus, or fever. The local hospital treated him with hemostatic drugs and blood transfusion for 3 d, but the bleeding did not stop. He was then transferred to our hospital (physical exam: T 37.0 ℃, P 86/min, BP 14/8 kPa). Patient looked acutely ill and anemic, with bleeding in the right eye. From that eye, one Haemadipsa japonica (Hj), about 1.0 cm × 0.2 cm, was removed. A corneal ulcer was found with visual disturbance. The pupils were round and equal in size and reactive to light. His neck was soft, and lungs and heart were normal. His abdomen was flat and soft, and the liver and spleen were not palpable. No mass was present on palpation. There was pain on pressure and rebound tenderness over the left lower abdomen. Bowel sounds were normal. There was a small amount of fresh blood around the anus, and no external or internal hemorrhoids were found by endoscopy. There was one Hj (1.5 cm × 0.2 cm) in the rectum, about 8 cm distant from the anus. In order to avoid additional bleeding, we treated the patient with non-operative methods, including blood transfusion, oral hemostatic drugs, and honey. On the second day after treatment, the bleeding stopped. The final diagnosis was lower gastrointestinal tract hemorrhage caused by Hj. The patient recovered and was discharged from the hospital after 6 d.

DISCUSSION

Hj, *Desmodium racemosum (Thunb) DC*, *Hedysarum racemosum Thunb*, grows in the dark and moist forest along the mountain valley and gully of Guangdong and Guangxi Provinces and other coastal areas. It is approximately 1.5 cm × 0.2 cm, and it has a sucker as teeth. It can attach on the skin surface of the human body and sucks blood. It can swing a distance of up to 1-2 meters. When it swings to the skin surface, it can climb into and hide in the human cavity organs, such as nose, anus, vagina, and external auditory canal. Once attached, it sucks the blood, and its toxin can destroy the coagulation mechanism. Common hemostatic drugs have no effect on this organism. According to a folk recipe, honey may cause Hj to lose its activity, having a hemostatic effect when perfused into the affected cavity organ. This method was used in our patient with satisfactory results.

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