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AIMS AND SCOPE

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

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COMMENTARY

Current research and development of integrated traditional Chinese and Western Medicine in gastroenterology

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Ke-Ji Chen, Professor in integrated Traditional Chinese and Western medicines, especially Qing Rcyal medicine and anti-ageing herbal medicines having 100 papers published with several China national/Ministry of Public Health academic awards the Albert Einstein world award and Taiwan Li-Fu TCM award, an academician of Chinese Academy of Sciences and Chinese Academic Degree Council (Medicine), a standing member of the committee of Chinese association for Science and Technology, the President of the Chinese Association of Integration of Traditional and Western Medicines and Vice-President of Geriatrics Society of Chinese Medical Association, a WHO consultant on Traditional medicine and advisor for Phytother Res (United Kingdom) and Abstr Chin Med (Hong Kong).

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Abstract

Traditional Chinese medicine (TCM) is one of China's scientific treasures. We are very fortunate to apply modern scientific knowledge and methodology towards reorganizing our inherited TCM and to then promote the integration of TCM and Western medicine (TCM-WM). Over the 45 years, many remarkable achievements have been made in this regard and we are looking forward to the next achievements in the coming century; however, many challenges remain to the establishment of TCM-WM as a trusted and oft used approach for improving human health.

Key words: Gastroenterology; Integrated Traditional Chinese Medicine (TCM); Western Medicine

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DEVELOPING NEW AND HIGHLY-EFFECTIVE MEDICINES

To date, 12807 kinds of traditional Chinese medicines (TCMs) and botanical medicines exist in China. Investigating new drugs from those resources, based on the TCM theory and long-term clinical experience, is a promising endeavor. Previous new drugs identified by this approach include Artemisine, anisodamine, biphenyl diester, trichosanthin, tetrahydropalmatine, ligustrazine, tanshinone, puerain, Erycibe obtusifolia alkaloid agrimophol, alizarin, ilexonin A, tetrandrine, total glycerides of tripterygium wilfordii, and ferulic acid. Among these, artemisine is capable of killing plasmodia of pernicious malaria rapidly and has been promoted as a malaria treatment by the World Health Organization (WHO).

In the 1980s, the United States, the United Kingdom and Japan developed new medicines from natural materials with beneficial therapeutic activities such as anti-tumor and anti-viral (for HIV). There are two different attitudes in the medical field regarding this trend. Some people believe that this is one of the ways to develop new medicines, while others entertain the entirely different view that this may serve to convert TCMs into the "Western medicine" practice. I personally believe that we should insist on having an open mind toward various considerations in scientific research. To develop new and highly effective medicines, we should not only pay attention to their economic value at home but also their competitive ability in the international market.

To reach this goal, we must take advantage of the advanced scientific and technological methods available to us. First, we should define the effective components, molecular structure and contents of Chinese herbal medicines. Second, we should define not only the general pharmacological features of each but also the effective mechanism (s) related to the whole body at the molecular level. Third, we should obtain the definite data of pharmacokinetics as well as strict evidence on the toxicology profile. In this manner, we can gain more competitive opportunities in the international market. We have already explored new immunopotentiators based on the TCM theory, such as ganoderma-polysaccharide, polyporus umbellatus-polysaccharide, and astragalus polysaccharide; some of these medicines were found to tonify body functions. Those research findings have made remarkable contributions to our country and to the traditional Chinese scientific culture. I believe that in the first 20 years of the coming century, we will develop more new drugs that will be better than Artemisine.

TOWARDS IMPROVING AND CREATING PHARMACEUTICS IN CHINESE MEDICINES

Pharmaceutical study of sustained or control released tablets, specifically for skin- and mucosa-targeting drugs, has been made. Now, we should enhance these findings to develop new medicines, such as coated control release tablets, bone tent tablets, free-floating-in-the-stomach tablets and osmosis pump tablets, to name a few. Furthermore, we also have studied targeting drugs that are based

upon delivery *via* lipid or lipid protein, micro-capsule, micro-sphere, and magnet micro-globe; additionally, the delivery approach of bullet drug combined with monoclonal antibody has been studied. The development of those medicines is progressing rapidly.

Most Chinese herbal medicines are provided in the form of pills, powders, plasters, pellets, and tablets. There are also a few provided as injections, sprays and suppositories. The Center for Drug Evaluation of the Ministry of Health in China approved 217 patent drugs in 1993, only 10 of which were classified as second class; all others were classified as third and forth class. Chinese medicines have now spread throughout the world, but very few patients drink herbal tea and most report disliking it. The granules and powders of TCMs, however, are welcomed in America and Japan. In the international market, 90% of Chinese compound formulas are claimed by Japan and not by China. Taiwan has also been producing so-called scientific Chinese medicines, which have been accepted easily for various syndromes. I hope our medical researchers can enhance the studies on physio-pharmacology and bio-pharmacology in order to correct this problem and to make the Chinese medicines more popular internationally and thereby improve our national export business.

TOWARDS STUDY OF THE CHINESE COMPOUND FORMULAS

The clinical application of a compound formula is the main trait and advantage of treatments based on Chinese medicines. The method of investigating any particular compound formula relies on modern science and technology to elucidate the mechanism underlying the medicine's function and, ultimately, to clarify the theory of TCM. Over the past 45 years, we have carried out vast research on more than 230 compound formulas. The research on Shengmaisan (pulse-producing powder), baoyuantang (invigorating original-qi pill) and da chengqitang (drastic purgative decoction) has provided experimental or clinical pharmacodynamic data that confirms the consistency of the TCM theory and its application. The research on the compatibility of compound formula has also proven its superiority; for instance, in the buzongyiqitang formula (decoction for reinforcing middle-jiao and replenishing qi), the chaihu (radix bupleuri) and shengma (rizoma cimicifugae) are the activators. In Japan, researchers are similarly focusing on compound formulas, such as xiaocaihutang (minorbupleurum decoction), and guizhifulingwan (cinnamon twing and poria pill); their approaches are very systematic and not behind our own efforts.

Further research on compound formulas is necessary, as they will explain the functioning mechanism and enrich the theory of TCM. It is very difficult to set up experimental animal models that precisely and accurately coincide with any human syndrome or disease. Therefore, the objectives of developing new experimental models and methods must be very clear and effective. Meanwhile, the compound formula research—from the principal herb to the auxiliary herb, from the raw materials to the effective components—is also stressed in Japan. This approach represents one productive way to aim towards development of new medicines, and should be encouraged.

TOWARDS PROMOTING RESEARCH ON BIANZHENG SHIZHI

Bianzheng shizhi, which is the diagnosis and treatment based on overall analysis of the patient's symptoms and signs, is the outstanding science of TCM. According to this theory, TCM can treat patients individually and directly to combat the particular syndromes. The scientific research on the essence of a syndrome forms the basis of the TCM theory. The syndromes are classified as basic, compound, multi-compound, comprehensive, partial organ, exogenous pathogenic factors, latent or overt syndromes, *etc.* Research on the essence of a syndrome is often performed from the aspect of its being combined with several different diseases, as the same syndrome may appear in different diseases. The clinical effect of a syndrome needs to also be considered from the perspectives of macro- and micro-observation indexes in combination. If the treatment is too flexible, it would be difficult to precisely pinpoint the exact profile of the syndrome. Therefore, it is paramount to abide by the basic plan that involves having a fixed formula based on the type of disease the patient is suffering with and then applying flexibility according to the different syndrome to perform the random-controlled research efforts. Researchers, therefore, need to closely follow-up related international progress in their field and obtain the results from others worldwide. This will promote productive competition with others at an international level, and help us to further align with the western medical standard that is stricter.

In the study of the essence of Chinese syndrome, we have already achieved tremendous successes in setting up experimental whole animal models of the various syndromes, including cold, heat, qi-deficiency, blood-deficiency, yin-deficiency, yang deficiency, yang-exhaustion, blood-stasis and hyperactivity of liver-yang syndromes, *etc.* Some models have even been developed that combine a particular syndrome with a particular disease. We have also made substantial progression towards building models of the syndromes in isolated organs or cultured cells.

I believe that in the coming century the research on TCM theory will have a meaningful break-through that will be facilitated by the systematic study of the syndrome to develop the TCM system; furthermore, the findings from these studies will promote new theories of the current medical science and enrich the worldwide medical system, enhancing the development of new drugs.

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EXPERIMENTAL PAPERS

Oncoprotein expression and inhibition of apoptosis during colorectal tumorigenesis

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Abstract

AIM: To study bcl-2 and p53 protein expression and inhibition of apoptosis during colorectal tumorigenesis.

METHODS: Expression of bcl-2 and p53 was detected by immunohistochemical staining of 45 colorectal adenomas, 61 colorectal carcinomas, and 15 pathologically-confirmed normal colorectal biopsies.

RESULTS: The bcl-2 and p53 protein expression was uniformly negative in the normal mucosa specimens, whereas bcl-2 and p53 positive rates were significantly higher in the adenoma and carcinoma specimens ($P < 0.01$). Strong bcl-2 expression was often present in areas of severe dysplasia. In the colorectal adenoma specimens, expression of p53 increased with increasing size and dysplasia, being higher in adenomas ≥ 20 mm in diameter than in adenomas < 10 mm in diameter (77.8% *vs* 35.0%, $P < 0.05$). p53 protein expression was correlated with differentiation and Duke's staging. A significant inverse correlation was found between immunostaining of bcl-2 and p53 in adenomas but not in carcinomas. Furthermore, carcinomas with a high percentage of bcl-2 positive cells were significantly more likely to have low rates of apoptosis.

CONCLUSION: Bcl-2 expression appears to be an early event in colorectal tumorigenesis that can inhibit apoptosis. p53 expression plays an important role in the development and malignant change of colorectal adenoma. Bcl-2 and p53 may represent useful markers of cell apoptosis.

Key words: Colorectal neoplasms; Protein p53; Gene expression; Apoptosis; Bcl-2

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INTRODUCTION

Colorectal cancer is a common malignant tumor and it is believed to arise from adenoma. A recent study showed that colorectal tumorigenesis is related not only to the balance of tumor genes and tumor suppressor genes, but also to cell apoptosis. Cell apoptosis is an important mechanism in cancer biology[1]. Although both of these genes are associated with the process of apoptosis, their relationship in the process has not yet been extensively investigated. Only recently has it been observed that bcl-2 can inhibit apoptosis triggered by wild-type p53[1].

Therefore, we analyzed bcl-2 expression in normal, adenoma and malignant colorectal epithelia and determined the time of bcl-2 activation and its relationship to alteration of p53 expression.

MATERIALS AND METHODS

Tissue samples

Sixty-one paraffin-embedded surgical samples of colorectal carcinoma, 45 colorectal adenomas removed by biopsy or polypectomy and 15 pathologically-confirmed normal colorectal biopsies obtained by colonoscopy were collected from patients being treated at the Guangzhou Military general Hospital. The biopsied tissues were fixed in 10% formalin and embedded in paraffin. Serial sections were made for immunohistochemical or histochemical hematoxylin-eosin (HE)-based staining of bcl-2 and p53 respectively. Colorectal adenoma and carcinoma were diagnosed according to the published criteria[2].

Immunohistochemistry

An immunohistochemical method involving a labeled streptavidin biotin (LSAB)[3] was used to detect bcl-2 and p53 protein in de-paraffinized tissue sections. The primary anti-bcl-2 mAb and anti-p53 mAb DO7 (1:20 and 1:40 respectively; Dako Corporation, Carpinteria, CA, United States) and the appropriate murine anti-human secondary antibodies (LSAB K-9002; Dako Corporation) were used.

Table 1 Relationship between bcl-2 and p53 in clinico-pathological features of colorectal adenoma and carcinoma

Parameter	*n*	bcl-2		p53	
		positive	%	positive	%
Adenoma size in mm					
< 10	20	17	85.0	7	35.0
10-20	16	9	56.3	10	62.5
≥ 20	9	7	77.8	7	77.8[a]
Histological type					
Tubular	20	14	70.0	10	50.0
Tubulovillous	7	5	71.4	4	57.1
Villous	18	14	77.8	10	55.6
Dysplasia					
Mild	20	13	65.0	8	40.0
Moderate	18	15	83.3	10	55.6
Severe	4	4	100.0	3	75.0
Malignant change	3	1	33.3	3	100.0[c]
Serosal invasion					
-	24	17	70.80	11	45.8
+	37	28	75.70	23	62.1
Well differentiated	13	10	76.90	4	30.8
Moderately differentiated	29	19	65.50	14	48.3
Poorly differentiated	12	11	91.67	10	83.3[c]
Mucinous	7	5	71.43	6	85.71
Lymph node metastasis					
-	28	19	67.9	13	46.4
+	33	26	78.8	21	63.6
Duke's staging					
A	17	12	70.6	5	29.4
B	9	5	55.6	7	77.8[e]
C	20	17	85.0	11	55.6
D	15	11	73.3	11	73.3[a]

[a]$P < 0.05$, mild dysplasia *vs* malignant change; [c]$P < 0.05$, well differentiated *vs* poorly differentiated; [e]$P < 0.05$, Duke's stage B or D *vs* Duke's stage A.

Controls

A colorectal neoplasm with a high level of cytoplasmic bcl_2 and nuclear p53 immunoreactivity was used as a positive control for these oncoproteins. Negative control slides were processed with PBS alone, instead of the primary antibody, but included all other steps of the procedure.

Apoptotic index

The percentage of apoptotic cells was determined by microscopic examination (magnification of × 400) of HE-stained sections for the 61 colorectal carcinomas. For each slide, five fields of non-necrotic areas were examined and 100 nuclei in each field were scored as normal or apoptotic in appearance. Morphological features used to identify apoptotic nuclei included overall shrinkage and homogeneous dark-staining basophilia and acidophilia in cytoplasm[4].

Data statistics

Data were analyzed with the chi-square test, with exact probabilities using the 2 × 2 chi-square test, and correlation coefficient test. Statistical significance was defined as $P < 0.05$.

RESULTS

Expression of bcl-2

In histologically normal mucosa, bcl-2 staining was observed in basal epithelial cells of the colonic crypts overlying the muscularis mucosa, but was absent in the superficial portions of the mucosa. Bcl-2 staining in basal epithelial cells was intense and localized to the cytoplasm. In adenomas, dysplastic cells in adenomatous colonic glands showed diffuse positive staining for bcl-2. Severe epithelial dysplasia displayed intense staining. The bcl-2 positive rate was significantly higher in adenomas (73.3%) and carcinomas (73.8%) than in normal tissue (0%) ($P < 0.01$), but no significant difference was found between the staining in the adenomas and carcinomas. The expression of bcl-2 was not related to clinical and histopathologic features of the adenomas and carcinomas.

Expression of p53 protein

Expression of p53 protein was located in the cell nucleus, as evidenced by brown staining. Normal mucosa was uniformly negative for nuclear p53 immunostaining. The p53 positive rate was significantly higher in adenomas (53.3%) and carcinomas (55.7%) than in normal tissue ($P < 0.01$), but no significant difference was found between the staining in the adenomas and carcinomas. Expression of p53 increased with the size of adenoma and degree of dysplasia, and was higher in adenomas with ≥ 20 mm diameter than in adenomas with < 10 mm diameter ($P < 0.05$). The expression of p53 in adenomas was related to dysplasia ($P < 0.05$). In colorectal carcinoma, the expression of p53 was related to the differentiation and Duke's staging (Table 1).

Table 2 Correlation between bcl-2 and p53 staining[1] in adenomas and carcinomas

Pathology	*n*	Correlation coefficiency, *r*
Adenomas	45	-0.295
Carcinomas	61	-0.112

[1]Positive label index.

Expression of bcl-2 and p53 proteins in colorectal tumors

A significant inverse relationship was found between the immunostaining of bcl-2 and p53 in the adenomas ($P < 0.05$) but not in the carcinomas ($P > 0.05$) (Table 2).

Apoptotic index (AI)

The mean AI of 61 colorectal carcinomas was 2.76 ± 1.75 (range, 0.2%-10%). A significant inverse relationship was observed between the AI and the percentage of cells that were positive for bcl-2 ($r = 0.74$, $P = 0.0001$).

DISCUSSION

The bcl-2 proto-oncogene is a known inhibitor of apoptosis and may therefore allow for an accumulation of genetic alterations that propagates upon cell division. Over-expression of the *bcl-2* gene was first described in follicular lymphoma and was then shown to result from a chromosomal 14:18 translocation. Our results indicate that basal epithelial cells of the normal colonic crypts express the bcl-2 protein, but its expression was found to be absent in superficial portions of the mucosa. The bcl-2 positive rate was significantly higher in adenomas (73.3%) and carcinomas (73.8%) than in normal tissue (0%) ($P < 0.01$), but no significant difference was seen between the expression in adenomas versus carcinomas, suggesting that abnormal *bcl-2* gene activation is an early event in neoplastic development or progression. Thus, expression of bcl-2 may not be related to clinical and histopathologic features of colorectal cancer.

The p53 tumor suppressor gene is the most commonly mutated gene in human cancers and is a frequent abnormality found in colorectal cancers[5]. Our results showed p53 staining was higher in adenomas and carcinomas, with only minimal to undetectable in non-tumorous mucosa. Moreover, expression of p53 in the adenomas increased with the increasing size and extent of dysplasia. Expression of p53 in adenomas of ≥ 20 mm in diameter was higher than in adenomas of < 10 mm in diameter, and was higher in tissue with malignant changes than in those with mild dysplasia. In colorectal cancers, the expression of p53 was correlated with the differentiation status and Duke's stage. Our results are consistent with those previously reported by Kaklamanis[6], wherein nuclear p53 staining was detected in 42%-67% of adenomas[7,8]. Furthermore, our results showed a progressive increase in p53 alteration during colorectal tumorigenesis. Although p53 mutation is thought to be a late event in colorectal tumorigenesis, we suggest that it may be more common during malignant change of the polyps.

Spontaneous apoptosis is a feature of some human cancers and may regulate cell growth[9]. We found that the spontaneous apoptotic rate in colorectal carcinomas ranged from 0.2% to 10%. Moreover, our results indicated that an inverse relationship existed between the AI and bcl-2 expression in carcinomas. This result indicates that bcl-2 acts *in vivo* as an inhibitor of apoptosis in colorectal cancers.

Interestingly, bcl-2 and p53 staining was found in our study to be inversely correlated in adenomas but not in carcinomas. p53 nuclear staining was increased in adenomas at the areas of severe dysplasia, which showed mild staining for bcl-2. This result sug-

gests a potential down-regulation of bcl-2 by mutant p53 in pre-malignant polyps. Similarly, an inverse correlation between bcl-2 and p53 staining has been reported in breast cancers and colorectal tumors[10,11]. Hence, a potential molecular basis for this effect may involve a p53-dependent negative response element on the *bcl-2* gene through which p53 can directly or indirectly down-regulate bcl-2 expression, so that a colorectal epithelia malignant change may occur by inhibiting cell apoptosis.

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New combined therapy for multiple organ failure in abdominal surgery

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Abstract

AIM: To report 15 cases of abdominal-surgical multiple organ failure (MOF) treated successfully by a new combined therapy using four high doses in large volume and one nutritional support (FHDOS), consisting of one short period high dose of anisodaminum in a large volume, followed by one short period high dose of dexamethasone in a large volume, one high dose of disinfectant antibiotics in a large volume and one high dose washing liquor in a large volume to the abdominal cavity, and finally by applying support of nutrition metabolism.

METHODS: The study group consisted of 15 patients (10 women and 5 men; average age: 42.7) who were hospitalized in our hospital. All patients were given FHDOS as follows. First, a short period of high doses of anisodaminum in large volume delivered *via* intravenous injection of 40 mg once and with an optional 40 mg added 30 min later according to condition; the total amount may reach 120-240 mg a day, or intravenous injection of 40 mg every 15 min until the condition comes under control. Second, a short period of high doses of dexamethasone in large volume delivered *via* intravenous injection of 100-200 mg once; this remedy could be continued for 1-3 d and the amount could be decreased according to condition. Third, high doses of disinfectant antibiotics in large volume with select use of antibiotics according to clinical condition of Gram staining status; it was necessary to repeatedly culture the bacteria and make adjustments according to the result and status of drug-resistance to prevent dual infections from occurring. Fourth, high doses of washing liquor (normal saline at 6000-8000 mL to wash the abdominal cavity) in large volume. Finally, one full support of nutrition metabolism.

RESULTS: All the patients treated by FHDOS survived after the rescue treatment without any complication.

CONCLUSION: MOF should be prevented, if possible, by stopping or controlling the injury, removing as much necrotic tissue as possible, improving blood flow and oxygen consumption, supporting metabolism, and preventing infection or by initiating treatment early and adequately. FHDOS is a combined therapy and plays a key role in treating MOF.

Key words: Abdomen/surgery; Multiple organ failure/therapy; Combined modality therapy

Yue MX. New combined therapy for multiple organ failure in abdominal surgery. *World J Gastroenterol* 1996; 2(1): 6-8 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i1/6.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i1.6

INTRODUCTION

The syndrome of multiple organ failure (MOF) is the final common pathway for a number of clinical problems, including severe multiple system trauma, complications following surgery, infections such as peritonitis, inflammatory processes such as pancreatitis, and severe illness with limitations in organ function or cardiac output secondary to aging, arteriosclerosis or chronic diseases[1]. MOF has widely attracted clinicians' attention, and has emerged as a hot research focus of international medical circles. However, no MOF treatment has yet to provide satisfactory results. The average mortality of the cases suffering from failure of two organs is 69%, of three organs is 85%, and of four organs is 100%[2].

Therefore, countries all over the world are carrying out tremendous research on issues concerning MOF. We report here our experience with 15 patients with abdominal-surgical MOF and who were treated with a new combined therapy.

MATERIALS AND METHODS

Materials

The study group consisted of 15 patients (10 women and 5 men; average age: 43.7 years, age range: 11-93 years) who were hospitalized in our hospital. All the cases were diagnosed as MOF according to the diagnostic standards proposed by Baue[2], Eiseman[3], Knaus *et al*[4], Chen[5]and Yue[6]. Among this group, 9 cases suffered from failure of two organs, 5 from failure of three organs and 1 from failure of four organs. Underlying diseases included serious compound wound caused by a collapse accident (n = 1; which had resulted in injury and massive hemorrhaging (total: 4400 mL) from the liver, spleen, kidney and lungs, and various fractures), car accident (n = 1; which had resulted in serious compound wound and subsequent colon fistula, perineal-rec-

tal diaphragm abscess, golden staphylococcal septicemia), acute large and small intestine twist-induced necrosis with delayed diagnosis (n = 1), acute cholangitis (n = 4; which had resulted from gallstones and overflowing peritonitis; abscesses in the thoracic and abdominal cavities along with septicemia (n = 1; which had resulted from dystocia and cesarean operation), colon perforation and overflowing peritonitis coupled with delayed diagnosis for 5 d (n = 1), liver and abdominal cavity abscesses and septicemia (n = 1; which had resulted from local hormone injection), acute hemorrhagic-necrotic pancreatitis complicated by ARDS and intestinal failure (n = 1), and compound wound caused by traffic accident or falling from high buildings (n = 4). The clinical course for all 15 cases had very clear initiating factors that resulted in MOF; among them were serious compound wound (n = 6), serious infection (n = 8), and acute large-range (2.8 meters) small intestine necrosis coupled with delayed diagnosis (n = 1).

Methods

All patients underwent surgery to resolve the initiating factor and then were given "four high doses in large volume and one support" (FHDOS), a new combined therapy that consisted of the following. First, a short period high dose of anisodaminum in large volume delivered by intravenous injection of 40 mg once, with an optional 40 mg added 30 min later according to the patient's condition; the total amount could reach 120-240 mg per day or intravenous injection of 40 mg every 15 min until the condition came under control. Second, a short period of high dose of dexamethasone in large volume delivered by intravenous injection of 100-200 mg once; this step could be continued for 1-3 d and the amount could be decreased according to the patient's condition. Third, high dose of antibiotics in large volume using select antibiotics according to the patient's clinical condition and findings from Gram staining test; this step required repeated culturing of bacteria to make adjustments in the procedure according to the culture results and development of drug-resistant strains in order to prevent opportunistic infections. Fourth, high dose abdominal cavity washing liquor with large volume; this step consisted of normal saline applied to wash the abdominal cavity with 6000-8000 mL. Finally, support of full nutrition metabolism [parenteral (TPN) and enteral (TEN) nutrition] was applied.

RESULTS

All the patients survived after the rescue treatment without any complication. Follow-up revealed that 8 patients returned to work normally, 4 retired but were able to perform normal housework activities, and 2 continued their schooling; the oldest patient died of cancer at the age of 93, 5 mo after study participation.

DISCUSSION

All the patients in this study, having been in dangerous conditions following surgery, survived due to the combined treatment measures mentioned above and management in the Intensive Care Unit. In cases of infection, once the lethal pathogen was removed the condition reversed and the patients were cured. In general, the clinical observation of these patients showed that abdominal-surgical MOF has distinctive clinical characteristics. First, abdominal-surgical MOF does not show any special early clinical symptoms. Second, there are obvious initiating factors resulting in MOF. Third, when MOF is consequential to another condition, the patient's status worsens very quickly. Fourth, if the initiating factors are not removed, death will result. Fifth, oftentimes effective surgery is required to thoroughly resolve the lethal factors, such as ruptured abdominal organs and massive hemorrhaging, abscesses in the abdominal cavity, acute obstructive and festering cholangitis caused by gallbladder stones, acute hemorrhage-necrosis pancreatitis, and wide-range abdominal-internal organs necrosis. Sixth, the cases often represent very difficult technical challenges and surgeons should try to concentrate their energies towards treating the most serious disease, so that the patient's condition will stabilize and they can then carry out the operative treatment. Seventh, only under the close monitoring provided in the Intensive Care Unit and along with the strong supportive measures can the operation be performed safely. Eighth, the patients with abdominal-surgical MOF have hypermetabolism and need metabolic support. Ninth, once the lethal factors are resolved, the conditions will reverse and the patient may finally be cured.

The three main causes of death of abdominal-surgical patients with MOF are microcirculation failure, uncontrolled severe infection, and hypermetabolism. Our clinical observation indicated that anisodaminum has much better anti-shock effects and in improvement of microcirculation than achieved with dopaminum, which can expand the blood vessels of visceral organs. During the operations, there were no occurrences of low blood pressure and bleeding, and the recovery from cases of septic shock was relatively smooth. The underlying functional mechanism involves the combination of cardiac vascular effects, energy metabolism protection, calcium ion effect and anti-oxygen free radical effect. The short period and high dose of dexamethasone in large volume delivered by intravenous injection saves on the amount of infusion given, gains precious time for blood transfusion and the operation, lessens complications of organ edema, stabilizes the cells to avoid damage induced by free radicals due to occurrence of septic shock, and prevents or slows down the further development of organ failure. The use of high doses of disinfectant antibiotics in large volume and abdominal cavity washing with high doses of washing liquor in large volume are helpful in preventing and treating infection. The final step of full nutritional and metabolic support can correct damage involving the cell biochemistry damage, and ensure supplies of nutrition for organ metabolism through TPN and TEN methods.

The clinical application showed that the various steps of the "FHDOS" therapy work in coordination and complement each other. For example, infection predisposition, which may occur after intravenous injection of high-dose dexamethasone in large volume, can be prevented by the delivery of high-dose disinfectant antibiotics in large volume. Abdominal-surgical MOF has no specific clinical manifestation at the early stage, when surgeons are concentrating on treating sepsis or other serious disease. It may be too late to cure MOF after it becomes obvious. The "FHDOS" therapy described herein involves the author's experience with its application as a rescue method and involved adoption of combined and extraordinary doses in order to safely operate on the patients due to its resolution of lethal pathogenic infections and rescue of patients who were critically ill.

Most of the patients in this study had normally functioning organs before their diseases occurred. It is recommended that clinicians pay great attention to the clinical manifestation of MOF, so as to diagnose MOF early and try to slow or halt its development. Ultimately, however, MOF should be prevented because of its particularly high mortality rate. Thus, prevention of MOF and prevention or early treatment of infection are both paramount to clinical care. Nutritional support to prevent complications is also necessary. If TPN is necessary, special solutions are available for the liver, kidneys or lungs. Stopping or controlling any organ injuries, including removing as much necrotic tissue as possible, improving the blood flow and oxygen consumption, supporting metabolism, and preventing infection or treating it early and adequately is necessary. The FHDOS approach is practical and effective; when MOF develops, it plays a key role as treatment.

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CLINICAL ARTICLES

Thresholds of gastroesophageal reflux for diagnosis of esophageal reflux diseases

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Abstract

AIM: To establish the optimal thresholds of pH variation (pH fluctuations and reflux episodes) for separating physiological and pathological gastroesophageal reflux (GER), and to evaluate their significance for GER diagnosis.

METHODS: Twenty-four hour intraesophageal pH monitoring and endoscopy were performed in 400 patients with GER symptoms and in 100 healthy controls.

RESULTS: The percentages of the time with pH fluctuations in patients with and without esophagitis, and in healthy controls were, on average, 12.65%, 9.5% and 2.76% in 24 h, respectively, and the respective percentages of the time with reflux episodes in the same groups in 24 h were, on average, 3.12%, 2.04% and 0.18%, respectively. Using a receiver-operating-characteristic curve analysis, < 6.7% of the time with pH fluctuations and 0.1% of the time with reflux episodes were defined as the combined thresholds for physiological versus pathological reflux. The sensitivity of the combined thresholds for the detection of GER in patients with and without esophagitis was 96.7% and 90.0%, respectively, and the specificity for the diagnosis of patients with abnormal GER disease was 100%.

CONCLUSION: pH fluctuations and reflux episodes, when evaluated together, are more useful for classifying patients with GER; the combined thresholds yield higher diagnostic accuracy for assessing patients with GER disease.

Key words: Gastroesophageal reflux/diagnosis; Esophagitis/diagnosis; Hydrogen-ion concentration

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INTRODUCTION

Twenty-four hour intraesophageal pH monitoring is the technique of choice for measuring gastroesophageal reflux (GER)[1-5]. Most studies have suggested a certain percentage of the recording time with pH below 4 to be set as a threshold of normality[6,7]. In these studies, only episodes of pH changes > 2 units (*e.g.* from around pH 6 to 4) have been considered. However, reflux events that do not fulfill the above criteria have been ignored when evaluating the contributions of some pathogenic factors to the development of esophagitis. The aims of the present study were to establish the optimal thresholds for both reflux episode and pH fluctuations, and to assess their value in the diagnosis of GER disease.

MATERIALS AND METHODS

Materials

Four-hundred patients (280 men, 120 women) were enrolled in the study. All patients had experienced the GER symptoms of regurgitation, heartburn and/or chest pain for at least 1 year (Table 1). None of these patients had undergone previous upper gastrointestinal surgery and none had taken H2-blockers or H-/K+ATPase (proton-pump) blockers during the 2 wk prior to endoscopy and pH-metry. The control group consisted of 100 (60 men, 40 women) healthy subjects aged 20-year-old to 72-year-old, with a mean age of 49-year-old. None of the controls had experienced any GER symptoms, such as chest pain, dysphagia and heartburn. A few subjects had experienced occasional regurgitation. This study was conducted in accordance with the Declaration of Helsinki and was approved by the participating hospitals.

Methods

Twenty-four hour esophageal pH monitoring was carried out in accordance with the method described elsewhere[8,9]. Briefly, subjects were advised to take a standard meal of approximately 9199.5 KJ (2200 kcal) during 24 h intraesophageal pH monitoring. A glass pH electrode with an incorporated potassium chloride reference (In gold electrode, No 440) was introduced *via* the naso-esophageal route and positioned with the tip at 5 cm above the gastroesophageal junction that had been identified by the pH meter. The output from the pH probe was recorded on a solid-state recorder (Autroni-

Table 1 Anamnestic data and endoscopic findings

Parameter	Gastroesophageal reflux patients with esophagitis (n = 228)	Gastroesophageal reflux patients without esophagitis (n = 172)	Healthy controls (n = 100)
Age, yr	52 ± 18.3	46 ± 7.5	48 ± 9.4
Sex, male/female	158/70	122/50	60/40
Duration, yr	12.4 ± 3.2	8.1 ± 3.8	
GER symptoms, %	100	100	0
Smoker, %	54.3	44.2	35
Alcohol consumer, %	25	20.3	22
Hiatal hernia, %	18.4	11.0	2.0
Grade of esophagitis, %			
Ⅰ - Ⅱ	72.3	0	0
Ⅲ - Ⅳ	27.7	0	0

Table 2 Reflux episodes and pH fluctuations in gastroesophageal reflux patients ($\bar{x} \pm s$)

Group	pH fluctuations			Reflux episodes		
	n	Duration, min	Time, % 24 h	n	Duration, min	Time, % 24 h
Gastroesophageal reflux patients with Esophagitis						
Total	67 ± 46	191.7 ± 118.9	12.7 ± 6.5[b]	22 ± 31	45.4 ± 110.6	3.1 ± 7.5[b]
Day	55 ± 34	124.0 ± 97.5	8.2 ± 4.6[b]	15 ± 13	19.1 ± 58.7	1.3 ± 2.7[b]
Night	12 ± 8	50.1 ± 35.4	3.3 ± 4.5[b]	7 ± 10	22.8 ± 64.7	1.5 ± 4.8[b]
GER patients without Esophagitis						
Total	50 ± 18	130.4 ± 57.5	9.5 ± 2.8[b]	14 ± 14	27.4 ± 68.3	2.0 ± 5.0[b]
Day	41 ± 14	94.0 ± 35.7	6.8 ± 3.5[b]	11 ± 7	18.8 ± 45.2	1.4 ± 12.9[b]
Night	9 ± 8	36.3 ± 26.4	2.6 ± 3.1[b]	3 ± 6	8.6 ± 24.7	0.6 ± 2.2[b]
Healthy controls						
Total	23 ± 16	41.3 ± 30.7	2.7 ± 2.0	3 ± 3	3.0 ± 5.7	0.2 ± 0.4
Day	20 ± 11	37.3 ± 24.1	2.5 ± 1.9	2 ± 2	2.0 ± 2.4	0.1 ± 0.2
Night	3 ± 5	4.1 ± 3.9	0.3 ± 0.4	1 ± 1	1.0 ± 2.1	0.1 ± 0.1

[b]$P < 0.01$ *vs* healthy controls.

Table 3 Correlations between the percentages of the time with pH fluctuations/reflux episodes and the total duration and numbers of episodes

Group	Total duration		Total number	
	r	P	r	P
Gastroesophageal reflux patients with esophagitis				
Time (%) with pH fluctuations	0.92	< 0.01	0.53	< 0.01
Time (%) with reflux episodes	0.67	< 0.01	0.65	< 0.01
GER patients without esophagitis				
Time (%) with pH fluctuations	0.88	< 0.01	0.29	> 0.05
Time (%) with reflux episodes	0.42	< 0.05	0.45	< 0.01
Controls				
Time (%) with pH fluctuations	0.98	< 0.01	0.82	< 0.01
Time (%) with reflux episodes	0.62	< 0.01	0.46	< 0.05

[*]Total duration: Duration of pH fluctuations + duration of reflux episodes; Total numbers: Numbers of pH fluctuations + numbers of reflux episodes.

cord CM 18), which the patients carried on a belt. Data were electronically analyzed by means of a self-designed computer program. The parameters recorded included the frequency and duration of the 24 h reflux episodes and pH fluctuations. The pH fluctuation was defined as a decrease from pH < 4 to pH 2 lasting more than 20 s, and the reflux episode was a decrease in pH < 2 lasting more than 20 s. All study subjects underwent upper gastrointestinal endoscopy during the week before pH-metry. Esophagitis was graded from Ⅰ to Ⅳ according to Savary and Miller[10]. Only a few patients presented with grades Ⅱ and Ⅲ, and therefore all patients were grouped as Ⅰ and Ⅱ or Ⅲ and Ⅳ, respectively.

Statistical analysis

The Wilcoxon rank sum test was used to compare the percentages of the time with both types of pH variations (pH fluctuations and reflux episodes) for the three groups (*i.e.* Ⅰ and Ⅱ, Ⅲ and Ⅳ, healthy controls). Spearman's rank correlation was used to analyze the relationships between total recording time and pH < 4 and the time (%) with both types of pH variations. Differences between GER patients with/without esophagitis and healthy controls in the distribution of pH fluctuations and reflux episodes were analyzed by means of the chi-square test. Using receiver operating characteristic (ROC) curves[11,12], the optimal threshold values were identified for each parameter and combined parameters (pH fluctuations and reflux episodes). For these thresholds, the percentages of true-positive decisions (sensitivity) and false-positive decisions (100% minus specificity) were calculated and charted. The more the line curved towards the point of 100% true positivity and 0% false-positivity, the better the diagnostic value of the test; therefore, lowering of the threshold decreased sensitivity but increased specificity, and vice versa.

RESULTS

Anamnestic data and endoscopic findings are presented in Table 1. Table 2 shows the frequency, duration and percentage of the time with pH fluctuations and reflux episodes in GER patients with and without esophagitis and in healthy controls. The percentages of the time with pH fluctuations in GER patients with and without esophagitis and in healthy controls were, on average, 12.7%, 9.5% and 2.7%, respectively, while percentages of the time with reflux episodes in the same groups were, on average, 3.1%, 2.0% and 0.2%, respectively. Both patients with and without esophagitis showed statistically significant differences compared to the healthy controls ($P < 0.01$). There was no significant difference between the patients with esophagitis and those without esophagitis for comparison of percentage of the time with pH fluctuations/reflux episodes; total duration and frequency of episodes are listed in Table 3.

The distribution of the duration and frequency of pH fluctuations and reflux episodes in GER patients with and without esophagitis and in healthy controls is shown in Figure 1. The duration of pH fluctuations in GER patients with and without esophagitis and in healthy controls was 80.8%, 82.6% and 93.2%, respectively, whereas the duration of reflux episodes in the same groups was 19.2%, 18.4% and 6.8%, respectively. There was a significant difference between the patients with/without esophagitis, and healthy controls (Figure

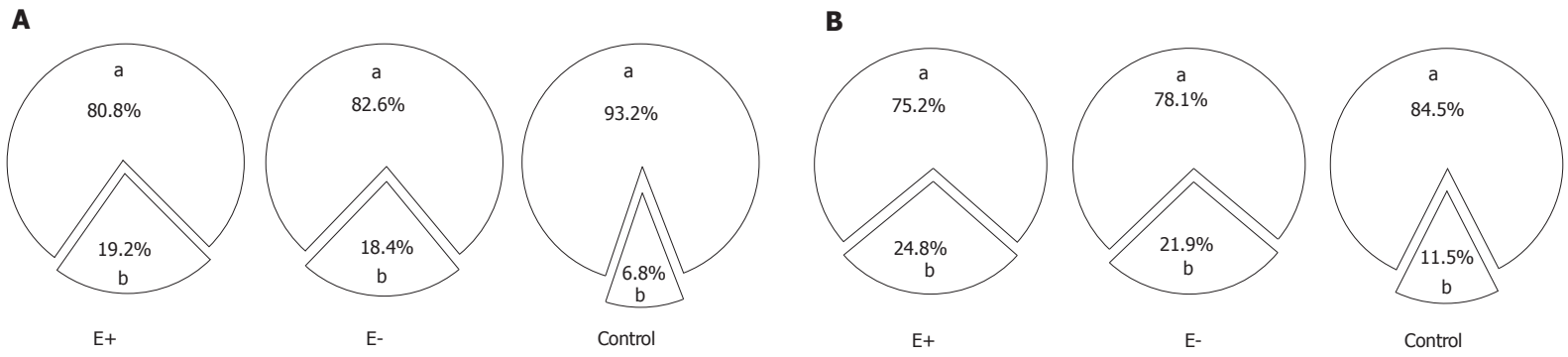

Figure 1 Distribution of the duration (A) and frequency (B) of pH fluctuations (a) and reflux episodes (b) in gastroesophageal reflux patients with (E+) and without (E-) esophagitis and in healthy controls.

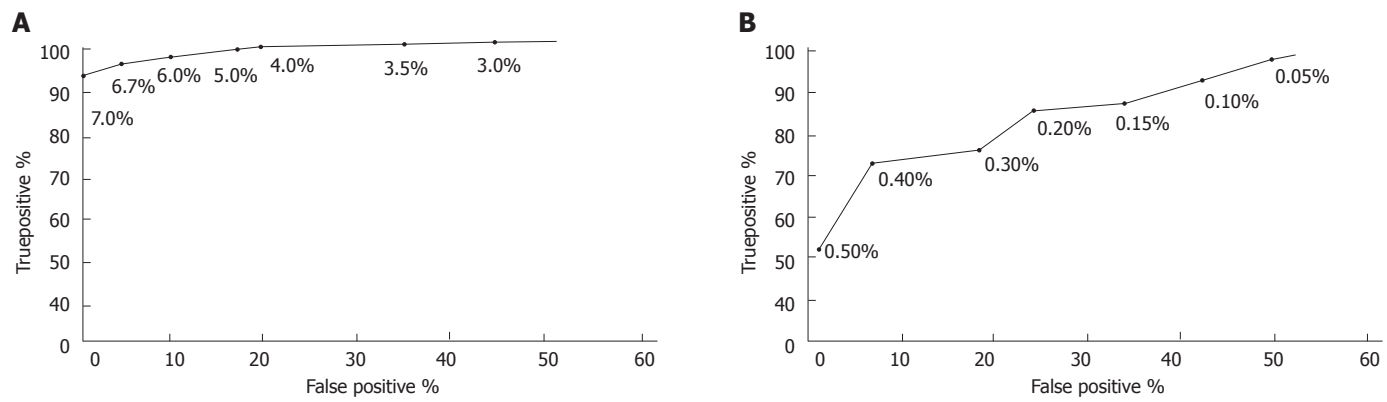

Figure 2 Receiver operating characteristic curve analysis for thresholds of pH fluctuations (A) and reflux episodes (B). The true- and false-positive rates for thresholds of pH fluctuations (A) and reflux episodes (B) are shown.

Table 4 Sensitivity and specificity of pH fluctuations and reflux episodes for the diagnosis of abnormal gastroesophageal reflux

Threshold, %		Sensitivity, %		Specificity, %
		gastroesophageal reflux patients with esophagitis	gastroesophageal reflux patients without esophagitis	
pH fluctuations	< 6.7	93.7	93.3	97.7
Reflux episodes	< 0.1	96.7	90.0	45.2
Combination		96.7	90.0	100

*Combination: pH fluctuation < 6.7% + reflux episode < 0.1%.

1A, $P < 0.01$). The frequencies of pH fluctuations and reflux episodes in the three groups are shown in Figure 1A. The difference between the patients with/without esophagitis and healthy controls was notable ($P < 0.01$).

Figure 2 shows the true- and false-positive rates for different thresholds of pH fluctuations and reflux episodes by means of ROC curve analysis. As shown in Table 4, if only < 0.1% of reflux episodes was used as the threshold, the sensitivity for diagnosis of abnormal GER was 96.7% in GER patients with esophagitis and 90.0% in GER patients without esophagitis, but the specificity was only 45.2%. Similarly, when < 6.7% of pH fluctuations was used as the threshold to define pathological reflux, the sensitivity was 93.7% in GER patients with esophagitis and 93.3% in GER patients without esophagitis; the specificity for diagnosing abnormal GER was 97.7%. When < 6.7% of pH fluctuations for 24 h was combined with < 0.1% of reflux episodes for 24 h as the combined thresholds to define pathological reflux, the sensitivity for diagnosing GER patients with and without esophagitis was 96.7% and 90.0%, respectively, and the specificity for the patients with GER disease increased to 100%.

DISCUSSION

Over the past few years, 24 h intraesophageal pH monitoring has been increasingly used to investigate suspected GER patients. Until now, though, thresholds for the detection of pathological GER have not been standardized. Having defined < 4.2% of time with pH < 4 during 24 h as a threshold between physiological and pathological GER, DeMeester *et al*[13] obtained a sensitivity for the detection of acid reflux of 90.3% and a specificity for excluding acid reflux of 90%. The thresholds defined by Schindlbeck *et al*[14] were 10.5% of time with esophageal pH < 4 for the upright position and 6.0% for the supine position, and the sensitivity and specificity obtained were 93.3% and 92.9%, respectively.

The main problem is whether the sensitivity and specificity of 24 h intraesophageal pH monitoring for the diagnosis of abnormal GER can be further improved. It is evident that improving the sensitivity and specificity for the diagnosis of GER disease depends upon the ability to establish optimal thresholds between pathological and physiological reflux. The greater the difference between the patients with pathological reflux and the subjects with physiological reflux in distribution of the data, the better the sensitivity and specificity of the threshold. In our study, we found that reflux episodes, as characterized by a sudden, appreciable decrease in pH, accounted for 3.1%, 2.0% and 0.2 % of recording time, respectively, while in GER patients with and without esophagitis and in healthy controls, pH fluctuations, as characterized by smaller pH variations (*i.e.* ≤ 2 pH units below pH 4), accounted for 12.7%, 9.5% and 2.7% of recording time, respectively. There was a significant difference between GER patients with abnormal GER and healthy controls in percentages of time with reflux episodes and pH fluctuations. The pH fluctuations, in comparison with the reflux episodes, showed a better correlation with total duration of the pH fluctuations and reflux episodes. In addition, in the distribution of the duration and frequency of both types of pH variations, the rates of duration and frequency of reflux episodes in GER patients with and without esophagitis were significantly greater than in healthy controls, whereas the rates of duration and frequency of pH fluctuations were significantly smaller

than those in healthy controls. Thus, there may be a significant difference between patients with GER disease and healthy controls in the distribution of the pH fluctuation and reflux episode data. Thus, pH fluctuation and reflux episodes can be used to differentiate between pathological and physiological reflux.

Defining normal limits of the given data is important for establishing an optimal threshold to categorize pathological reflux. One approach is to take the mean value ± 2 standard deviations of the control subjects. Analysis of the present data indicates that this method is not appropriate, as our data were not normally distributed. The means and standard deviations were created to describe normally distributed data. Another way to define normal limits is the ROC curve analysis. The ROC curve is not dependent on normal distribution. Using ROC curve analysis, we selected < 6.7% of pH fluctuations and < 0.1% of reflux episodes as the cut-off points between physiological and pathological reflux; thus, if both values were above the thresholds, the GER was defined as pathological. The results indicated that the combined thresholds of pH fluctuations and reflux episodes yield higher diagnostic accuracy in the evaluation of GER disease than when a single threshold is used; additionally, our results appear to be better in terms of both sensitivity and specificity than those obtained by other investigators[13,14].

In conclusion, pH fluctuations and reflux episodes are two different kinds of esophageal pH variations. The use of both parameters, the thresholds of which are < 6.7% and < 0.1% of recording time, yields higher diagnostic accuracy in assessing patients with GER disease.

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CLINICAL ARTICLES

Pixu syndrome grading and its relationship with D-xylose and BT-PABA absorption in 183 patients

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Abstract

AIM: To study the grading of pixu (spleen deficiency) syndrome and the scientific basis of its relationship with absorption of D-xylose and BT-PABA.

METHODS: The present study included 115 cases of chronic superficial gastritis, 15 cases of chronic atrophic gastritis, 19 cases of peptic ulcer, and 34 cases of chronic colitis. All cases were diagnosed by endoscopy and biopsies. Chronic gastrointestinal diseases were categorized into the following six types: spleen and stomach asthenia syndrome (including asthenia and cold); disharmony between liver and stomach; damp and heat of spleen and stomach (bowel) syndrome; spleen-stomach-yin deficiency; blood stasis; and spleen-kidney-yang deficiency. Grading of these syndromes were made with concomitant estimation of D-xylose and BT-PABA tests.

RESULTS: Compared with healthy controls, the patients with chronic gastrointestinal diseases showed diminished urinary level of D-xylose and of BT-PABA ($P < 0.05$), with the exception of those with damp and heat of spleen-bowel syndrome. The excretory rate of D-xylose was nearly normal, while the levels of D-xylose and BT-PABA were lower than in the healthy controls ($P < 0.05$-0.01). In regard to the grading of spleen deficiency and the disharmony between liver and stomach syndromes, the excretory rate of D-xylose decreased gradually ($P < 0.01$) as the severity of symptoms increased; in patients with disharmony between liver and stomach syndrome, the excretory rate of BT-PABA also decreased ($P < 0.01$) as symptoms worsened. These data provide scientific evidence for appraisal of the pathophysiology of these syndromes.

CONCLUSION: Changes of D-xylose can reflect the specificity of pixu syndrome, whereas changes of BT-PABA can reflect the specificity of disharmony between liver and stomach syndrome.

Key words: Spleen asthenia; Gastrointestinal diseases; D-xylose; BT-PABA

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INTRODUCTION

Patients with pixu (spleen deficiency) syndrome have lower levels of D-xylose and BT-PABA than healthy individuals[1]. This finding indicates that these patients have functional impairment of absorption and assimilation. Systemic studies have not been carried out to elucidate the relationship between the symptoms of pixu syndrome, disharmony between liver and stomach syndrome and the levels of D-xylose and BT-PABA. We started such an investigation in 1990 and have now achieved some new enlightenment.

MATERIALS AND METHODS

Materials

This study included 115 cases of chronic superficial gastritis, 15 cases of chronic atrophic gastritis, 19 cases of peptic ulcer, and 34 cases of chronic colitis. All cases were diagnosed by endoscopy and mucosa biopsies. Among them, 79 were males and 104 females. The ages ranged from 13-year-old to 66-year-old, with a mean age of 46-year-old. The course of illness ranged from 0.5 years to 21 years, with an average of 8.7 years.

Methods

Grading of symptoms was made according to the theory of traditional Chinese medicine, by which chronic gastrointestinal diseases were categorized into six types, namely spleen and stomach asthenia, disharmony between liver and stomach, damp and heat of spleen-stomach (bowel), spleen and stomach-yin deficiency, blood stasis, and spleen-kidney-yang deficiency. The items of differentiation were different in each syndrome, and only the significant ones were selected. High scores were given to those chronic gastrointestinal diseases with symptoms in common, such as stomach ache, poor appetite, epigastric and abdominal fullness, loose bowel movement, and constipation; the scores were 5 points for mild symptoms, 6.5

Table 1 Relationship between concentration of D-xylose and BT-PABA [mean ± *s*, % (*n*)]

Group	D-xylose	BT-PABA
Normal	26.37 ± 0.70 (32)	74.57 ± 1.47 (36)
Chronic superficial gastritis	20.70 ± 1.18 (115)	49.15 ± 1.64 (115)
Chronic atrophic gastritis	18.33 ± 2.67 (14)	56.35 ± 4.84 (14)
Peptic ulcer	17.46 ± 1.81 (19)	46.72 ± 3.22 (19)
Chronic colitis	21.21 ± 2.16 (34)	52.11 ± 2.81 (34)

Table 2 Relationship between excretory rate of D-xylose and level of BT-PABA [mean ± *s*, % (*n*)]

Group	D-xylose	BT-PABA
Normal	26.37 ± 0.70 (32)	74.57 ± 1.47 (36)
Disharmony between liver and stomach	19.35 ± 1.69 (57)	48.15 ± 2.45 (57)
Spleen and stomach (cold) asthenia	19.95 ± 1.44 (57)	53.11 ± 2.12 (57)
Damp and heat of spleen and stomach (bowel)	24.17 ± 2.21 (35)	50.38 ± 2.55 (35)
Spleen and stomach-yin deficiency	19.86 ± 2.73 (14)	51.47 ± 5.47 (14)
Spleen and kidney-yang deficiency	17.84 ± 4.89 (9)	44.72 ± 6.45 (9)
Blood stasis impeding meridians	16.57 ± 3.26 (10)	47.70 ± 4.48 (10)

Table 3 Relationship between various grades of spleen asthenia and the level of D-xylose or BT-PABA [mean ± *s*, % (*n*)]

Index	Mild	Moderate	Severe
D-xylose	21.91 ± 2.42 (16)	19.86 ± 1.73 (49)	16.81 ± 2.62 (15)
BT-PABA	51.22 ± 3.09 (16)	52.75 ± 2.56 (49)	49.78 ± 5.10 (15)

Table 4 Relationship between grade of the main symptoms of pixu and the D-xylose excretory rate [mean ± *s*, % (*n*)]

Main symptoms	Pixu syndrome grade		
	Mild	Moderate	Severe
Poor appetite	25.64 ± 3.28 (8)	20.50 ± 1.93 (34)	16.31 ± 2.89 (11)
Epigastric fullness	22.29 ± 3.89 (8)	20.68 ± 2.16 (25)	19.18 ± 3.24 (8)
Loose bowel movement	27.36 ± 3.32 (5)	20.07 ± 2.29 (27)	16.31 ± 2.89 (11)
Fatigue and shortness of Qi	23.01 ± 3.65 (7)	19.79 ± 2.33 (28)	17.21 ± 3.72 (8)

Table 5 Relationship between the grade of symptoms of pixu and the level of BT-PABA [mean ± *s*, % (*n*)]

Main symptoms	Pixu syndrome grade		
	Moderate	Mild	Severe
Poor appetite	53.29 ± 4.53 (8)	53.76 ± 2.83 (34)	51.93 ± 5.37 (11)
Epigastric fullness	50.60 ± 5.22 (8)	53.81 ± 3.74 (25)	45.39 ± 5.60 (8)
Loose bowel movement	58.36 ± 5.53 (5)	51.73 ± 3.48 (27)	51.92 ± 5.37 (11)
Fatigue and shortness of Qi	51.59 ± 4.28 (8)	52.23 ± 3.42 (28)	49.21 ± 6.81 (8)

points for moderate symptoms and 8 points for severe symptoms, with 10 points given for signs of tongue and pulse concord with each syndrome. Cases that were difficult to differentiate were excluded from the analysis.

Measurement of D-xylose was made by Jin's modified method[2]. BT-PATA was measured by Zhou's method[3].

RESULTS

The urinary levels of D-xylose and BT-PABA in chronic gastrointestinal diseases were lower than those in normal controls (Table 1).

Except in the damp and heat of spleen and stomach (bowel) group, the level of D-xylose was close to normal; the levels of D-xylose and BT-PABA in other syndromes were all significantly lower than normal (Table 2). Symptoms of spleen asthenia consist of spleen and stomach-yin deficiency and spleen and kidney-yang deficiency. The excretory rate of D-xylose decreased as the symptoms worsened; nevertheless, the level of BT-PABA showed no statistically significant changes (Table 3).

The main symptoms of patients with spleen asthenia were poor appetite, epigastric fullness, loose bowel movement, fatigue and shortness of Qi. The D-xylose excretory rate was lower than normal (26.37% ± 0.70%; Table 1). The D-xylose excretory rate decreased gradually as the severity of symptoms increased. The level of BT-PABA was also lower than normal (74.57% ± 1.47%; Table 1). However, there was no significant change in the relationship between the severity of symptoms and the level of BT-PABA (Tables 4 and 5).

DISCUSSION

Our study showed that patients with pixu had functional impairment in absorption and assimilation. The urinary levels of D-xylose and BT-PATA of chronic gastrointestinal diseases were lower than those of normal controls. By employing the theory of traditional Chinese medicine, we found that the urinary level of D-xylose in damp and heat syndrome of spleen and stomach (bowel) was close to that of the normal controls. After herbal treatment, the level of D-xylose in damp and heat syndrome decreased. The excretory rates of D-xylose in the other types were all increased, and those of BT-PATA were increased as well. This finding indicated that the absorptive functions of the small intestine were improved.

The symptoms of pixu were scored among three degrees: mild, moderate and severe. The excretory rate of D-xylose decreased gradually in all three, whereas the urinary level of BT-PABA did not show any remarkable changes. After herbal treatment, the level of D-xylose and that of the BT-PABA increased with improvement of symptoms. This finding indicated that herbal treatment was effective for all three degrees of pixu symptomology. In disharmony of liver and stomach syndrome, the excretory rate of D-xylose was different from that of spleen deficiency syndrome. The level of BT-PABA decreased gradually in all the degree subgroups though. After herbal treatment, the excretory rate of D-xylose decreased in mild cases but increased in moderate and severe cases, and particularly for the latter two. The above results suggested that change in D-xylose level may reflect the characteristics of spleen deficiency syndrome, whereas change in BT-PABA may reflect those of dishar-

mony between spleen and stomach syndrome. In spleen deficiency syndrome, the D-xylose level decreased gradually in accordance with the severity of symptoms, and BT-PABA showed no concordant changes; for disharmony between spleen and stomach syndrome, the reverse was true. One syndrome relates to deficiency and the other to excess, and as such the clinical manifestations vary.

In patients with chronic gastrointestinal diseases, the disharmony between liver and stomach syndrome was found to occur in early and intermediate stages, and to produce a relatively milder condition. The decrease of BT-PABA reflects diminution of pancreatic exocrine function. Spleen deficiency syndrome was found to occur in moderately advanced and advanced stages, and the patients' vitality was lowered; the pathogenic state is not too excessive and the condition is more serious, as reflected by diminution of D-xylose. This finding indicated the absorptive function of the small intestine and digestion are impaired in the late stage, when malabsorption occurs. Hence, disharmony between liver and stomach syndrome comes first and spleen deficiency syndrome comes late. From the point of view of modern medicine, it can also be well explained that digestion occurs earlier and malabsorption of nutrients ensues subsequently.

We found that D-xylose testing can be used not only as a marker of relative specificity of pixu syndrome, but also to determine the four main symptoms of disharmony between liver and stomach syndrome. This may represent a new method for studying of syndromes in traditional Chinese medicine.

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Significance of serum tumor markers CA50 and CEA in gastric cancer

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Abstract

AIM: Cancer antigen 50 (CA50) and Carcinoembryonic antigen (CEA) are well-described human tumor-associated antigens, utilized clinically in management of gastrointestinal cancer cases. In this study, we compared these markers in sera from patients with malignant and benign digestive tract diseases.

METHODS: Using a side-phase radioimmunoassay, CA50 and CEA serum levels were measured in 33 control subjects and 86 patients with either gastric cancer (n = 34), gastric ulcer (n = 27) or chronic atrophic gastritis (n = 25). Carcinoma of the stomach was found in the antrum (n = 22), the body (n = 3) and the fundus (n = 9), and according to histopathological findings was divided into adenocarcinoma (n = 21), squamous cancer (n = 4) and undetermined (n = 9). Gastric ulcer, when present, appeared in the antrum (n = 18), the body (n = 3) and the fundus (n = 9). Chronicatrophic Gastritis cases were all associated with intestinal metaplasia.

RESULTS: The normal ranges established for CA50 and CEA in the control group were 16.26-6.14 kU/L and 3.12-1.03 μg/L respectively. In patients with gastric cancer, serum levels of CA50 (112.67 ± 38.36 kU/L) and CEA (10.28 ± 3.76 μg/L) were elevated significantly (P < 0.01), the former being < 22 kU/L in 18 of 34 patients (53%; range: 5-550 kU/L) and the latter being < 5 μg/L in 19 of 34 patients (55.8%; range: 0.5-17.4 μg/L). A statistically significant correlation was found between the levels of CA50 and CEA (r = 0.648, P < 0.01). The serum levels of CA50 (46.4 kU/L *vs* 25.9 kU/L, P < 0.01) and CEA (6.85 μg/L *vs* 2.43 μg/L, P < 0.01) were much lower in patients with gastric ulcer or chronic atrophic gastritis (P < 0.05).

CONCLUSION: CA50 and CEA are indicators for advanced gastric cancer, and postoperatively their serum levels may decrease considerably. Overall, there is such a close correlation between these two factors that in clinical practice they might be of great value for the diagnosis of gastric cancer.

Key words: Gastroesophageal reflux/diagnosis; Esophagitis/diagnosis; Hydrogen-ion concentration

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INTRODUCTION

Cancer antigen 50 (CA50) is a monoclonal antibody raised against another human colorectal carcinoma cell line COLO 205[1,2]. It reacts with an epitope present on two carbohydrate moieties, the sialylated Lewis blood group antigen and the sialosyl-lactoetraose, lacking the fucosyl residue of the sialylated Lewis antigen[1,3]. Normally, the sialylated Lewis is the dominant CA50 ganglioside in epithelial carcinomas[4]. The sialosyl-lactoetraose has been found in small amounts in various carcinomas, defined as Lewis[5]. The CA50 is expressed on cell surfaces as glycolipids and glycoproteins. In serum, the antigen is associated with a high molecular weight carbohydrate-rich mucin fraction[6]. The CA50 level is high in many patients with digestive tract malignancies. The highest frequencies of elevated level are found in ovarian cancer patients (70%) and biliary cancer patients (60%), but elevated values have also been found in patients with colorectal, gastric and liver cancer[1]. Increased serum concentrations of CA50 have also been observed in some benign diseases.

Carcinoembryonic antigen (CEA) is an oncofetal protein originally described in 1965, which has become one of the most commonly used serum tumor markers and is frequently elevated in patients with colonic, pancreatic, lung, and breast cancers[7]. The extent of elevation of CEA in colon cancer is related to the patient's overall tumor burden[2,8]. However, the usefulness of CEA is limited by the marker's overall low sensitivity in patients with minimal disease extent and by effects of smoking and benign gastrointestinal diseases on its specificity[1,9]. Nonetheless, CEA is a marker approved for monitoring the recurrence of colon cancer. It has also been proposed for a similar purpose for other gastrointestinal tract cancers and breast and lung cancers.

In the present study, the serum levels of CA50 and CEA were determined in patients with digestive tract diseases and those with digestive tract cancers.

Table 1 Serum Cancer antigen 50 and Carcinoembryonic antigen in gastric diseases (mean ± SD)

Group	CA50, kU/L	CEA, μg/L
Gastric cancer	112.67 ± 38.36[b]	10.28 ± 3.76[b]
Gastric ulcer	21.42 ± 9.57[a]	4.48 ± 1.09
Atrophic gastritis	20.82 ± 8.95[a]	4.52 ± 1.45
Controls	16.26 ± 6.14	3.12 ± 1.03

[b]$P < 0.01$ *vs* controls and benign gastric diseases; [a]$P < 0.05$ *vs* controls.

MATERIALS AND METHODS

Materials

The study consisted of: 34 patients with gastric cancer in an advanced stage, including 12 women and 22 men with a mean age of 38.2 years (s: 10.4); 27 patients with gastric ulcer in active stage, including 11 women and 16 men with a mean age of 39.4 years (s: 9.3); 25 patients with chronic atrophic gastritis associated with intestinal metaplasia (IM), including 11 women and 14 men with a mean age of 41.2 years (s: 10.6). Carcinoma of the stomach was found in the antrum (n = 22), the body (n = 3) and the fundus (n = 9), and was classified by histopathological results as adenocarcinoma (n = 21), squamous cancer (n = 4) and undetermined (n = 9). Gastric ulcer, when present, was in the antrum (n = 18), in the body (n = 3) and in the fundus (n = 9). The study also included 33 healthy subjects, including 9 women and 24 men with a mean age of 38.6 years (s: 9.7) who served as controls. Four mL of fasting blood were collected and the serum was immediately separated by centrifugation, frozen and stored at -20 ℃ until use in analysis.

Methods

Commercially available solid radioimmunoassay (RIA) was used for quantitative determination of the serum CA50 concentrations, as previously described in detail[10]. The recommended cut-off value of 22 kU/L was used. CEA assays were performed using a solid-phase two-site immunoradiometric assay (The general Hospital of Chinese PLA, Beijing, China)[11]. The samples reacted with beads dual-coated with a capture monoclonal antibody directed against one CEA antigenic site and with a radiolabelled marker monoclonal antibody. The radioactivity bound was measured with a gamma counter and was proportional to the concentration of CEA in the test sample. The recommended cut-off value was 5 μg/L. Both coefficients of variation (CV) were controlled to less than 10%. The smallest amount of tumor markers detectable with 95% confidence was 1 kU/L for CA50 and 0.1 μg/L for CEA.

Statistical analysis

The levels of the tumor markers are given as mean ± SD. The unpaired Student's t-test was used for comparison of the data for controls and patients. Correlations were calculated on the values of CA50 and CEA with linear regression analysis. $P < 0.05$ was considered significant.

RESULTS

The values of CA50 and CEA in the series of patients are summarized in Table 1. In patients with gastric cancer, the CA50 level was elevated in 18 of 34 patients (53%; range: 5-1550 kU/L). Benign gastric diseases were also associated with an increased concentration in 2 of 27 patients with gastric ulcer (7.4%; range: 5-276 kU/L) and in 3 of 25 patients with chronic atrophic gastritis (12%; range: 5-285 kU/L), whereas none of the normal subjects had an increased CA50 concentration. The CEA level was elevated in 19 of 34 patients with gastric cancer (55.8%; range: 0.5-17.4 μg/L) and in 2 of 27 patients with gastric ulcer (7.4%; range: 0.1-8.6 μg/L) and in 2 of 5 of patients with chronic atrophic gastritis (8%; range: 0.2-8.7 μg/L), whereas none of the normal subjects had an increased CEA concentration.

A statistically significant correlation was found between the CA50 and CEA levels (r = 0.648, $P < 0.01$) in patients with gastric cancer. The postoperative serum levels of CA50 (46.4 ± 25.9 kU/L, $P < 0.01$) and CEA (6.85 ± 2.43 μg/L, $P < 0.01$) were much lower post-operative (n = 21) than pre-operative. However, changes in the levels of CA50 ($P < 0.05$) and CEA ($P > 0.05$) were also observed in patients with gastric ulcer or chronic atrophic gastritis.

DISCUSSION

The results show that fasting serum CA50 and CEA levels are changed in gastric cancer. Both CA50 and CEA values are indicators for advanced gastric cancer, and after surgery, the values of CA50 and CEA may decrease. Overall, there was a good correlation between the CA50 and CEA levels. In clinical practice, the greatest value of CA50 and CEA is in the diagnosis of gastric cancer. We have not calculated the sensitivities and specificities of the CA50 and CEA tests in this study, but when we used cut-off values based on normal subjects, as expected, a good correlation was found between the serum concentrations of CA50 and CEA; although, some patients showed a clear difference in the serum levels. Both markers are usually elevated in the same patients, and a combination of the tests would therefore be of no benefit[2-4,12].

Originally, the broader reactivity of the CA50 antibody was thought to be an advantage for Lewis-negative subjects, who normally do not express sialyated Lewis but may synthesize sialosyllactotetraose[1]. However, concentrations of CA50 and CEA in the membrane fraction tended to be higher in normal tissue than in carcinoma, whereas the cytosol-to-membrane ratio was significantly higher in carcinoma. For CA50 and CEA, the phenotypic pattern of malignant transformation seems to involve a different intracellular distribution rather than a quantitative change. CEA, a most popular and useful tumor marker for cancer of digestive organs, is frequently positive in sera of colorectal cancer patients who had no subjective complaint or physical sign. This experience supported employment of CEA as a routine screening test for colorectal cancer[6-8,13].

CA50 and carbohydrate antigen 19-9 (CA19-9) perform best for the diagnosis of pancreatic cancer. SOC analysis has confirmed previous data, showing only a marginal difference between the markers in this disease. Both tests have shown higher sensitivity than CEA[6]. The histologic type of a cancer may have limited relevance in cancer screening, although known carcinogenic exposures seem to have some specificity in inducing some particular histologic gastric cancer types. However, marker specificity for certain histologic cancer types may be more important from the diagnostic viewpoint. The markers or their combinations studied here show no significant association with any histologic cancer type, with the exception of the combination of CEA and CA50 for adenocarcinomas[2].

The measurement of CA50 in serum and pleural fluid by immunoradiometric assay was presented in 45 (27 malignancy and 18 tuberculosis) patients with pleural effusion. The mean CA50 level in malignant effusion (89.26 ± 122.32 kU/L) was significantly higher than that in tuberculous effusion (5.18 ± 8.65 kU/L) ($P < 0.01$). CA50 levels of pleural fluid above an arbitrary level of 20 U/mL were found in 78% of malignant fluids and in 6% of tuberculous fluids. The serum CA50 value from 27 patients with malignant effusion (58.67 ± 85.85 kU/L) was also higher than that from 18 patients with tuberculous effusion (6.18 ± 8.37 kU/L) ($P < 0.01$). Serum CA50 levels above the same level were found in 58% of patients with malignant fluid and in 6% of patients with tuberculous fluid. The results suggest that the measurement of CA50 in pleural effusion may be helpful in the differential diagnosis between tuberculous and malignant effusions[12].

A new cell line (BRC-230) was established from a surgical speci-

men of primary ductal infiltrating breast carcinoma. The epithelial nature of this cell line was confirmed by ultrastructural analysis and demonstrated the retention of structural properties characteristic of the original tumor. The BRC-230 cell line was then shown to induce tumors in athymic Crl: nu/nu (CD-1)BR nude mice. It possessed an abnormal karyotype with a model chromosome number between 60-61 with eight recurrent marker chromosomes, and it presented a doubling time of 30.5 h. Scatchard analysis demonstrated that both primary tumor and BRC-230 cells are estrogen and progesterone receptor negative. Immunoenzymatic and radioimmunoassays show a production of marker antigens (CEA, TPA, CA125, CA15-3, CA19-9) that are similar in the patient's serum and BRC-230 cells. The *in vitro* drug sensitivity assay of the cell line and of the parental tumor tissue shows overlapping results to all tested anti-blastic drugs. BRC-230 cells are resistant to 4-idroperoxy-cyclophosphamide, idarubicinol, mitoxantrone, etoposide, 4'-epidoxorubicin, and doxorubicin, showing a multiple drug resistance phenotype. Amplification or rearrangement of HER2/neu, Ha-ras, and C-myc genes is observed neither in the original tumor nor in BRC-230 cells; the *mdr-1* gene is also present in a single copy. The authors concluded from these studies that the BRC-230 cell line maintains the same characteristics as the original tumor and may provide us with a good model to study *in vitro* the biology of drug resistance of breast cancer[13].

Significant increases in the serum levels of CA125 and CA19-9 were observed over one month prior to the removal of an ovarian adenofibroma, and the serum levels of CA125 and CA19-9 decreased rapidly after surgery. The surface of the tumor at surgery showed marked inflammation, probably induced by the necrosis produced by torsion. Pathologically, most of the tumor was necrotic, and histoimmunochemical staining of the viable cells was weak for CA125 but intense for CA19-9. Clinicopathological observations suggested that CA125 and CA19-9 might be stimulated in the cells by inflammation or that originally existing CA125 and CA19-9 were released from the tumor cells following the cell necrosis[14].

Most of the recently developed tumor antigens detected by monoclonal antibodies are sugar chains and frequently associated with blood group substance. Using immunohistochemical studies, we evaluated the clinical usefulness and significance of the serum assay of CA-50 classified as a type Ⅰ sugar chain, sialyl SSEA-1 as a type Ⅱ sugar chain, and ST-439 with an undetermined structure, as well as their clinicopathological significance. In addition, the value of measurement of CA19-9, ST-439, and SLX in pure pancreatic juice (PPJ) was investigated. Furthermore, the clinical usefulness of K-ras mutation at codon 12 in PPJ was studied. The incidence of serum CA19-9 among tumor markers was highest in pancreatic cancer (81%), but relatively high in benign diseases. On the other hand, both serological and immunohistological studies showed that sialyl SSEA-1 and ST-439 were highly specific for the tumor, whereas they appeared in serum or tumor with less frequently than CA19-9 or CA50 carrying the type Ⅰ sugar chain. The accuracy of the tumor markers (CA19-9, sialyl SSEA-1, and ST-439) for pancreatic cancer was almost equal to and even higher than (77% to 80%) that of CEA (69%). However, a highly positive correlation between sialyl SSEA-1 and ST-439 was revealed as well as among type Ⅰ sugar chains in malignant diseases. These data suggest that a combination assay with CA19-9 or a similar tumor marker, sialyl SSEA-1 or ST-439, and CEA would be appropriate for the screening of pancreatic cancer. When the cut-off value was set as the x- ± 2s of the controls, significantly elevated concentrations of CA19-9 in PPJ were found in the secretory phase in 90% of the patients with pancreatic cancer and 66% of the patients with chronic pancreatitis. Although increased concentrations of CA19-9 in PPJ have no cancer specificity, measurement of CA19-9 in PPJ can be used as a sensitive marker for some pancreatic disorders. On the other hand, concentrations of ST-439 and SLX in PPJ are significantly increased only in pancreatic cancer, and their positive rates are 50% and 40%, respectively. While they have a high tumor-specificity, their positive rates are not as high as initially expected[15].

The immunohistochemical expression of CA19-9, epithelial membrane antigen (EMA), and CEA was studied in 103 tissue samples of gallbladder cancer and 25 samples of non-tumoral gallbladder lesions. CA19-9 and EMA were positive in > 90% of cancers and in none of the non-tumorous lesions. Dupan-2 expression was observed in 100% of the non-tumorous lesions and 78% of the cancers. CEA expression was observed in 12% of the non-tumorous lesions and 89% of the cancers. The magnitude of immunohistochemical staining was moderate or intense for all antibodies, except Dupan-2. No differences were observed in the location of positive staining in superficial or deep parts of the tumor. In these lesions, the positive staining was cytoplasmic with a granular and irregular pattern; on the contrary, in the non-tumorous lesions, staining was seen in the apical parts of the cell. The calculated sensitivity, specificity and positive predictive value for CEA were 89%, 88% and 96% respectively. If future studies disclose a good correlation between serological and immunohistochemical detection of this antigen, its determination would be potentially useful in clinical settings[16].

Our results indicate that serum CA50 and CEA markers are different between malignant and benign stomach diseases. CA50 and CEA can be indicators of advanced gastric cancer, and after surgery the CA50 and CEA levels may decrease. Overall, there is a good correlation between the CA50 and CEA levels. In clinical practice, the greatest value of CA50 and CEA lies in the diagnosis of gastric cancer.

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CLINICAL ARTICLES

Alterations of red blood cell immunoadherence function in hepatitis B patients

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Abstract

AIM: To investigate the alterations of red blood cell (RBC) immunoadherence function in patients with hepatitis B.

METHODS: RBCC3bRR, RBCICRR and serum CIC levels were measured in 42 patients with acute and chronic hepatitis B at active and convalescence stages.

RESULTS: RBCC3bRRs at the active/acute stage of hepatitis were decreased, with 13.54% ± 5.23% in acute hepatitis, 7.61% ± 4.12% in acute fulminant hepatitis, and 16.18% ± 6.10% in chronic hepatitis, all of which were lower than the value in normal persons (18.12% ± 3.91%). At the quiescent/recovery stage of hepatitis, the RBCC3bRRs were increased significantly. The changes of RBCICRR and serum CIC level were contrary to those of RBCC3bRR.

CONCLUSION: RBC immunoadherence function is decreased in acute and chronic hepatitis, and the decrease is in direct proportion to severity of the diseases.

Key words: Hepatitis, viral, human/immunology; Erythrocytosis/immunology; Antigen-antibody complex/blood

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INTRODUCTION

Red blood cells (RBCs) have a multitude of immunological functions, the most important being immunoadherence which has garnered much research attention in recent years. In previous studies[1,2], we observed that the RBC immunoadherence function as significantly reduced in rats with acute liver damage induced by D-galactosamine. In the present study, the RBC immunoadherence function was detected in patients with acute and chronic hepatitis B by determining the RBC C3b receptor yeast rosette rate (RBCC3bRR) and the RBC immune complexes rosette rate (RBCICRR).

MATERIALS AND METHODS

Materials

Forty-two patients with acute and chronic hepatitis B participated in the study. The diagnosis had been made according to the diagnostic criteria for hepatitis revised by the National Viral Hepatitis Conference held in Shanghai in 1990. Among the patients were 14 with acute hepatitis (AH; including 9 males and 5 females with mean age of 39.5 years), 7 with acute fulminant hepatitis (AFH; including 5 males and 2 females with mean age of 35.4 years), 21 with chronic hepatitis (CH; including 17 males and 4 females with mean age of 36.3 years). All patients had evidence of HBV infection. Blood specimens were obtained from the patients at the active (or acute) stage and the convalescent stage. Fifteen healthy blood donors were recruited for participation as normal controls (NC; mean age: 35.6 years).

Methods

RBCC3bRR test: Heparin-anticoagulated blood specimen (1 mL) was mixed with Fiscol liquor and was centrifuged to separate the RBCs. The RBCs were then washed three times with normal saline and resuspended in the same (1.25 × 10 cells/mL). Next, 0.5 mL of complement-sensitized yeast (1 × 10/mL) was added into 0.5 mL of the RBC suspension, and then incubated at 37 ℃ in a constant-temperature water bath for 30 min. After being diluted with 1 mL of normal saline, the RBC sample was fixed with 0.3 mL of 0.25% glutaraldehyde. An aliquot of the prepared RBC suspension was smeared on a slide and stained with Wright's stain. The number of RBCs combining with two yeasts or more was calculated among 200 RBCs and represented the RBCC3bRR.

RBCICRR test: This procedure was performed the same as described above, except that the yeast used was not sensitized by complement. The circulating immune complexes (CIC) test was then performed on the serum samples by using the polyethylene glycol precipitation method.

Table 1 Changes in RBCC3bRR, RBCICRR and serum CIC in hepatitis

Group	RBCC3bRR, %		RBCICRR, %		Serum CIC, U/L	
	A stage	C stage	A stage	C stage	A stage	C stage
AH	13.54 ± 5.23	17.47 ± 3.82	6.46 ± 2.44	4.73 ± 2.21	470.5 ± 97.3	285.3 ± 57.7
AFH	7.61 ± 4.12	15.38 ± 3.55	9.10 ± 3.83	5.32 ± 1.68	534.7 ± 173.8	227.1 ± 76.4
CH	13.96 ± 5.01	15.42 ± 5.13	7.23 ± 1.94	5.78 ± 4.88	401.9 ± 137.4	357.2 ± 90.1
NC	18.12 ± 3.91		4.61 ± 1.12		216.6 ± 34.2	

A: Active; C: Convalescent; AH: Acute hepatitis; AFH: Acute fulminant hepatitis; CH: Chronic hepatitis NC: Normal controls.

RESULTS

RBCC3bRR change in hepatitis

The RBCC3bRRs were lower at the active stage of AH and CH, compared to the convalescent stage of each. The more severe the disease, the lower the RBCC3bRR. There was no significant difference between AH and CH. The RBCC3bRR increased gradually as the patients recovered. Of the 7 patients with AFH, 3 died of acute liver failure; RBCC3bRRs in the 4 survivors also significantly increased. In the CH group, however, the increase was much slower.

RBCICRR changes in hepatitis

The RBCICRRs were higher in the patients with hepatitis than in the NCs (in the order of AFH > CH > AH > NC), which was inversely proportional to the changes observed for the RBCC3bRR (r = -0.863, $P < 0.05$).

Serum CIC level

The serum level of immune complexes was higher in patients with hepatitis than in the NCs, which was correlated strongly with RBCICRR changes (r = 0.824, $P < 0.05$) but negatively with RBCC3bRR changes (r = -0.959, $P < 0.01$)(Table 1).

DISCUSSION

The present study demonstrated that RBCC3bRRs in AH and CH are decreased and the degree of decrease is closely related to the severity of liver damage. When the patients recovered or were in the quiescent stage, RBCC3bRR became elevated. Therefore, RBCC3bRRs can be used to assess the severity and prognosis of hepatitis conditions.

It has been verified that there are type Ⅰ complement receptors (CR1) on the surface of RBCs, through which the RBCs combine with C3b-opsonized immune complexes and bring them to macrophages in the liver and other organs and finally are eliminated[3,4]. RBCC3bRR and RBCICRR tests are reliable and convenient for detecting the RBC immunoadherence function[5]. The decrease of RBCC3bRR observed in this study suggests that the number of CR1 is reduced and its function is diminished.

In a recent *in vivo* study[1,6], we observed that the RBC immunoadherence function in acute liver damage was reduced significantly. The degree of the depression was strongly related to the increase of hepatic and serum lipid peroxidate (LPO) level. *In vitro* study also showed that LPO could reduce RBCC3bRRs. Antioxidant therapy with vitamin E protected against the observed reduction. The reason for this finding may be that LPO can combine with amino acids of the CR1 protein, causing intra- and inter-cross linking, and thereby destroying the normal structure of CR1 and leading to the decrease in RBC immunoadherence function.

In the present study, we found that the CIC was increased in relation to the decrease of RBCC3bRR. Because CR1 plays an important role in eliminating CIC, and about 95% of total C3b receptors in circulation are on the RBC surface, the increase of RBCICRR reflects in part the increase of CIC. The mechanism underlying the decrease in RBC immunoadherence function may be a complicated one. Further study will be beneficial to understand the relation of these findings to the prevention and treatment of hepatitis.

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Bacterial infections in cirrhotic patients with hepatocellular carcinoma

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Abstract

AIM: To establish the prevalence of bacterial infection in cirrhotic patients with hepatocellular carcinoma (HCC).

METHODS: All 719 cirrhotic patients with HCC were investigated retrospectively for occurrence of bacterial infections.

RESULTS: The incidence of bacterial infection was 15.4% (111/719). According to Child-Pugh classification, the incidences of bacterial infection in Class A, B and C were 2.3%, 8.0%, and 26.4%, respectively. The bacterial infection incidence increased with the severity of cirrhosis, and severe bacterial infections usually occurred in Child-Pugh class B and C patients.

CONCLUSION: The susceptibility of HCC patients to bacterial infection is mainly due to the underlying cirrhosis and not to the HCC itself.

Key words: Liver cirrhosis; Liver neoplasms; Bacterial infection; Hepatocellular carcinoma (HCC)

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INTRODUCTION

The predisposition of cirrhotic patients to bacterial infections is well documented[1-5]. However, the susceptibility to bacterial infections according to the severity of liver disease has not yet been evaluated. There has been no published report of bacterial infections in patients with hepatocellular carcinoma (HCC), except for cases of spontaneous bacterial peritonitis (SBP)[6]. In China, the majority of HCC reportedly occurs in patients with cirrhosis.

We undertook the present study with three aims: (1) to establish the prevalence of bacterial infections in HCC patients; (2) to evaluate the susceptibility of HCC patients to bacterial infections, according to the severity of the liver disease; and (3) to assess the clinical and bacteriological features of bacterial infection in cirrhosis with HCC.

MATERIALS AND METHODS

We retrospectively investigated 719 cirrhotic patients with HCC admitted to our hospital from September 1985 to July 1995. Altogether, 543 males and 176 females participated in the study, aged between 50 years to 68 years. The diagnosis of cirrhosis with HCC was verified by liver biopsy, ultrasonography, computerized tomography, and/or angiography. All patients were confirmed to have underlying cirrhosis. The grading of the severity of the liver cirrhosis was made according to the Child-Pugh classification. Hepatitis B and C sera were examined, and 78% of the cases tested were found to have hepatitis C antibody but the rest remained untested. The schistosomal circumoval precipitin test (COP), indirect hemagglutination test (IHT) and enzyme linked immunoassay (ELISA) were performed. Diagnosis of primary biliary cirrhosis was made based on the histopathology and the positive result for anti-mitochondrial antibody (AMA). Complete physical examinations, standard laboratory tests, chest X-ray, and cultures of blood, urine, and other body fluids were performed whenever signs of infection became apparent. A diagnosis of bacteremia, urinary tract infection, or bacterial enteritis was made according to positive culture. SBP was diagnosed as ascitic PMN > 250 × 10^6 cells/L or by positive culture.

RESULTS

Incidence of bacterial infection relevant to Child-Pugh classification

To investigate the effect of hepatic functional reserves on the incidence of bacterial infection, we divided the patients into three groups: 173 patients were in class A, 201 in class B, and 345 in class C. Differences were not significant among the three groups. Table 1 shows that the rate of bacterial infection increased with the severity of cirrhosis.

Etiology of cirrhosis and bacterial infection

Susceptibility to bacterial infection was compared in patients with varied etiologies of cirrhosis. Differences were not significant among the five groups as to average age and to distribution of patients according to Child-Pugh classification. The incidence of bacterial infection in patients with HCC was 18.7% (38/203) for schistosomiasis cirrhosis, 14.0% (26/186) for hepatitis B cirrhosis, 14.2% (41/289) for non-A, non-B hepatitis, and 13.3% (2/15) for primary biliary cirrhosis (Table 2). The difference in rates of bacterial infec-

Table 1 Child-Pugh classification and incidence of bacterial infections in patients with hepatocellular carcinoma

Child-Pugh class	*n*	No. of bacterial infections	%
A	173	4	2.3
B	201	16	8.0[a]
C	345	91	26.4[b]
Total	710	111	15.4

[a]$P < 0.05$ *vs* class B; [b]$P < 0.01$ *vs* class A or B.

Table 2 Etiology of cirrhosis and incidence of bacterial infections

Etiology of cirrhosis	*n*	Bacterial infection	
		Rate[1]	%
Schistosomiasis	203	38	18.7
Hepatitis B	186	26	14.0
Non-A, non-B hepatitis	289	41	14.2
Primary biliary cirrhosis	15	2	13.3
Other causes	26	4	15.4

[1]No significant differences, as analyzed by χ^2 test.

Table 3 Organisms isolated from infectious ascites in cirrhotic patients with hepatocellular carcinoma

Organism	Blood	Fluid	Sputum	Urine	Other	Total	%
Gram-positive						68	33.3
S. aureus (MRSA)	5 (2)	3 (1)	6 (3)	2	7	23 (6)	11.3
Enterococcus	2	3	3	4	4	16	7.8
S. epidermidis	3	1	2	1	2	9	4.4
S. pneumoniae			4		3	7	3.4
Others	1	2	8	1	1	13	13
Gram- negative						122	59.8
E. coli	4		5	8	6	27	13.2
Pneumoniae	6	4	6	2	3	20	9.8
P. aeruginosa	3	3	7	2	3	20	9.8
Enterobacter	1	5	2	6	1	10	4.9
Serratia	2		2	1	2	9	4.4
Cepacia	4			2	3	9	4.4
Others	3	2	8	9	5	27	13.2
Candida sp.	3	1	5	2	3	14	6.9

tion was not significant among the five groups (by χ^2 test).

Bacteriology

There were 260 cases of bacterial infections among 111 patients, and 204 yielded isolates of bacteria. The isolates consisted of 59.8% (122/204) of Gram-negative bacilli, *Escherichia coli* and *Klebsiella pneumoniae*, with *Pseudomonas aeruginosa* being the most frequent. The most common Gram-positive isolate was *Staphylococcus aureus*, with 26.1% (6/23) of the *S. aureus* isolates being methicillin-resistant (MRSA). Blood cultures yielded one organism in all bacteremic patients. Cases of mixed *S. aureus* and *K. pneumoniae* were also seen. Among 32 cases of bacteremia, 16 were spontaneous, 8 were associated with SBP, 3 were associated with urinary tract infection, 3 were associated with infections from intravenous cannulation, and 2 were associated with pneumonia. Positive cultures were obtained in 15 (68.2%) of the 22 total SBP cases. A single bacterium was often recovered in patients with SBP (66.7%, 10/15). *E. coli* and *P. aeruginosa* were the two most common organisms. The remaining 7 SBP patients had culture-negative neutrocytic ascites (CNNA). In the 42 cases of pneumonia, 57 bacteria were isolated, among which *K. pneumoniae*, P. aeruginosa, and *S. aureus* were most frequent. Seventy-five percent (30/40) of the organisms isolated from urine were Gram-negative bacteria, usually of enteric origin (Table 3).

DISCUSSION

The high susceptibility of cirrhotic patients to bacterial infections is due to decreased function of the hepatic reticuloendothelial system, diminished opsonic activities, low levels of serum complement, and impaired monocytic and neutrophil phagocytic functions. Rimda *et al*[4] reported that the elimination rate constant of technetium^{99m}sulfur colloid (k-Tc) is a good index for risk evaluation, as it shows a direct correlation with the plasma elimination rate constant of indocyanine green (k-ICG). In our patient series, the rate of bacterial infection in cirrhotic patients increased with severity of cirrhosis, according to the Child-Pugh classification; a similar pattern was also observed in HCC patients. The susceptibility of HCC patients to bacterial infection is related mainly to the underlying cirrhosis rather than to HCC itself.

The occurrence of bacterial infection in patients with liver diseases is difficult to ascertain from the literature[1]. The high susceptibility to bacterial infection of patients with cirrhosis may be related to the severity of the liver disease rather than to the causes of cirrhosis. Severe bacterial infections, in particular bacteremia, pneumonia and SBP, were more commonly seen in Child-Pugh class B and C patients, who tended to have a high mortality rate.

Infection of the urinary tract, bacteremia and pneumonia are the most common sources of bacterial infection, although patients with ascites are particularly prone to SBP[4]. Pneumonia was the most common bacterial infection in our patient series and was associated with a mortality of 26%. One study reported that infections in soft tissue were the most common source of bacteremia, and Gram-positive organisms were isolated in 69%[2]. Half of the bacteremic episodes of our patients were spontaneous and were caused by bacteria normally present in the intestinal flora, as previously reported by others[4-6]. These spontaneous infections in patients with cirrhosis may be due to the passage of the intestinal bacteria from the bowel into the portal circulation. Clinically apparent foci of infection were found in half of the bacteremic cases. SBP was the most common source of bacteremia, followed by urinary tract infection and then by infection from intravenous cannulation. Half of the urinary tract infections were catheter-related.

Our study indicated that bacterial infection was a major problem in HCC. Despite the rapid initiation of appropriate antibiotic therapy, a high mortality rate was observed. The observed high incidence of and high mortality from bacterial infection in patients with advanced chronic liver disease emphasized the need to consider prophylactic parenteral antibiotics therapy as gut sterilization with oral antibiotics in these high-risk patients. Most of the microorganisms isolated in

our study were highly sensitive *in vitro* to imipenem and ofloxacin, either of which may represent an effective first-line therapy or prophylactic therapy for bacterial infection in cirrhosis with HCC.

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Clinical significance of immunohistochemical study of p53 protein in colorectal carcinoma

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Abstract

AIM: To determine the clinical significance of p53 protein expression in colorectal carcinoma.

METHODS: The expression of p53 protein was examined in 92 colorectal carcinomas using the monoclonal antibody PAb 1801. Correlation between p53 protein expression and prognosis in colorectal carcinoma was analyzed using the log-rank test.

RESULTS: The frequency of p53 protein expression was 57.61%, corresponding with Dukes' stage of bowel cancer. Analysis of survivor data demonstrated that the survival rate of the colorectal carcinoma with positive staining for p53 protein was lower than that of group with negative staining.

CONCLUSION: Expression of p53 protein is correlated with poor prognosis in colorectal carcinoma.

Key words: Colorectal neoplasms; Oncogenes; Protein p53; Immunohistochemistry

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INTRODUCTION

p53 gene mutations and differential p53 protein expression occurs in most human tumors[1-5]. In the case of breast carcinoma, the immunoreactivity of p53 protein has been shown to correlate with poor prognosis[5,6]; however, whether p53 protein expression is correlated with prognosis of colorectal carcinoma remains controversial.

MATERIALS AND METHODS

Clinical data

Ninety-two patients with colorectal carcinoma were included in this study. The mean age at operation was 55.53 ± 13.10 (range: 23-82)-year-old, and the cohort consisted of 49 males and 43 females. The distribution of these tumors according to the Dukes' staging system was 17 cases of stage A, 33 cases of stage B, and 42 cases of stage C. The histological classification of tumors was based on previous criteria[7], and consisted of 9 well-differentiated adenocarcinoma, 62 moderately-differentiated, and 21 poorly-differentiated. Sixty-three of the patients were followed up for 4 years after operation.

Immunohistochemistry

Immunoperoxidase staining was performed on sections of formalin-fixed paraffin-embedded tumor blocks using monoclonal antibody derived from fusion of BALB/C splenocytes with NB-1 mouse myeloma cells, specific for human p53 protein with binding near the N terminals (clone PAb 1801; Oncogene Science Inc, United States). For the staining procedure, 5-micron sections were deparaffinized, rehydrated and placed in a solution of 1% H_2O_2 in methanol to inactivate endogenous peroxidase. After washing with tap water and PBS (2 × 5 min each), the sections were incubated with 10% normal goat serum for 30 min to reduce nonspecific staining. The specimens were then incubated with 1:50 PAb 1801 in a moist chamber overnight at 4 ℃. The sections were washed with PBS, incubated with a 1:400 dilution of biotinylated rabbit anti-mouse IgG (Dako A/S, Denmark) at 37 ℃ for 30 min, and covered with a 1:400 dilution of avidin-HRP (Dako A/S) at 37 ℃ for 30 min. After a final wash with PBS, the antibody staining was visualized by exposure to diaminobenzidine in TBS containing fresh hydrogen peroxide (0.01% H_2O_2). As a last step, the slides were lightly stained in Mayer's hematoxylin. Negative controls were prepared by replacing the primary antibody with PBS.

Statistical analysis

Correlations between the p53 protein expression and clinico-pathologic features were determined by using the chi-square test with Yates' correction. Survival data was analyzed by using the log-rank test. $P < 0.05$ was considered to be statistically significant.

RESULTS

The p53 immunostaining was localized to the nuclei of neoplastic

Table 1 p53 protein expression in colorectal cancer

Dukes' stage	*n*	p53 (+), *n* (%)	Differentiation	*n*	p53 (+), *n* (%)
A	17	6 (35.30)	well	9	4 (44.44)
B	33	19 (57.58)	moderate	62	37 (59.67)
C	42	28 (66.67)	poor	21	12 (57.14)

$P < 0.05$ *vs* Dukes' stage C. Survival rate of patients with p53 positive staining was compared with that of patients with p53 negative staining. Patients with p53 positive colorectal carcinomas showed a significantly shorter survival period as compared with those without p53 ($P < 0.05$).

cells. Normal colorectal mucosa and the control group were completely negative for p53 protein. The majority of p53 protein in colorectal carcinomas showed a uniform immunostaining pattern throughout the carcinomatous tissues for all or nearly all malignant cells. In 92 patients with colorectal carcinoma, 53 (57.6%) had positive immunostaining for p53 protein. Positive staining for p53 protein was found in 35.3% (6/17) of patients with Dukes' A, 57.6% (19/33) of Dukes' B and 66.7% (28/42) of Dukes' C. The proportion of positively reacting colorectal tumors increased as the Dukes' staging progressed, and the incidence of p53 expression in Dukes' stage C was significantly higher than that in Dukes' stages A and B ($P < 0.05$). However, no relationship between p53 protein expression and tumor differentiation was found ($P > 0.05$).

DISCUSSION

The *p53* gene mapped on chromosome 17 short arm contains 11 exons and 10 introns[8,9]. Among these, exons 5 through 8 are considered a mutant hot spot due to the inclination of those sequences towards mutation[9]. The *p53* gene is an important tumor suppressor gene. The wild-type *p53* gene can suppress tumor cell growth and reverse the transformed phenotype[10,11]. Baker *et al*[12] reported that when cells were transfected with the wild-type *p53* gene they formed colonies 5- to 10-fold less efficiently than cells transfected with the mutant *p53* gene. The p53 protein, encoded by the *p53* gene, functions in negative growth regulation to control cell proliferation[13]. This is role is based in part on its ability to bind DNA[14]. Most mutations that occur in any of the four evolutionarily conserved domains alter the conformation of the p53 protein[15], which result in DNA binding that is weaker than that of the wild-type p53. Mutant p53 proteins have an increased half-life, and they are more stable than the wild-type p53 in tissue and can be more readily detected by immunohistochemistry. Expression of the p53 protein has been demonstrated immunohistochemically in a number of human malignancies, including carcinomas of colon[1], breast[2] and lung[3]. In breast cancer, p53 expression is reportedly related to estrogen, growth factor receptor status and tumor infiltration, as well as to poor prognosis[5,6]. Therefore, p53 expression in breast cancer represents as a new marker for judging prognosis. However, opinions regarding its precise role in determining prognosis are varied among the authors in the literature.

Using the log-rank test to analyze the follow-up data of 64 cases, we found that survival rate of patients with colorectal carcinoma who showed positive staining for p53 protein was lower than the group of patients showing negative staining. The question that comes to the forefront then is: why does p53 expression correlate to poor prognosis in colorectal carcinoma? We believe that the p53 protein detected by immunohistochemistry may include almost all mutant forms of the p53 protein, and that the overall ability of this detected p53 profile to bind DNA and suppress cell proliferation was decreased compared with a profile of primarily or exclusively the wild –type form. Therefore, the colorectal carcinoma showing positive p53 protein staining has more malignant potential and a related shorter survival period than that with negative p53 protein staining.

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CLINICAL ARTICLES

Studies on surgical operations and prognosis of extrahepatic bile duct cancer

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Abstract

AIM: To perform a clinical evaluation of the surgical procedures of extrahepatic bile duct cancer and their influence on prognosis.

METHODS: A total of 55 patients with pathologically and clinically verified extrahepatic bile duct cancer treated in our department between January 1984 and December 1993 were analyzed retrospectively. Clinical courses, with respect to the surgical procedures, investigated by follow-up and the survival period was assessed.

RESULTS: Among the 55 patients, 24 received surgery that involved the upper third of extrahepatic biliary tract, 12 involving the middle third, and 19 involving the lower third. The diagnosis of bile duct cancer was confirmed histopathologically in 42 of the patients, with a clear predominance of adenocarcinoma (97.6%). Eleven (26.2%) of the patients received curative resection, 30 received palliative procedures (*i.e.* biliary-enteric bypass (n = 14) and external drainage (n = 16)), 6 received permanent percutaneous transhepatic cholangio-drainage (PTCD) alone, and 8 received exploratory laparotomy only or conservative treatment. Forty-eight patients (87.3%) were followed-up. The overall mean survival period was 10.8 ± 9.7 mo ($\bar{x}$ ± s); patients with curative resection had the longest survival period (21.4 ± 16.7 mo, $P < 0.01$) and highest survival rate ($P < 0.05$). A significant survival difference was observed for patients with biliary-enteric anastomosis as compared with those who had external drainage, *etc.* ($P < 0.05$), but there was no significant difference in survival period between patients who had preoperative PTCD (n = 23) and those who did not (n = 26) ($P < 0.05$).

CONCLUSION: Curative resection is the treatment of choice for suitable patients with extrahepatic bile duct cancer; biliary-enteric anastomosis is preferable for those with unresectable tumor in order to improve prognosis and quality of life.

Key words: Bile duct neoplasms/surgery; Prognosis; Portoenterostomy; Hepatic

INTRODUCTION

Despite the widespread application of new imaging techniques in diagnosis of bile duct cancer, the prognosis remains discouraging[1-3]. As a result of difficult early-detection and the low resectability rate of such tumors, only a few reports are available on the relationship between the surgical procedures and the prognosis of extrahepatic bile duct cancer. This study made an effort to evaluate the influence of various surgical procedures on survival and prognosis of patients with extrahepatic bile duct cancer by retrospective analysis in order to identify the best choice of management of the disease.

MATERIALS AND METHODS

A total of 55 consecutive patients with extrahepatic bile duct cancer were surgically treated between January 1984 and December 1993. This group included 30 men and 25 women, with a mean age of 65.0 years (range: 39.2-85.0 years). Taking into account the general condition of the patients and stage of the disease, we grouped the 55 cases according to the subsequent treatment, as follows: (1) curative resection, (2) biliary-enteric anastomosis, (3) operational external drainage, (4) permanent percutaneous transhepatic cholangio-drainage (PTCD), and (5) exploratory laparotomy only or conservative treatment. All patients were followed up routinely.

Statistical analysis

All the statistical analyses were made by Student's t-test and Student-Newman-Keuls test for comparison between the groups. Survival curves were constructed by the Kaplan-Meier method[4] and were compared by the log-rank test[5]. $P < 0.05$ was considered to be of statistical significance.

RESULTS

Diagnosis

Among the 55 patients, diagnosis of extrahepatic bile duct cancer was confirmed histopathologically in 42, with a clear predominance of adenocarcinoma (97.6%), including 24 infiltrative type, 6 nodular type and 11 papillary type. The other 13 patients were diagnosed clinically by imaging procedures such as ultrasonography, PTC, ERCP

Table 1 Tumor sites and surgical procedures in 42 patients with extrahepatic bile duct cancer

Tumor site	*n*	Curative resection, *n* (%)	Palliative procedures, *n* (%)		Laparotomy
			Biliary-enteric bypass	Stent insertion	
Upper third	17	2 (11.8)	5	10 (88.2)	-
Middle third	11	3 (27.3)	3	4 (63.6)	1
Lower third	14	6 (42.9)	6	2 (57.1)	-
Total	42	11 (26.2)	14	16 (71.4)	1

Table 2 Mean survival period of the total 55 patients with extrahepatic bile duct cancer according to treatment

Group	Treatment	*n*	Mean survival period ($\bar{x} \pm s$, mo)
1	tumor curative resection	11	21.4 ± 16.7^{b}
2	biliary-enteric bypass	14	12.2 ± 6.8^{a}
3	operative stent insertion	16	7.4 ± 3.4
4	permanent PTCD	6	4.3 ± 1.5
5	conservative treatment or exploratory laparotomy only	8	2.3 ± 1.8

$^{b}P < 0.01$ *vs* groups 2-5; $^{a}P < 0.05$ *vs* groups 3-5. PTCD: Percutaneous transhepatic cholangio-drainage.

Table 3 Mean survival period with or without percutaneous transhepatic cholangio-drainage prior to operation

Preoperative PTCD	*n*	Mean survival period (mean ± SD, mo)
Yes	23	12.5 ± 6.6
No	19	15.2 ± 13.4

$P > 0.05$. PTCD: Percutaneous transhepatic cholangio-drainage.

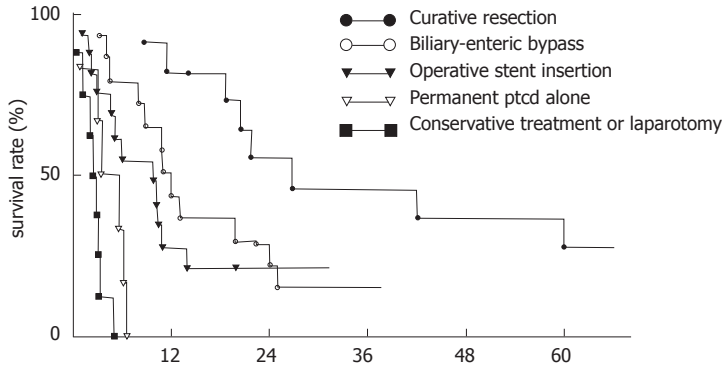

Figure 1 Kaplan-Meier survival curves for 55 patients with extrahepatic bile duct cancer. $^{a}P < 0.05$ for curative resection *vs* biliary-enteric bypass. $^{b}P < 0.01$ for curative resection *vs* operative stent insertion, curative resection *vs* permanent PTCD alone, conservative treatment or exploratory laparotomy only. PTCD: Percutaneous transhepatic cholangio-drainage.

or CT. Of these, 24 (43.6%) involved the upper third of extrahepatic biliary tract, 12 (21.8%) the middle third, and 19 (34.6%) the lower third, according to Longmire's classification[6].

Treatment

In group 1, 11 patients underwent curative resection, including left hemihepatectomy and biliary-enteric anastomosis ($n = 2$), resection of tumors with biliary-enteric anastomosis ($n = 3$), and Whipple's procedure ($n = 6$). The overall curative resection rate was 26.2%, of which the lower third cholangiocarcinoma had the highest resective rate (42.9%) and the upper third tumor had the lowest resective rate (11.8%). In group 2, 30 patients with unresectable tumors underwent palliative procedures (accounting for 54.6% of all patients and with 71.4% receiving surgical exploration), including biliary-enteric bypass ($n = 14$; *i.e.* Roux-en-Y cholangiojejunostomy ($n = 9$), cholangio-duodenostomy ($n = 1$), choledochojejuno-intubation internal drainage ($n = 1$), cholecystojejunostomy ($n = 2$) and cholecystoduodenostomy ($n = 1$)) and biliary external drainage by operative stent insertion ($n = 16$; *i.e.* extrahepatic ($n = 6$) or intrahepatic biliary T-tube intubation ($n = 7$), and hepatobiliary U-tube intubation ($n = 3$)). In group 3, 29 patients underwent PTCD, including preoperative PTCD ($n = 23$) and permanent PTCD alone ($n = 6$). In group 4, 1 patient underwent exploratory laparotomy only. In group 5, 7 patients received conservative treatment (Table 1).

Follow-up study

The range of follow-up was 1-62 mo. Seven patients were lost to follow-up. Five patients are still alive and disease-free. Forty-eight (87.3%) patients have data that are evaluable. The overall mean survival period was 10.8 ± 9.7 mo (range: 8 d to 5.2 years). The 1/2-, 1-, 2-, 3- and 5-year survival rates were 68.8%, 39.6%, 20.8%, 6.3% and 2.1%, respectively. Long-term survival following various treatment procedures is shown in Table 2 and Figure 1. Patients with curative resection had the longest mean survival period (21.4 ± 16.7 mo, $P < 0.01$) as well as the highest rate ($P < 0.05$). Biliary-enteric anastomosis gave better outcome and longer survival period than operative stent insertion, *etc.* ($P < 0.05$). There was no significant difference in the mean survival period and the survival rate between patients with operative stent insertion and permanent PTCD, conservative treatment or exploratory laparotomy only ($P > 0.05$).

The mean survival period of patients with preoperative PTCD ($n = 23$) was 12.5 ± 6.6 mo in contrast to those ($n = 19$) without which had a period of 15.2 ± 13.4 mo (Table 3). There was no significant difference between the two groups ($P > 0.05$).

DISCUSSION

Prognosis and resectability rate

Despite great advances having been made for diagnostic techniques and surgical procedures in hepatobiliary surgery, patients with extrahepatic bile duct cancer, and in particular hepatic hilar cancer, still have a poor prognosis, with less than 5%-30% surviving 5 years. The vast majority of patients died in the first year[1-3]. The cancer is frequently infiltrative and sclerotic in nature, tending to involve the hilum of the liver. As a consequence, resectability rates under 20% are common[1]. A national clinical survey in China determined the overall resectable rate of extrahepatic bile duct cancer to be 11.1%-33.3%, with 10.4% for carcinoma of the upper third of the extrahepatic bile duct[7]. However, it had also been reported that the resectable rate of carcinoma of the upper third might reach 50%[8-10].

Our data showed that the overall resectable rate of extrahepatic bile duct cancer was 26.2%, with 11.8% for carcinoma of the upper third. This finding should be ascribed to the late diagnosis and advanced lesions in our case series; fifty-four (98.2%) of these patients developed icterus at 1-6 mo prior to the operation, and 52.7% had weight loss and 32.7% developed ascites. In addition, lymph node metastasis (42.2%) and hepatic metastasis (25.5%) were present during laparotomy. Therefore, early diagnosis and operation are essential.

Prognosis and treatment procedures

Among therapeutic modalities, resection remains the best choice of treatment of these tumors[1-4,8-13]. In this case series, 42 patients received surgical exploratory operation, of whom 11 underwent curative resection and 30 underwent palliative procedures, with 6 receiving permanent PTCD alone and 7 receiving conservative medical treatment. The overall mean survival period of all patients was 10.8 ± 9.7 mo. Curative resection gave the best chance of prolonged survival and the highest survival rate, as shown in Table 2 and Figure 1. The results are consistent with the above-mentioned reports in the literature.

Patients with curative resection had better general condition, as well as being treated at an earlier stage. However, because the lesion has access to the hepatic artery and portal vein, as well as frequent liver invasion, curative resection is not possible and the surgeon is faced with choosing the most appropriate form of palliation[1,14]. Our data indicated that biliary-enteric anastomosis gave

longer survival period than operative stent insertion and permanent PTCD, *etc.* (Table 2). Biliary-enteric anastomosis has a low operative mortality rate, and less post-operative complications, such as cholangitis, which improves quality of life. Besides, there was no significant difference in survival period and survival rate between patients with permanent PTCD alone and conservative medical treatment or exploratory laparotomy only ($P > 0.05$).

PREOPERATIVE PTCD

There is considerable debate as to whether preoperative PTCD diminishes postoperative morbidity and reduces the operative mortality rate in patients who submit to surgery for obstructive jaundice. Several early reports showed that decompression and external drainage of the biliary tract can reduce the serum bilirubin level and improve operative morbidity and mortality[15,16]. However, prospective randomized controlled clinical trials from McPherson *et al*[17], Pitt *et al*[18] showed negative results.

In the current study, there was no statistically significant difference found for survival period of patients with and without preoperative PTCD ($P > 0.05$), which is consistent with the above-mentioned reports. Huang did not emphasize on this aspect[8]. The reasons were: (1) PTCD might be complicated with biliary infection resulting in loss of chance for radical operation; (2) septal occlusion may develop and impede successful drainage; (3) a decrease in serum bilirubin will not restore hepatocyte function; and (4) many PTCD-related complications are possible, such as bile leakage, loss of electrolytes, bile peritonitis, intra-abdominal bleeding, pain and catheter dislodgement[8]. Therefore, we consider that PTCD prior to surgery should be performed with caution.

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Clinico-pathologic significance of neuroendocrine cells in gastric cancer tissue

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Abstract

AIM: To elucidate the biological and clinicopathological significance of neuroendocrine (NE) cells in gastric carcinoma (GC).

METHODS: One hundred and eighty nine patients with various histological types of GC were observed using light microscopy, histochemistry, immunohistochemistry and electron microscopy. Of these, 127 patients were followed up.

RESULTS: Chromogranin A (CgA)-positive GC was demonstrated in 85 cases (45.0%). The types of NE cells in GC were probed using 9 kinds of hormone antibodies, and 49 cases (67.2%) presented more than one hormone. NE cells were found more often in poorly differentiated GC than in well differentiated GC ($P < 0.01$). Expression of some kinds of hormone was related to the differentiation and histological types of GC. bombesin, calcitonin ($P < 0.01$), gastrin and serotonin ($P < 0.05$) were more highly expressed in poorly differentiated cases than in well differentiated ones. Nineteen cases with metastatic foci in the regional lymph nodes were found to have CgA-positive cancer cells. The presence of HCG in metastatic lymph nodes was more often observed than that of other hormone ($P < 0.01$). The survival rate of patients with NE cell-positive GC was 38.9%, and of NE cell-negative GC was 52.7%. Five of 7 patients (71.4%) with somatostatin-positive GC were still alive at the follow-up (range: 33-66 mo), but 4 patients with HCG-positive GC had died (range: 12-29 mo).

CONCLUSION: NE cells occur more frequently in poorly differentiated GC. Certain hormones appear to be related to metastasis and prognosis.

Key words: Neuroendocrinology; Stomach neoplasms pathology; Immunohistochemistry; Hormone

Wang LP, Yu JY, Shi JQ, Liang YJ. Clinico-pathologic significance of neuroendocrine cells in gastric cancer tissue. *World J Gastroenterol* 1996; 2(1): 30-33 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i1/30.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i1.30

INTRODUCTION

Gastric carcinomas (GCs) contain endocrine-differentiated cancer cells as an integral tumor component. In this study, various neuroendocrine (NE) cells were examined using immunohistochemical staining and electron microscopy.

MATERIALS AND METHODS

Resected GC specimens ($n = 189$) and normal stomach specimens ($n = 5$) obtained from autopsy were collected from the general Hospital of Beijing Commanding Area during the period of 1987 to 1990. The patients (154 men, 35 women) were aged 21 to 83 years (mean, 55.9 years). The specimens were cut along the greater curvature. Four-five blocks were taken for each specimen including tumor and regional lymph nodes. The blocks were fixed in 10% formalin and embedded in paraffin. Fifteen serial sections (5 micron each) were cut from each block. Hematoxylin and eosin, alcian blue-periodic acid (AB-PAS), and orcein/AB staining were carried out. The immunohistochemical staining was visualized with the ABC procedure (ABC kit, K355; Dako, United States). The primary antibodies and positive control are listed in Table 1. All antibodies are polyclonal except for the monoclonal SERO (antibodies from Dako, Copenhagen, Denmark, except for bombesin (Bom) which was from Cambridge Research Biochemical Limited, England). Peroxidase was revealed using DAB. Control of the specificity of the immunoreaction was performed by incubating consecutive sections with non-immune serum instead of the primary antiserum or with the specific antiserum that had been preabsorbed with an excess of respective antigens.

The fresh GC specimens obtained *via* surgery and biopsy were fixed immediately by incubating in 3% sodium salt-buffered glutaraldehyde solution for 4 h, followed by rinsing with 0.17 mol/L sodium carboxylate buffer, dehydrating through an alcohol series, embedding in Epon-Araldit, and making semi-thin sections for orientation and ultra-thin sections thereafter. The sections were double-

Table 1 Tumor sites and surgical procedures in 42 patients with extrahepatic bile duct cancer

Code	Antigen	Working dilution	Positive control
A430	Chromogranin a (cga)	0.3194444	Pancreas
A586	Gastrin (gast)	0.25	Gastric antrum
A566	Somatostatin (ss)	0.3194444	Pancreas
M758	Serotonin (5-ht)	1:20	Small bowel
A576	Calcitonin (ct)	0.5972222	Medullary cancer
A231	Hcg	0.5972222	Pituitary gland
A571	Acth	0.5972222	Pituitary gland
A619	Pancreatic polypeptide (pp)	2.8194444	Pancreas
A565	Glucagon (glu)	0.5972222	Pancreas
CA08210	Bombesin (bom)	1:20000	Duodenum

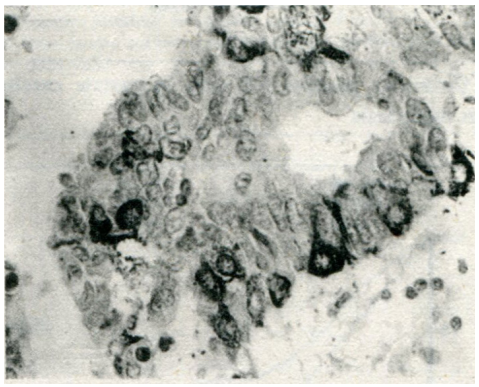

Figure 1 Triangular, flat, or cylindrical neuroendocrine cells with Chromogranin A positivity at the infranuclear spaces. ABC method, magnification × 400.

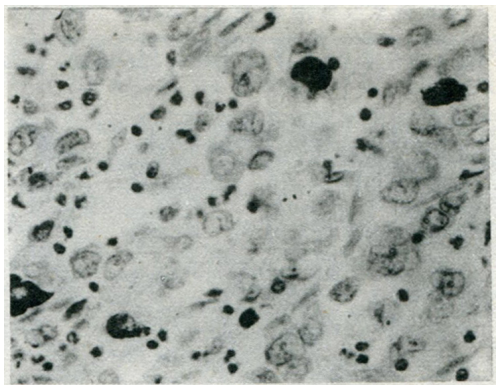

Figure 2 HCG-positive cells in poorly differentiated gastric carcinoma. ABC method, magnification × 400.

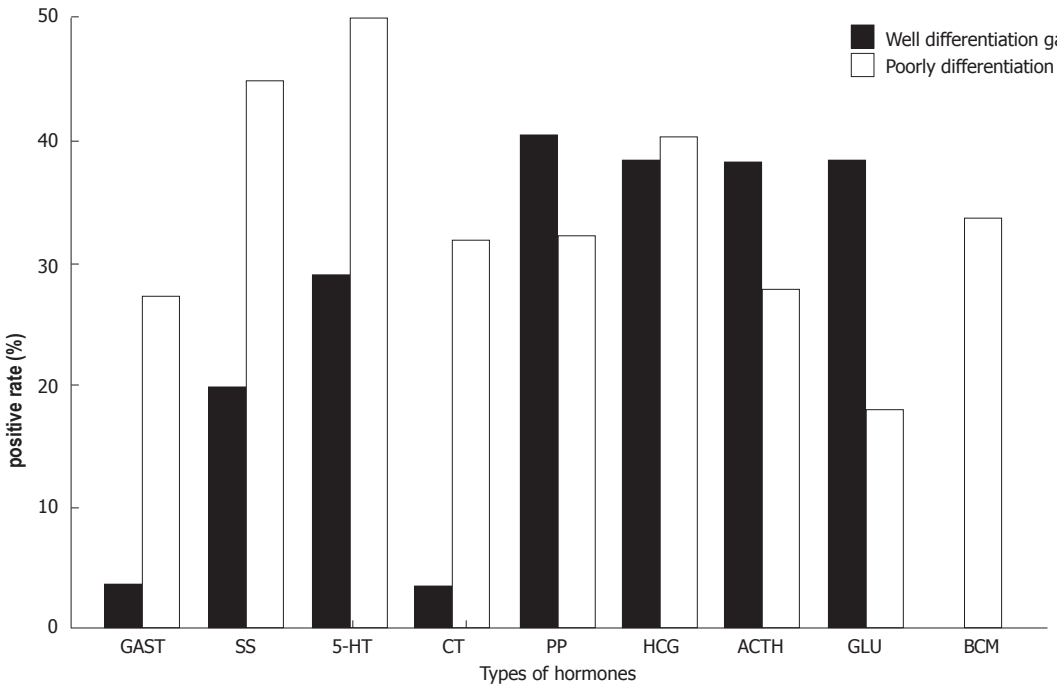

Figure 3 Hormone containing neuroendocrine cells in gastric carcinoma and cancer differentiation.

stained with lead citrate and uranium acetate for observation under a JEM-1200EX electron microscope.

Statistical analysis

Statistical significance was determined using the chi-square test for the measurements of NE cells in GC. Kaplan-Meier survival curve was evaluated using the log-rank test.

RESULTS

Eighty-five of 189 total GCs showed variable extent of chromogranin A (CgA) staining, 73 of which were observed by immunohistochemical methods with 9 types of hormonal antibodies. Only 4 of these expressed CgA without any hormonal expression, while 20 contained only one kind of hormone, 18 two kinds, 12 three kinds, 9 four kinds, 7 five kinds, 1 six kinds and 2 seven kinds. Forty-nine of the 73 cases contained more than one kind of hormone. The 9 hormones found in the GCs were as follows: serotonin (5-HT) (n = 31), HCG (n = 28), pancreatic polypeptide (PP) (n = 26), ACTH (n = 22), calcitonin (CT) (n = 17), glucagon (Glu) (n = 16), somatostatin (SS) (n = 16), Bom (n = 15) and gastrin (GAST) (n = 13). The positive cells were triangular, flat or cylindrical in shape, with characteristic positive staining at the infranuclear spaces (Figure 1). The positive staining varied considerably in the NE cells, which were found to be scattered all over (Figure 2) and penetrating the deeper layers of the gastric wall or restricted within a small area of the tumor.

The 189 cases of gastric adenocarcinoma were grouped as well-differentiated or poorly-differentiated. There were more NE cells in the first group than in the second (Figure 3, $P < 0.01$). GAST, 5-HT, Bom and CT were more highly expressed in the second group. In 72 of 161 cases, metastasis of the lymph nodes was found; moreover 32 of 88 (36.4%) GC cases showing CgA negativity and 40 of 73 (54.8%) showing CgA positivity also presented with metastasis in lymph node ($P > 0.05$). Nineteen out of the 40 cases with lymph nodes metastatic foci showed CgA positivity, with HCG positivity in 13 cases, which was more frequent than the other hormones (Figure 4, $P < 0.01$).

One-hundred-and-twenty-seven cases were followed up and sur-

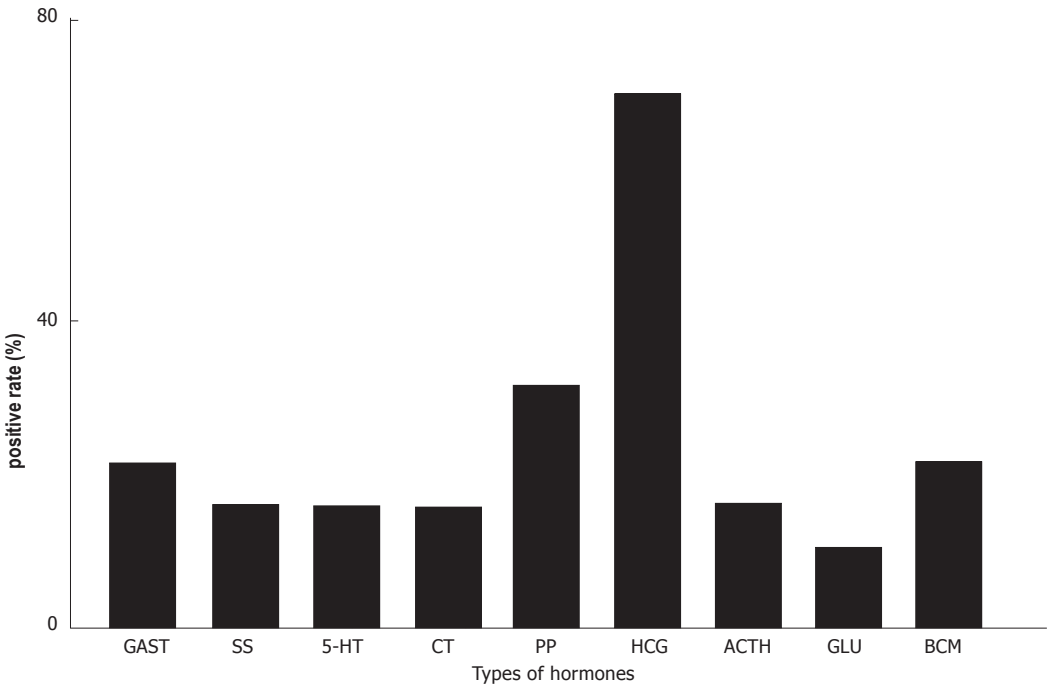

Figure 4 Hormone-positive neuroendocrine cells in gastric carcinoma with lymph nodes metastasis.

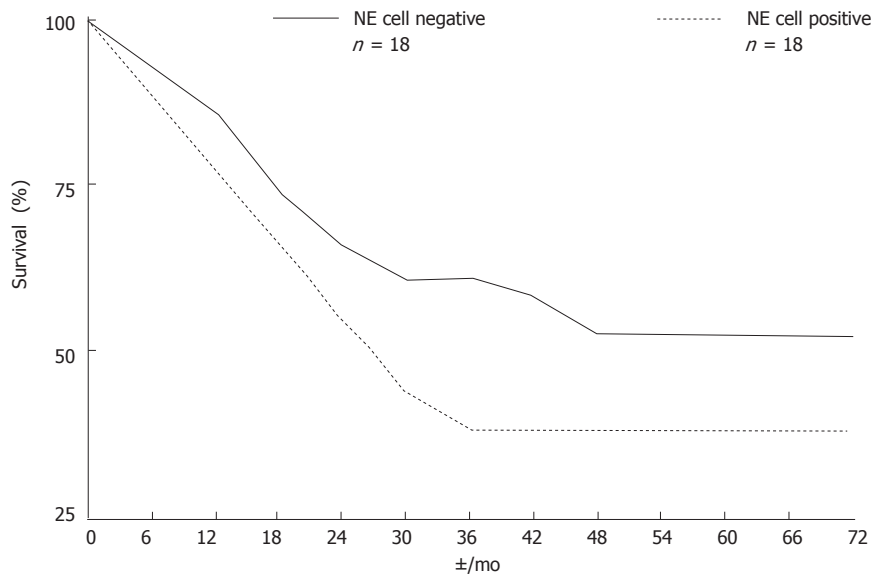

Figure 5 Survival curve of 54 gastric carcinoma patients.

vival state was available for 54 patients, 36 of whom were NE (CgA) cell positive. The range of follow-up was 39.6-73.2 mo (mean, 56.0 mo) (Figure 5). The survival rate was 48% for all the cases, including 52.7% for those with NE cell negative and 38.9% for those with CgA positivity ($P > 0.05$). Five of 7 patients (71.4%) with SS-positive carcinomas survived between 36 mo and 66 mo, while all 4 cases with HCG-positive cells died between 12 mo and 30 mo.

Under the electron microscope, the NE cells with dense granules were easily recognized in 8 of 20 cases. The NE cell presented dysplasia, irregular nucleus and large nulceolus. NE cells were divided into three types by endocrine granules: type Ⅰ (3 cases), showing small pleomorphic granules and granule diameters of 100-200 nm (Figure 6), similar to enterochromaffin cells; type Ⅱ (6 cases), showing either vesicular granules with an irregular dense core or a round and relatively homogeneous to coarsely granular dense core surrounded by a membrane (Figure 7), with granule diameter of 100-250 nm and similarity to enterochromaffin-like cells; type Ⅲ (1 case), showing vesicular granules of larger diameter (150-350 nm). Eight of the 20 cases were CgA positive by immunohistochemistry.

DISCUSSION

The presence of NE cells in GC has long been established. Endocrine differentiation is not a rare phenomenon in conventional gastric carcinoma (3.1%-53.3%). Using immunohistochemistry and electron microscopic examinations, with combination of 10 antibodies, we found that 85 of 189 cases of GC (44.9%) contained NE cells. 5-HT was the most frequent hormone antigen. Previous studies have suggested that endocrine-positive carcinomas are more frequent in poorly-differentiated, advanced and diffuse GC than in well-differentiated, intestinal type and early GC.[1]. In our study, the frequency was 54.2% to 32.9% ($P < 0.01$). It was interesting that we found some relationship between different types of hormones and histological types of tumor. Bom ($P < 0.01$), CT ($P < 0.01$), GAST ($P < 0.05$), and 5-HT ($P < 0.05$) were more frequent in poorly-differentiated cancers; in contrast, GLU was more frequent in well-differentiated cancers. Some reports pointed out that GC with NE cells tended to advance slowly and was associated with longer survival than GC without NE cells. In other studies, however, GC with NE cells was shown to occur much more frequently in advanced GC and to have poor prognosis.

The mortality of the cases with NE cells and without NE cells was 61.1% and 47.2%, whereas the lymph node metastasis rate in these cases was 54.8% and 36.4% respectively. The poor prognostic tendency in GC patients with NE cells was apparent, although the difference was not significant by statistical analysis. The different types of hormone might have some relation with tumor metastasis and prognosis. In our study, 19 of 40 cases with lymph node metas-

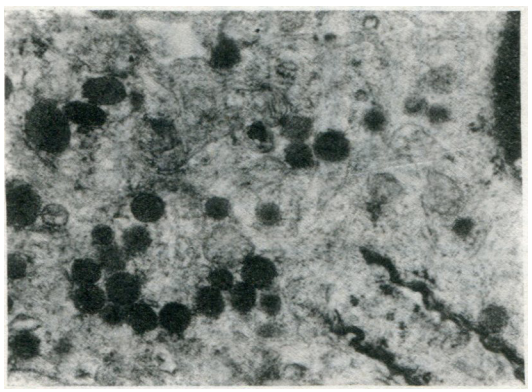

Figure 6 Type Ⅰ neuroendocrine cell with 100-200 nm-sized pleomorphic granules. Transmission electron microscope, magnification × 20000.

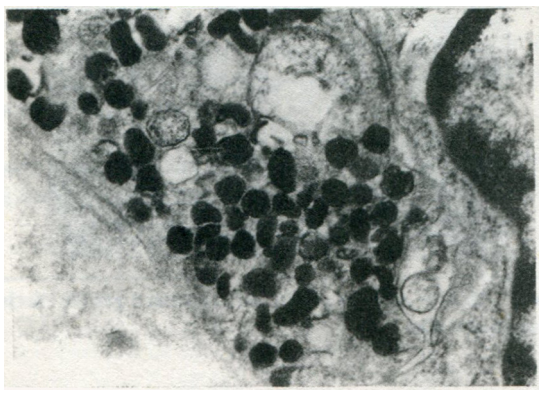

Figure 7 neuroendocrine cell showing round granular dense core surrounded by a membrane. Transmission electron microscope, magnification × 20000.

tasis showed NE cells and 13 of those 19 cases (68.4%) were HCG positive in metastatic foci, which was significantly higher than that for other hormones (10%-31.6%) ($P < 0.01$). Four cases with HCG positivity died (range, 12-38 mo) and 5 cases with SS positivity survived (range, 36-66 mo).

Some peptides can act as growth factors stimulating proliferation of GC cells, such as GAST[2,3], Glu[4], CT and Bom[5]. Production of some hormones might be a marker of prognosis, such as HCG[6], which was not found in normal gastric mucosa. It has been reported that GC patients with high levels of HCG in the serum or a high density of HCG-positive cells in tumor tissue had a poor prognosis. Other hormones, such as SS, can be inhibitory to the growth of tumor cells. SS has been found to inhibit the growth of several experimental cancers, many of which are endocrine-dependent. The presence of receptors for gastrointestinal hormones, such as GAST and VIP[7], has been reported for GC cell lines. Some *in vitro* evidence of tumor cell growth regulation by autocrine and paracrine mechanisms led us to presume the existence of a paracrine interaction between NE cells in GC. Our findings suggest that some hormones, such as GAST, Bom, CT, HCG and Glu, may act as promoters of tumor growth and may be able to stimulate the growth of GC. On the other hand, such hormones as SS may be able to inhibit tumor growth. The relation of some hormones with GC, such as ACTH and PP, remains unclear.

In conclusion, we think that there are different NE cells producing different hormones in GC that may play different roles in the cancer pathogenesis. Further investigation is required to elucidate the significance of relations between GC and hormonal substances.

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Comparison between biopsy and brilliant blue chromo-endoscopy in gastric cancer

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Abstract

AIM: To increase the detection rate of early gastric cancer and the positive rate of biopsy, brilliant blue (BB) chromo-endoscopy and routine endoscopy were used to examine 54 patients with gastric cancer.

METHODS: The BB staining method and steps involved the examinee being injected intramuscularly with 20 mg of Anisodamini Hydromidum, taking oral BB solution and being examined by endoscope 40-60 min later. All of the examinees were also subjected to a routine endoscopic examination.

RESULTS: Sixteen of the total 54 patients were diagnosed with early gastric cancer; the detection rate of early cancer was 29.4%. Among those, 15 cases were discovered by BB chromo-endoscopy and only 5 cases were made by the routine endoscopy. The sensitivity of BB chromo-endoscopy was significantly higher than the routine endoscopy for early gastric cancer ($P < 0.01$). In addition, the BB chromo-endoscopy had a higher positive rate of biopsy than the routine endoscopy (70.4% (38/54) to 94.4% (51.54), $P < 0.01$)

CONCLUSION: The BB chromo-endoscopy is more useful than routine endoscopy for following-up gastric precancerous lesions.

Key words: Stomach neoplasms/diagnosis; Biopsy; Gastroscopy

Su L, Shi M, Liu B, Hong MY, Pan HZ. Comparison between biopsy and brilliant blue chromo-endoscopy in gastric cancer. *World J Gastroenterol* 1996; 2(1): 34-35 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i1/34.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i1.34

INTRODUCTION

Gastric carcinoma is typically diagnosed at a late stage and therefore has a poor prognosis, as reflected by the low 5-year survival rates which are reported at 30%-40%. In contrast, the 5-year survival rate for *in situ* adenocarcinoma of the stomach, which is more frequently detected at the early stage, is 95%. Detection of an early gastric carcinoma lesion, however, is difficult by traditional diagnostic methods. Therefore, many gastroenterologists and oncologists have sought the development and application of different methods in the hopes of raising the detection rate of early gastric cancer and the biopsy positive rate.

This paper presents the results of our investigation that was conducted from 1988 to 1994 on cases of gastric malignant lesions diagnosed by brilliant blue (BB) chromo-endoscopy and biopsy.

MATERIALS AND METHODS

Subjects

During the last 5 years, we examined a total of 458 patients with gastric cancer and gastric benign lesions and followed-up patients with gastric precancerous lesions using the BB chromo-endoscopy procedure as well as the routine endoscopy procedure (*i.e.*, GIF or video-endoscope). We collected the data in order to summarize the total 54 cases of gastric cancer that were diagnosed by both of the methods.

Methods

Each patient was first injected with 20 mg Anisodamini Hydromidum (to suppress gastric motility and acid secretion), and then drank a cup of BB solution that was composed of 150 mg of BB, 20 mg of g-chymotrypsin, 1.2 g of sodium bicarbonate, 5 mL of dimethypolysiloxane and 105 mL of distilled water. Afterward, the patient lay down on a bed equipped for rotation in a counterclockwise manner, so that the body would be shifted from prone to supine position for 10 min, with a 30-s pause in each; the objective of this physical manipulation was to facilitate distribution of the solution over the whole surface of the gastric mucosa. Forty to 60 min later, the endoscopic examination was performed.

RESULTS

Staining appearances of gastric mucosa

Normal mucosa showed a clearly observable gastric areola ditch, for which the areola appeared as a light blue regular network. Benign lesions, such as an ulcer or polyp, showed a light blue coloration on the surface of the polyp or on the edge of the ulcer, with normal areola construction. Malignant lesions showed clearly with a reddish coloration and a notable lack of normal areola construction.

Detection rate of early gastric cancer

Of the 54 patients that presented observable staining with BB, 17 were diagnosed as early gastric cancer. All of them were treated by operation, and the findings of which included involvement of the mucosa (n = 9) or the submucosa (n = 7) and extension to the muscle of the stomach wall (n = 1). Ultimately, 16 cases were demonstrated as early cancer, among which 1 was classified as stage Ⅱa, 4 as stage Ⅱb, 1 as stage Ⅱa + Ⅱc, 3 as stage Ⅱc, and 7 as stage Ⅲ or Ⅱc + Ⅲ. The detection rates of early gastric cancer by both endoscopic procedures was 29.6% (16/54).

It is noteworthy that the tumors of the 3 cases with Ⅱb cancer were situated in a benign gastric ulcer; staining with BB showed three flat red lesions near the ulcer. Four specimens were obtained from the lesion by biopsy; histological examination indicated that two were signet-ring cell cancer and the other was adenocarcinoma. The patients were treated by operation. The ulcer itself was demonstrated to be a benign lesion in each case, but the proximal mucosa (1.5-2.0 cm) was determined to be cancerous.

Five cases of early gastric cancer were diagnosed by routine endoscopy and confirmed by biopsy. Among them, 1 case had not been demonstrated by biopsy of BB endoscopy. Of 16 patients with early gastric cancers, 15 were discovered by BB endoscopy, yielding a sensitivity rate of 93.8% (15/16); the 5 cases discovered by routine endoscopy indicated a sensitivity rate of 31.3% (5/16). Thus, the BB chromo-endoscopy was more sensitive for discovering early cancer than the routine endoscopy ($P < 0.01$, by the McNemar χ^2 test).

All 54 patients were examined by BB chromo-endoscopy and biopsy specimens were obtained from the red-stained area. Fifty-one of those were determined to be malignant lesions by histopathology, with the remaining patients being diagnosed as early cancer (n = 1) and advanced gastric cancer (n = 2). Thus, the biopsy positive rate was 94.4% (51/54). The 54 biopsy specimens obtained by routine endoscopy showed 37 as malignant lesions by pathology; the remaining specimens were classified as early cancer (n = 11) and advanced cancer (n = 6). The positive rate of biopsy by BB chromo-endoscopy for gastric cancer was clearly superior to the routine endoscopy according to the statistical significance findings by the McNemar χ^2 test.

DISCUSSION

The routine endoscopy procedure has been widely used throughout clinics to examine and follow-up gastric lesions. However, some small lesions, especially the flat-type of early cancer, are very easy to miss by routine endoscopy. The magnifying endoscope can help to increase the detection rate of such early gastric cancers. Yokoyama, using magnifying endoscopy, has checked 84 cases of depressed-type early gastric cancer and found the positive rate to be 81.4%, with the rate from chromo-endoscopy as 84.9% and that from routine endoscopy as only 65%. Moreover, the positive rates of the magnifying and chromo-endoscopy procedures were significantly higher than that of the routine endoscopy procedure ($P < 0.01$). Chromo-endoscopy is an accurate, simple method and no special equipment is required. Ikeda reported an intra-arterial dye (IAD) method for diagnosing stomach cancer, and endoscopically diagnosed 40 cases of gastric cancer (15 early, 25 advanced) with it. Seventeen cases were similarly examined using a pharmaco-angiographic technique before dye injection (PIAD). The author concluded that these techniques permitted more precise endoscopic delineation of the size and extent of cancerous lesions, particularly those that were otherwise obscured by overlying intact epithelium. Herein, we describe our clinical efforts with chromo-endoscopy in examination of 54 cases of gastric cancer. Ultimately, 16 cases of early cancer were discovered, which included 3 remarkable cases of Ⅱb early cancers situated proximal to benign gastric ulcers ($P < 0.01$).

Because some malignant lesions are similar to benign lesions, especially micro gastric cancers, they are likely to be missed if the target region for biopsy is incorrectly chosen. Direct brushing, selective lavage and biopsy imprint can reportedly raise the positive rate of routine endoscopy from 70%-80% to 85%-90.7%[1], and the positive rate of re-biopsy to 91.8%[2], and even up to 93.6% when more biopsy specimens (> 8) are taken. The combined use of three techniques (endoscopy, biopsy and cytology) can reportedly increase the positive diagnosis rate to 95.4%. Our study showed that the BB chromo-endoscopy procedure is significantly more sensitive, at 94.4% (51/54), than the routine endoscopy procedure ($P < 0.01$).

Some pathologists have purported that moderate and severe dysplasia may coexist with the initial stages of cancer[3], while others have demonstrated that dysplasia regresses in the majority of cases and that progression occurs in only a minority of cases[4]. Indeed, among 46 patients who had severe dysplasia for more than 3 years, only 4 developed gastric carcinoma. In our study, early cancer coexisted with moderate or severe dysplasia and benign gastric ulcers coexisted with Ⅱb type early cancer. The BB chromo-endoscope is a safer and simpler procedure than that of other dye-based endoscopies. BB is also an additive material of food stuffs that is approved for human consumption by the World Health Organization, having no poisonous effect. Thus, the BB-based procedure provides a simple means of detection for gastric lesions. We recommend that the BB chromo-endoscope procedure should be used for frequent checking of precancerous lesions in order to facilitate the convenient and sensitive discovery of early gastric cancer.

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CLINICAL ARTICLES

Anti-rejection therapy with tripterygium woifordii and low-dose cyclosporine for small bowel transplantation in pigs

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Abstract

AIM: To determine whether anti-rejection therapy with tripterygium wolfordii (TW) and low-dose cyclosporine (CsA) is better than treatments with large-dose CsA for small bowel transplantation.

METHODS: Two-step segmental small bowel transplantation was performed in pigs and followed by treatment with either no, low-dose or high-dose CsA, which was followed by TW, a traditional Chinese medicine, or not.

RESULTS: The transplanted pigs receiving no CsA developed organ rejection, as did the pigs who received the low-dose CsA treatment alone; the mean survival time of the grafts was 12 ± 8.2 ± 7 d and 12 ± 4.2 ± 6 d respectively. Of the 4 transplanted pigs receiving the high-dose CsA for 100 d and then the TW treatment, 2 required euthanasia for severe pneumonia that developed on day 92 and 97 respectively, and the other 2 survived more than 348 and 327 d respectively. Of the 5 transplanted pigs receiving low-dose CsA for 100 d and then the TW treatment, all survived for 243 ± 2.90 ± 9 d and none succumbed to infection.

CONCLUSION: We are the first to use TW in small bowel transplantation and to show that TW can be a powerful and effective anti-rejection agent when applied in conjunction with the standard immunosuppressant CsA.

Key words: Tripterygium wolfordii intestine; Small/transplantation cyclosporine graft rejection

Li N, Li JS, Liao CX, Li YS. Anti-rejection therapy with tripterygium woifordii and low-dose cyclosporine for small bowel transplantation in pigs. *World J Gastroenterol* 1996; 2(1): 36-40 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i1/36.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i1.36

INTRODUCTION

The successful application of small bowel transplantation as a treatment for short gut syndrome is reliant on postoperative administration of the immunosuppressive agent cyclosporine A (CsA). While CsA is commonly used to prevent organ rejection and graft versus host disease (GVHD) in various transplantation cases, small bowel transplantation cases require much larger doses (15-20 mg/kg) to achieve the same results[1-6]. However, in general, allograft recipients should be maintained on the lowest possible doses of immunosuppressive agents in order to avoid development of lethal infections and lympho-proliferative disorders, as well as to minimize organ toxicity caused by the drug. In an overview of small bowel transplantation that was published in 1990, Wood[4]concluded that clinical intestinal transplantation would have to await further refinements in immunosuppression before it reaches its maturity as a successful treatment. We, however, have performed successful segmental small bowel transplantations in pigs by using low-dose CsA in combination with tripterygium wolfordii (TW), a traditional Chinese medicine, since Mar. 1992.

MATERIALS AND METHODS

Surgical technique

Two animals were prepared for small bowel transplantation involving one another. Each animal thus served as both graft donor and recipient. The two-step segmental small bowel transplantation was performed as described earlier[5]. Briefly, in the first-step operation (heterotopic transplantation), a segment of 5 m in length (approximately 40% of the entire intestine) was isolated. The mid portion of this segment (1 m in length) was reserved for use as a donor organ and the other 4 m of intestine was discarded. After the vascular bed and intes-

Table 1 Technical failures

Group	Animal No.	Survival days	Cause of death
I	1	5	Venous thrombosis
	3	1	Venous thrombosis
	5	0	Anesthetic
	9	1	Transplant intussusception
	10	32	Anesthetic, Rejection?
II	11	1	Venous thrombosis
	13	4	Transplant intussusception
	16	4	Arterial thrombosis
	17	7	Arterial thrombosis
III	19	1	Venous thrombosis
	22	0	Anesthetic
	26	0	Anesthetic
IV	29	3	Venous thrombosis
	31	4	Venous thrombosis
	33	6	Arterial thrombosis

Table 2 Immunosuppression and survival

Group	Immunosuppressant	Animal No.	Survival days	Cause of death
I	None	2	14	Rejection
		4	10	Rejection
		6	10	Rejection
		7	16	Rejection
		8	14	Rejection
II	High-dose CsA	12	92	Infection
		14	398	Survival
		15	97	Infection
		18	> 419	Survival
III	Tw + low-dose CsA	20	> 274	Survival
		21	> 356	Survival
		23	166	Ileus
		24	135	Unknown
		25	> 285	Survival
IV	Low-dose CsA	27	16	Rejection
		28	14	Rejection
		30	10	Rejection
		32	10	Rejection
		34	12	Rejection

TW: Tripterygium woilfordii.

tinal lumen were irrigated, the allograft artery and vein were anastomosed orthotopically to the recipient superior mesenteric artery and vein in an end-to-end fashion. Both ends of the graft were exteriorized with the stomas, and the native bowel continuity was restored. The second-step operation (orthotopic transplantation) was performed 4-6 wk after the heterotopic transplantation, if both the recipient and graft had survived. The heterotopic graft was freed from the enterostomies. Both ends of the graft were anastomosed with the recipient bowel in an end-to-end fashion.

Animal groups

Thirty-four outbred white pigs weighing 20 ± 1.5 kg were randomly assigned to one of four groups (below) for study. Genetic diversity in the population was assured by using donor recipient pairs from separate litters, except for Group Ⅰ in which the donor-recipient pairs came from the same litters.

Group Ⅰ (n = 10): control group, received no immunosuppressant.

Group Ⅱ (n = 8): CsA group, received CsA at 15 mg/kg intramuscularly provided on the day before the first-step operation and continuing for 60 d, with dosage being reduced to 10 mg/kg at 1 wk until day 60 post-transplantation and then again to 7.5 mg/kg until day 100 post-transplantation.

Group Ⅲ (n = 8): TW + low-dose CsA group, received TW at 2 mg/kg orally for 3 d prior to the first-step operation and for lifelong use post- transplantation, and also received CsA at 7.5 mg/kg intramuscularly for 7 d post-transplantation, with dosage being reduced to 5 mg/kg from day 8 to day 60 post-transplantation and again to 2.5 mg/kg until day 100 post-transplantation.

Group Ⅳ (n = 8): low-dose CsA group, received CsA alone (without TW) *via* administration as described for Group Ⅲ.

Group Ⅴ (n = 7): TW group, consisting of animals that survived more than 100 d from Groups Ⅱ and Ⅲ who then received only TW at 2 mg/kg orally.

Postoperative monitoring

Animals were evaluated daily postoperatively. Clinical characteristics and appearance of the exteriorized stomas of the transplanted intestines were recorded. If the graft mucosal biopsies were found to contain necrotic tissue, the animals were sacrificed. All recipient animals were autopsied upon death.

Serum protein, aspartate aminotransferase, alanine aminotransferase, total bilirubin, blood urea nitrogen and serum creatinine were measured on postoperative day 40 and 80.

Whole blood CsA levels were measured on postoperative day 5, 10, 20, 40, 60, 80 and 100, on a sampling schedule just prior to administration of daily CsA. The radioimmunoassay kit for CsA measurement was purchased from Abbott Inc. (United States).

While the grafts were in a heterotopical position, mucosal biopsies of the transplant were obtained at 2-d intervals, with a suction biopsy instrument. The specimens were fixed in 10% phosphate-buffered formalin, embedded in paraffin, and serially sectioned (6 microns) for analysis by staining with hematoxylin and eosin and light microscope.

Statistical analysis

All data are expressed as the mean ± SD. Survival times were compared using rank sum analysis, and for other parameters the Student's test and variance analysis was used.

RESULTS

Of the total 34 transplants that were conducted, 15 (44.1%) of

Table 3 Mean survival period with or without percutaneous transhepatic cholangial drainage prior to operation

Group, *n*	Postoperative days							
	5	10	20	40	50	80	60	100
Group Ⅱ, 4	1476 ± 11.6[b]	902 ± 79[b]	894 ± 89[b]	896 ± 86[b]	878 ± 87[b]	495 ± 109[b]		-425, 388
Group Ⅲ, 5	429 ± 79	304 ± 33	298 ± 34	301 ± 39	289 ± 36	157 ± 30	155 ± 35	
Group Ⅳ, 5	446 ± 63	304 ± 48						

[b]$P < 0.01$ *vs* group Ⅲ and Ⅳ.

Table 4 Results of liver kidney functions in Groups Ⅱ and Ⅲ

Biochemistry	Preoperative	POD 40		POD 80	
	Control	Group Ⅱ	Group Ⅲ	Group Ⅱ	Group Ⅲ
ALT (nkat)	280 ± 40	429 ± 89[a]	350 ± 53	629 ± 257[a]	327 ± 87
AST (nkat)	410 ± 90	771 ± 119[a]	690 ± 132	1267 ± 444[a]	410 ± 114
LDH (U/L)	301 ± 72	445 ± 69	370 ± 79	567 ± 147[a]	333 ± 140
AKP (nkat)	840 ± 184	942 ± 319[a]	837 ± 177	1304 ± 593	750 ± 196
Bil (μmol/L)	11.3 ± 5.5	17.1 ± 7.4	16.1 ± 5.7	31.2 ± 23.7	14.7 ± 7.4
BUN (mmol/L)	3.2 ± 0.8	3.2 ± 0.4	3.7 ± 1.1	4.3 ± 0.7	3.4 ± 0.8
Cr (μmol/L)	89 ± 26	28 ± 34	95 ± 27	93 ± 27	83 ± 20

[a]$P < 0.05$ *vs* preoperative and group Ⅲ. POD: Postoperative days.

Table 5 Results of serum biochemistry in group Ⅳ

Biochemistry	Preoperative	Postoperative days		
	(Control)	130	160	190
Sodium (mmol/L)	153 ± 8	150 ± 8	153 ± 10	153 ± 9
Potassium (mmol/L)	5.7 ± 1.0	5.9 ± 0.9	5.9 ± 1.0	5.8 ± 1.1
Chloride (mmol/L)	101 ± 5	100 ± 4	101 ± 5	102 ± 3
Total protein (g/L)	87.8 ± 6.8	85.3 ± 8.1	84.3 ± 4.1	85.0 ± 5.1
Albumin (g/L)	37.2 ± 5.9	36.0 ± 3.3	37.0 ± 2.8	37.0 ± 2.2
Globulin (g/L)	50.6 ± 4.4	49.3 ± 5.5	47.3 ± 2.2	48.0 ± 5.4
A/G	0.74 ± 0.14	0.74 ± 0.06	0.78 ± 0.06	0.78 ± 1.1
ALT (nkat)	280 ± 40	304 ± 37	283 ± 101	300 ± 53
AST (nkat)	410 ± 90	496 ± 184	450 ± 175	484 ± 168
LDH (u/L)	301 ± 72	289 ± 65	273 ± 66	278 ± 74
AKP (nkat)	840 ± 184	859 ± 97	805 ± 104	824 ± 127
Bilirubin (μmol/L)	11.3 ± 5.5	13.5 ± 5.3	12.4 ± 5.1	12.6 ± 4.3
BUN (mmol/L)	3.2 ± 0.8	3.5 ± 0.8	3.4 ± 0.9	3.5 ± 0.9
Cr (μmol/L)	89 ± 26	88 ± 17	86 ± 22	92 ± 28

Survival > 200 d ($n = 5$).

the animals died of technical failures, including anesthetic accidents, (Table 1) within the first 7 d post-transplantation; all these animals were excluded from analyses. If the 4 animals that died as a result of anesthetic accident were excluded, the rate of technical failure was 36.7% (11/30).

Immunosuppression and survival

The immunosuppressive regimens used and corresponding survival figures are presented in Table 2. The animals in Group Ⅰ ($n = 5$) that received no immunosuppression, as well as those in Group Ⅳ ($n = 5$) that received low-dose CsA, died of rejection on post-transplantation days 12.8 ± 2.7 and 12.4 ± 2.6, respectively. At autopsy, it was apparent that the transplanted bowel had deteriorated into a hard, whitish, irregular mass. Histological analysis showed typical signs of rejection that were specific to the transplanted organ, and no obvious changes were observed in the liver, spleen or lymph nodes of the recipients.

The animals in Group Ⅱ ($n = 4$) that received high-dose CsA survived for 251.5 ± 181.5 d post-transplantation. In this group, 2 animals were sacrificed on post-transplantation days 92 and 97, respectively, due to severe pulmonary infection; the remaining 2 pigs survived to post-transplant day 398 and 419, respectively. The animals in Group Ⅲ ($n = 5$) that received TW + low-dose CsA survived 243.2 ± 90.9 d ($P < 0.05$ compared to Group Ⅳ). In this group, 3 animals remained alive until post-transplantation day 274, 285 and 356, respectively, 1 animal died suddenly at post-operative day 135 of no identifiable cause (despite autopsy evaluation), and 1 animal succumbed to obstruction of the native intestine on post-transplantation day 166. Although 2 pigs in Group Ⅱ and 2 in Group Ⅲ died between post-transplantation day 92 and 166, there was no evidence of acute or chronic rejection at autopsy.

Pathology

The histological structure of the transplanted intestines was normal for the animals in both Group Ⅱ and Group Ⅲ, comparable to that of the native intestine. The specimens from Group Ⅰ and Group Ⅳ showed signs of rejection, including decreased mucosal depth accompanied by atrophic villi, reduced glandular structures and degeneration of arteriole in the laminal propria with infiltration of mononuclear cells. As rejection progressed, patch erosion of the mucosal surface occurred along with loss of epithelial cells. Finally, the mucosal structure disappeared entirely and mucosal biopsy specimens consisted solely of necrotic tissue. The earliest pathological indication of rejection was seen at 7.6 ± 1.7 d after transplantation. Mucosal necrosis of the transplanted intestine emerged at 12.4 ± 2.6 d after surgery. Results of whole blood CsA and serum biochemistries are listed in Tables 3-5.

DISCUSSION

Prognosis and resectability rate

Rejection is the main challenge to success of intestinal transplantation. In the 1960s and 1970s, azathioprine was the major drug used to combat rejection of the donated organ; yet, during this period there were almost no reports of successful intestinal transplantation. In the 1980s, CsA became the most popular and effective anti-rejection agent in use, and the survival rate of small bowel graft was greatly prolonged[6]. Although reports of successful small bowel transplantation were present in the literature after 1988[7], the long-term survival rate remained low—less than 36% of the animals survived for 100 d[3]. Here, we demonstrate for the first time that

TW is a powerful anti-rejection agent, as evidenced by all 5 of the pigs in our study who received TW along with low-dose CsA surviving for more than 100 d.

Although CsA has been widely accepted as an effective immunosuppressive agent for small bowel transplantation, large doses have been required to prevent rejection and GVHD. Grant[2] applied a dosage of 8 mg/kg CsA and 1 mg/kg prednisolone for pigs receiving total intestinal transplantation, but all the pigs in that study died of acute rejection within 15 d. According to Ricour[8] and Revillon[9], 14 pigs with blood CsA level < 750 μg/L (equal to a CsA plasma concentration of 250 mg/L) died of rejection within 8-75 d after the operation. Pritchard's experiment[10] revealed that 4 of the pigs with blood CsA concentration of 200 μg/L died within 15 d after operation because of acute rejection, with the other 6 animals with blood CsA level of 380 μg/L dying within 29 ± 18 d and still another 8 pigs with CsA concentration reaching 610 μg/L dying within 34 ± 33 d. This evidence indicated that CsA itself was unable to inhibit rejection if blood level was < 750 μg/L.

We obtained similar results as above, with 4 pigs in Group Ⅱ surviving without rejection when the CsA blood level was maintained > 800 μg/L for 60 d; acute rejection manifested in the 5 pigs that received half the dosage of CsA (blood level 300-500 μg/L). Thus, we concluded that the minimal effective concentration of CsA was -750 μg/L, which is equivalent to a daily injection of 10 mg/kg of CsA. Although large dosage of CsA can inhibit rejection of the transplanted small bowel, few animals in our study survived for 100 d and most succumbed to severe infection. In Grant's study[2], a daily intravenous administration of CsA at 20 mg/kg was provided for intestinal transplanted pigs followed by oral ingestion of CsA (25 mg/kg) 3 wk later. Unfortunately, all of the animals in that study died of infection within 28 d. In another study, Kaneko[11] gave intravenous injection of CsA (15 mg/kg) to intestinal transplanted pigs for 30 d followed by oral CsA at 30 mg/kg; as a result, 69% (9/13) of these animals died within 38 ± 14 d due to infection. Similarly, Kimura[3] reported that among 11 recipient pigs of segmental jejunal transplantation receiving 10 mg/kg of CsA intramuscular injection, 7 animals (63.6%) died of infection, 1 died of rejection, and only 4 pigs survived for 100 d. Pritchard[10] reported that pigs of segmental jejunal transplantation were given 25 mg/kg of CsA orally and 40% of the animals died of infection, with only 2/47 surviving for 100 d. In our experiment, 4 pigs in our group Ⅱ suffered from lung infection at 35-50 d after the operation and 2 of those animals died. We, thus, concluded that large-dose CsA could not only inhibit rejection but also increase risk of severe infection.

Large-dose CsA can cause liver and kidney damage. The nephrotoxicity of CsA is widely acknowledged. Clinical data have revealed that CsA causes severe kidney damage if its concentration is maintained > 800 ng/mL for a long period. In our study, instances of kidney damage were not prominent; this finding may reflect species variation. However, animals in group Ⅱ showed a certain degree of liver damage; specifically, blood ALT, AST and LDH on post-transplantation days 40 and 80 were significantly higher than those of other groups; autopsy of the 2 animals that died in group Ⅱ showed degeneration and necrosis of liver cells in the lobules. Thus, we concluded that large dosage of CsA was not the ideal regimen to avoid rejection.

TW, a traditional Chinese medicine, is an anti-inflammatory, antibacterial, anti-fertilization and immunosuppressive agent. It is already in wide use for the treatment of autoimmune diseases, but has not yet been generally extended towards the prevention of rejection after major organ transplantation. We are the first to use TW to treat small bowel transplantation and find that TW is an effective anti-rejection agent. Ultimately, our results suggest that TW can partly replace CsA in the prevention and treatment of acute rejection in the early stage after small bowel transplantation and, in the later period, can be used alone to protect against chronic rejection.

Combined drug therapy can help to protect against rejection, thereby reducing related side effects and achieving better outcome. In the field of intestinal transplantation, the effective blood concentration of CsA can cause not only liver and kidney damage, but also severe infection[13]. In our study, combined drug therapy was adopted in group Ⅲ and the dose of CsA was only half that administered to the animals in group Ⅱ. Although rejections were effectively controlled in both groups, all of the 4 pigs in group Ⅱ manifested infection within 35-50 d after the transplantation. Both liver function test and biopsy revealed liver cell damage, and only 2 of the animals survived for 100 d. All of the 5 pigs in group Ⅱ survived more than 100 d, and 2 of them manifested transient mild lung infection but did not show any signs of liver damage. Thus, combination treatment of TW with low-dose CsA appeared to be an effective and reasonable regimen against rejection. It could produce the same effect as that of large dosage of CsA without causing significant infection and liver damage, supporting a high rate of long time survival.

TW is a toxic herb and its toxicity is dose-related. The symptoms of chronic toxicity in experimental animals include hair shedding, weight loss, white blood cell count reduction and damage to the heart, liver and kidney. In our study, 7 pigs in group Ⅳ took TW for as long as 135-419 d without developing any of these symptoms.

We postulated that the mechanism underlying the immunosuppressive effect of TW involves inhibition of cytokines. Our primary study has shown differences in IL-2 and IL-6 levels in animals of groups Ⅱ and Ⅲ, suggesting that TW may act *via* inhibition of IL-2 and IL-6 production; further investigation is warranted.

The regime of TW + low-dose CsA will be very cost effective. It is commonly known that CsA is very expensive, but TW is much cheaper. The daily cost of TW per kg body weight is only 1/10 of that of CsA and thus is promising for clinical care.

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p53 gene mutations in primary gastric cancer

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Abstract

AIM: To analyze the relationshio between *p*53 gene mutations and these parameters including DNA ploidy in Chinese primary gastric cancers.

METHODS: Mutations of the *p*53 gene in exon5-8 were examined in 20 cases of priary gasric cancer by PCR-SSCP (Polymerase Chain Reaction-Single Strand Conformation Polymorphism) analysis.

RESULTS: Mutations were detected in 8 (40%) cases: 2 cases in exon5-6, 2 cases in exon7, 4 cases in exon8. These mutations were detected from stage 0 to stage Ⅲ. No significant association was found between *p*53 gene mutations and the clinicopathological parameters such as macroscopic classification, degree of histological differentiation, depth of tumour invasion and lymphonod metastasis. In addition, 66.7% (6 of 9) of aneuploidy tumours had p53 mutations and only 18.2% (2 of 11) of diploid tumours had mutations.

CONCLUSION: These results suggest that *p*53 gene mutations are related to DNA ploidy alterations and that *p*53 gene is one of the important tumour suppressor genes in human gastric cancer.

Key words: Genes, *p53*; Stomach neoplasms; Mutation; Polymerase chain reaction

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INTRODUCTION

Human gastric cancers represent some of the most frequently occurring tumors worldwide. In China, cancer mortality is the highest among cases of gastric cancers and research on this topic is prevalent, with the latest studies focusing on the genetic changes that support gastric cancer development and progression. Studies of gastric tumors have revealed several genetic alterations. Besides mutations in known oncogenes, such as ras and cerb B2, mutations in tumor suppressor genes, such as p53, have also been reported. However, the relationship between p53 mutations and the histopathological parameters of gastric tumors, such as macroscopic classification, stage, degree of differentiation and depth of tumor invasion, remain unknown. In addition, the role of p53 mutations that are related to gastric tumor parameters within the upcoming integrative approach to patient care involving traditional Chinese and Western medicine has not been investigated.

This study was designed to investigate structural alterations in exons 5 though 8 of the *p53* gene in 20 cases of gastric cancer by using PCR-single strand polymorphism (SSCP) analysis, a rapid and sensitive method for detecting mutations and polymorphisms in a given sequence[1]. Ultimately, the SSCPs that were detected were compared with the histopathological parameters of the patients' gastric tumors and spleen Qi status to understand how to adopt p53 molecular data to the integrative care approach.

MATERIALS AND METHODS

Tumor Samples

Fresh tumor tissues and adjacent normal gastric mucosa were by surgical resection from patients treated for gastric cancer at the Fourth Affiliated Hospital of Hebei Medical University, Shijiazhuang, China. All samples were immediately snap-frozen in ice-cold ethanol upon excision and stored at -80 ℃ until use in analysis.

Each excised tissue specimen was prepared as a single-cell suspension. After filtration, the nuclei were treated with 0.1% RNase and stained with ethidium bromide. DNA ploidy was determined by DNA flow cytometry using a FACS 420 (Becton-Dickinson Co., United States). The DNA index (DI) was calculated as the relative DNA content using the following formulas: for diploid tumors, DI = 1.0 ± 2cv; for aneuploid tumors, DI ≠ 1.0 ± 2cv (cv ≤ 5%).

Preparation of genomic DNA

Genomic DNA was extracted from each tumor and patient-matched normal gastric mucosa specimen, respectively, by treating with sodium dodecyl sulfate Pronase K and phenol

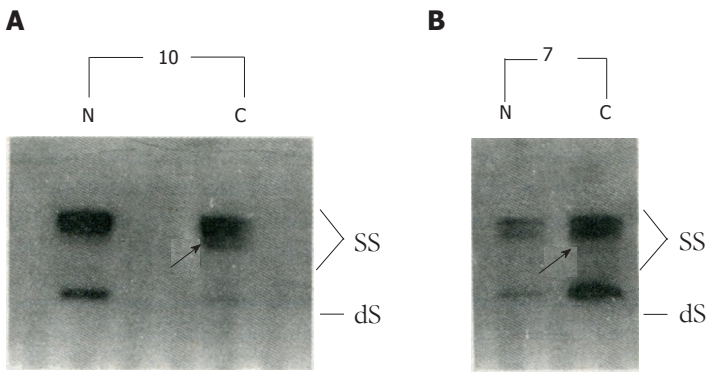

Figure 1 Polymerase Chain Reaction-Single Strand Conformation Polymorphism analysis of DNA from gastric cancers. A: Exons; B: Exon 5-6 of the *p53* gene. Arrows, mutations. N: Normal tissues; C: Cancer tissues; SS: Single strand; dS: Double strand. Aneuploid 619 (66.7).

Table 1 p53 mutations in gastric cancer

Case	Stage[1]	Mutated exon
4	ⅢⅠa	5-6
6	Ⅱ	7
7	Ⅰb	5-6
8	Ⅱ	7
10	Ⅰb	8
13	0 (carcinoma *in situ*)	8
15	Ⅱ	8
20	ⅢⅠa	8

[1]According to the U.L.C.C. staging system.

chloroform. PCR-SSCP analysis. Exons 5-6, 7 and 8 of the *p53* gene were amplified individually by PCR and presence of mutations was analyzed by the SSCP method[13]. The reaction mixture (total volume, 20 μL) consisted of 300 ng of genomic DNA, 0.5 μL *Taq* polymerase (Promega, United States), 3.70 MBq (2.5 μCi) 32 PdCYP, exon-specific primer pair (50 pmol/L each; see below), and 125 mmol/L of each deoxynucleotide triphosphate. The PCR amplification was carried out in 30 cycles of 90 s at 94 ℃, 90 s at 55 ℃, and 120 s at 72 ℃. Each completed reaction mixture was diluted 10-fold with formamide-based dye solution (95% formamide, 20 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). A 10 μL aliquot of the diluted reaction mixture was then heated for 5 min at 90 ℃ and resolved by electrophoresis using a 6% neutral polyacrylamide gel containing 5% glycerol (13) and 30 W for 4-6 h at room temperature. The resolved amplicons in the gel were visualized by exposure to X-ray film at -80 ℃ for 12-24 h.

The synthetic oligonucleotide primers were: Exon 5-6 (sense) 5′-GGAATTCCTCTTCCTGCAGTAC-3′ and (antisense) 5′-GGAATTCAGTTGCAAACCAGACCTCAGG-3′; Exon 7 (sense) 5′-GGAATTCTCCTAGGTTGGCTCTGAC-3′ and (antisense) 5′-GGAATTCAAGTGGCTCCTGACCTGGA-3′; Exon 8 (sense) 5′-GGAATTCCTATCCTGAGTAGTGGTAA-3′ and Exon 8 (antisense) 5′-GGAATTCCTGCTTGCTTACCTCG-3′.

RESULTS

PCR-SSCP analysis detected *p53* gene mutations in gastric cancer specimens from 8 (40%) of the patients (Figure 1; Table 1), with 2 of the mutations located in exons 5-6, 2 in exon 7, and the remaining 4 in exon 8. The 8 tumors represented stages 0 to Ⅲ. There was no significant correlation found between the PCR-SSCP detected p53 mutations and the clinic-pathological parameters of these tumors, including for the macroscopic classification, degree of differentiation, depth of tumor invasion, and lymph node involvement (Table 2).

DNA flow cytometry indicated that 11 of the tumor specimens were diploid and 9 were aneuploid. Two of the diploid tumors (18.2%) and 6 of the aneuploid tumors (66.7%) had p53 mutation (χ^2, $P < 0.05$).

Table 2 Histopathological parameters and p53 mutation in gastric cancer

Histopathological parameter	p53 mutation (%)
Macroscopic classification	1/3 (33.3)
Early gastric cancer	
Advanced gastric cancer	
Borrman Ⅰ	0/1 (0)
Borrman Ⅱ	2/3 (66.7)
Borrman Ⅲ	4/11 (36.4)
Borrman Ⅳ	1/2 (50)
Degree of differentiation	
Well	4/7 (57.1)
Poor	4/13 (30.8)
Depth of tumor invasion	
Mucosa, submucosa layer	1/3 (33.3)
Muscular layer	6/13 (46.2)
Serous coat	1/4 (25)
Lymph node metastasis	
Presence	4/13 (30.8)
Absence	4/7 (57.1)
Dna ploidy alteration	
Diploid	2/11 (18.2)
Aneuploid	6/9 (66.7)

DISCUSSION

The *p53* gene is located on chromosome arm 17p13, and mutation and loss of heterozygosity (LOH) in this genetic sequence have been reported for many types of cancers[2], including colon, brain, ovary, breast, lung and esophagus. The original reports of *p53* gene abnormalities generally regarded this event in gastric cancer as a late event[3,4]; however, recent research has suggested involvement at much early stages in tumorigenesis[1,5]. In our current study, *p53* gene mutations were commonly detected in gastric cancer specimens of stage 0 to Ⅲ. Intriguingly, case 10 of our series that presented with a mucinous adenocarcinoma arising from ulcer showed *p53* gene mutation. Thus, alterations of the *p53* gene may play an important role in the development of gastric cancer, including canceration of an ulcer.

The frequency of p53 mutations in our case series was 40%. This result may be lower than the real figure, however, because we did not attempt to detect mutations in other parts of the *p53* gene sequence (*i.e.* outside of exons 5-8), partly as a limitation of the procedure used, for which the relatively short length of genomic DNA (approximately 200 bp) produces the best effect for observing SSCP. This limitation also represents a particular challenge for the exonic region encompassing exons 5-6, for which the genomic DNA is longer (374 bp) and detecting point mutations may be more difficult and risk of false negative results is higher.

It has been reported that p53 mutation is detected frequently in cases of metastatic gastric cancer but rarely in the primary tumors of gastric cancer[6,7]. However, in the present study, no correlation was found between the presence of p53 mutations and the clinic-pathological parameters, including lymph node metastasis[1,4], so that our data agree with the previous reports. In addition, our study found that p53 mutations mainly occurred in aneuploid tumors, similar to the data reported by Tamura *et al*[4]. Therefore, we concluded that the *p53* gene may fit within the polygene model of gastric cancer and play an important role in regulating the cell cycle during tumorigenesis.

Furthermore, our study found no instance of LOH of the *p53* gene, which does not agree with other reports[1,8]. The possible reason for this discrepant finding is that LOH may only be detected by PCR-SSCP when the genomic DNA is strongly heterozygous (3) and the rate of heterozygosity of the *p53* gene is reportedly very low[9]. Finally, because the types and loci of *p53* gene mutations are not able to be determined by PCR-SSCP analysis, we are preparing to perform sequencing in the next phase of our research on this important topic.

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CLINICAL ARTICLES

Clinical and experimental study on gastric mucosal pathology, gene expression, cAMP and trace elements of pixu patients

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Abstract

AIM: To investigate the relationship between spleen deficiency substance, spleen deficiency and gastric cancer.

METHODS: We adopted the IBAS 2000 image analysis system and the 501B SEM with 9100/60 energy chromatic dispersing X-ray analysis instrument, along with histologic chemistry and radioimmunoassay to investigate the ultramicrostructure, intestinal metaplasia subtypes, cAMP, DNA, trace element series and their oxides of patients with gastric cancer.

RESULTS: The incidence rates of gastric cancer, incomplete colonic intestinal metaplasia (IM) and background lesion (P < 0.05 0.001). Additionally, the patients with incomplete IM and those with small bowel IM have lower levels of cAMP, Zn, Cu, ZnO and CuO and higher levels of p53 gene expression in gastric mucosa than patients with complete IM and those with colonic IM (P < 0.05-0.001). However, the levels of p53 DNA, cAMP, Zn, Cu, ZnO and CuO in gastric mucosa of incomplete colonic IM tissue are not remarkably different from those in gastric cancer tissue.

CONCLUSION: Gastric diseases in patients with spleen Qi deficiency or Qi stagnation have the tendency of turning into cancer. There is a close relationship between the incidence of incomplete colonic IM and gastric cancer.

Key words: Spleen asthenia; Gastric mucosa; DNA cyclic; AMP trace elements

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INTRODUCTION

The incidence of gastric carcinoma is highest among all malignant tumors. Early gastric cancer has no distinct symptoms; when discovered, it usually has developed to intermediate and late gastric carcinoma, both of which represent serious threats to a patient's life. Observations in clinical practice and pathologic tissue examination have suggested that "spleen deficiency" of traditional Chinese medicine and gastric mucosa intestinal metaplasia have a very close relationship with gastric carcinoma[1]. This study sought to determine the underlying mechanism of evolution for these three diseases, from both quantitative and qualitative aspects, and to provide experimental evidence to help promote earlier diagnosis of gastric cancer and guide clinical prevention and treatment approaches. Comprehensive statistical analysis of the data helps to provide insight into this important topic.

MATERIALS AND METHODS

Observational study

Sixty-seven patients who underwent fiber-optic gastroscopy, histopathologic examination and/or partial stomach resection and were diagnosed with gastric cancer or benign gastric diseases (including gastric ulcer and duodenal bulb ulcer) were divided into two groups according to spleen Qi deficiency or Qi stagnation (excess).

Deficiency/stagnation of spleen energy

Fifty of the total 67 patients in the study had intestinal metaplasia. Among the 31 cases of spleen Qi deficiency (22 males, 9 females; average age: 45 years, age range: 22-74 years) there were 11 cases of gastric ulcer, 12 cases of duodenal bulb ulcer, and 8 cases of gastric cancer. Seventeen of the patients in this group had intestinal metaplasia. Among the 36 cases of spleen Qi stagnation (22 males, 11 females; average age: 52 years, age range: 29-68 years) there were 10 cases of gastric ulcer, 6 cases of duodenal bulb ulcer, and 20 cases of gastric cancer. Thirty-three of the patients in this group had intestinal metaplasia.

Methods of study

The gastric mucosa specimens taken from each patient were divided

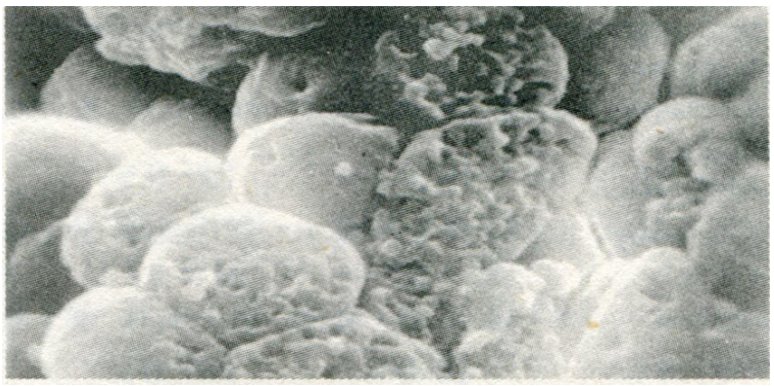

Figure 1 Focal superficial inflammation, partial rupture of the epithelial cytomembrane, and exposure of the cytoplasmic granule.

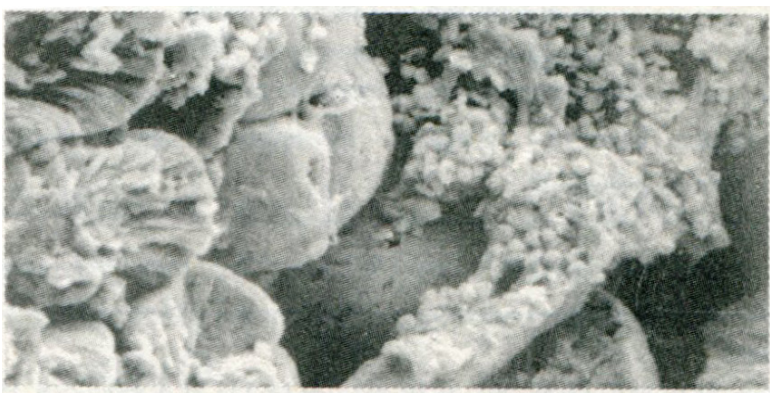

Figure 2 Focal intestinal metaplasia, with microvilli outside of cells, unclear intracellular granules and partial degradation of epithelial cells.

into non-focal and focal disease according to pathological examination; the levels of bioactive substances in these two tissue types were measured to determine the change in mucosa patterns. The ultramicroscopic structure of the gastric mucosa, plus the levels of trace elements and their oxides within, were evaluated by scanning electron microscope and ESI, as previously described[2,3]. The levels of cAMP and cGMP were assayed from 20 mg moist gastric mucosa tissue, with the former assayed by a radio-immune competitive protein binding method and the latter by a radio-immune diaphragm separation method. The weight unit was pmol/20 mg. To assay DNA expression in the gastric mucosa specimens, we first prepared a mucosal smear, stained it with Feulgen, and then made microscopic examination using an IBAS 2000 image analysis system equipped with a TV camera and processing computer. The integrated optical density (IOD) values of the assayed cellular karyons were calculated digitally and compared to the level of DNA. When more than 100 karyons from each patient were randomly assayed, the results were printed out in the form of a histogram.

To assay the body's cellular immune function, we performed the tritium thymidine lymphocyte conversion test, with units of measurement at cpm/0.2 mL in whole blood. Results from histochemical staining allowed for the gastric mucosa specimens to be classified for presence of intestinal metaplasia. Staining with ABpH/PAS was performed as well. If only acid mucin was found in the epithelium the tissue was classified as having complete intestinal metaplasia. If there was a neutral mucin finding, the tissue was classified as incomplete intestinal metaplasia. By staining with HiD/AB pH 2.5, if there was sulfur mucin found in the epithelium, the tissue was classified as colonic intestinal metaplasia, but if there was no sulfur mucin found then the tissue was classified as small intestinal metaplasia. By staining with HiD/AB pH 2.5/PAS, intestinal metaplasia was classified into the following 4 types: complete, incomplete, colonic and small.

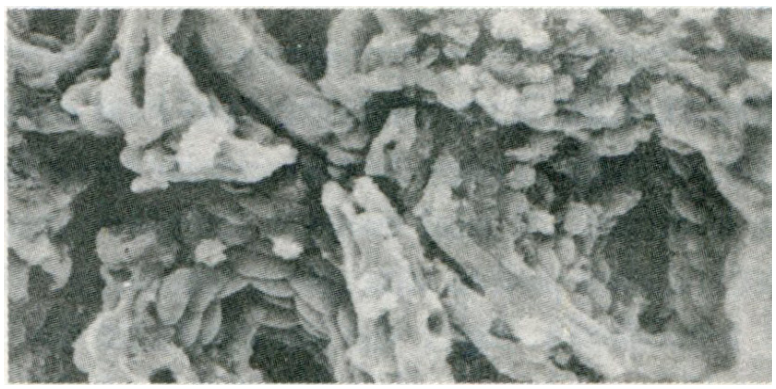

Figure 3 Focal atrophic inflammation, with a large area of cells with maintained contour but with ruptured cytomembranes and exposed cytoplasmic granules. There were only a few red blood cells present.

Figure 4 Micro-ulcer, with mucosa scaled in the ulcer area, clear granule and normal cells in the periphery. There was a small amount of red blood cells and white blood cells present.

RESULTS

Gastric mucosa patterns

Change in gastric mucosa structural patterns is one of the features of local structural state alteration related to gastric disease with spleen deficiency. Scanning electron microscopic observation of duodenal bulb ulcer, gastric ulcer, and gastric cancer, we found that in addition to the typical characteristic lesions there existed different degrees of chronic inflammation (Figure 1), focal intestinal metaplasia (Figure 2), focal atrophic gastritis (Figure 3) and micro-ulcerations (Figure 4) in both the non-focal areas of the body of the stomach and the gastric antrum mucosa. This phenomenon represented the "background lesion", and there were distinct differences in the incidence rates of such corresponding to the different spleen deficiency types ($P < 0.05$; see Table 1). Among the 18 cases of duodenal bulb ulcer, there were 7 of complete and 2 of incomplete small intestinal metaplasia and 2 of complete colonic intestinal metaplasia. Among the 21 cases of gastric ulcer, there were 3 of complete and 5 of incomplete small intestinal metaplasia, as well as 3 of complete and 4 of incomplete colonic intestinal metaplasia. Among the 28 cases of gastric cancer, there were 3 of incomplete small intestinal metaplasia, 5 of complete and 15 of incomplete colonic intestinal metaplasia. There were distinct differences in the incidence rates of intestinal metaplasia and gastric cancer between the two spleen deficiency types ($P < 0.001$; see Table 2).

Gastric mucosa trace elements and their oxides

ESI was used to assay the trace element series and their oxides in normal and focal tissues in the gastric mucosa of 41 patients with gastric disease and spleen Qi deficiency or stagnation. For each specimen, ESI automatically detected elements above atomic number 12, in the range of 0.1-0.001 mm^2. The computer calculated the weight percentage of each element in the element series, which was

Table 1 Comparison of pathologic changes in non-focal gastric mucosa between the different spleen deficiency types(*n*, %)

Pathologic changes in non-focal gastric mucosa	Deficiency of spleen energy (*n* = 31)	Spleen deficiency with energy stagnation (*n* = 36)		
	Gastric antrum cases (%)	Body of stomach cases	Gastric antrum cases	Body of stomach cases
Focal chronic superficial inflammatory lesion	31 (100.0)	31 (100.0)	36 (100.0)	36 (100.0)
Focal chronic atrophic inflammatory lesion	19 (61.3)	12 (38.7)	32 (88.9)	29 (80.6)
Focal intestinal metaplasia	12 (38.8)	13 (41.9)	25 (69.4)	30 (83.3)
Micro-ulcer	18 (58.1)	9 (29.0)	17 (47.2)	28 (77.8)

Table 2 Comparison of incidence rates of gastric cancer, intestinal metaplasia and intestinal metaplasia subtypes between the different spleen deficiency types (*n*, %)

	Gastric cancer	Intestinal metaplasia	Small intestinal metaplasia		Colonic intestinal metaplasia	
			Complete	Incomplete	Complete	Incomplete
Deficiency of spleen energy (*n* = 31)	8 (25.81)	15 (48.39)	6 (19.35)	5 (16.13)	2 (6.45)	2 (6.15)
Spleen deficiency with energy stagnation (*n* = 36)	20 (55.56)	33 (91.66)	4 (11.11)	5 (13.89)	7 (19.44)	17 (47.22)

Table 3 Quantitative changes of cAMP, DNA, Zn, Cu, ZnO and CuO levels in gastric mucosa from the various intestinal metaplasia groups

Intestinal metaplasia	cAMP	DNA	Zn	ZnO	Cu	CuO
1 Non-intestinal metaplasia	15.63 ± 1.06 (*n* = 15)	10.95 ± 1.32 (*n* = 9)	3.27 ± 0.61 (*n* = 14)	1.62 ± 0.64 (*n* = 14)	4.66 ± 0.62 (*n* = 14)	2.67 ± 0.53 (*n* = 14)
2 Small intestinal metaplasia	9.55 ± 1.62 (*n* = 20)	15.62 ± 5.04 (*n* = 13)	2.44 ± 0.44 (*n* = 11)	0.71 ± 0.10 (*n* = 11)	3.51 ± 0.66 (*n* = 11)	1.62 ± 0.32 (*n* = 11)
P value	< 0.001	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001
3 Colonic intestinal metaplasia	4.35 ± 0.93 (*n* = 22)	29.99 ± 12.41 (*n* = 17)	1.51 ± 0.51 (*n* = 16)	0.31 ± 0.09 (*n* = 16)	2.48 ± 0.68 (*n* = 16)	1.07 ± 0.09 (*n* = 16)
NO 1:3 *P*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
NO 2:3 *P*	< 0.001	< 0.00	< 0.001	< 0.0	< 0.001	< 0.001

Table 4 Quantitative changes of cAMP, DNA, Zn, Cu, ZnO and CuO levels in gastric mucosa of the different intestinal metaplasia subtypes

		Complete	Incomplete	*P* value
Colonic intestinal metaplasia	cAMP	5.96 ± 0.42 (*n* = 9)	2.74 ± 0.64 (*n* = 13)	< 0.001
Small intestinal metaplasia	cAMP	12.31 ± 1.51 (*n* = 10)	6.78 ± 1.80 (*n* = 10)	
P value		< 0.001	< 0.001	
Colonic intestinal metaplasia	DNA	19.53 ± 9.55 (*n* = 6)	35.71 ± 9.79 (*n* = 117)	< 0.01
Small intestinal metaplasia	DNA	11.06 ± 0.99 (*n* = 9)	20.17 ± 4.78 (*n* = 4)	
P value		< 0.01	< 0.05	< 0.01
Colonic intestinal metaplasia	Zn	1.77 ± 0.48 (*n* = 6)	1.25 ± 0.32 (*n* = 10)	< 0.05
Small intestinal metaplasia	Zn	2.82 ± 0.50 (*n* = 6)	2.06 ± 0.41 (*n* = 5)	< 0.05
P value		< 0.01	< 0.01	
Colonic intestinal metaplasia	ZnO	0.57 ± 0.10 (*n* = 6)	0.25 ± 0.08 (*n* = 10)	< 0.001
Small intestinal metaplasia	ZnO	1.01 ± 0.20 (*n* = 6)	0.41 ± 0.16 (*n* = 5)	< 0.001
P value		< 0.001	< 0.05	
Colonic intestinal metaplasia	Cu	2.78 ± 0.70 (*n* = 6)	2.11 ± 0.40 (*n* = 10)	< 0.05
Small intestinal metaplasia	Cu	3.86 ± 0.51 (*n* = 6)	3.11 ± 0.50 (*n* = 5)	< 0.05
P value		< 0.05	< 0.01	
Colonic intestinal metaplasia	CuO	1.56 ± 0.13 (*n* = 6)	0.58 ± 0.05 (*n* = 10)	< 0.001
Small intestinal metaplasia	CuO	2.41 ± 0.43 (*n* = 6)	0.83 ± 0.21 (*n* = 5)	< 0.001
P value		< 0.001	< 0.001	

expressed as comparative weight unit (WT%). The corresponding oxide and its WT% were also assayed synchronically at the same point. We set 15 points respectively for the normal and focal tissues, which were repeatedly and synchronically assayed 15 times, and obtained an average value of WT%. The average value of WT% of the sum of the normal and focal tissues was designated as the comprehensive value.

Twenty elements, including Na, Mg, Al, Si, P, S, K, Ca, Fe, Cu, Zn, Ti, Ni, Cr, *etc.*, and their corresponding oxides were assayed in the gastric mucosa of 41 patients. The WT% change of Zn, Cu, ZnO and CuO were the most distinct. In normal tissue, the WT% of Zn was 3.31 ± 1.26, of Cu was 4.64 ± 1.94, of ZnO was 1.48 ± 0.71, and of CuO was 2.39 ± 0.98. In focal tissue, the WT% of Zn was 1.44 ± 0.85, of Cu was 2.42 ± 0.92, of ZnO was 0.73 ± 0.41, and of CuO was 1.35 ± 0.48. All of these differential values showed statistical significance ($P < 0.001$). In the normal gastric mucosa of 15 cases of benign gastric disease due to deficiency of spleen energy, Zn was 4.39 ± 0.33, Cu was 6.28 ± 0.54, ZnO was 2.11 ± 0.13, and CuO was 3.28 ± 0.25; in the focal tissue, Zn was 1.99 ± 0.01, Cu was 3.05 ± 0.12, ZnO was 1.04 ± 0.08, and CuO was 1.73 ± 0.26. There were distinct differences between the two tissues ($P < 0.001$). In the normal gastric mucosa of 15 cases of benign gastric disease due to spleen deficiency with energy stagnation, Zn was 3.23 ± 0.37, Cu was 4.84 ± 0.89, ZnO was 1.51 ± 0.38, and CuO was 2.34 ± 0.35; in the focal tissue, Zn was 1.57 ± 0.07, Cu was 2.24 ± 0.23, ZnO was 0.84 ± 0.14, and CuO was 1.48 ± 0.10. Again, there were distinct differences between the two tissues ($P < 0.001$). In the normal gastric mucosa of 10 cases of gastric cancer due to spleen deficiency with energy stagnation, Zn was 1.97 ± 0.41, Cu was 2.77 ± 0.95, ZnO was 0.44 ± 0.04, and CuO was 1.10 ± 0.39; in focal tissues, Zn was 0.50 ± 0.22, Cu was 1.63 ± 0.85, ZnO was 0.09 ± 0.03, and CuO was 0.72 ± 0.31. There were distinct differences between the two tissues as well ($P < 0.001$). There were also distinct differences between the spleen Qi types (P < 0.01-0.001). The comprehensive value of WT% was also remarkably different between the spleen Qi types, between the disease types, and between the intestinal metaplasia subtypes (see Tables 3 and 4).

Gastric mucosa cAMP

The level of gastric mucosa cGMP showed no distinct quantitative change. In the normal tissue of 28 cases of deficiency of spleen energy, the level of gastric mucosa cAMP was 14.86 ± 1.97, and in focal tissue was 6.10 ± 1.13; there was a distinct difference between the two tissues ($P < 0.001$). In the normal tissues of 29 cases of spleen deficiency with energy stagnation, the cGMP level was 8.87 ± 1.73, and in the focal tissue was 2.89 ± 1.11; there was a distinct difference between the two tissues ($P < 0.001$). There were also distinct differences between the spleen Qi types ($P < 0.05$-0.001). The comprehensive quantity of gastric mucosa cAMP was also remarkably different between the spleen deficiency types, between the disease types, and between the intestinal metaplasia subtypes (see Tables 3-5).

Table 5 Quantitative changes of cAMP, DNA, Zn, Cu, ZnO and CuO levels in gastric mucosa of the different spleen deficiency types and gastric diseases

		Deficiency of spleen energy	Spleen deficiency with energy stagnation	*P* value
Gastric cancer	cAMP	8.34 ± 1.23 (n = 8)	4.15 ± 1.36 (n = 14)	< 0.001
Benign gastric disease	cAMP	12.62 ± 1.87 (n = 20)	7.61 ± 1.84 (n = 15)	< 0.001
P value		< 0.001	< 0.001	
Gastric cancer	DNA	26.54 ± 3.94 (n = 4)	36.11 ± 9.75 (n = 11)	< 0.05
Benign gastric disease	DNA	10.72 ± 0.92 (n = 15)	13.84 ± 3.52 (n = 9)	< 0.05
P value		< 0.05	< 0.01	
Gastric cancer	Zn	1.60 ± 0.01 (n = 1)	1.24 ± 0.23 (n = 10)	< 0. 001
Benign gastric disease	Zn	3.19 ± 0.17 (n = 15)	2.40 ± 0.83 (n = 15)	< 0.001
P value		< 0.01	< 0.001	
Gastric cancer	ZnO	0.71 ± 0.02 (n = 1)	0.27 ± 0.17 (n = 10)	< 0.001
Benign gastric disease	ZnO	1.57 ± 0.11 (n = 15)	1.18 ± 0.33 (n = 15)	< 0.001
P value		< 0.001	< 0.001	
Gastric cancer	Cu	2.68 ± 0.03 (n = 1)	2.20 ± 0.57 (n = 10)	< 0.05
Benign gastric disease	Cu	4.66 ± 0.33 (n = 15)	3.63 ± 1.21 (n = 15)	< 0.01
P value		< 0.001	< 0.001	
Gastric cancer	CuO	1.36 ± 0.03 (n = 1)	0.91 ± 0.19 (n = 10)	< 0.001
Benign gastric disease	CuO	2.51 ± 0.26 (n = 15)	1.91 ± 0.43 (n = 15)	< 0.001
P value		< 0.001	< 0.001	

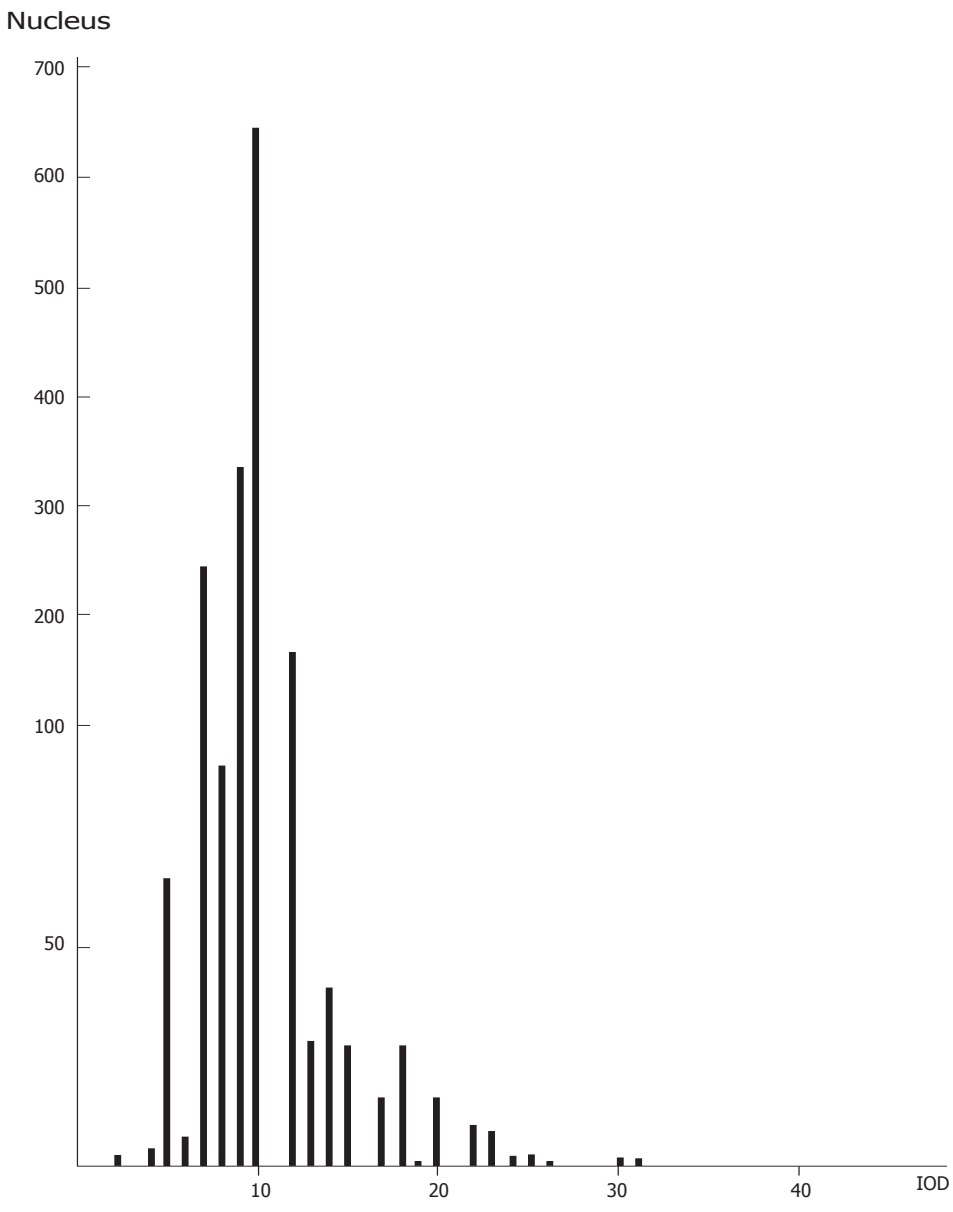

Figure 5 Distribution histogram of DNA levels in gastric mucosa from 15 cases of benign gastric disease due to deficiency of spleen energy.

Gastric mucosa DNA

In 24 cases of benign gastric disease, the level of DNA was 11.89 ± 2.73, while in 15 cases of gastric cancer it was 33.55 ± 9.58; there were distinct differences between these two diseases (P < 0.001). In 19 cases of deficiency of spleen energy, the level was 14.04 ± 6.75, and in 20 cases of spleen deficiency with energy stagnation it was 26.09 ± 13.44; there were distinct differences between the two spleen deficiency types (P < 0.01). DNA was remarkably different between the spleen deficiency types, between the disease types, and between the intestinal metaplasia subtypes (see Tables 3-5).

The DNA histogram of benign disease due to spleen deficiency with energy stagnation was similar to that of gastric cancer and different from that of benign gastric disease due to deficiency of spleen energy (see Figures 5-7).

Organismal cellular immunity

In 35 cases of benign gastric disease, the ^{3}H-TdR LCT was 21404 ± 15962, and in 16 cases of gastric cancer it was 9946 ± 10498. In 27 cases of deficiency of spleen energy, it was 28578 ± 15101, and in 24 cases of spleen deficiency with energy stagnation it was 7093 ± 6887. In 73 cases of healthy people, it was 38083 ± 18296. The differences between each group were significant (P < 0.05-0.001).

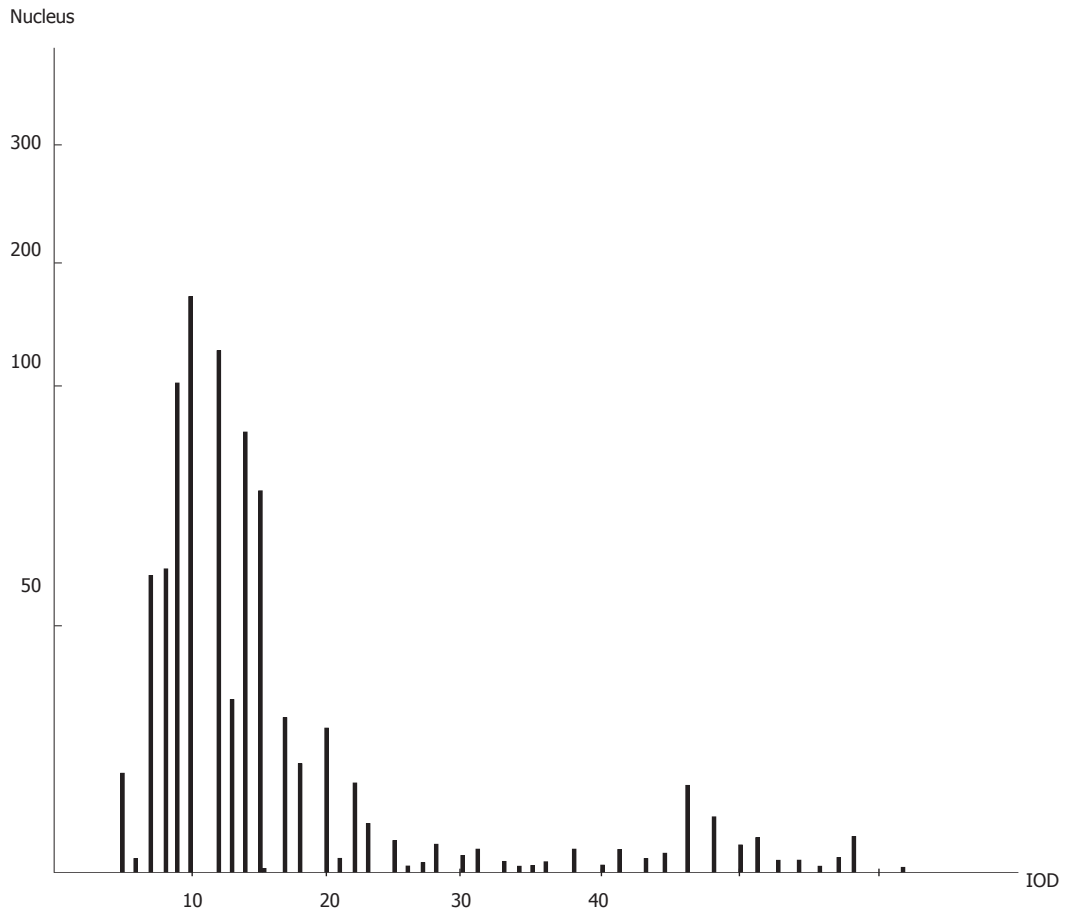

Figure 6 Distribution histogram of DNA levels in gastric mucosa from 9 cases of benign gastric disease due to spleen deficiency with energy stagnation.

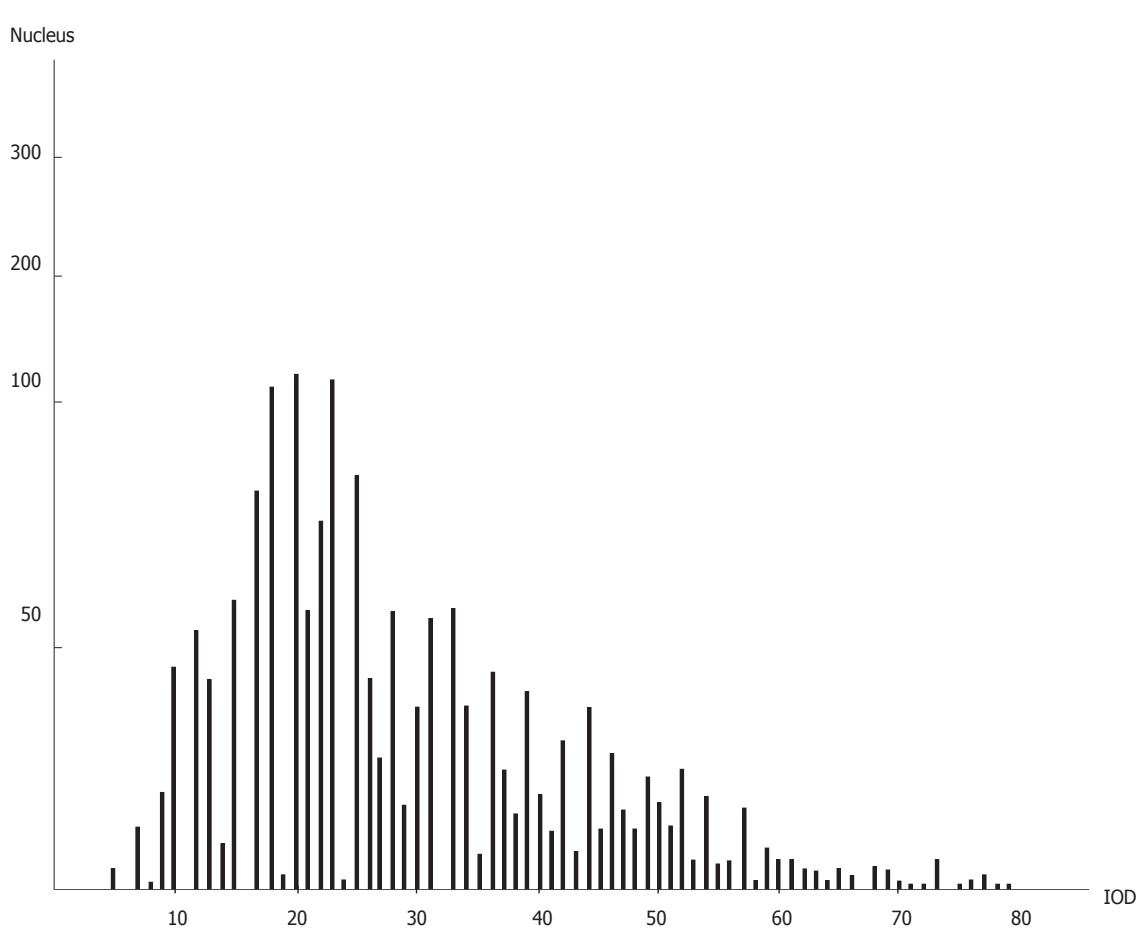

Figure 7 Distribution histogram of DNA levels in gastric mucosa from 11 cases of gastric cancer.

Collectively, the above data show that the levels of cAMP, Zn, Cu, ZnO and CuO in gastric mucosa from patients with gastric disease due to spleen deficiency decreased in the order from deficiency of spleen energy to spleen deficiency with energy stagnation, from normal to focal tissue, from complete to incomplete intestinal metaplasia, and from small to colonic intestinal metaplasia. Meanwhile, DNA increased in the above order ($P < 0.05$-0.001). The incidence rates of "background lesion" in non-focal gastric mucosa and incomplete colonic intestinal metaplasia increased from deficiency of spleen energy to spleen deficiency with energy stagnation, and from benign

gastric disease to gastric cancer (P < 0.05-0.001). The incidence rates of incomplete colonic intestinal metaplasia, gastric cancer and "background lesion" of gastric disease due to spleen deficiency with energy stagnation were all higher than those in patients with deficiency of spleen energy (P < 0.05-0.001). The levels of cAMP, DNA, Zn, Cu, ZnO and CuO in the incomplete colonic intestinal metaplasia tissue of gastric mucosa showed no distinct differences from those detected in gastric cancer tissues (see Tables 3-5).

The cellular immune function of patients suffering from gastric cancer and spleen deficiency with energy stagnation was also poor.

DISCUSSION

The quantitative change of cAMP, Zn, Cu, ZnO and CuO in gastric mucosa is the material foundation for spleen deficiency types and cellular cancerization, as well as for the physiopathologic and histopathologic reaction of gastric disease due to spleen deficiency. The body's cellular immune function of gastric disease due to spleen deficiency, the quantitative changes of cAMP, Zn, Cu, ZnO and CuO in gastric mucosa tissue, and the concomitant changes between "background lesion" in non-focal gastric mucosa and intestinal metaplasia subtypes can be considered the body's systemic, local and phasic dynamic resistance of physiology against pathology the occurs during the disease process, which manifests as the synchronic changes in degrees and levels of the body's metabolic state, structural state and functioning state.

When the levels of Zn and Cu decline in the organism, the synthesis and activities of various Zn- and Cu-dependent enzymes will be inhibited and the metabolism of sugar, lipids, proteins, nucleic acids and vitamins will be disturbed subsequently. Indeed, when the activity of adenylate cyclase does not coordinate with that of cAMP diesterase, the synthetic metabolism of cAMP is lower and the level of cAMP in tissue becomes decreased; moreover, the translation and transcription of genes related to the metabolism of nucleic acids through phosphorylase kinase and protein kinase is disturbed, which in turn causes mismatch of DNA base pairing, disrupts cellular heredity, and influences cellular division and differentiation, thereby creating damage in the epithelial cells of gastric mucosa. In addition, this mechanism may promote ulcer formation (due to atrophy of the glandular body) and increased regeneration of the epithelial cells, the latter of which presents increased opportunities for gene mutation and may result in intestinal metaplasia and anaplasia, even cancerization.

In this study, the ratio of the speed of multiplication of cellular division was inverse to cAMP density in cells and direct to DNA density. The quantitative changes of cAMP and DNA were particularly distinct in incomplete colonic intestinal metaplasia cells and cancer cells. The ^{3}H-TdR LCT decreased when the metabolic paths of lymphocytes were influenced, and transcription was inhibited by the quantitative changes of Zn and cAMP.

Functional state changed abnormally due to the changes of metabolic state and structural state, and symptoms of spleen deficiency occurred in conjunction. As the disease developed, the degree of synchronic change and level of the body's systemic, local and phasic metabolic state, structural state and functioning state differed; the evolution and transformation of symptoms differed, as well. If the state developed to kidney-related changes in degrees, deficiency of spleen energy evolved to positive splenonephric deficiency. If it developed to the degree and level of energy stagnation hypostasis, it evolved to spleen deficiency with energy stagnation. If it developed to the physiologic state, the condition was moving towards recovery.

Symptoms have relation to the manifested disease, and its extent. Thus, symptoms may be useful for comprehensively diagnosing the degrees and levels of the three disease characteristics (entirety, locality and phasity) and the three states by using a quantitative change range based upon multilevel correlative indexes. Each ZANG and FU (vivscera) stated by the traditional Chinese Visceral Manifestation Theory is actually a systematic level that is based on "comprehensive function" associated with some anatomical structure. When various kinds of diseases occur, as repercussions of the body in its entirety adjusting to an individual heredity diathesis, the disease's impact on each "comprehensive functional unit" (with viscera as its systematic level) results in the clinical phenomena of "different diseases with the same symptoms", "the same disease with different symptoms" and "the evolution of symptoms" as the degrees and levels that are different for entire, local and phase reactions.

The histogram of DNA in gastric disease tissue due to spleen deficiency with energy stagnation was similar to that of gastric cancer; the incidence rate of incomplete colonic intestinal metaplasia in the gastric mucosa of this spleen deficiency type was high. As for the incomplete colonic intestinal metaplasia, the DNA content was found to be abnormally increased. Sulfuric acid mucin was detected in many samples and the detective rate of CEA was also found to be increased with regard to changes in cellular heredity substances, mucosal histochemistry findings and antigenicity[4]. The levels of cAMP, Zn, Cu, ZnO and CuO in the tissues of these cases were more or less the same as those in gastric cancer tissue, indicating that this kind of intestinal metaplasia is undifferentiated and possibly prone towards progressing to cancer (so it is possibly an indicator of impending cancerization). In clinical practice, more attention should be paid to the cancerization of gastric disease due to spleen deficiency with energy stagnation; the intestinal metaplasia subtypes, cAMP, DNA, Zn, Cu and CEA should also be monitored over time in the gastric mucosa of patients with gastric disease due to spleen deficiency with energy stagnation, as the findings may be helpful towards making an early diagnosis of gastric cancer.

Duodenal bulb ulcer due to spleen deficiency, and gastric ulcer focality in gastric tissue of gastric cancer are special lesions of the disease, and often exist as "background lesions" of different degrees in the non-focal gastric mucosa and may be a common feature of phase structural state of the symptom-disease combination. Although partial surgical excision of the stomach can remove these special lesions, the procedure may not entirely remove the "background lesion". The residual "background lesion" may then become the original pathologic foundation for residual gastric disease, while the flow of gall and duodenal juices related to the operation mode may also inducing residual gastric disease. Therefore, we recommend avoiding operative treatment for benign gastric disease that has not cancerized or has no expected cancerization trend, especially in cases where there is no serious complication or risk of such. The operation method should be selected carefully according to the comprehensive treatment principles, and postoperative treatment measures should be designed according to an integrative approach for traditional Chinese medicine and Western medicine in order to best prevent and cure residual gastric disease.

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Evaluation of multiparameter histologic diagnosis of gastric cancer

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Abstract

AIM: To evaluate multiparameter histologic diagnosis of gastric cancer (GC).

METHODS: Expression of the MGa23c7 antigen and the cerbBa22 receptor was detected in specimens of GC by ABC immunohistochemistry. The DNA content of specimens was determined by flow cytometry (FCM). The data of the multiparameter methodology were analyzed statistically.

RESULTS: The expression rates of MGa23c7 antigen and cerbBa22 receptor were observed in gastric tubular adenocarcinoma (GTA; 100% and 56% respectively), adenocarcinoma (AC; 58% and 50%) and normal gastric mucosa (NGM; 20% and 33%). The DNA heteroploid rate was 63% (12/19) in GTA, 75% (3/4) in AC and 0% (0/8) in NGM. In detecting GTA with FCM and the ABC method, the histological parameter analysis showed that sensitivity was 82% ± 3%, specificity was 88%, predictive value was 95% ± 4% and accuracy was 84%. Both methods were complementary.

CONCLUSION: Multiparameter diagnosis and studies of histomorphology and molecular biology may be a useful approach towards histologic diagnosis of GC.

Key words: Stomach neoplasms/diagnosis; Adenocarcinoma/diagnosis; Immunohistochemistry; Flow cytometry

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INTRODUCTION

Early diagnosis of gastric cancer (GC) is an important focus of the research field examining digestive tract tumors. The single parameter diagnosis is rather limited, and involves either histology or serology. Differences in laboratory test sensitivity and antibody specificity are common limitations to accurate tumor diagnosis. In this study, the self-made antihuman gastric tubular adenocarcinoma (GTA) monoclonal antibody (mAb) MG3c7 and the antihuman oncogene antibody cerbB-2 were used to investigate a series of paraffin-embedded GC tissues by ABC immunohistochemistry; the DNA content was analyzed by flow cytometry FCM as well. The aim of the study was to evaluate histological diagnosis of GC in clinical application by way of multiparameter analysis.

MATERIALS AND METHODS

Histological specimens

Specimens of GC (n = 51), gastritis (GS; n = 17) and normal gastric mucosa (NGM; n = 10, obtained through biopsy) were obtained from the surgically-resected samples and confirmed by pathological diagnosis. The paraffin-embedded tissue sections were evaluated by immunohistochemistry and tissue cell suspensions were detected by FCM to determine DNA content.

Reagents and instruments

The antihuman gastric tubular adenocarcinoma mAb MG-3c7 was made by our laboratory. The working dilution of antibody was 1:200 after purification. The ABC reagent, antihuman oncogene cerbB-2 IgG (1:200) and anti-rabbit IgG-HRP (1:40) were obtained from the Institute of Zhong Da Medical Applied Research (Shanghai, China). The fluorescein active cell selector (FACS, 420 type) was provided by the Institute of Cancer Research (Hebei Province, China).

Methods

Immunohistochemistry. Lactonase was dispelled from the sections after deparaffinization and re-hydration. Afterwards, the sections were blocked by 5% BSA and divided into 2 groups for respective incubation with McAb MG-3c7 or the oncogene cerbB-2 for 3 h at room temperature or overnight at 4 ℃. This was followed by reaction for 1 hour with ABC reagent and anti-rabbit IgG-HRP. The color reaction was visualized by DAB under microscopy, after which hematoxylin staining was completed and the sections were dehydrated, hyalinized and mounted.

FCM. The paraffin-embedded tissues were deparaffinized, re-hydrated, washed and shred to pieces. The cell suspension was then passed through a 300-millipore nylon sieve, centrifuged and fixed with 70% ethanol. Fluorescence staining of DNA interpolated with ethidium bromide and chicken red cells (as the internal refer-

Table 1 Distributions of MG-3c7 and CerbB-2 in gastric cancer tissues

Type	*n*	MG-3c7				*n*	cerbB-2			
		+ + +	+ +	+	-		+ + +	+ +	+	-
TACN	30	12	13	5		23	5	7	1	10
AC	12	3	2	2	5	6		1	2	3
SRCC	2				2	3				3
MAC	7				7	2			1	1
GS	4				3	4			2	2
NGM	10				8	6			2	4

TACN: Tubular adenocarcinoma of nipple; AC: Adenocarcinoma; SRCC: Signet-ring cell carcinoma; MAC: Mucous adenocarcinoma; GS: Gastritis; NGM: Normal gastric mucosa.

Table 2 Results of positive coincidence rate determined by three markers

Type	cerbB-2	MG-3c7	DNA
	Positive rate (%)	Positive rate (%)	2c rate (%)
TACN	13/23 (56)	30/30 (100)	12/19 (63)
AC	3/6 (50)	7/12 (58)	3/4 (75)
MAC	1/2 (50)	0/2 (0)	2/2 (100)
SRCC	0/3 (0)	0/7 (0)	
GS	2/4 (50)	1/4 (25)	0/17 (0)
NGM	2/6 (33)	2/10 (20)	0/8 (0)

TACN: Tubular adenocarcinoma of nipple; AC: Adenocarcinoma; SRCC: Signet-ring cell carcinoma; MAC: Mucous adenocarcinoma; GS: Gastritis; NGM: Normal gastric mucosa.

ence standard) was carried out by staining for half an hour; the cells were then evaluated by FACS. The cell fluorescence photon appeared on the multichannel pulse analyzer in a combined point and digital pattern. The CV value remained steady between 3% and 5%. Ten-thousand cells from each specimen were determined and all of the data were analyzed statistically.

Experimental judgment standard

The stained cells were scored as negative or in accordance with the section background (-), with 1%-9% of cells showing light yellow coloration or weak positive reaction judged as (+), 10%-49% of cells showing yellow-brown coloration or positive reaction judged as (++), and 50%-100% of cells showing dark brown coloration or strong positive reaction judged as (+++). The DNA index (DI) G0/1 peak/channel average value of tumor tissues G0/1 peak/channel average value of normal tissues was determined according to the following:

Referring to the method of Fallenicus, *etc.* DI = 1 ± 0.1 was diploid (2c) and the excess was heteroploid.

Standard of multiparameter diagnosis: Sensitivity = TP/(TP + FN) × 100%.

Specificity = TN/(TN + FP) × 100%.

Predictive value = TP/(TP + FP) × 100%.

Accuracy = (TP + TN)/(TP + FN + TN + FP) × 100%.

TP: True Positive cases. FN: False Negative cases.

TN: True Negative cases. FP: False Positive cases.

RESULTS

The expression and distribution profiles of MG-3c7 antigen and cerbB-2 receptor in the different types of GC are shown in Table 1. The results of the positive coincidence rate of GC tissues determined by ABC and FCM for the three different markers are shown in Table 2.

The comparison of the positive judgment parameters determined by MG3c7 by immunohistochemistry and DNA content by FCM are shown in Table 3.

Table 3 Comparison of the positive judgment parameters determined by ABC and flow cytometry

Parameter	GC			GTB		
	A	B	A+B	A	B	A+B
Sensitivity	72.5	72.5	72.5	100.0	72.5	82.5
Specificity	80.0	100.0	88.8	80.0	100.0	88.8
Predictive value	94.8	100.0	96.9	94.8	100.0	95.4
Accuracy	73.7	73.7	75.2	95.0	68.9	84.0

A: Positive rate in immunoenzymology and histochemistry; B: Heteroploid rate in flow cytometry; A+B: Positive rate in immunohistochemistry and heteroploid rate in flow cytometry.

DISCUSSION

It is not unusual to make a clinical diagnosis of GTA with many markers and methods in histology or serology. We performed immunohistochemical staining with the self-made antihuman GTA mAb MG-3c7 and antihuman oncogene antibody cerbB-2.

The changes of DNA diploid status were determined by FCM in GC tissues. It was possible to evaluate the same specimen *via* different methods at the same time. We hope to make an early diagnosis by using analysis and comparison of the multiparameters as well as with complementation of laboratory tests. As a whole, we can combine histomorphology with molecular biology for the diagnosis of GC.

The mAb MG-3c7 reagent was generated from BALB/c mice that had been immunized, and fused with the NGCC-8310 cell line of human GTA. It was found to be specific to the GTA histo-antigen.

The function of cerbB-2 is very similar to that of the EGF receptor. Its over-expression is related to the occurrence of human tumor. Cell division and proliferation is based on DNA content. As normal cells transform into malignant ones, the DNA content and the expression rate of heteroploid status are elevated accordingly. Therefore, abnormal DNA content is a biological marker for diagnosis of malignant tumor.

It was shown in the current study that the mAb MG-3c7 was strongly sensitive to GTA and that its positive coincidence rate might reach 100%. The positive rate of cerbB-2 and heteroploid rate of DNA content were 56% and 63% respectively. The markers of MG-3c7, cerbB-2 and DNA showed rates of 80%, 50% and 68% for diagnosis of GC. Immunohistochemical staining showed that cerbB-2 was distributed in the cytomembrane and MG-3c7 was located in the cytoplasm. According to the different specificity profiles in histological analysis, we were able to observe the expressions of distinctly related antigens in the same tissues. As tumor markers, cerbB-2 and MG3c7 were found to be in direct proportion to the tumor differentiation degree. However, it was shown in the analysis on DNA diploid status that the poorer the tumor was differentiated, the higher the DNA heteroploid positive rate. The results showed that tumor malignant behavior, as well as its growth and decline, could be studied from different perspectives and have impact on cell immunology and molecular biology. No specific tumor marker has yet been obtained for the clinical diagnosis of GC. Therefore, we believe that this study provided very important knowledge towards making multiparameter analysis for the diagnosis of tumors.

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REVIEW

Effect of erythromycin on gastric emptying

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INTRODUCTION

Motility is a general term that embraces all movements of the gastrointestinal tract, such as smooth muscle contraction, intraluminal pressures, and transit time of chyme throughout the bowel. These movements are modified by the actions of specialized segments of the intestine and the sphincters, and the whole system is integrated by neural and humoral levels of control. Motility is best understood teleologically, *i.e.* as a process that facilitates other more fundamental functions of the gut.

The stomach has three major functions: accommodating meals of varying volumes; grinding solid food into small particles; and facilitating the process of gastric emptying.

The last function serves as an important control of the load of chyme presented to the small bowel for digestion and absorption. Thus, the gastric fundus exhibits "receptive relaxation, " which is a decrease in basal tone that is mediated by vagal reflexes and that reduces pressure in gastric body and fundus during the meal. Receptive relaxation provides accommodation for a meal, and food remains in the stomach for acid-peptic digestion to proceed. Later in the postcibal period, basal tone returns; this increase in intraluminal pressure facilitates emptying of the liquid phase of mixed meals[1-3].

Of all disorders of gastroduodenal motility, vomiting is the major manifestation and is the forceful expulsion from the upper gastrointestinal tract. When projectile, the symptom suggests mechanical obstruction, but less disturbed progression of chyme may produce effortless regurgitation. Vomiting can be either central or peripheral in origin, *e.g.*, intra-abdominal stimulation of the vomiting reflex (peritonitis) or mechanical obstruction of the upper gut (*e.g.*, pyloric stenosis).

Nausea and vomiting may be regarded as the body's defense against toxins. The vomiting reflex is initiated by the vomiting center in the medulla, which receives messages from various sources.

Vomiting caused by local irritation of chemoreceptors in the gut mucosa is mediated by afferent vagal and sympathetic pathways to the vomiting center. Toxins that reach the circulation after absorption (or intravenous injection) stimulate the chemoreceptor trigger zone (CTZ) in the area postrema, a circumventricular area which acts as a window in the blood-brain barrier, and neuronal afferents pass messages from here to the vomiting center. The area postrema receives input impulse from vagal afferents and also has neural links with the nucleus tractus solitarius, which is the main site for peripheral input from vagal and sympathetic afferents. Afferent neural impulses from the fauces also pass *via* the nucleus tractus solitarius to the vomiting center. The vomiting reflex is preceded by a prodromal vasovagal phase characterized by pallor, sweating and bradycardia, followed by elevation of the epiglottis and soft palate. Motor impulses activated by the vomiting center then result in relaxation of the stomach and the lower esophageal

sphincter and contraction of the pylorus, diaphragm and thoracic and abdominal muscles, resulting in expulsion of the stomach contents through the mouth.

Nausea is not necessarily a slow-level stimulation of the vomiting reflex. Vomiting may occur without nausea. Nausea is also more difficult to assess than vomiting, as will be discussed later.

Normally, progression of food through the small intestine is slow and steady, and an extensive spreading out of chyme allows its maximal contact with the digestive-absorptive surface. Although the "head" of a barium meal may reach the terminal ileum in 1 to 2 h, meal transit time (for 50% of a meal) is slower and the "tail" is even more delayed. This pattern of transit through the small bowel has led to the unsubstantiated proposal that "intestinal hurry" is a cause of malabsorption and diarrhea. Although appealing, the concept has received little experimental scrutiny. The only documented examples of intestinal hurry are those of "short bowel syndrome", seen after extensive resection of the small bowel, and in the malabsorption that accompanies jejunoileal bypass. In these conditions, the major defect is inadequate contact between chyme and the digestive-absorptive surface. Stanghellini *et al*[4] reported that patients with both ulcer and non-ulcer dyspepsia show interdigestive and postprandial antral hypomotility.

For disorders of gastroduodenal motility, the primary goals of therapy are[1] improving gastroduodenal emptying, intestinal decompression, restoration or maintenance of fluid and electrolyte balance, and treatment of the cause.

Most instances of adynamic ileus are transient, and a medical approach can achieve all three goals. However, when the obstruction is mechanical, initial decompression and removal of the cause of obstruction usually requires surgery.

PATHOPHYSIOLOGY ON GASTROINTESTINAL EMPTYING

Up to now, although gastric emptying disorders are relatively uncommon, they are potentially devastating conditions resulting from pathophysiologic motor disturbances. Rapid gastric emptying of liquids is the hallmark of dumping syndrome and occurs after operations, including vagotomy. Vagal denervation abolishes receptive relaxation and accommodation in the proximal stomach (the storage site for ingested liquids) resulting in increased intragastric pressure that forces liquids through an ablated or bypassed pylorus. Dumping symptoms may occur in up to 50% of postgastrectomy patients, but most patients are treated satisfactorily by dietary manipulation or, in the rare incapacitated patient, by the long-acting somatostatin analogue octreotide. Reconstructive gastric surgery may rarely be indicated to slow gastric emptying and alleviate dumping syndrome. Re-operative procedures include pyloric reconstruction after pyloroplasty, generation of small intestinal pouches, interposing of isoperistaltic and antiperistaltic jejunal segments, and the Roux-en-Y gastrojejunostomy. Interposed jejunal loops and the Roux-en-Y gastrojejunostomy provide the most satisfactory results.

Delayed gastric emptying may occur in the acute postoperative period or be a late complication of gastric surgery. Loss of vagal input to the gastricantrum and resection of the antrum with vagotomy may produce an atonic stomach or atonic gastric remnant, respectively, which fails to grind and propel solids into the small intestine. Scintigraphic imaging of both the liquid and solid components of a meal is a valuable diagnostic adjunct. Gastric ileus occurring in the early postoperative period generally resolves within 6 wk after operation, and the temptation to re-operate on a non-obstructed stomach should be avoided.

Pharmacologic therapy of chronic gastric stasis with the benzamide prokinetic agents (metoclopramide, cisapride, renzapride), domperidone, or the motilin agonist erythromycin may be effective initially but long-term results are still undefined, and post-vagotomy and post-gastrectomy patients have not been studied adequately[5]. The effects of erythromycin on different parts of the rabbit intestine have been reported[6]; in particular, the effect of erythromycin on the rabbit intestinal smooth muscle was investigated and was compared to that of motilin. Whole isolated segments from the duodenum, jejunum, ileum and ascending colon, as well as strips from the circular and longitudinal smooth muscle of the ascending colon, were used. Erythromycin was found to possess a concentration-dependent contractile effect on the above intestinal parts but to different degrees of intensity. The order of the sensitivity of the intestinal parts to erythromycin was reported as duodenum = jejunum > ascending colon > ileum. The circular smooth muscle of the ascending colon was found to be more sensitive than the longitudinal smooth muscle; moreover, a similar contractile effect, but at lower concentrations, and the same regional specificity were observed with motilin.

During phases Ⅱ and Ⅲ of the migrating motor complex, there is an increase in plasma motilin level that is synchronous with phasic and tonic contractile activity of the lower esophageal sphincter and of the stomach. The action of motilin on human lower esophageal sphincter is proposed to be mediated by cholinergic mechanisms. Recently, it has been shown that erythromycin was a motilin agonist. That study evaluated the pharmacological effects and the mechanisms of action of intravenous erythromycin on esophageal motility in humans. Healthy volunteers were studied three times at 7-d intervals in a randomized, double-blind fashion. Subjects were first studied for 10 min before drug administration. Afterwards, they received an intravenous injection of placebo or atropine (12 mg/kg), in a blind and random manner, followed by a 20 min continuous intravenous infusion of placebo or erythromycin (150 mg). The difference (delta) between lower esophageal sphincter pressure and the duration, amplitude and velocity of peristaltic contractions during the control period and after administration of drugs was compared. Erythromycin significantly increased ($P < 0.05$) the lower esophageal sphincter pressure to 2234.4 ± 625.1 Pa (16.8 ± 4.7 mmHg) compared to placebo -3.9 ± 186.2 Pa (-0.029 ± 1.4 mmHg). Erythromycin significantly decreased peristaltic contraction velocity compared to placebo ($P < 0.05$). The effects of erythromycin on lower esophageal sphincter pressure were completely blocked by previous administration of intravenous atropine. Erythromycin increased the number of fundic contractions compared to the placebo, but this effect was not blocked by prior atropine administration[7,8].

USEFULNESS OF ERYTHROMYCIN FOR GASTROINTESTINAL EMPTYING DISORDERS

Postoperative gastroparesis

Erythromycin has recently been shown to exert a great effect on gastroduodenal motor activity. This prokinetic action may be clinically useful to patients with gastrointestinal hypomotility, such as diabetic or postoperative gastroparesis. A diabetic patient who underwent antrectomy Billroth Ⅱ for gastric cancer was presented; severe gastroparesis appeared after surgery and nasogastric aspiration could not be removed, although the patient was treated with metoclopramide and glucose, the water and electrolyte imbalance and nutritional status were corrected. Forty-one days after the first operation, a second gastrectomy with Billroth Ⅱ reconstruction was performed because of suspicion of anastomotic narrowing, which was not confirmed at surgery. Fourteen days later, *i.v.* erythromycin (200 mg/4 h) was started owing to persistent gastroparesis. Six days after treatment, the patient tolerated oral ingestion. Prokinetic drugs constitute the specific therapy for gastroparesis. Metoclopramide is the most commonly used, although its efficacy is limited. In the last few years, erythromycin has been proven to have a powerful effect on gastroduodenal motility. This effect is mediated, at least in part, by its motilin-stimulating activity that accelerates gastric emptying; our patient completely recovered with it. Recent results of erythromycin therapy in patients have been promising, despte the difficult management involved[9].

Diabetic gastroparesis

To compare the effects of erythromycin and metoclopramide on gastric emptying and improving symptoms of gastroparesis in diabetic patients with delayed gastric emptying, Erbas *et al*[10] used a study group consisting of 13 patients with symptoms of severe gastroparesis and delayed gastric emptying. Gastric emptying was evaluated using a radionuclide method, and gastrointestinal symptoms were scored. The patients were given either erythromycin (250 mg 3 times/d) or metoclopramide (10 mg 3 times/d) in random order for 3 wk, and after a washout period of 3 wk they were crossed-over to the other medication for another 3 wk. Parameters of gastric emptying were assessed before treatment and after both erythromycin and metoclopramide. The half-time of gastric emptying in diabetic subjects was 110 (77-120) min before treatment. At 60 and 90 min, the median value of residual isotope activity was 66.5% (55%-83.5%) and 55% (43%-74.3%) respectively. The half-time decreased to 55 min (28.6-115) after 3 wk of treatment with erythromycin and percentages of meal retention in the stomach at 60 and 90 min were 49.9% (38.4%-70%) and 40.5% (29.7%-60%) respectively. After taking metoclopramide, the median value of half-time was 67 (15-115) min and percentages of meal retention at 60 and 90 min were 51% (34.5%-93.9%) and 42% (24%-71.2%) respectively. When compared with baseline values, a significant difference in gastric emptying parameters was found after both erythromycin and metoclopramide treatment. A significant improvement of the total score for gastrointestinal symptoms was observed with both drugs, but this improvement was more pronounced with erythromycin. The data suggest that erythromycin, a motilin receptor agonist appears to stimulate intestinal motility and seems to be an alternative agent for the treatment of gastroparesis caused by diabetic autonomic neuropathy[10].

Gallbladder motor function is impaired in some patients with diabetes. It has been suggested that the abnormalities of gallbladder motility are confined to those patients with autonomic neuropathy. Erythromycin, a motilin receptor agonist causes gallbladder contraction in both normal subjects and patients with gallstones and impaired gallbladder emptying. The effect of erythromycin on gallbladder motility in 7 diabetic patients with autonomic neuropathy, 6 diabetic patients without autonomic neuropathy, and 17 normal subjects was studied using ultrasound. There was no significant difference in the gallbladder fasting volume between the three groups, but the diabetic patients with autonomic neuropathy had impaired postprandial gallbladder emptying compared with normal subjects (percentage emptied $\bar{x} \pm s$, 40% ± 10.3% *vs* 64% ± 2.8%, $P < 0.01$) and the patients with autonomic neuropathy (48% ± 7.7%) (NS). Erythromycin produced a dramatic reduction in gallbladder fasting volume in diabetic patients with autonomic neuropathy, compared with either normal subjects or diabetic patients without autonomic neuropathy (percentage reduction of 62% ± 4.6% in patients with autonomic neuropathy *vs* 37% ± 17.6% in those without autonomic neuropathy, and 26% ± 7.3% in the normal subjects; $P < 0.02$), and returned gallbladder emptying to normal in all patients with impaired emptying. The pronounced effect of erythromycin in diabetic autonomic neuropathy suggests denervation supersensitivity and that the action of erythromycin on the gallbladder is neurally modulated[11].

Gastroesophageal reflux disease

Erythromycin and some of its analogues stimulate gastrointestinal smooth muscle contractions. Because gastroesophageal reflux disease (GERD) in humans is in part caused by a reduction in lower esophageal sphincter (LES) pressure, one study aimed to investigate the effect of LY267108 (an erythromycin-A analogue with no significant antimicrobial activity) on LES function. In ketamine-anesthetized cats, LES pressure was recorded using a Dent sleeve. In cats, LY267108 increased LES pressure, as did motilin and erythromycin-A. Neither LY267108, erythromycin-A, nor motilin altered LES relaxation in response to a swallow. LY267108 increased LES pressure in cats in which the basal LES pressure was lowered experimentally by perfusing the distal esophagus with HCl (0.1 N for 3 d) or following isoproterenol (3.0 mg/kg *i.v.*). In summary, LY267108 increases LES pressure not only in normal cats, but also in animals with an experimentally-induced decrease in LES pressure, but it does not affect the relaxation of LES in response to a swallow. The results suggest that LY267108 may be useful in treating GERD because of its ability to increase LES pressure and thus present a barrier for gastroesophageal reflux[12].

Motilin induces phase Ⅲ activity of the gastrointestinal tract. Erythromycin has a motilin-like effect on the stomach and significantly increases the LES pressure in normal volunteers. This investigation was performed to evaluate the effects of erythromycin on esophageal function in patients with GERD. Esophageal manometry was performed

in 10 GERD patients before and after intravenous infusion of 500 mg of erythromycin; values were expressed as x- ± s. LES pressure increased from 1848.7 ± 385.7 Pa (13.9 ± 2.9 mmHg) at baseline to 3843.7 ± 478.8 Pa (28.9 ± 3.6 mmHg) after infusion of erythromycin ($P < 0.01$). The duration of contractions in the proximal, middle, and distal esophagus was significantly prolonged from 3.5 ± 0.4 s, 3.8 ± 0.4 s and 4.1 ± 0.5 s to 4.2 ± 0.2 s, 4.6 ± 0.5 s and 5.6 ± 0.6 s respectively after infusion of erythromycin ($P < 0.05$ for each comparison). Erythromycin did not affect esophageal body contraction amplitude or velocity, or the upper esophageal sphincter. Serum motilin decreased slightly after the administration of erythromycin.

The authors concluded the following[11]. Erythromycin profoundly stimulates the defective LES in patients with GERD; this appears to be a direct motilin agonist-like effect rather than being mediated by release of endogenous motilin. Erythromycin has less effect on the esophageal body, although it does prolong the duration of esophageal contractions.

ADVERSE EFFECTS OF ERYTHROMYCIN

It is long known that erythromycin may cause unpleasant gastro-intestinal side-effects, such as nausea and vomiting. Recent studies, however, show that at low dosage erythromycin may have a beneficial effect. Erythromycin induces the migrating motor complex in the fasted state and after a meal it accelerates gastric emptying. Although largely preliminary, studies in pathological conditions are being conducted on its effects on esophageal, small intestinal, colonic and biliary tract motility.

Erythromycin is certainly a powerful gastrokinetic agent. Its antibiotic properties are a disadvantage, but more powerful derivatives devoid of antibacterial properties may soon become available, and they form a new family of prokinetics[8].

Erythromycin is generally well tolerated at the recommended dose of 400-600 mg daily. The renewed interest in macrolide antibacterials with expanded indications for clinical use, as well as their markedly increased usage, justifies the continuous search for new compounds designed to offer the patient not only enhanced bioavailability but also a reduced incidence of adverse effects. Macrolides are an old and well-established class of antimicrobial agents that account for 10% to 15% of the worldwide oral antibiotic market. Macrolides are considered to be one of the safest anti-infective groups in clinical use, severe adverse reactions being rare. Newer products with improved features have recently been discovered and developed, maintaining or significantly expanding the role of macrolides in the management of infection. This review deals with the tolerability of the clinically available macrolide antibacterials. With the exception of drug interactions, adverse effects have been analyzed during the last 40 years in many thousands of adult and pediatric patients. Recently developed derivatives have been compared with the older compounds, and the expected and well-assessed adverse effects have been set apart from those which are unusual, very rare or questionable.

Gastrointestinal reactions represent the most frequent disturbance, occurring in 15% to 20% of patients on erythromycins and in 5% or fewer patients treated with some recently developed macrolide derivatives that seldom or never induce endogenous release of motilin, such as roxithromycin, clarithromycin, dirithromycin, azithromycin and rikamycin (rokitamycin). Except for trioleandomycin and some erythromycins administered at large dosage and for long periods of time, the hepatotoxic potential of macrolides, which rarely or never form nitrosoalkanes, is low for josamycin, medecamycin, miocamycin, flurithromycin, clarithromycin and roxithromycin; it is negligible or absent for spiramycin, rikamycin, dirithromycin and azithromycin. Transient deafness and allergic reactions to macrolide antibacterials are extremely unusual effects and have definitely been shown to be more common following treatment with the erythromycins than with the recently developed 14-, 15- and 16-membered macrolides.

There have been case reports in the literature of 51 patients during the last 30 years who experienced uncommon or dubious adverse effects after treatment with older compounds and in whom there appeared to be strong evidence of a causal relationship with the drug. Only 3 cases had an unfavorable outcome, and these were patients administered erythromycin lactobionate intravenously at either too rapid a rate or at too large a dose. Targets of these occasional reactions are generally the heart, liver and central nervous system. Other unusual organ pathologies are related more to immunomediated disorders than to primary parenchymal toxicity, or to the rarely serious consequences of macrolide-induced alterations in intestinal microflora[13].

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RIVIEW

Fat-storing cells and liver fibrosis

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INTRODUCTION

In normal liver tissue, fat-storing cells (FSCs, also known as Ito cells) lie between endothelial cells and hepatocytes in the space of Disse. One of the most characteristic features of these cells is the presence of cytoplasmic lipid droplets. Desmin has been the best specific and reliable marker for detection of FSCs up to now. Recently, however, more attention has been paid to the relationships between FSCs and liver fibrosis[1,2].

A prominent role of FSCs in human and experimental fibrogenesis has long been suspected based on the identification of transitional cells with regions of active liver injury that have features of both FSCs and fibroblasts. Transitional cells have also been termed myofibroblasts, the latter defined morphologically by the presence of prominent microfilaments, ruffled nuclear membrane, and contractile activity. Liver transitional cells/myofibroblasts additionally have hypertrophied rough endoplasmic reticulum and are associated with increased pericellular collagen. They are proliferative *in situ* and retain expression of desmin. The theory of FSC origin of myofibroblasts was supported by the expression of α-smooth muscle actin (α-SMA) upon activation both in culture and *in vivo*; α-SMA is typical of myofibroblasts[2].

In summary, FSCs activation can be defined as the acquisition of a myofibroblastic phenotype with enhanced fibrogenic and proliferative activity.

BIOLOGICAL FUNCTIONS OF FSCS

Extracellular matrix (ECM) synthesis

A combination of immunoelectron microscopy, *in situ* hybridization and Northern blot analysis of purified liver cell populations has shown that FSCs are major sources of matrix components in both normal and fibrotic livers[3]. When set up in primary culture, FSCs remain quiescent for 4-5 d, then undergo dramatic phenotypic changes towards a myofibroblastic appearance. They proliferate and express both basement membrane and interstitial matrix proteins at high levels. The results from immunoelectron microscopy also suggest that FSCs can synthesize ECM components, such as collagens, fibronectin, laminin, undulin, tenascin, perlecan, *etc.* Sinusoidal cells contain gene transcripts for α1 (I), α2 (I), α1 (Ⅲ) and α1 (Ⅳ) procollagens and laminin B1 chain. Unfortunately, the current *in situ* hybridized technique can not provide sufficient resolution to establish firmly which sinusoidal cell populations contain the signals[4]. However, non-parachymal cells containing these transcripts in experimental models of fibrosis have morphological and phenotypic features of FSCs. Undulin, found in recent years, is a large ECM glycoprotein that is associated with dense interstitial connective tissue. With double-staining for cell type-specific antigens, Milani *et al*[5] showed undulin expression in mesenchymal cells, which were identified by immunostaining for vimentin. A number of cells expressing a small amount of undulin RNA located on the fibrotic septa and cirrhotic nodules also stained for α-SMA. Several investigators[6]have used Northern blot hybridization to demonstrate ECM mRNAs in freshly isolated cell fractions to avoid the error caused by phenotypic changes of FSCs. Geerts have shown that isolated FSCs contain strong transcripts for α1 (Ⅲ) procollagen and type Ⅳ collagen. Maher *et al* demonstrated that FSCs isolated from animals with experimental liver injury showed a dramatic increase in procollagen [WTBZ] α1 (Ⅲ) and α1 (I) transcripts. Although other sinusoidal cells and hepatocytes could produce some ECM components, the FSCs are quantitatively more important.

Collagenases and collagenase inhibitors synthesis

The collagenases, which are matrix metalloproteinases, have been grouped into interstitial collagenase, type [WTBZ] Ⅳ collagenase, and stromelysin. In the liver, FSCs are the major source of interstitial collagenase that degrades type Ⅰ and Ⅲ collagens. There are two types of Ⅳ collagenase, one produced by fibroblasts (72000) and the other derived from macrophages (92000). The activated FSCs secrete the 72000 type Ⅳ collagenase to degrade basement membrane collagen and denatured collagen (gelatin)[7]. Metalloproteinase activity is regulated in part by the tissue inhibitors of metalloproteinases (TIMPs), TIMP-1 and TIMP-2, and by general proteinase scavengers such as α-2 macroglobulin. FSCs in culture synthesize TIMP-1 and express α-2 macroglobulin, which by inhibit-

ing collagenase activity and hence collagen degeneration may result in increased collagen accumulation. Release of metalloproteinase inhibitors by activated FSCs may contribute to progressive fibrosis by preventing degradation of interstitial collagen[2,7].

Cytokines synthesis

Transforming growth factor β (TGFβ) is the most important cytokine in liver fibrosis. FSCs express TGFβ1 mRNA in the CCl4-induced rat model of liver fibrosis. FSC clones (CFSC) derived from CCl4-induced rat liver tissue are heterogeneous with regard to the expression of cytokines and various ECM components. While clone CSFC-2G expresses low basal levels of α1 (Ⅰ) procollagen, TGFβ and IL-6 mRNAs, clone CFSC-5H expresses high levels of these mRNAs. Inagaki *et al*[6] suggested that CFSC-5H cells have been already activated and express type Ⅰ collagen due to an autocrine stimulation with TGFβ. However, although CFSC-2G cells are activated and probably contain the required nuclear transcriptional factors, they need paracrine stimulation with TGFβ to produce type Ⅰ collagen. FSCs are believed to be the major source of circulating insulin-like growth factor-1 (IGF-1) and FSCs express IGF binding proteins and their mRNAs[8]. Cultured human FSCs can express the genes encoding for the platelet-derived growth factor (PDGF) A and B chains. The gene expression of these two PDGF chains is associated with the secretion of bioactive PDGF. Moreover, FSCs are able to synthesize and release platelet-activating factor (PAF) and its 10-acyl analogue, a fluid-phase and cell-associated mediator of inflammation having potent effects on hepatic circulation and metabolism[9]. FSCs also express a 47 kD collagen-binding heat shock protein, which closely correlates with the transition of FSCs and development of hepatic fibrosis. Some investigators have demonstrated that FSCs can release other soluble factors, such as macrophage-colony stimulating factor and monocyte chemotactic peptide 1, heparin binding growth factor-2, *etc.* Several cytokines, including TGFβ, PDGF, IL-1 and IGF-1, are mitogenic for FSCs but the most important profibrogenic factor appears to be TGFβ[2]. TGFβ1 induces the synthesis of fibronectin, laminin, collagens, proteoglycans and TIMPs. In addition, TGFβ1 down-regulates the expression of stromelysin and interstitial collagenase. The key function of TGFβ1 is to activate phenotypic changes that occur in FSC primary cultures, from a 'resting' state toward a myofibroblastic-like state. Interestingly, TGFβ has been shown to increase the steady-state levels of its own mRNA in FSCs, which forms positive feedback effects[11]. DNA synthesis and proliferation of FSCs is stimulated by PDGF, EGF, TGFβ, basic FGF, IL-1 and TNFα. PDGF may play a critical role during the activation of FSCs. Both stimulatory and inhibitory effects of TNFα on collagen synthesis in FSCs have been described. More interestingly, however, TGFβ has been shown to strongly inhibit EGF or TGFβ-induced proliferation of FSCs. The interferons (IFNs), which are potent inhibitors of FSC activation and proliferation. Therefore, the functional imbalance of up- and down-regulated cytokines on FSCs may affect fibrogenesis.

Other functions

Integrins are the main receptors of ECM. Integrin β1 chain expression is found in sinusoidal cells in liver tissue under conditions of alcoholic hepatitis and cirrhosis. Unfortunately, concrete cell types can not be identified[10]. The FSCs also have functions in storing and metabolizing vitamin A under normal and fibrotic conditions.

MEDIATION OF HEPATIC FIBROSIS

Interferons

IFNs are potent antifibrotic agents both *in vivo* and *in vitro*. Jimenez *et al* showed that both α- and γ-IFNs caused a dose-dependent inhibition of collagen synthesis in quiescent human fibroblasts. In experimentally-induced fibrotic livers, γ-IFN treatment of Schistosoma-infected mice was found to affect collagen accumulation and to decrease the steady state procollagen α1 (Ⅰ) and α1 (Ⅲ) mRNA levels[11]. Capra *et al*[12] demonstrated that in some patients with chronic viral hepatitis and cirrhosis, who had been treated with recombinant γ-IFN 2a for 6 mo at least, aminotransferase activity, serum procollagen Ⅲ peptide and laminin levels had significantly decreased, while the scores for inflammation and necrosis were significantly lower than those in the control group. The data suggest that γ-IFN treatment may decrease stimuli for fibrogenesis by reducing liver inflammation and necrosis, thus preventing progression to cirrhosis. Substantial data have been reported showing that α- and γ-IFNs are potent inhibitors of FSCs activation *in vitro*. These factors have marked effects on FSC proliferation and significantly reduce the steady state levels of mRNAs for α-SMA, collagens and fibronectin. In addition, IFNs can modulate other cytokines so as to affect the FSC activities indirectly.

Vitamin A

Retinyl palmitate is the predominant storage form of vitamin A in FSCs. It is possible to infer that a decrease in retinyl palmitate *per se* may be a facilitating factor in hepatic fibrosis by lipocytes. Senoo *et al* have demonstrated that the administration of vitamin A inhibits CCl4-induced fibrosis, and another study has shown that vitamin A treatment inhibits the rate of collagen synthesis by FSCs[13]. There are some conflicting data suggesting that chronic hypervitaminosis A results in hepatic cirrhosis. Up to now, the majority of authors have suggested vitamin A can inhibit proliferation and transition of FSCs in fibrosis. We believe that the dose of vitamin A is the key to determining up- or down-regulation on FSCs.

Polyunsaturated lecithin (PUL)

Lieber *et al*[14] have shown that oral supplementation of the diet with a purified soybean PUL extract can prevent the development of septal fibrosis and cirrhosis in baboons fed ethanol. Their previous study, which involved withdrawal of the PUL from the ethanol-diet in three baboons, showed progression of perivenular and/or interstitial fibrosis to septal fibrosis and then to cirrhosis in all three baboons after 18-21 mo. The mechanism for the remarkable effect of PUL in preventing liver fibrosis remains unknown. Regarding the effects of PUL on collagen synthesis, it was noted that the percentage of transformation of lipocytes to transitional cells was decreased by PUL in the livers of the alcoholic-fed baboons. In cultured rat FSCs, PUL can increase collagenase activity of the cells. PUL has also been shown to have excellent bioavailability in patients with no known side effects[7].

Chinese herbal medicines

We have investigated the therapeutic action of *Cordyceps sinensis* (CS) on CCl4-induced hepatic fibrosis in Wistar rats. Serum procollagen levels in rats treated with CS were much lower than those in the control group ($P < 0.01$). In liver tissue, degeneration and necrosis of hepatocytes, fibrosis and deposition of type Ⅰ, Ⅲ and Ⅳ collagens in the Disse space were all less than observed in the controls. Moreover, the number of desmin-positive cells, after treatment for 12 wk, was fewer than that in the control group ($P < 0.01$). Our ultrastructural data showed that CS could inhibit proliferation and transition of FSCs to myofibroblasts. It is suggested that CS can effectively prevent and cure experimental hepatic fibrosis, and that the mechanism may involve inhibition of FSCs by CS[15]. Sun *et al*[16] have demonstrated that tetrandrine, a purified extract from the Chinese herb Stephania tetradra S. Moore, can prevent and treat rat hepatic fibrosis, *via* the possible mechanism of FSC inhibition. Their later clinical study provided the same results. Our investigation on FSC primary culture has confirmed that tetrandrine exerts an inhibitory effect on proliferation and transition of FSCs (data not shown). Some other Chinese herbs have been identified and studied in experimental animals and patients, such as *Salvia miltiorrhiza*, peach kernel, *etc.*, but the mechanisms need to be studied.

Others

Prednisone is a widely utilized inhibitor of TNF production, and colchicine is used frequently in various liver diseases. These agents can decrease TNF receptor density and protect against TNF toxicity in mice *in vivo*. Multiple agents, such as antibodies against IL-1, IL-1 receptor antagonists, and TNF, have been used to affect the activities of FSCs indirectly.

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CASE REPORTS

Light and electron microscopic observation of malignant gastric leiomyoblastoma

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CASE REPORT

A 57-year-old male worker suffered from recurrent dull pain over the upper abdomen for 2 years and presented with worsening over the recent past 3 mo. The pain was described as persistent, irregular and without radiation but accompanied by loss of appetite, remarkable emaciation, anemia and weight loss of 10 kg. The patient was admitted on February 29, 1988.

The findings included: P.E., no positive findings; red blood cell count, 1.5 × 10^{12}/L; Hb, 40 g/L; stool occult blood, positive. X-ray examination showed a filling defect of about 6 cm in diameter with a 2 cm niche over the lesser curvature below the cardia. A 0.5 cm niche was also found over the pyloric canal. The X-ray diagnosis was protuberant lesion of the upper gastric body on the lesser curvature. Gastroscopy showed a 3 cm ulcerative lesion with projected margin near the cardia. Biopsy diagnosis was gastric ulcer, but a second gastroscopic biopsy diagnosis was gastric leiomyobastoma.

The patient underwent an exploratory operation under general anesthesia on March 3, 1988. Proximal gastrectomy and gastroesophagostomy were carried out smoothly with uneventful recovery. The pathological findings included a mass of 8 cm × 7 cm × 6 cm in size, with an ulcer about 4 cm in diameter (Figure 1). The tumor was covered with gastric mucosa and showed partial necrosis, ectoplasm and round or oval nuclei, around which there was sharp transparent halo (Figure 2) interposed with a small amount of collagen fibers. There were 5 karyokinetic phases observed per high power field. Masson staining revealed muscle cells and myoblastic cells as red and the interstitial cells as green. The plasma was negative by PAS staining. Two of the three lymph nodes from the right side of the cardia were found to be occupied by metastasis of the leiomyoblastic cells.

Electron microscopy showed that the nuclei were spindle-shaped, with both clear and dark cells in apposition. Many pinosomes were observed on the surface of the membrane of the clear cells (Figure 3). The dark cells were shown to penetrate into the clear cells, which were abundant in the rough endoplasmic reticulum with a remarkable amount of myofilaments and dense patches within the dark cells (Figure 4) and abundant organelles within the clear cells. Golgi complex and rough endoplasmic reticulum were well developed with cystic extension (Figure 5).

DISCUSSION

Leiomyoblastoma is also termed Bizarre leiomyoma. In 1962, Stout[1] reported 69 cases of Bizarre leiomyoma and first termed this condition as leiomyoblastoma or Bizarre leiomyoma, which has been used universally since then. The gastrointestinal tract and esophagus are especially prone to developing leiomyoblastomas. Some other organs and tissues, such as uterus, vulva, retroperitoneum, mediastinum, lungs, urinary bladder, prostate and subcutaneous tissue, can also develop this disease. In the stomach, the tumor is often found over the antrum and the body, and the cardia. The present case was located near the cardia. One-hundred-and-fifty cases reported by Salajar[2] had a mean age of 56.6 years. Tumor size ranged from 0.5 cm to 20 cm in diameter and the heaviest one weighed 3500 g. In general, the tumor is single, with only 6.8% presenting as multiple.

Preoperative diagnosis

Since the preoperative diagnosis is not easy, most cases are diagnosed pathologically after operation. The biopsy could be taken directly from the tumor since the ulceration of the mucosal surface usually exposes the tumor. Recently, Paparzin[3] reported the first case of leiomyoblastoma in the lung, which was found by bronchoscopy. Our case was grossly diagnosed as ulcerative carcinoma by gastroscopy. The first biopsy failed to reach the tumor and was diagnosed as an ulcer, but the second time the specimen was accurately taken and accurate diagnosis of leiomyoblastoma of the stomach was made pathologically. This is the first case of leiomyoblastoma diagnosed by gastroscopic biopsy in our country. It is clear that it is

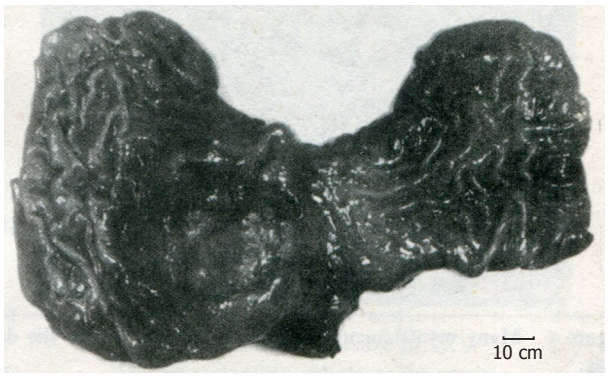

Figure 1 A stomach mass located at the cardia on the lesser curvature, having 8 cm × 7 cm in volume, projecting into the gastric lumen with a 4 cm ulcer.

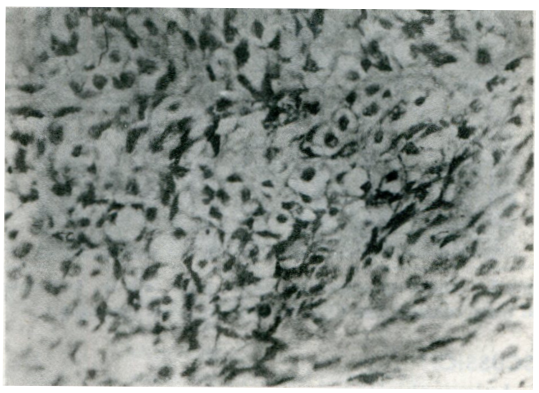

Figure 2 Round and polygon-shaped tumor cells interposed with small amount of collagen fibers, with round central nuclei and clear perinuclear zones.

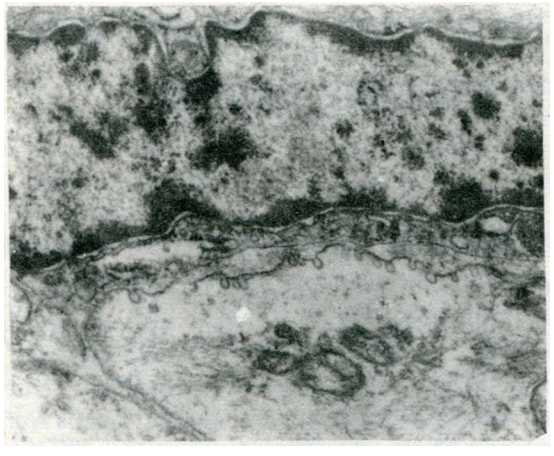

Figure 3 Both clear and dark cells with spindle-shaped nuclei are in apposition, with many pinosomes on the membrane of the clear cells.

possible to diagnose the disease accurately through multiple biopsies before operation.

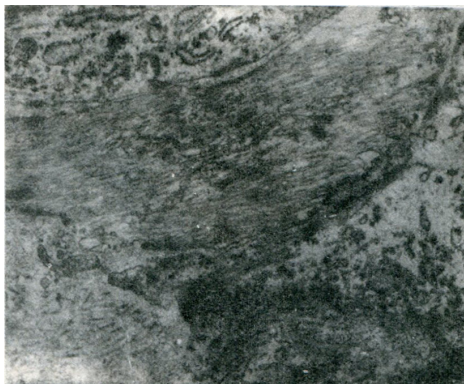

Figure 4 Many myofilaments and dense patches within the dark cells.

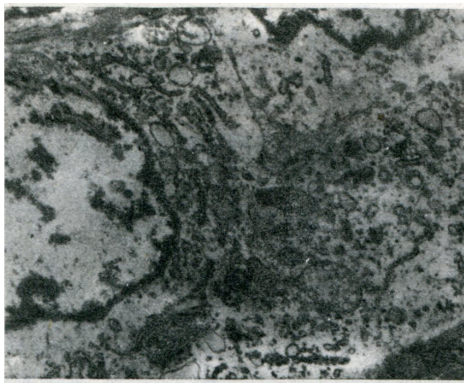

Figure 5 Abundant organelles within clear cells, *i.e.* well developed Golgi complex and rough endoplasmic reticulum followed by cystic extension.

Evaluation of malignancy

Three criteria, including course of the disease over 6 mo, diameter of the tumor larger than 10 cm, and the karyokinetic phase over 10 per high power field, suggests unfavorable prognosis. In the present case, the tumor had 2 years' duration and the size was 8 cm in diameter with 5 karyokinetic phases per high power field and metastasis to 2 lymph nodes. According to the above-mentioned criteria, this patient's prognosis might not be too bad. But according to other reports, a tumor with 5 karyokinetic phases per high power field and a size of 6 cm in diameter should be considered as malignant. Thus, our case's prognosis is certainly not favorable.

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COMMENTARY

Optimal treatment of peptic ulcer: Perspectives beyond the year 2000

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is the most common chronic disease afflicting humans. It is estimated that around 50% of the world's population suffers from this infection. The prevalence of *H. pylori* infection is low in developed countries (20%-40% of healthy adults), but it is much higher in developing countries. In China, epidemiological studies have shown that the prevalence of *H. pylori* infection varies from 50% to 90% in different cities and villages[1]. The prevalence is higher in aged individuals, and in those with low socioeconomic status and living under poor sanitation and crowded living conditions. Many studies have shown that *H. pylori* is closely related with gastritis, peptic ulcers, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. It has also been suggested that *H. pylori* may be a causative agent of Ménétrier's disease.

Several years ago, the principal treatment of patients with duodenal and gastric ulcer included the use of various H_2-receptor blockers and proton pump inhibitors. Although these agents are able to heal a high percentage of ulcers, the recurrence rate is very high after cessation of treatment[2]. Since the discovery of *H. pylori* by Warren and Marshall in 1982, innumerable basic and clinical studies have been carried out over the past 14 years. Many of these studies have indicated that *H. pylori* plays an important etiological role in the pathogenesis of peptic ulcer. Now, nearly all cases of duodenal ulcer and the majority of cases of gastric ulcer can be regarded as infectious diseases and the strategy for treatment of peptic ulcer has completely changed.

It is believed that the proverb "no acid, no ulcer" will still be valid beyond the year 2000, but it should be modified to: "no *H. pylori* and no acid, no ulcer". Peptic ulcer has a recurrent course. If a patient received no treatment after the ulcer was healed, duodenal ulcer recurrence rate was reportedly 72% within 12 mo, but dropped to 25% following maintenance therapy with H_2-receptor antagonist. It was a very exciting finding that showed the duodenal ulcer recurrence rate was only 2%-3% when *H. pylori* was eradicated, and that this rate was maintained for at least 7 years after treatment with a low incidence of reinfection. It was thus concluded that peptic ulcer can be cured if the associated *H. pylori* is eradicated. The follow-up study of patients with bleeding peptic ulcer showed that the relapse rate of bleeding was 33% in those with persistent *H. pylori*, and 0% in those with eradicated *H. pylori*.

In February 1994[3], the National Institute of Health in the United States organized a consensus development conference on *H. pylori* in peptic ulcer disease. This effort brought together specialists in gastroenterology, surgery, infectious diseases, epidemiology and pathology, as well as members of the public, to discuss many questions related to *H. pylori* and peptic ulcer disease. Finally the consensus panel concluded that ulcer patients with *H. pylori* infection require treatment with antimicrobial agents in addition to antisecretory drugs, whether the administration begins upon first presentation of the illness or upon recurrence. It is doubtless that nearly all patients with peptic ulcer, except for those suffering from Zollinger-Ellison syndrome and NSAID-induced peptic ulcer, should be treated with antimicrobial agents and acid suppressants, such as H_2-receptor blocker or proton pump inhibitor.

How to eradicate *H. pylori* has emerged as a hot topic that is under investigation all over the world. In 1990[4] the Working Party of the 9th World Congress of Gastroenterology in Sydney recommended a triple therapy [colloidal bismuth subcitrate (CBS) at 120 mg q.i.d., tetracycline hydrochloride at 500 mg q.i.d. or amoxicillin at 500 mg q.i.d., and metronidazole at 400 mg t.i.d. given for 2 wk] as the standard therapy for *H. pylori* infection, giving an eradication rate of 80%-90%. However, many patients are intolerant to this treatment because of its related side effects and poor compliance. In addition, development of metronidazole-resistance is an important factor influencing the efficacy of the standard triple therapy.

Many other regimens have been reported in the literature for eradication of *H. pylori*, and these may be categorized into two main groups: bismuth-based therapies and proton pump inhibitor-based therapies. In China, both are used for eradication of *H. pylori*. We would like to mention that furazolidone is an anti-ulcer agent that has been in routine use in China for more than 20 years[5]. In the early 1970's, many doctors in Shanghai, Beijing and other cities prescribed furazolidone for refractory peptic ulcer and achieved consistently good results. In 1978, healing of ulcer by treatment

Figure 1 Professor Shu-Dong Xiao.

with furazolidone was confirmed with evidence. Then, in 1982 and 1983, open controlled studies and double-blind controlled studies were carried out in China. The ulcer healing rates were 73%-75% or 80%-83% after 2 wk or 4 wk of treatment with furazolidone. At that time nobody knew why furazolidone was effective for peptic ulcer, and particularly for the refractory peptic ulcer, and only a few patients relapsed after the ulcer was healed (Figure 1).

In 1989, we used furazolidone monotherapy (furazolidone at 100 mg t.i.d. for 21 d) to treat *H. pylori*-associated dyspepsia[6] and the eradication rate was 55.5%. The minimal inhibitory concentration (MIC) of furazolidone for *H. pylori* was found to be < 0.2 mg/L. Furazolidone is not expensive and is also very effective for eradication of *H. pylori* and ulcer healing. Serious side effects, such as skin rashes and polyneuritis, may occur occasionally if furazolidone is used in high doses and for long-term. However, if we use furazolidone at a low dose and for several days, development of serious side effects is uncommon. It is important to remember, though, that furazolidone is contraindicated in patients with glucose-6-phosphate dehydrogenase deficiency.

In 1994, a multi-center collaborative study was carried out in Shanghai. We used dual and triple therapies with furazolidone and the bismuth compound to treat *H. pylori*-associated dyspepsia and peptic ulcer[7]. The dual therapy consisted of CBS at 120 mg and furazolidone at 100 mg q.i.d. for 14 d, and the triple therapy consisted of CBS at 120 mg, furazolidone at 100 mg, and metronidazole at 200 mg q.i.d. for 10 d. The results showed that with the dual therapy, the healing rates of duodenal ulcer and gastric ulcer were 80% and 77.7%, respectively. With the triple therapy, the healing rates of duodenal ulcer and gastric ulcer were 86.1% and 77.3%, respectively. There was no significant difference in the healing rates of duodenal ulcer or gastric ulcer achieved with either the dual therapy or the triple therapy. With the dual therapy, the overall eradication rate of *H. pylori* was 73%, and with the triple therapy, the overall eradication rate of *H. pylori* was 77.8%. The difference was not statistically significant between the two groups, but there were milder side effects in the patients who received the triple therapy.

Now many studies have shown that some regimens are able to achieve a high eradication rate of *H. pylori*. Sung *et al* reported that a 1-wk regimen of the triple therapy (bismuth subcitrate, tetracycline and metronidazole) produced a successful eradication of *H. pylori* in 95% of patients. In a prospective randomized trial, *H. pylori*-associated duodenal ulcer patients were treated with either the triple therapy alone or in further combination with omeprazole. Four weeks after cessation of the treatment, duodenal ulcers were healed in 92% of patients who received the triple therapy alone and 95% of patients who received the triple therapy plus omeprazole. Bazzoli reported on a 1-wk treatment regimen composed of omeprazole at 20 mg once daily or b.i.d., clarithromycin at 250 mg b.i.d., and tinidazole at 500 mg b.i.d. or metronidazole at 400 mg b.i.d., and showed that this regimen eradicated *H. pylori* infection in > 90% of treated patients. Unpublished data from our group have demonstrated that a 1-wk triple therapy (CBS at 240 mg b.i.d., or lansoprazole at 30 mg q.d., clarithromycin at 250 mg b.i.d., and furazolidone at 100 mg b.i.d.) achieved an eradication rate of *H. pylori* of > 90%. In addition, our clinical trial has also demonstrated that a bismuth-based dual therapy (CBS at 240 mg b.i.d. plus josamycin at 1000 mg b.i.d.) achieved a 69.9% eradication rate of *H. pylori*. Josamycin and clarithromycin are macrolide antibiotics. Similar to clarithromycin, josamycin possesses high activity against *H. pylori*, even in an acidic environment. Our study showed that when *H. pylori* strains developed resistance to clarithromycin or josamycin, they might not be eradicated with clarithromycin or josamycin-based dual or triple therapy.

H. pylori is relatively easy to suppress, but much more difficult to eradicate. New regimens for eradication of *H. pylori* are still under investigation. An ideal *H. pylori* eradication therapy has not been found yet. Hopefully, the use of low-dose, short-term triple therapy (consisting of one antisecretory agent and two antibiotics) with high efficacy (90% eradication rate), low incidence of side effects and convenient administration (twice daily for only 7 d) will be acceptable as an optimal treatment for peptic ulcer associated with *H. pylori* infection. We are confident that optimal treatment of peptic ulcer will be found beyond the year 2000, and we predict that peptic ulcer might not run a chronic and recurrent course any more. Peptic ulcer is a disease that can be cured if the associated *H. pylori* is eradicated.

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COMMENTARY

Update on the recent progress in chronic hepatitis B therapy in China

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INTRODUCTION

Chronic hepatitis B is prevalent in China and certain South-Eastern Asian countries, and great efforts have been made to search for an effective therapeutic regimen. More experimental and clinical studies have emerged recently, providing insightful and useful results based on Chinese populations; the most important of these studies are presented here.

Chronic hepatitis B is primarily caused by interaction of the replication of the hepatitis B virus (HBV), the impaired immuno-modulating function of the host, the release of host-derived cytokines, inflammatory mediators, free radicals, and the microcirculatory impairment of the host-target organ, the liver. The end results are hepatic inflammation, degeneration and fibrosis, and necrosis of hepatocytes. In treatment, elimination of serum HBV DNA is of primary importance. In a study of 1016 serum specimens from patients with chronic hepatitis B and a few cases of hepatic cirrhosis, serum HBV DNA was detected by PCR in all kinds of combinations of HBV markers, among which positive serum HBV DNA was most common in the samples showing positivity for hepatitis B surface antigen (HBsAg), anti-HBs and hepatitis B e antigen (HBeAg) (86%, 259/301) and was not infrequent in the samples showing positivity for HBsAg, anti-HB core protein (c), anti-HBe (13.2%, 421/311), although the latter still indicated the presence of viral replication. Anti-HBs was positive in one case and negative in one case, with all other five classified as HBVM, but still having positivity for serum HBV DNA. The cases of recrudescence of obscure origin are most probably due to reactivation of viral replication.

ELIMINATION OF SERUM HBV DNA AND HBeAg

Besides interferon-α, there are several Chinese herbal polysaccharides that are effective in eliminating serum HBV DNA and HBeAg. These include Cordeceps, Grifola umbellata and Mocre, and their elimination rates are reported as 60%-68% and 53%-68% for HBV DNA and HBeAg respectively. The related adverse effects are insignificant.

Cordeceps sinensis polysaccharide coated with liposome[1]

This herbal polysaccharide was administered at a dosage of 400 mg b.i.d. orally for 3 mo. After one course of treatment, CD_4 increased, CD_8 decreased, CD_4/CD_8 ratio increased, and natural killer cell (NK) activity was enhanced ($P < 0.001$). Rates of negative seroconversion were as follows: HBV DNA: 68.4%; HBeAg: 53.2%; Pre-S_2: 85%; HBsAg: 19.2%. Anti-HBe became positive in 20%. Follow-up for 1 year showed that the rate of negative seroconversion of HBV DNA increased to 73.6%, and that of HBeAg to 66.6%. Serum albumin and prothrombin times reverted to normal, gamma-glutamyltransferase (GGT) levels improved, and bilirubin and alanine aminotransferase (ALT) levels returned to normal or reduced in 73% and 90%, respectively. The mechanisms of action are many. First, Cordeceps has an immuno-modulating effect, as shown by changes in T-cell subsets and enhanced NK activity, and liposome itself has an immune-enhancing adjunct effect. Second, liposome is hepatophilic and can serve as a carrier for targeting and can be phagocytized by monocytes/macrophages. Third, the herbal polysaccharide is released intermittently with prolonged effect and produces no observable immunogenic effect or toxicity. In comparison, Cordeceps coated with liposome is more effective than Cordeceps without the liposome coating.

Grifola umbellata polysaccharide combined with hepatitis B vaccine[2]

A randomized controlled study was conducted with groups of 100 patients of similar age, sex, course of illness and hepatic functional status. All HBV-infected participants showed positivity for serum HBV DNA, HBeAg and anti-HBc-IgG. Liver biopsy was performed in 28 patients of the treated group, and 10 of those patients underwent a second biopsy; only 18 individuals in the control group underwent liver biopsy. Grifola umbellata polysaccharide was given at 40 mg im daily for 20 d, then discontinued for 10 d and repeated again over a total period of 3 mo. Concomitantly, hepatitis B vaccine was given at a dosage of 30 μg sconce every 2 wk, also for 3 mo. The rates of negative seroconversion of HBV DNA and HBeAg were 67.5% and 60%, (*vs* control group, $P < 0.005$ and 0.01 respectively), the rate of positive seroconversion of anti-HBe was 10% (*vs* 3% in control group, $P < 0.05$). Ten of the 28 patients in the treated group

Figure 1

showed varied improvement in hepatic pathology. Normalization of serum ALT was found in 95.9%. Follow-up was made for an average of 9 mo (ranging from 6 mo to 6 years). The rate of negative seroconversion of HBV DNA remained at 56% and that of HBeAg increased further to 66%. Transient low grade fever occurred in 5 cases, minimal axillary lymph node enlargement occurred in 15 cases, and slight skin rash occurred in 4 cases with no need to discontinue the treatment. Another study showed that better effect was achieved with a combined treatment than with Grifola umbellata alone, the reason for this difference remains unknown at present (Figure 1).

Mocrea

Two capsules of Mocrea were given twice daily for 3 mo, and 5 out of total 38 patients received 3 capsules twice daily beginning at the third month, in a randomized control study. After completion of the treatment course, rates of negative seroconversion of HBV DNA and HBeAg were both 63.2%, with those of HBsAg and anti-HBc at 18.2% and 10.5% respectively; the rates of positive seroconversion of HBV DNA and HBeAg remained at 57%, that of HBsAg elevated to 25.7%, and those of anti-HBe and anti-HBs increased to 45.7% and 8.6% (*vs* control group, $P < 0.01$)[3]. In addition, after completion of the treatment course, patients' appetite increased, weakness and hepatic pain disappeared, the enlarged liver regressed, the slight jaundice disappeared, serum ALT fell to normal level in 80%-95% of the patients; in general, the results were much better than in the controls. Immunologically, CD_4 increased, CD_8 decreased, CD_4/CD_8 ratio increased and NK activity increased.

Transfusion of autologous and heterologous lymphokine-activated killer (LAK) cells[4,5]

Around ten papers have reported on transfusion of LAK cells. One paper reported the LAK cells were incubated with 10000 U or 20000 U of IL-2, with or without Ara-AMP, and then all were compared with controls; the limitation of this study was small sample size. The rates of negative seroconversion of HBV DNA were 58% (16/27) and 58% (21/36) respectively in both treated groups, and those of HBeAg were 29% and 42% respectively (*vs* control groups, $P < 0.01$); duration of the reversion of serum ALT to normal level was shortened in the treated group. LAK cell activity was increased by the presence of IL-2, in a dose-dependent manner, and was much lower in patients with chronic hepatitis B. Ara-AMP, when given as the sole treatment, was also effective. In another article using heterologous LAK cells, the rates of negative seroconversion of HBV DNA were 73.7% (19 cases) and 26.6% (15 cases) compared with the effect of autologous LAK cells; the rates of HBeAg were 63% and 46.6% respectively and positive seroconversion of anti-HBe were 47% and 40% respectively. Both groups were given LAK cells once per week, altogether six times, and the response in all was elevated temperature after transfusion, with increased CD_4 and CD_4/CD_8 ratio. Thus, IL-2 appears to increase the activities of both Tc and NK cells.

Dane particles, when incubated with monocytes, can cause degenerative and necrotic changes, resulting in eventual disruption of these cells; in this experiment, HBcAg and HBeAg were detected by immune-electron microscopy with suppression of erythrocytes *in vitro*, demonstrating that HBV could infect and replicate within the blood cells. It was proposed that cell membrane fluidity was involved first and followed by loss of normal cellular function with alteration of surface receptor function. Many patients relapsed after completion of interferon-α therapy, which may have resulted from the release of HBV DNA from the peripheral blood mononuclei, supporting the idea that elimination of serum HBV DNA (as a therapeutic approach) is of great importance[6].

ALLEVIATION OF INFLAMMATION AND IMPROVEMENT OF HEPATIC MICROCIRCULATION

Monocytes/macrophages and neutrophils can all produce TNF after being infected with HBV. TXB_2 has been found to be expressed at higher than normal levels in patients with chronic hepatitis B who present with moderate or severe disease activity; the level of 6-keto-PGF_{1a}, on the contrary, was lower than normal, and the TXB_2/6-keto-PGF_{1a} ratio was elevated and platelet aggregation remained high. In cases of chronic hepatitis presenting as mild disease activity, the reverse was true except that the platelet aggregation was slightly higher than normal. It is believed in more severe cases, that the serum levels of IL-1, IL-6 and TNF can all increase, thereby causing impairment of hepatic microcirculation and leading to hepatocyte damage. Besides, liberated oxygen free radicals can further promote liver damage through their lipid peroxidation effects on the hepatocyte membrane, resulting in hepatic dysfunction. A number of effective drugs have been found to ameliorate the above changes and these will be presented below.

Glycerrhyzin mimics the action of glucocorticoid in ameliorating inflammation but does not have the latter's side effects. It can reduce the serum levels of IL-6 and TNF, and it is capable of inhibiting the mRNA of procollagen I and III.

Tetramethylpyrazine can inhibit TXB_2 synthetase and increase PGI_2 production, so as to maintain the balance of TXB_2/PGI_2; in addition, it is also an antioxidant.

Dan Shen (Salvia miltiorrhiza) is a useful drug, exerting not only antioxidant effects but also causing increases in hepatic blood flow, improvements in hepatic microcirculation and inhibition of platelet aggregation. It can protect hepatocytes from toxic injury induced by CCl_4 and its long-term administration can promote synthesis of RNA of hepatocytes, increase glycogen content in hepatocytes and lower the hepatic lipids.

Sedum sarmentosum can significantly inhibit the disease-related decrease of glutathione and increase production of malondialdehyde (MDA) in experimental liver injury[7]. It protects liver cells by inhibiting lipid peroxidation and has long been used clinically as an adjuvant therapy for chronic hepatitis B.

Astragalus is also an immune-modulating agent, as well as antioxidant.

Mocrea can ameliorate the disease-related features of ballooning, vacuolation, eosinophilic degeneration and spotty necrosis involving hepatocytes. It can also help to maintain the integrity of the mitochondrial and endoplasmic membrane system[8], lower the serum ALT level, and increase the serum albumin level and the total protein level in rat liver damaged by CCl_4.

Hepatocyte stimulating substance (HSS) can promote the synthetic functions of hepatic cells and suppress replication of HBV DNA. In patients with chronic hepatitis B in the active stage, the IL-2 activity and IL-2R cell number was found to be increased profoundly after HSS treatment, and as a result the production of IL-2 and expression of IL-2R returned to normal levels; hence, HSS is believed to have a cellular immuno-enhancing effect[9].

ANTI-HEPATIC FIBROSIS THERAPY

Animal models of hepatic fibrosis are useful for screening therapeutic drugs. The models can be induced by bovine serum albumin, human serum albumin, D-galactosamine, CCl_4, alcohol and schistosoma infestation. Both bovine and human serum albumin are well recognized for their abilities to cause liver fibrosis through formation of immune-complexes (*i.e.* the type Ⅲ immunologic reaction), which

are sufficiently reflective of the mechanism of hepatic fibrosis in human viral hepatitis.

There are several elaborated herbal products that produce a definite effect on the alleviation of hepatic fibrosis, and these include Cordeceps, semen persicae and tetrandrine.

Cordeceps mycelium has been shown to increase the collagenase activity in both experimental animals and humans with schistosomal hepatic fibrosis. The deposition of total and subsets of collagen Ⅰ and II in the rat liver was much less after treatment with Cordeceps than in the untreated controls, and these effects were comparable to those obtained with colchicine treatment ($P > 0.05$). The microscopy-based study revealed that Cordeceps could decrease the infiltration of inflammatory cells and increase the phagocytic function of Kupffer cells of the fibrotic liver. The human serum albumin animal model showed that the deposition of intrahepatic IgG-immune complexes along the hepatic sinusoids diminished significantly. The underlying mechanism likely involves the following: an increase in phagocytic function of phagocytes and Kupffer cells, as well as of scavenging of the IgG-immune complexes; inhibition of cell necrosis and inflammation, as well as release of the relevant cytokines; stimulation of collagenase activity, and promotion of lysis and resorption of collagen. In another study, Cordeceps and colchicine were both found to markedly reduce the serum procollagen level and collagen Ⅰ, Ⅲ and Ⅳ deposition in liver tissues, as well as the number of desmin-positive cells, which were much less than those in controls; finally, the pathologic changes were identical to those obtained with Cordeceps and colchicine respective treatments. In the condition of hepatic fibrosis, collagen Ⅲ appeared early and collagen Ⅰ appeared late. Both Cordeceps and colochicine treatments led to inhibition of the proliferation and transformation of Ito cells, thereby inhibiting the synthesis and perisinusoidal deposition of collagen. Such effects caused by Cordeceps have been confirmed clinically. Furthermore, Cordeceps produced no adverse effect, unlike colchicine which produced side effects, suggesting Cordeceps as having better future prospects[10,11].

When extract of semen persicae was given to rats with experimental fibrosis induced by CCl_4, the number of Ito cells that secreted collagen Ⅰ and laminin changed, which led to capilarization of the sinusoids, interfering with exchange of nutrients and waste products between hepatocytes and the sinusoids. Desmin is an immunohistochemistry marker used to differentiate Ito cells from other cell types. Apparently, extract of semen persicae inhibits the activation and proliferation of Ito cells, and it can increase hepatic blood flow and enhance collagenase activity, which is the main mechanism of its actions in ameliorating hepatic fibrosis[12,13]. Combined therapy using extract of semen persicae and Cordeceps mycelium was applied in one study of 6 patients with hepatic cirrhosis due to viral hepatitis B. The semen persicae was given at a dose of 1.5 g in 500 mL of 5% glucose infusion every other day and the oral Cordeceps was given at a dose of 1.5 g twice daily, both for a course of 3 mo. Repeat peritoneoscopy evaluation after treatments showed that the hepatic consistency and coloration had improved, edema of falciform ligament and ascitic fluid had disappeared, and varices had diminished in caliber. Liver biopsy at the same sites, performed prior to and after the treatments, showed diminished perisinusoidal collagen Ⅰ and Ⅲ by immunohistochemistry in 3 of the patients; in addition, the collagen bundles had become thinner, and the perisinusoidal and intercellular fibronectin had decreased in amount in 2 of the patients, with the other 4 patients showing no change. These findings indicated that this therapy reduced portal venous hypertension accompanied by an increase in hepatic blood flow, which occurred primarily through increased collagenase activity and degradation of collagen metabolism, leading to eventual improvement in the hepatic microcirculation. In most of the patients, the degenerative changes and inflammatory cellular infiltration rate were diminished, resulting in improvement of hepatic consistency and coloration. The decrease of variceal caliber and subsidence of edema agreed with the ultrasonic findings. These experimental and clinical data indicate inactivation of the Ito cell, inhibition of collagen synthesis, promotion of collagenase activity and the immune-modulating effect of the two drugs[14].

Tetrandrine, like semen persicae, had been used as one constituent of decoction in treatment of liver cirrhosis in China for 2000 years. In the animal model of hepatic fibrosis induced by CCl_4, the serum markers PIIIP and hyaluronic acid (HA) were measured, immunohistochemical staining of collage Ⅰ, Ⅲ and Ⅳ and electromicroscopy of hepatic cells were performed as well. The number of Ito cells and the changes in their ultrastructural organelles were used as the parameters for evaluation. Tetrandrine was given to 22 rats of the CCl_4 model group and to 115 patients with chronic hepatitis B. The patients received 50 mg twice daily for 6 mo as one course, for a total of 2-5 courses, and were followed-up for 6, 12, 24 and 36 mo. Glucoronic acid tablets were used as control.

A comparative analysis of tetrandrine and verapamil was performed in rats. Both treatments demonstrated only modest collagenous proliferation and inflammatory cellular infiltration, with none of the rats developing cirrhosis. Electromicroscopy revealed that the treatments diminished number of Ito cells and the mean surface area of rough endoplasmic reticulum and mitochondria. In a group of patients, tetrandrine treatment led to remarkably reduced serum levels of PIIIP and HA (*vs* control group, $P < 0.01$); moreover, the longer the observation period, the more marked the decrease of the two markers, with the order following as 6 mo < 12 mo < 24 mo < 36 mo. A follow-up study was done with 93 of the patients, and it was found that the fibrotic lesions at 36 mo and at 24 mo after the treatment were much less extensive than those measured prior to treatment. The hepatic fibrosis resolved completely in 14 patients (15.4%), collagen deposition attenuated in 54 (58.1%), inflammation improved in general, and 26.5% of the study population remained unchanged[15,16]. Possible mechanism of actions for these effects are various. First, tetrandrine is also a calcium channel blocker, and it may have produced the effects by modulating the intracellular Ca levels, thereby ameliorating the inflammation. Second, this herbal medicine has antioxidant action. Third, this herbal medicine inhibits LTB_4, TXB_2 and cytokines such as IL-1, TGF-B and TNF, which may induce production of fibronectin, laminin proteoglycan and collagen, all of which are known to interact and lead to fibrosis.

In conclusion, Chinese herbal medicines have multi-actions on multi-systems and tissues, but rarely induce significant adverse effects. Therefore, they are preferred for use in treatment by many of the Chinese doctors who were trained in Western medicine. In the author's viewpoint, these medicines can be used in combination or in succession with Western medicine, in order to achieve an optimum effect, in particular, at the earlier stage of the disease.

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EXPERIMENTAL PAPERS

Effects of glutamine on gut structure and function in endotoxemic rats

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Abstract

AIM: To study the effects of glutamine-supplemented parenteral nutrition on protein metabolism, small intestinal mucosal morphology, and barrier function in endotoxin-treated rats.

METHODS: Thirty-five male Wistar rats were divided randomly into four groups: group A, normal control; group B, enteral nutrition (EN); group C, non-glutamine total parenteral nutrition (TPN); group D, glutamine TPN. Endotoxemia was induced by continuous intravenous infusion of endotoxin at a dose of 2 mg/kg per day throughout the 5-d study period. The small intestinal bacterial translocation rate and contents of mucosal protein, DNA, superoxide dismutase (SOD), malondialdehyde (MDA), adenosine triphosphate (ATP) and secretory immunoglobulin A (sIgA) were determined in mucosal homogenates. Mucosal thickness and villous height were measured with light microscope, and thickness of microvillus was measured with electron microscope.

RESULTS: The bacterial translocation rates of group B and group D were lower than that of group C ($P < 0.01$). Group D showed increased protein content and DNA content in the small bowel ($P < 0.01$), and, unlike the other groups, maintained the height of intestinal villi, the thickness of mucosa and the whole small intestine ($P < 0.01$). Group D showed increased intestinal mucosal contents of ATP and sIgA and decreased contents of SOD and MDA, compared with group C ($P < 0.01$). All parameters returned to normal levels in group B with EN, which also showed higher villous height and mucosa thickness than group A ($P < 0.01$).

CONCLUSION: Glutamine can improve gut metabolism, decrease the extent of mucosal atrophy and assist in the maintenance of the mucosal barrier function. Early use of TPN plays an important physiological role in the small bowel.

Key words: Glutamine; Septicemia; Parenteral nutrition; Intestine, small; Bacteria

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INTRODUCTION

Increasing attention has been focused on the role of the gut as a reservoir of bacteria and their endotoxins (ET), both of which may enter the circulation by crossing over a compromised gut mucosal barrier and thereby initiating a septic process and multi-organ failure. Functional defects of the gut mucosal barrier occur under various conditions, such as hemorrhagic shock, thermal injury, major operation, or sepsis. Maintenance of a healthy gut requires a variety of therapeutic measures.

The development of total parenteral nutrition (TPN) has offered a means to provide adequate calories and protein to these patients. In spite of its many advantages, TPN has been shown to lead to intestinal mucosal atrophy in both animals and man[1,2]. This atrophy is considered to be due in large part to a deficiency of glutamine (GLN), the principal energy substrate for intestinal mucosal cells, which is absent from standard intravenous amino acid solutions[3,4]. Several studies have, in addition, suggested that a deficiency of GLN in TPN may cause a breakdown of the intestinal mucosal barrier and subsequent bacterial translocation[5,6]. The addition of GLN to TPN solutions reduces atrophy of the intestinal mucosa[2,7] and helps maintain the mucosal barrier[8].

The purpose of this study was to investigate whether the provision of GLN could stimulate mucosal metabolism, reduce mucosal atrophy and maintain the gut barrier function during endotoxemia in rats.

MATERIALS AND METHODS

Animals and study procedure

Thirty-five male Wistar rats, weighing 200-220 g, were allowed *ad libitum* intake of water and standard rat food. After 16 h of fasting, all rats were anesthetized with intraperitoneal injections of sodium pentobarbital (40 mg/kg). The neck and interscapular region of the rats were shaved and prepared in a sterile manner for catheterization. A silastic catheter (inner diameter of 0.6 mm, outer diameter of 1.0 mm) was inserted through the external jugular vein to reach the superior vena cava. The catheter was tunneled subcutaneously to the mid-scapular region. A flexible spring guarded the catheter, which was then hooked up to an infusion pump. The

Table 1 General conditions

	Group A	Group B	Group C	Group D
Weight loss, g	-	20.50 ± 4.10	29.00 ± 6.45	15.30 ± 4.12[b]
Death, *n*	-	2	3	2
BTR, % (*n*/total)	-	33.3 (3/9)	77.8 (7/9)	40 (4/10)[b]

[b]$P < 0.01$ *vs* group C.

Table 2 Intestinal protein and DNA contents (mg/g)

	Group A	Group B	Group C	Group D
Jejunum protein	161.79 ± 36.75	159.92 ± 43.20	142.79 ± 26.14	155.51 ± 15.88[b]
Ileun protein	141.62 ± 37.90	150.82 ± 23.68	140.20 ± 26.48	173.99 ± 1.77[b]
Jejunum DNA	14.16 ± 3.60	13.10 ± 2.83	9.55 ± 2.97	15.02 ± 3.57[b]
Ileum DNA	14.53 ± 3.87	12.15 ± 2.95	8.47 ± 2.39	14.32 ± 4.15[b]

[b]$P < 0.01$ *vs* group C.

Table 3 Intestinal mucosal adenosine triphosphate, secretory immunoglobulin A, superoxide dismutase and malondialdehyde contents (/g)

	Group A	Group B	Group C	Group D
Jejunum ATP, mmol	2.78 ± 0.16	2.03 ± 0.17	1.78 ± 0.13	2.23 ± 0.20[b]
Ileum ATP, mmol	2.54 ± 0.1	2.09 ± 0.31	1.56 ± 0.22	2.14 ± 0.12[b]
Jejunum sIgA, μg	597 ± 79	439 ± 97	311 ± 73	460 ± 81[b]
Ileum sIgA, μg	546 ± 87	387 ± 65	289 ± 56	408 ± 84[b]
Jejunum SOD, Nu	92 ± 12	327 ± 21	365 ± 32	302 ± 29[b]
Ileum SOD, Nu	101 ± 14	307 ± 24	347 ± 27	286 ± 34[b]
Jejunum MDA, nmol	6.07 ± 1.1	13.09 ± 5.33	17.11 ± 5.21	11.67 ± 4.70[b]
Ileum MDA, nmol	5.13 ± 1.3	10.44 ± 2.48	14.54 ± 3.82	9.80 ± 2.52[b]

[b]$P < 0.01$ *vs* group C. ATP: Adenosine triphosphate; sIgA: Secretory immunoglobulin A; SOD: Superoxide dismutase; MDA: Malondialdehyde.

rats were maintained in individual metabolic cages. The nutrient solution was infused by pump at a constant rate of 2 mL/h.

Endotoxemia in the rats was induced by intravenous infusion of endotoxin (Lipopolysaccharide, *Escherichia coli* O 127: B8, Sigma Chem Co, St. Louis, MO, United States) at a dose of 2 mg/kg·d in TPN solution or, for controls infusion of normal saline, from the morning of day 1 and throughout the 5-d study period.

Experimental groups

Following catheterization, all rats received intravenously 0.9% saline solution at 1.0 mL/h and *ad libitum* access to food and water for 24-30 h to allow for complete recovery from the effects of anesthesia before randomization to the experimental treatment groups. Rats were allocated randomly to four groups: group A (n = 6), normal control; group B (n = 9), enteral nutrition (EN); group C (n = 10), non-GLN solution; group D (n = 10), GLN-TPN solution (2.5 g/100 mL) group. The PN solution used in groups C and D was isonitrogenous and isocaloric. The daily dose of amino acids infused was 2.5 g of nitrogen per kilogram body weight, and the amount of non-protein calories given was 1045 KJ/kg per day as 50% glucose and 10% intralipid (energy index 1:1). Multivitamins and electrolytes were also included in the TPN solutions.

Endotoxemia was induced in groups B, C, and D by intravenous infusion of ET at 2 mg/kg·d in 0.9% saline solution or in TPN for 5 d. At the end of the period, the animals were sacrificed by exsanguination, and samples of the small intestine and mesenteric lymph nodes were properly treated.

General observations

All rats were weighed before or after surgery and at the time of tissue sampling. Rats with any catheter complications or infusion pump malfunction were excluded from the study. At the time of death, the intra-abdominal viscera were closely inspected before exsanguination.

Bacterial translocation rate (BTR)

Immediately after anesthesia, the rats were placed on a sterile field and the ventral abdominal wall was cleaned with 70% isopropyl alcohol and opened with sterile instruments. Several mesenteric lymph nodes (MLNs) were isolated from the ileocolic junction and put into sterilized glass tissue grinders. After manual grinding, 200 μL of the homogenate was plated onto general agar plates and agar plates with 5% sheep's blood. These plates were then incubated at 37 ℃ for 48 h. Bacterial colonies were stained by Gram's method and identification of bacterial species was performed using standard microbiologic methods.

Intestinal measurements

Following MLN excision, the rats were sacrificed by cardiac exsanguination. Ten-centimeter segments of proximal jejunum and distal ileum were then rapidly excised, opened along the antimesenteric border, and the mucosa was blotted with tissue paper and then weighed. All samples were placed at -70 ℃. The mucosae of the jejunal and ileal segments were scraped off from the specimens using a glass slide and homogenized in a blender at 30000 rpm for 30 s. The homogenates were centrifuged for 15 min at 3000 × *g* and the supernatants assayed spectrophotometrically for protein[9], DNA[10] and adenosine triphosphate (ATP)[11]. The contents of superoxide dismutase (SOD), malondialdehyde (MDA) (the kits were supplied by Nanjing Railway Medical College) and secretory immunoglobulin a (sIgA) were measured by simple agar diffusion test. Values are expressed per gram of intestinal tissue.

Histologic study of intestines

Following intestinal sampling for intestinal measurements, 10-cm sections of the proximal jejunum and ileum were cut, opened along the antimesenteric border, pined flat onto wax, and fixed in Boin's solution. Tissues were subsequently embedded in paraffin, and 5-μm sections were taken and stained with hematoxylin-eosin. All slides were coded and morphologically interpreted in a "blind" fashion.

Statistical analysis

Analysis of the bacterial translocation data from rats was performed using χ^2 analysis. Intestinal measures are expressed as $\bar{x} \pm s$ of the sample and comparisons between treatment groups were made using one-way analysis of variance. A P-value below 0.05 was considered statistically significant.

RESULTS

General observations

All rats lost weight over the 5-d study period (Table 1), but rats in groups B and D did not differ in body weight loss, although these two groups had less weight loss compared with rats in group C. The mortality rate was 33% (3 of 9) in group B, 30% (3 of 10) in group C and 20% (2 of 10) in group D; the between-group differences did not reach statistical significance.

BTR

Bacterial translocation data are presented in Table 1. There was a significant increase of the BTR in the MLN of group C (7 of 9) ($P < 0.01$). There was no difference in the BTR between groups B (3 of 9) and D (4 of 10).

DNA and protein contents

The intestinal mucosal DNA and protein values are listed in Table 2. Group D rats had higher mucosal DNA and protein contents of all segments of gut compared with group C ($P < 0.01$).

ATP, SIgA, SOD and MDA

The results of intestinal mucosal measurements are listed in Table 3. Group D had higher mucosal ATP and sIgA contents of the jejunum and ileum, but lower intestinal SOD and MDA, as compared with group C ($P < 0.01$). The differences between groups B and D had no statistical significance.

Histologic observations

Data of the histologic cross-sections of the jejunum and ileum of

Table 4 Intestinal histologic changes (μm)

	Group A	Group B	Group C	Group D
Jejunum layer	692.25 ± 11.37	715.26 ± 17.43	605.77 ± 17.63	751.86 ± 14.88[b]
Ileum layer	652.20 ± 37.20	679.39 ± 7.12	502.30 ± 10.06	551.31 ± 12.32[b]
Jejunum mucosa	568.20 ± 69.72	674.55 ± 10.02	519.60 ± 9.31	579.08 ± 11.26[b]
Ileum mucosa	474.40 ± 27.31	513.52 ± 25.72	376.34 ± 9.25	423.79 ± 7.24[b]
Jejunum villi	364.12 ± 6.42	471.50 ± 6.71	322.57 ± 3.09	379.28 ± 8.42[b]
Ileum villi	241.37 ± 8.25	304.20 ± 6.1	202.37 ± 674	249.26 ± 3.78[b]

[b]$P < 0.01$ *vs* group C.

all groups are presented in Table 4. Bowel wall thickness, mucosa thickness and villus height were all increased compared with those in group C ($P < 0.01$). Between groups B and D there was no statistically significant differences. Group D had higher microvilli than group C, and group B had the highest microvilli.

DISCUSSION

The present experimental model of endotoxemia induced in rats by constant infusion of lipopolysaccharide with TPN was designed to mimic the clinical condition. A 20% mortality rate during the 5-d course of study indicated a moderate septic insult in this experimental model. In terms of the results of BTR and the gut morphologic observations, this model is suitable for observing the effects of GLN on the small intestinal structure and function in endotoxemia rats.

Many studies have confirmed atrophy of the intestinal mucosa in patients with long-term intravenous nutrition. In a series of experiments, investigators have tried to optimize parenteral nutritional formulas by supplementation with GLN to support the intestinal mucosa. O'Dwyer *et al*[2] reported that when a standard amino acid solution was supplemented with 2 g of GLN per 100 mL, jejunal cellularity increased significantly compared with the same solution supplemented with 2 g of glycine per 100 mL. In addition to PN, exogenous endotoxin further impairs gut mucosal metabolism[12]. In our study, group D (with GLN-TPN) showed increased small intestinal protein and DNA contents, and significantly maintained the level of intestinal villi, the thickness of mucosa and of the whole small bowel wall. These findings suggested that GLN administration may exert a nutritional effect on the gut mucosa, even in endotoxemia. The exact mechanism remains unclear; however, the following explanations may be made. First, GLN serves to promote cellular differentiation of the intestine[13], and increased GLN uptake and consumption by the intestine supply fuel for oxidation and also nitrogen; the latter may support nucleotide biosynthesis. Second, GLN may act as a secretogogue to stimulate the release of gut peptides, which have nutritional effects on the mucosa[14]. Both of these processes would support cellular replication and maintain gut structure.

GLN is the principal fuel for maintenance of gut metabolism, structure and function, particularly during critical illness[15]. GLN provides nitrogen for the synthesis of protein, purines, pyrimidines, and ATP, through which it improves gut mucosal metabolism and transport. Our study showed that group D (with GLN) had increased mucosal ATP content in all segments of the gut. In addition, group D had significantly increased mucosal sIgA content of the intestine, as compared with group C. SIgA is considered the major effector of the gut-associated lymphoid tissue. Endotoxin infusion may destroy the tight junction barriers of the intestinal epithelium and induce bacterial translocation[5]. SIgA can bind to bacteria, prevent their adherence to the epithelium and subsequent translocation. Therefore, GLN is thought to preserve immune barrier function[16], as shown by a decreased BTR in group D.

Moreover, rats in group D had a significantly decreased mucosal SOD and MDA contents, compared with those in group C. SOD and MDA are related to the content of free oxygen radicals and the degree of damage to cells caused by free oxygen radicals in the body. Endotoxin damaged the mucosa barrier of gut directly, it also activated xanthine oxidase to release free oxygen radicals, which further destroy gut mucosa. Provision of exogenous GLN can reduce the injury induced by free oxygen radicals and can promote the healing of damaged mucosa. It is important to guide clinical treatment of small bowel ischemia and to prevent enteric perforation in endotoxemia.

To our knowledge, this is the first report in the literature documenting the influence of GLN-supplemented TPN on intestinal ATP, sIgA, SOD and MDA. GLN can preserve not only the morphology and structure by increasing mucosa DNA and protein of the jejunum and ileum but also the mucosal barrier function by increasing mucosa ATP and SIgA and decreasing SOD and MDA contents.

It is very interesting that Group B (with EN) had the highest villi, thickest mucosa and small bowel wall among all the groups, together with increased mucosa contents of ATP and SIgA, decreased SOD and MDA contents, and significantly decreased BTR; these findings are similar to those of group D (with GLN). It has been previously shown that there is abundant GLN in the general food rich in protein and EN provides sufficient GLN. What is more, EN is the primary stimulus for gastrointestinal cell growth. Although our experimental rats were under the endotoxemic state, the small bowel still had considerable absorption function. EN can improve the structure and function of gut, and protect mucosa barrier function in endotoxemic rats. Our study confirms that use of EN plays an important physiological role in the small bowel.

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Relationship between differentiation syndromes in stomach disease and *Helicobacter pylori*

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Abstract

AIM: To demonstrate the relationship between *Helicobacter pylori* (Hp) infection and differentiation syndromes in stomach diseases.

METHODS: Prospectively, 317 patients with stomach disease were randomly selected for study recruitment. All patients were diagnosed endoscopically and the diagnosis was confirmed by biopsy. All cases were classified according to the syndromes of traditional Chinese medicine (TCM) at the same time.

RESULTS: There were 213 cases with Hp (+) and 104 cases with Hp (-). Among the groups classified according to syndromes of TCM, the Hp (+) rate in syndrome of stagnation of qi and heat was highest (76.8%), while the pure asthenic cold was lowest (57.5%). Most of the Hp (+) cases (52.7%) had incoordination of liver and stomach. Less patients suffered from pure asthenic cold in the Hp (+) group than in the Hp (-) group (19.7% *vs* 29.8%, $P < 0.05$). The incidence of stomachache in the Hp (+) group was 80.0%, which was higher than that in the Hp (-) group (70.6%) ($P < 0.05$).

CONCLUSION: Hp infection may be one of the objective indexes for syndrome of stagnation of qi and heat. Heat allaying medicine can be used to treat the syndrome clinically.

Key words: Stomach disease; differentiation-treatment; *Helicobacter pylori*; Zheng; Helicobacter infections

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INTRODUCTION

In recent years, more attention has been paid to the relation between *Helicobacter pylori* (Hp) and gastritis and peptic ulcers. It has become one of the hot-points to eliminate Hp by traditional Chinese medicine (TCM). In order to provide valuable references for clinical treatment, we summarize herein the relationship between the differentiation of syndromes in stomach disease and Hp.

MATERIALS AND METHODS

Subjects

Of 317 cases enrolled in the study, 182 were male and 135 were female. Fifty-seven of the participants were < 30 years-old, 82 cases were 31-40 years-old, 60 cases were 41-50 years-old, 69 cases were 51-60 years-old, and 49 cases were over 61 years-old. Seventy-seven cases had a course of illness < 1 year, 83 cases had 1-5 years, 78 cases had 6-10 years, and 79 cases had > 10 years. The histories of patients with stomach disease were recorded and the patients were then classified into different groups according to the four diagnostic methods of TCM, prior to the endoscopy and biopsy being performed for pathologic diagnoses. Hp was detected using basic fuchsin staining and the urease test.

Classification according to differentiation of syndromes

Incoordination of liver and stomach: The clinical manifestations were stomach ache related to hypochondrium, acid regurgitation, frequent belching, preference for sighing, white or yellow and thin fur on tongue, and taut and slippery pulse.

Asthenic cold syndromes of spleen and stomach: This type is characterized by dull stomachache, preference of warmth and pressing, pain which can be relieved by food or worsened when hungry, intolerance of coldness, cold extremities, fatigue, loose stool, pale tongue with white and thin fur on tongue, and deep and feeble pulse.

Deficiency of stomach-yin: This type is characterized by dull and burning stomachache, dry mouth but unwilling to drink, poor appetite, feverish sensation in the palms and soles, dry stool, red tongue with little liquid, little fur or exfoliative fur on tongue, and thready pulse.

In order to advance the research, the group of incoordination of liver and stomach was divided into the following three subgroups according to secondary syndromes: pure stagnation of qi; combined stagnancy of heat; combined blood stasis. The group of asthenic cold syndrome of spleen and stomach was divided into the following two subgroups: pure asthenic cold; deficiency of middle-warm and stagnation of qi. The data obtained were classified into two groups

Table 1 Relationship between differentiation of syndromes in stomach disease and *Helicobacter pylori* infection

Classification	Rate of Hp (+), %	Hp (+)		Hp (-)	
		n	%	*n*	%
Ia	63.3	57	26.8	33	31.7
Ib	76.8	43	20.2	13	12.5
Ic	73.3	11	5.2	4	3.9
IIa	57.5	42	19.7	31	29.8
IIb	73.2	52	24.4	19	18.3
III	66.7	8	3.8	4	3.9

I: Incoordination of liver and stomach; Ia: Pure stagnation of qi; Ib: Stagnancy of heat; Ic: Blood stasis; II: Asthenic cold of spleen and stomach; IIa: Pure asthenic cold; IIb: Deficiency of middle-jiao and stagnation of qi; III: Deficiency of stomach-yin; Hp: *Helicobacter pylori*.

Table 2 Relationship between tongue characteristics and *Helicobacter pylori* in stomach disease

Characteristic	Hp (+), %	Hp(-), %
Pink	48.6	56.2
Red	29.9	24.8
Dark red	11.2	10.5
Enlarged and tender with teeth prints	6.5	6.7
Fissured	3.7	1.9

Hp: *Helicobacter pylori*.

Table 3 Incidence rate of different symptoms in stomach disease related to *Helicobacter pylori*

Symptoms	Group	
	Hp (+)	Hp (-)
Stomachache	80.0	70.5
Distension	64.8	57.7
Belching	63.8	64.1
Dry stool	44.8	25.0
Loose stool	36.2	53.6
Black stool	21.1	15.4
Acid regurgitation	27.1	23.1
Poor appetite	44.6	41.3

Hp: *Helicobacter pylori*.

(Hp positive and Hp negative), for analysis and comparison of the different types in Hp positive or negative groups.

RESULTS

Endoscopy and Hp

Three-hundred-and-five patients were diagnosed according to Western medicine as chronic gastritis, along with 44 cases of peptic ulcer, 5 cases of gastric cancer and 5 cases of other stomach diseases. There were 213 cases with Hp positivity (+) and 104 cases with Hp negativity (-), for a ratio of 2:1. In the Hp (+) group, active gastritis and ulcer were very common (106 cases or 75.1%), while in the Hp (-) group there were only 23 cases, comprising 22.0%.

Classification according to differentiation of syndromes in TCM

Among the various groups, the Hp (+) rate in syndrome of stagnation of qi and heat was highest (76.8%), and the next highest was the stagnation of qi and blood or hypofunction of middle-jiao and stagnation of qi. But there was no statistical significance for these comparisons ($P > 0.05$). The ratio of Hp (+) with pure asthnic cold was lowest (57.5%). In comparison of the constituents of Hp (+) and Hp(-), 52.1% of the Hp (+) group had incoordination of liver and stomach. While in the Hp (-) group, the percentage of incoordination of liver and stomach and of asthnic cold of spleen and stomach was 48.1%, respectively. More patients suffered from stagnation of qi and heat in the Hp (+) group (20.0%) than in the Hp (-) group (12.5%), but less patients suffered from pure asthenic cold in the Hp (+) group (19.7%) than in the Hp (-) group (29.8%). In these comparisons, there were statistically significant differences (Table 1, $P > 0.05$).

Inspection of tongue

Color of the tongue: The proportion of red tongue in Hp (+) was higher than in HP (-), while the pink tongue was on the contrary (Table 2).

Tongue coating: Yellow fur on the tongue was most common in the Hp (+) group, accounting for 47.2%. While in the Hp (-) group, the presence of white thin fur accounted for 47.3%. The rate of yellow and greasy fur was 18.1% for the Hp (+) group, which was far higher than that for the Hp (-) group ($P < 0.05$).

Pulse

A taut or slippery pulse was common in the Hp (+) group, accounting for 52.0%, which was far higher than that of the Hp (-) group (36.4%). The difference was statistically significant ($P < 0.001$). Thready, small and deep pulse (61.4%) was found in a large proportion of the Hp (-) group, which was significantly higher than that in the Hp (+) group (39.2%) ($P < 0.05$).

Analyses of symptoms (Table 3)

Stomachache: The incidence of stomachache in the Hp (+) group was 80.0%, which was higher than that in the Hp (-) group (70.6%); the difference was highly significant ($P < 0.05$). There was dull pain reported in both groups, but the incidence rates of burning pain, stabbing pain or colic were higher in the Hp (+) group than in the Hp (-) group.

Abdominal distension: The incidence rate of this symptom in the Hp (+) group was 64.8% and 57.7% in the Hp (-) group. The distension due to stagnation of qi was common in the Hp (+) group, accounting for 74.0%, and the incidence was higher than that of deficiency of stomach-yin or insufficiency of spleen (62.0% or 54.0% respectively).

Belching: There was no significant difference between the Hp (+) and Hp (-) groups. In the Hp (+) group, the incidence rate of belching due to stagnation of qi (65.0%) was slightly higher than to insufficiency of spleen and deficiency of stomach-yin (62.0%).

Abnormalities of stool: Many more patients suffered from dry stool in the Hp (+) group than in the Hp (-) group (44.8% *vs* 25.0%). On the contrary, in the Hp (+) group, less patients had loose stool than in the Hp (-) group (36.2% *vs* 53.6%, $P < 0.05$).

Black stool (hemafecia): The frequency of this symptom was higher in the Hp (+) group (21.1%) than in the Hp (-) group (15.4%). In the Hp (+) group, the black stool incidence was high due to insufficiency of spleen or stagnation of qi (24.0% or 19.0%).

Acid regurgitation: The incidence of this symptom was 27.7% in the Hp (+) group and 23.1% in the Hp (-) group. In the Hp (+) group, incoordination of liver and stomach was most common, accounting for 74.0%.

Poor appetite: This symptom occurred in 44.6% of the Hp (+) group and in 41.3% of the Hp (-) group. In the Hp (+) group, the incidence of poor appetite in asthenic cold, incoordination of liver stomach and deficiency of stomach-yin groups was 47.0%, 41.0% and 30.0%, respectively.

DISCUSSION

Based on the pathological view of TCM, the vital energy is emphasized as a guiding position in disease development. Besides, the pathogenic factors also play an important role. As the "RUMEN SHI QIN" put it, "*People didn't have the disease innately. Whether the disease comes exteriorly or interiorly, all were due to exogenous factors*." We regard Hp infection as an important factor in developing stomach disease, which belongs to the pathogenic factor in the category of TCM.

Analyses of the relation between Hp infection and differentiation of syndromes of TCM were carried out in this study. Among the various groups, the Hp positive rate was highest in stagnation of qi and heat, and lowest in pure asthenic cold. In the Hp (+) group, most cases were due to stagnation of qi and heat, and the symptoms of red tongue, yellow tongue coating (especially showing

yellow and greasy fur), taut and slippery pulse, stomachache, abdominal distension, dry stool, black stool, acid regurgitation, and poor appetite were more common. On the contrary, the symptoms of pink tongue, white and thin fur, thready pulse, loose stool, and pale complexion were common in the Hp (-) group. These findings demonstrated that more patients with Hp (+) suffered from excessive syndrome, heat syndrome, syndrome mixed with excess and deficiency, and syndrome with deficiency of primary aspect and excess of secondary aspect due to their active gastritis, peptic ulcer and the inflammation which was relatively more serious than that observed in the patients with Hp (-). Moreover, these findings agree with the previous research results for stomach disease. That is, the acute, active gastritis may be the pathogenic basis of damp heat, stagnation of qi, and blood stasis syndromes. Stagnation of qi and blood stasis syndromes may be commensurate to the inflammation, while asthenic cold of spleen and stomach may be commensurate to the chronic stage or covalescent stage of acute inflammation. Etiologically, we think that Hp, the "pathogenic factor", invaded the stomach and fought with the vital energy. It obstructed the qi and changed into heat or led to blood stasis. Along with the development of disease, the pathogenic factor would deplete the vital energy and promoted formation of the syndromes of mixture of excess and deficiency, deficiency of middle-jiao and stagnation of qi. If the vital energy was damaged, the asthenic cold of spleen syndrome would occur.

These findings collectively give insights into the treatment of patients with Hp (+). Since most of the patients who suffered from Hp were classified as having excessive heat, mixture of excess and deficiency of cold and heat, medicine (s) to allay heat and/or remove blood stasis should be prescribed to strengthen the patient's resistance and dispel the invading pathogenic factors. In this manner, the qi and blood can be regulated. Meanwhile, an herbal medicine that can inhibit or disinfect Hp should be selected according to an overall differentiation of syndromes and signs and according to disease conditions, so as to raise the therapeutic effect successfully.

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Stereological and ultrastructural research on rats with chronic gastritis and treatment based on the differential diagnosis of traditional Chinese medicine

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Abstract

AIM: We examined the stereological and ultrastructural changes of the gastric mucosa of rats with chronic gastritis so that the curative effects could be determined after respective treatments for Piweixuhan chronic gastritis (PXG, cold of insufficiency syndrome of the spleen and the stomach) and Ganweibuhe chronic gastritis (GBG, incoordination syndrome of the liver and the stomach) with Jianpiwenwei decoction (JWD, invigorating the spleen and warming the stomach) and Shuganhewei decoction (SHD, dispersing the stagnated liver-qi and regulating the stomach).

METHODS: Forty-five Wistar rats were randomly divided into the following 9 groups: N group, normal control group; PXG model group, induced by isoimmunization plus reserpine moulding method; JWD treatment group, PXG model rats treated with JWD and subdivided into 3 subgroups for treatment with large dose, moderate dose and small dose (10, 5 and 2.5 mL/kg, respectively); GBG model group, generated by isoimmunization plus electricity enraged method; SHD treatment group, which consisted of GBG model rats treated with SHD and was subdivided into 3 subgroups for treatment with large dose, moderate dose and small dose (as above). The JWD and SHD were Professor Jian-Xiong Zhao' s prescription that has been applied clinically for many years. The crude drugs' content in the decoction was 1600 g/L. The treatment groups and the model groups were respectively given oral decoction and NS once daily. After 7 wk, all the rats were sacrificed. Paraffin-embedded sections and ultrathin sections of the gastric mucosa from the body and the antrum of the stomach of each rat were made with conventional methods. The former sections (n = 5) were used to measure the thickness of gastric mucosa by the stereological weibel point-counting method, and the latter sections (n = 3) were used to observe the ultrastructural changes of gastric mucosa.

RESULTS: The thickness of gastric mucosa in the body was not significantly different between each group (P < 0.05), but in the antrum the mucosa showed obvious atrophy in the two model groups wen compared with the N group (P < 0.0 and, P < 0.05, respectively); the thickness in the treatment groups given the large dose and moderate dose was remarkably recovered in comparison to that in the model groups (P < 0.01 and P < 0.05, respectively). The main ultrastructural changes were atrophy and degeneration of the epithelial cells and mucous cells, and increased intercellular space, especially in the antrum. In general, these changes were similar in the two model groups. The treatment of large dose and moderate dose decreased the degree of mucosal injury or recovered the injury significantly.

CONCLUSION: The characteristics of pathological changes of chronic gastritis in rats were atrophy and degeneration of epithelial cells and mucous cells, which thereby led to atrophy of the gastric mucosa. After treatment with JWD and SHD, the atrophic lesion recovered significantly.

Key words: Zheng differentiation-treatment; Gastritis/therapy; Rats; Gastric mucosa/ultrastructure; Stereology

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INTRODUCTION

Chronic gastritis is a common disease, and the atrophic form is closely related to gastric cancer. It is important to further explore the stereological and ultrastructural changes related to this disease in order to accurately define its pathogeny, pathology, parameters for diagnosis and treatment. The aim of this study was to examine the stereological and ultrastructural changes of chronic gastritis using a rat model and to observe the therapeutic efficacy of Jianpiwenwei decoction (JWD, invigorating the spleen and warming the stomach) and Shuganhewei decoction (SHD, dispersing the stagnated liver-qi and regulating the stomach) in respective treatments based upon the presence of Piweixuhan (PXG, cold of insufficiency syndrome of the spleen and the stomach) and Ganweibuhe (GBG, incoordination syndrome of the liver and the stomach). Although some reports of ultrastructural observations in chronic gastritis[1-4] have been published previously, the results have not been completely

Table 1 Comparison of gastric mucosa thickness in each group ($\bar{x} \pm s$, mm)

Group, n = 5	Treatments, mL/kg	Gastric body	Gastric antrum
n	-	0.3952 ± 0.0635	02482 ± 0.0353
PXG	N.S., 10	0.3711 ± 0.0389	0.1615 ± 0.0458[b]
JWD	JWD, 10	0.3934 ± 0.0378	0.2430 ± 0.0438
JWD	JWD, 5	0.3889 ± 0.0250	0.2445 ± 0.0347[d]
JWD	JWD, 2.5	0.3738 ± 0.0570	0.2033 ± 0.0608
GBG	N.S., 10	0.3794 ± 0.0633	0.1798 ± 0.0447[a]
SHD	SHD, 10	0.3969 ± 0.0329	0.2425 ± 0.0244[c]
SHD	SHD, 5	0.3901 ± 0.0351	0.2388 ± 0.0401[c]
SHD	SHD, 2.5	0.3895 ± 0.0508	0.2176 ± 0.0228

[b]$P < 0.01$, [a]$P < 0.05$ *vs* N group; [d]$P < 0.01$, [c]$P < 0.05$ *vs* model groups. PXG: Piweixuhan chronic gastritis; JWD: Jianpiwenwei decoction; GBG: Ganweibuhe chronic gastritis; SHD: Shuganhewei decoction.

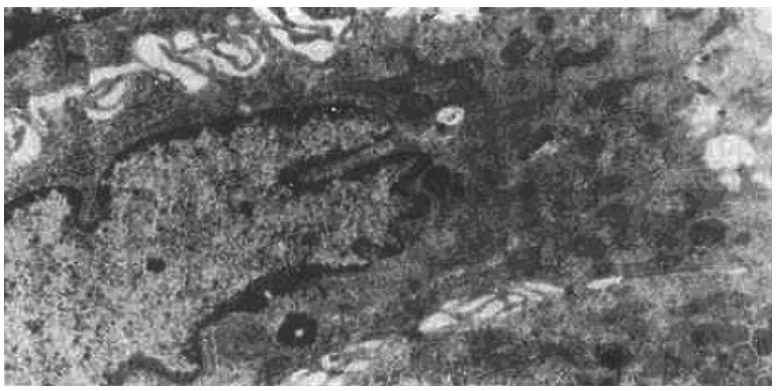

Figure 1 Mucosal epithelial cells show atrophy, with widened intercellular space and malformed nuclei.

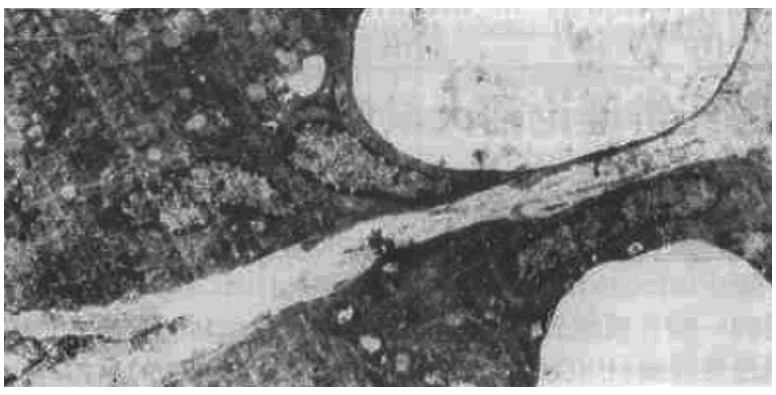

Figure 2 Mucous cells show atrophy and larger vesicles in the cytoplasm of the antrum tissues of the stomach.

consistent. Stereological and ultrastructural changes related to chronic gastritis, particularly in the rat model, have not yet been reported. In this paper, we will report our results from these types of observations to expand the overall body of data on chronic gastritis.

MATERIALS AND METHODS

Forty-five healthy adult Wistar rats, weighing 150 ± 10 g, were divided randomly into the following 9 groups: Normal control group (N group); PXG model group; GBG model group; 3 JWD treatment groups, administered large dose, moderate dose or small dose; 3 SHD treatment groups, administered large dose, moderate dose or small dose. All of the rats were fed routinely under the same condition. First, we established the chronic gastritis model, excluding rats in the N group, by means of isoimmunization[5], then we made the PXG model for the PXG model group and the 3 JWD treatment groups by means of the reserpine moulding method[6], and finally we made the GBG model for the GBG model group and the 3 SHD treatment groups by means of the electricity enraged method[5].

The treatment groups and the model groups were respectively given oral decoction and NS once daily for 7 wk. JWD and SHD, which were Professor Jian-Xiong Zhao's prescription that has been applied clinically for many years, were mainly composed of Sijunzi decoction and Sini powder, respectively. The decoctions were prepared according to the method described in the Chinese National Pharmacopoeia[7]. The crude drug content in each decoction was 1600 g/L.

After 7 wk of treatment, we euthanized all of the rats and obtained several specimens of gastric mucosa from the body and antrum, which were subsequently processed by routine methods to generate paraffin-embedded, ultrathin sections. The former specimen sections were stained with hematoxylin-eosin to allow for measurement of the thickness of gastric mucosa by the stereological method. In a square test system designed by us[8], $P_T = 368$, $L_T = 20$ mm, and we used the Weibel point-counting method[9] and the formula $\tau = L_T \cdot (\Sigma P_b)/(\Sigma I_a + \Sigma I_b)$[10]. In this formula, τ was thickness of gastric mucosa, P_b was the test point numbers in the mucosal section, and I_a and I_b were the intersection point numbers between the test lines and the mucosal surface and the basement side, respectively. All the data were processed statistically. The latter specimen sections were observed and photographed using a JM-100 CX electron microscope.

RESULTS

The stereological study showed that the thickness of gastric mucosa in the body was not significant different between each group ($P > 0.05$), but in the antrum the thickness of gastric mucosa was remarkably different. Specifically, the mucosal thickness of the two model groups was evidently diminished in comparison with that of the N group ($P < 0.01$ and $P < 0.05$), suggesting that the pathological changes were more severe in the antrum. The mucosal thickness in the treatment groups given the large dose and moderate dose recovered significantly, as compared to that in the model groups ($P < 0.01$ and $P < 0.05$); the efficacy of the small dose was not obvious ($P > 0.05$; Table 1).

The ultrastructural study indicated that the ultrastructure of mucosal epithelial cells and gastric glandular cells were nearly normal in the N group. There were a small number of G cells and ECL cells scattered throughout the glandular epithelium, with the former being distributed mainly in the antrum and the latter being mainly in the body. EC cells were also occasionally present in the antrum. Additionally, the mucosa of the two model groups showed a large number of acidophilic granulocytes and lymphocytes as well as a small number of plasmacytes that had infiltrated the tissue; in addition, the numbers of ECL cells was higher than the N group. The main ultrastructural changes were as follows: atrophied mucosal epithelial cell tissue, increased electron density and widened intercellular space, sparse surface microvilli, malformed nuclei, increased heterochromatin, decreased mucigen granules, and presence of fat droplets and vacuoles in the cytoplasm (Figure 1); in addition, the mucous cell tissues were also atrophied and showed widened intercellular space with lengthened microvilli on side surfaces and reduced presence of organelles. In the antrum specimens, in particular, the atrophied mucosa showed narrowing of the columnar or squamous cell epithelium, appearance of large vesicles in the cytoplasm and disappearance of mucigen granules (Figure 2); in the gastric chief cells, the rough endoplasmic reticulum (RER) was expanded to varying degrees, even to an extent that led to intracisternal sequestration, and some mitochondria were swollen and there was a general reduction in the presence of secretary granules. However, parietal cells showed only slight pathological changes, the main features of which being increased number of alveoli in the tubuloalveolar system, degenerated secretory channels in some cells, atrophied microvilli, and substantial shortening of channels with possible transition into vacuoles; finally, the perinuclear space was enlarged in a small number of cells. The above changes were basically similar between the two model groups, but the GBG group showed a slightly more severe degree of changes than the PXG group. After treatment, the injury in the groups treated with the large dose showed more improvement than that in the groups treated with the moderate dose; no effect of the small dose was observed.

DISCUSSION

In this study, the gastric mucosal thickness was measured and calculated by using the stereological method. The results revealed that the antral mucosa atrophied and became thinner with gastritis disease, as well as recovered significantly after treatment, but the changes of mucosa in the body were not obvious. Stereology a branch of applied mathematics[9], and it has been successfully adopted to study the relationship between structural stereoscopic morphology and its section, providing a much more precise and reliable way to measure and calculate microscopic images than the classical methods. The stereological method is simple and worth adopting to biological study, particularly for ultrastructural pathological changes of chronic gastritis, the findings of which to date have not been completely consistent[1-4].

Some researchers of gastritis have proposed that the major pathological changes related to the disease are swelling and vacuolation of the mitochondria. Indeed, these changes, along with RER enlargement, were found to occur in the epithelial and glandular cells of specimens in our current study; in addition, some of these changes involved G cells. However, the majority of the ultrastructural pathological changes that were observed in the rat models of chronic gastritis employed in our current study have not been reported so far. The major ultrastructural pathological changes of the two model groups we established were atrophy and degeneration of cells, with a particular involvement of the cells in the antrum, which thereby caused the mucosa of this region to become thinner. The mechanism underlying these observations may be that the isoimmunization stimulated the body to produce an immune reaction, which in turn injured the mucosal cells.

Our study also found that ECL cells were increased in mucosal epithelium. This cell type is known to secrete histamine[11] and to have a chemotactic extruding function that effects eosinophilic leucocytes. Therefore, it was not surprising to find a large number of eosinophilic leucocytes had infiltrated the gastric mucosa of our disease models. It is well known that the latter can take up immune complexes, inhibit immune reactions, stimulate release of histaminase to destroy and neutralize any histamine that has penetrated into the sub-epithelium, and reduce the extent of injury to tissue. The mucosal injury of our rat models did not lead to any serious necrosis of cells, but only changed the activity of some enzymes, reduced the cellular metabolic function and led to atrophy and degeneration of the cells. In addition, the findings from these models confirmed the above-mentioned morphological characteristics related to acidophilic leucocytes[12].

The ultrastructural pathologic changes of the two model groups were not completely consistent with those of the clinical patients that have been reported previously in the literature nor of those observed clinically by our team[1-4,13]. These discrepancies may be related to the following. First, the time of the animal model is shorter than that of humans, in whom the disease course is longer due to the presumed increased complexity of the human system. Second, unknown factors undoubtedly underlie the slightly different ultrastructural pathological changes of the two model groups.

Experimental results indicated that the JWD and SHD treatments using large and moderate doses can effectively treat the ultramicroscopic pathologic changes related to PXG and GBG. The mechanism lies in the likelihood that the decoction can not only regulate the body's immune function but also regulate the functions of the vegetative nervous system, the digestive system and the endocrine system, and thereby achieve a significant therapeutic efficacy.

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Histopathological study of hepatocellular carcinoma after transcatheter hepatic arterial embolization

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Abstract

AIM: To study the histopathological changes in hepatocellular carcinoma (HCC) after transcatheter arterial embolization (TAE).

METHODS: Histopathological analysis was made in 39 cases of liver neoplasms after TAE and 11 cases of liver neoplasms after digital selective angiography (DSA); data assessed included pathological type, histological grade, necrotic degree, encapsulation, times of treatment, vasculature injury and lymphocyte infiltration.

RESULTS: Following DSA, histological analysis identified 6 cases with 100% necrosis, 14 cases with 30%-95% necrosis, 19 cases with 0%-5% necrosis after TAE and 11 cases without necrosis. The presence of necrosis was related to the pathological type, encapsulation and vasculature injury, but not to the histological grade, times of treatment or lymphocyte infiltration of the liver neoplasms.

CONCLUSION: TAE is an effective therapy for late-stage HCC. Encapsulated HCC is a preferable indicator for TAE.

Key words: Carcinoma, hepatocellular/therapy; Carcinoma, hepatocellular/pathology; Embolization, therapeutic; Liver neoplasms/therapy; Liver neoplasms/pathology

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INTRODUCTION

In the first half of the 20th century, no remarkable progress was made towards advancing the clinical aspect of hepatocellular carcinoma (HCC) and the average survival of patients with HCC was around 2.5 mo. Between the 1970s and 1980s, alpha-fetoprotein (AFP) serosurvey was adopted for use in high-risk populations and the practice of small HCC resection opened a new era in clinical research of HCC, resulting in marked improvement of the 5-year survival. In the 1980s to 1990s, with the rapid progress of medical imaging and regional cancer therapies, substantial advances were made in the diagnosis and treatment of both small and large HCC. Surgical resection is still the first choice for treatment of HCC, but this operation is not always possible in cases with large mass of HCC. In addition, HCC frequently recurs after surgery. Among various chemotherapeutic modalities, transcatheter arterial chemoembolization (TAE) has demonstrated great potential for improving survival rate[1,2].

In China, HCC has been reported as the second most prevalent cancer killer in rural areas and as the third in cities, with 110000 patients killed annually and accounting for 40% of the HCC deaths in the world. In China, HCC deaths will probably surpass those of gastric cancer in the 21st century. The major clinical obstacle in further prolonging survival of HCC patients is recurrence and metastasis after resection, and re-resection is an effective approach to prolong survival. However, TAE might be useful for preventing and treating the recurrence.

TAE was initially applied to cases of unresectable HCC as a palliative treatment[3], but recently it has been used as a preoperative modality in operable cases. However, few reports present a significant number of resected HCC cases after TAE using histological methods[4-6], and it is not clear which type of HCC patients respond well to TAE.

MATERIALS AND METHODS

From 1988 to 1995, 307 patients with resectable HCC underwent hepatic resection in the Department of Surgery, Chinese PLA General Hospital. TAE was performed for 39 of these patients and digital selective angiography (DSA) was performed in 11 patients as control.

Angiographic studies of the selective celiac and superior mesenteric arteries were made before TAE. Subsequently, the following TAE procedure was performed before surgery. Chemoembolization with gelatin sponge particles soaked in contrast medium was conducted immediately after injection of 4-20 mL of iodized oil mixed with 12-36 mg of mitomycin C and/or 10-60 mg of amycin or adriamycin through the hepatic and collateral arteries supplying the tumors. The injection was made under fluoroscopic

Table 1 Pathology of resected tumors

Case no.	Age/sex	Pathological diagnosis	Differentiation	Encapsulated	TAE/DSA, time	Necrosis, %	Vascular damage	Lymphocyte infiltration
1	34/M			Yes	5	100	Yes	+++
2	41/F	HCC	P	Yes	1	30	Yes	++
3	50/F	HCC	M	Yes	2	50	Yes	+++
4	62/M	HCC	M	Yes	2	90	Yes	+++
5	25/M	HCC	M	Yes	4	95	Yes	++
6	56/M	HCC	M	Yes	3	0	No	+
7	47/M	HCC	M	Yes	5	0	No	+
8	30/M	HCC	M	Yes	2	90	Yes	+
9	27/M	HCC	M	Yes	3	90	Yes	+++
10	38/M			Yes	2	100	Yes	++
11	53/M	HCC	M	Yes	4	0	No	++
12	32/M			Yes	3	100	Yes	+
13	64/M	HCC	M	Yes	1	0	No	+++
14	45/M	HCC	P	Yes	1	0	No	+
15	67/M	HCC	M	Yes	1	30	Yes	+
16	55/M			Yes	1	100	Yes	+
17	38/M	HCC	W	Yes	1	0	No	+
18	29/M	HCC	P	Yes	3	95	Yes	+
19	50/M	HCC	P	Yes	3	80	Yes	+++
20	54/M	HCC	W	Yes	1	30	Yes	+++
21	42/F	HCC	M	Yes	1	40	Yes	+++
22	58/M	HCC	W	Yes	2	90	Yes	+++
23	54/M			Yes	2	100	Yes	+++
24	43/F	HCC	M	Yes	2	5	No	+++
25	46/M	HCC	M	Yes	1	0	No	++
26	44/M			Yes	2	100	Yes	+
27	52/M	HCC	P	Yes	1	5	No	+++
28	40/M	HCC	W	Yes	1	0	No	+++
29	56/M	HCC	M	Yes	3	50	Yes	+++
30	54/M	HCC	M	Yes	4	80	Yes	+++
31	17/M	MN	P	No	2	0	Yes	+
32	51/F	MN	M	No	1	0	No	+
33	49/F	MN	M	No	1	0	No	+
34	47/M	MN	W	No	1	0	No	++
35	42/M	CC	W	No	1	0	Yes	+
36	55/M	W	No	1	0	Yes	++	
37	56/M	HCC	P	No	1	0	No	+
38	43/M	MN	M	No	1	0	No	+
39	56/M	HCC	P	No	1	0	No	+
40	54/M	HCC	W	Yes	DSA	0	No	++
41	64/M	HCC	M	Yes	DSA	0	No	++
42	54/M	HCC	M	Yes	DSA	0	No	+
43	49/M	HCC	M	Yes	DSA	0	No	++
44	51/M	HCC	W	Yes	DSA	0	No	++
45	56/M	HCC	W	Yes	DSA	0	No	+
46	33/F	HCC	M	Yes	DSA	0	No	++
47	66/M	HCC	W	Yes	DSA	o	No	+
48	60/M	CC	W	No	DSA	0	No	+
49	61/M	HCC	W	Yes	DSA	0	No	+
50	55/M	MN	P	No	DSA	0	No	+

CC: Cholangiocarcinoma; HCC: Hepatocellular carcinoma; M: Moderately differentiated; MN: Metastatic neoplasm; P: Poorly differentiated; W: Well differentiated; DSA: Digital selective angiography; TAE: Transcatheter arterial chemoembolization.

control, permitting it to be slowed or discontinued if retrograde flow occurred.

All resected specimens were cut into slices 10-mm thick, after fixation in 10% formaldehyde solution. After macroscopic observation of the slices, each slice containing the tumor was sectioned for histological examination, and all lesions that differed in size or color from the surrounding parenchyma were sampled. All paraffin-embedded sample blocks were used for routine staining with hematoxylin and eosin.

Tumors were classified according to the following 7 features: presence or absence of a surrounding fibrous capsule; necrosis rate as an indicator of TAE response; histological type; differentiation of the tumors; time of TAE; damage of vasculature; lymphocyte infiltration. The extent of necrosis of tumors was measured for the largest cut surface area and expressed as a percentage. The tumor was assessed pathologically according to Peter's guidelines. The degree of lymphocyte infiltration was classified as follows: "+", a few lymphocytes; "++", moderate lymphocytes; "+++", dense lymphocytes.

RESULTS

The age distribution of the patient group ranged from 17-67 years-old (33-66 years-old for the control subjects), with a mean of 46.2 years-old (54.8 years-old for the control subjects). Male patients greatly outnumbered female patients, with a male to female ratio of 33:6. The pathology and TAE results are shown in Table 1.

Six encapsulated TAE-treated tumors developed complete necrosis (Case 1, 10, 12, 16, 23 and 26) (Figure 1). Histopathological diagnosis was not confirmed among these 6 cases because of the complete absence of carcinoma cells. Fourteen encapsulated TAE-treated HCC developed obvious necrosis involving 40% to 95% of the area (Case 2, 3, 4, 5, 8, 9, 15, 18, 19, 20, 21, 22, 29 and 30). No necrosis and < 5% necrosis were found in 12 cases of HCC (Case 6, 7, 11, 13, 14, 17, 24, 25, 27, 28, 37 and 39). All 7 TAE-treated cases of metastatic neoplasm and cholangiocarcinoma without encapsulation showed no necrosis (Case 31, 32, 33, 34, 35, 36 and 38). Eleven cases of DSA showed no necrosis.

The relationship between necrosis and related factors is shown in Table 2. No necrosis was found in 2 cases of cholangiocarcinoma, 5 cases of metastatic neoplasm, 9 cases without encapsulation (Figure 2), and 16 cases without vascular damage (Figure 3); moreover, the necrosis was not related to the differentiation of tumors, times of TAE or lymphocyte infiltration.

DISCUSSION

Since the initial report of TAE treatment for liver neoplasm was

Table 2 Necrosis and related factors

	Necrosis, %	Cases, *n*	Histology			Encapsulated		Vascular damage		Differentiation			TAE, time					Lymphocyte infiltration		
			HCC	CC	MN	Yes	No	Yes	No	W	M	P	1	2	3	4	5	+	++	+++
	100	6				6	0	6	0				1	3	1	0	1	3	1	2
TAE	30-95	14	14	0	0	14	0	14	0	2	9	3	4	4	4	2	0	3	2	9
	< 5	19	12	2	5	10	9	3	16	5	9	5	14	2	1	1	1	11	4	4
Controls (DSA)	0	11	9	1	1	9	2	0	11	6	4	1						5	6	0

CC: Cholangiocarcinoma; HCC: Hepatocellular carcinoma; M: Moderately differentiated; MN: Metastatic neoplasm; P: Poorly differentiated; W: Well differentiated; DSA: Digital selective angiography; TAE: Transcatheter arterial chemoembolization.

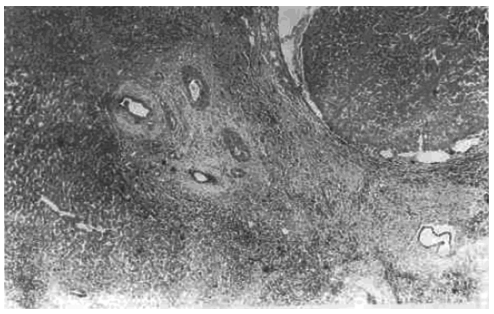

Figure 1 Complete necrosis of Hepatocellular carcinoma after transcatheter arterial embolization. HE, × 25.

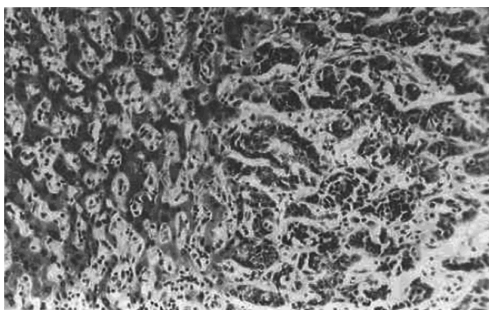

Figure 2 No necrosis of hepatocellular carcinoma without encapsulation. HE, × 50.

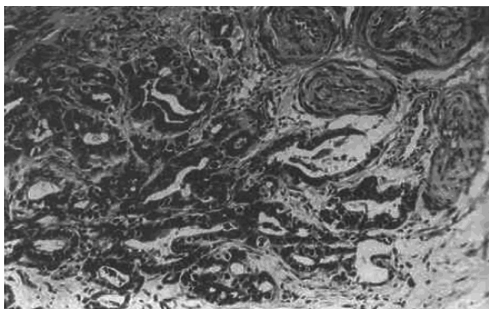

Figure 3 No necrosis of hepatocellular carcinoma even though obvious vascular damage is present. HE, × 50.

Figure 4 Complete necrosis of encapsulated hepatocellular carcinoma with obvious vascular damage. HE, × 25.

published in 1976[1], TAE has been considered a useful therapeutic procedure for HCC[2,3]. However, what happens in the carcinoma tissues after TAE and how to explain recurrence are questions that remain unclear and TAE alone does not constitute a sufficient therapy for HCC. Thus, histological investigation after TAE would be more beneficial for the evaluation of TAE in liver tumors.

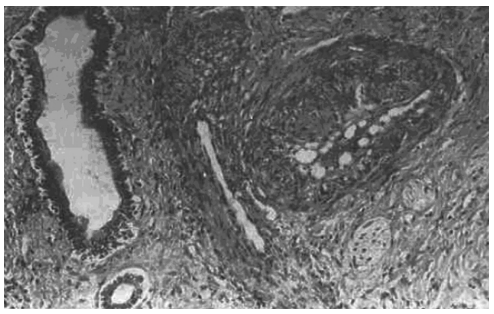

Figure 5 Complete obstruction of vascular lumen after transcatheter arterial embolization. HE, × 50.

It has been proposed in the previous studies that necrosis is related to encapsulation, invasion, satellite nodules, and portal vein blood supply of the tumor[4,5]. Our study has demonstrated that TAE produces a significant necrotic effect, as compared with the non-TAE treated group. All encapsulated HCC with vascular damage after TAE showed obvious necrosis (Figure 4). Conversely, HCC without encapsulation and vascular damage did not show sufficient necrosis after TAE. The main cause of tumor necrosis is related to vascular damage. Histologically, vascular damage presents as endothelium thickened by fibrous tissues, narrowed lumen, increased collagenous tissue in interstitium and occlusive vessels (Figure 5). These morphological changes are considered a result of TAE. Therefore, causing vascular damage in each case after TAE is the key point of TAE therapy for HCC.

In our study, 5 cases of metastatic neoplasm and 2 cases of cholangiocarcinoma did not show any necrosis; therefore, these types of liver tumor are not indicators for TAE therapy.

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Measurements of cell proliferation in esophageal and gastric cardia epithelia of subjects in a high incidence area for esophageal cancer

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Abstract

AIM: To determine the proliferation patterns of normal and different precancerous lesions of esophageal and gastric cardia epithelia by measuring levels of proliferating cell nuclear antigen (PCNA), Ki-67 and bromodeoxyuridine (BudR) incorporation.

METHODS: One-hundred-and-seventy-five esophageal biopsies and 45 gastric cardia biopsies were collected from symptom-free subjects in Huixian. Of these, 24 esophageal biopsies were incubated with BudR. The avidin-biotin-peroxidase complex (ABC) method was used to detect PCNA, Ki-67 and BudR. Quantitative data of the immunostaining results were recorded as the number of positive cells per mm^2 of the biopsied epithelium.

RESULTS: Intense immunostaining for PCNA, Ki-67 and BudR was observed in the cell nuclei of normal tissues and of tissues with different severities of precancerous lesions. For esophageal biopsies, the numbers of both PCNA and Ki-67 increased significantly as the epithelia progressed from normal to basal cell hyperplasia (BCH) and to dysplasia (DYS). The number of PCNA- and Ki-67-positive cells was three times higher than that of BudR in the same category of BCH. For cardia biopsies, the number of Ki-67-positive cells was lower in normal tissues and increased significantly from chronic superficial gastritis (CSG) to chronic atrophic gastritis (CAG) and to DYS.

CONCLUSION: The staining patterns for PCNA and Ki-67 were correlated with the histopathology of the esophagus and gastric cardia. These methods may be useful for screening subjects at high risk for esophageal and gastric cardia cancers and for monitoring the effect of chemoprevention. PCNA is relatively easy to analyze and may prove to be very useful in studies on esophageal cancer.

Key words: Esophageal neoplasms/pathology; Epithelial cells; Stomach neoplasms/pathology; Precancerous conditions

Wang LD, Zhou Q, Gao SS, Li YX, Yang WC. Measurements of cell proliferation in esophageal and gastric cardia epithelia of subjects in a high incidence area for esophageal cancer. *World J Gastroenterol* 1996; 2(2): 82-85 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i2/82.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i2.82

INTRODUCTION

Carcinoma of the esophagus (EC)[1] is a widely occurring disease in Linxian and Huixian of Henan Province in northern China and remains a leading cause of cancer-related deaths[2,3]. Because of poor prognosis of this disease, early diagnosis and prevention are of great importance. An early indicator of abnormality in persons predisposed to EC is the increased proliferation of esophageal epithelial cells, morphologically manifested as basal cell hyperplasia (BCH) and dysplasia (DYS)[1,4-6]. Gastric cardia adenocarcinoma (AC) seems to occur together with EC in many high incidence areas in China and other countries[7,8]. AC of the gastric cardia is an under-studied subject. The pathogenesis and cell proliferation of this disease have not been well characterized. There is evidence, however, that cardiac AC differs from cancer of the rest of the stomach in terms of time trend, risk factors and histopathogenesis[9-11].

Measurements of cell proliferation are being increasingly used to assess the effects of cancer prevention interventions[12-15]. Tritiated thymidine labeling of S phase cells is an established method for identifying proliferating patterns in tissue sections. Using this labeling technique, we found that the esophageal BCH and DYS had higher labeling indices than normal epithelium. These precancerous lesions also showed expansion of proliferating zones toward the

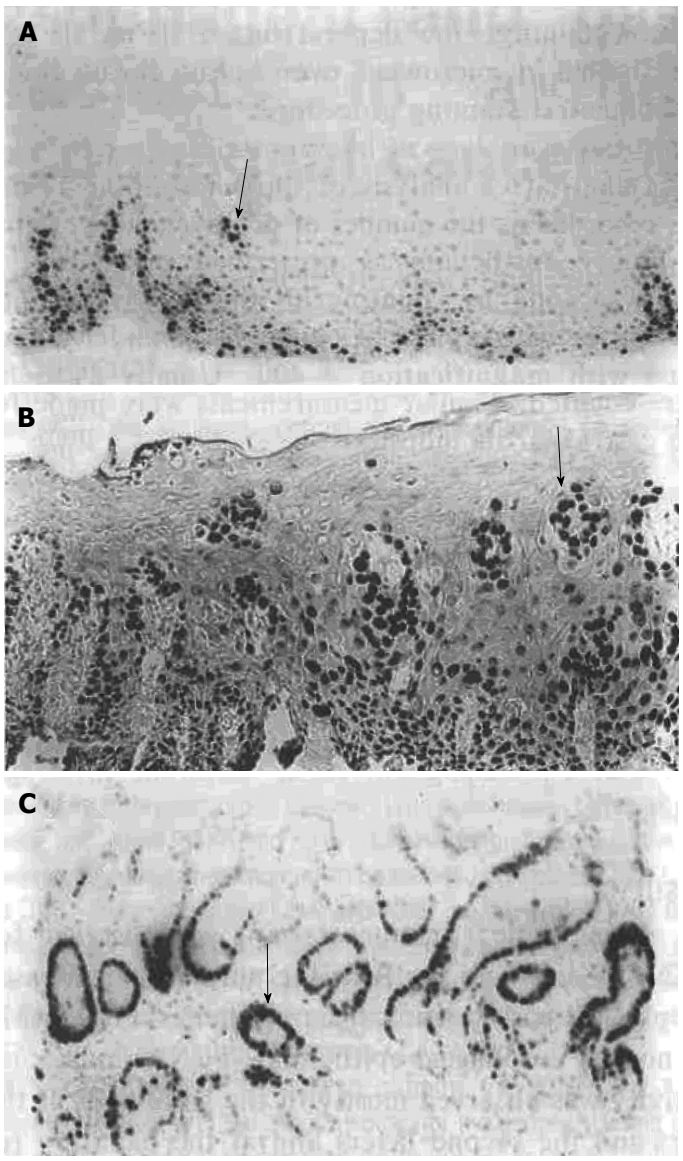

Figure 1 Immunostaining of Proliferating cell nuclear antigen in biopsied samples of esophageal and gastric cardia epithelia. Immunoreactivity is located in the nuclei of basal cells in the papillary region of the normal epithelia of esophagus (A, arrows) and the positive cells expand in the upper region of DYS (B). C: PCNA staining in gastric cardia epithelium with CSG (× 100). PCNA: Proliferating cell nuclear antigen; DYS: Dysplasia; CSG: Chronic superficial gastritis.

esophageal surface[3]. Recently, immunohistochemical identification of cell cycle-related proteins has been used for the assessment of cellular proliferation. The markers used include the proliferating cell nuclear antigen (PCNA), Ki-67 and bromodeoxyuridine (BudR). PCNA is a 36-kDa nuclear protein identified as an auxiliary protein of DNA polymerase δ. Its synthesis is sharply increased in late G1 phase and also increased during S phase, but is declined throughout G2 and M phases[16]. Ki-67, a nuclear antigen, is present in G1, S and G2 phases of the cell cycle[17]. BudR, a pyrimidine analogue of thymidine, is incorporated into DNA during cell replication in S phase and can be detected by anti-BudR antibody[18]. In the present study, we used PCNA, Ki-67 and BudR incorporation labeling index as parameters to characterize the cell proliferation patterns of human esophageal and gastric cardia epithelia with normal and different severities of precancerous lesions from symptom-free individuals in Henan, China.

MATERIALS AND METHODS

Tissue collection and processing

One-hundred-and-seventy-five esophageal biopsies and 41 gastric cardia biopsies were collected from symptom-free subjects in Huixian of Henan Province, China. Of the 175 esophageal biopsies, 24 were immediately incubated with BudR (1.5 mg/100 mL; Sigma) in a 95% basal medium with 10% fetal calf serum, with shaking at 37 ℃ for 1 h. All tissues were fixed with 80% alcohol, embedded in paraffin, and serially sectioned at 5 μm. The sections were mounted onto Histostick-coated slides. Three or 4 adjacent ribbons were collected for histopathological analysis (hematoxylin and eosin (H & E) staining) and immunohistochemical analysis.

Histopathological analysis

Histopathological diagnoses of esophageal epithelia were made according to the previously established criteria[7]. The normal esophageal epithelium contained 1-3 layers of basal cells; the papillae were confined to the lower half of the epithelium. In BCH, the number of proliferating basal cells was increased to more than 3 cell layers. DYS was characterized by partial loss of cell polarity and nuclear atypia. The following histopathological classifications were used for the gastric cardia epithelia: chronic superficial gastritis (CSG; inflammation manifested as a mild lymphocyte and plasma cell infiltration in biopsies from the gastric cardia); chronic atrophic gastritis (CAG; glandular morphology disappeared partially or completely in the mucosa and replaced by connective tissues; interglandular space infiltrated mainly by plasma cells and lymphocytes); and DYS (neoplastic features including nuclear atypia and/or architectural abnormalities confined to the gastric epithelium, without invasion)[19].

Immunohistochemical analysis

The avidin-biotin-peroxidase complex (ABC) method was used for PCNA, Ki-67 and BudR antigen detection. Briefly, after dewaxing, inactivating endogenous peroxidase activity, and blocking cross-reactivity with normal serum, the sections were incubated overnight at 4 ℃ with a diluted solution of the primary antibodies (1:200 for PCNA and Ki-67; 1:30 for BudR). Localization of the primary antibodies was achieved by subsequent application of a biotinylated anti-primary antibody, an avidin-biotin complex conjugated to horseradish peroxidase, and diaminobenzidine (Vectastain Elite Kit). Normal serum blocking and omission of the primary antibody were used as negative controls. Counterstaining with H & E was used only for BudR. For Ki-67 immunostaining, the deparaffinized tissue sections were boiled in a microwave oven before the immunohistochemical staining procedure.

Quantitative analysis of immunostaining results

Quantitative data of immunostaining results were recorded as the number of positive cells per mm^2 of biopsied epithelium, as described previously[20]. This was done by counting all the positive stained cells in the whole biopsied tissue sample under microscope with magnification of × 400. Usually, 24 fields were counted. Similar measurements were made for the gastric cardia specimens.

Statistical analysis

The $\bar{x} \pm s_{\bar{x}}$ of the PCNA, Ki-67 and BudR positive-immunostained cell number/mm^2 in the esophageal and gastric cardia specimens in each histologic category were calculated using univariate analysis. The ANOVA test followed by Fisher's PLSD test was used to assess the significance of differences ($P < 0.05$) among values of different histologic parameters.

RESULTS

Clear nuclear immunostaining was observed for PCNA, Ki-67 and BudR in the normal and diseased esophageal and gastric cardia epithelia (Figures 1-3). In normal esophageal epithelium, PCNA immunoreactivity was observed mostly in the basal cells at the first and second layers and at the papillary regions (Figure 1A). As the esophageal tissue progressed from normal to BCH and DYS, the PCNA positive immunostaining cells increased significantly in number ($P < 0.001$) and expanded to the upper part of the epithelium (Table 1, Figure 1B). A similar pattern of immunoreactivity was observed for Ki-67 (Figure 2). In samples with BCH and DYS, the number of PCNA positive cells appeared to be larger than that of Ki-67. The number of BudR-labeled cells was similar to that of PCNA and Ki-67 in the normal epithelium and appeared to be higher in DYS. However, the numbers of both PCNA and Ki-67 positive immunostaining cells were much larger (3.5-times) than that of BudR in the category of BCH (Table 1). In the gastric cardia, the number of PCNA-positive cells was smaller in

Table 1 Cell proliferation of esophageal and gastric cardia epithelia measured by proliferating cell nuclear antigen, Ki-67 and BudR in symptom-free subjects from high incidence area of northern China

Histological type	PCNA		Ki-67		BudR	
	No. of biopsies examined	Positive cells/mm^2 ($\bar{x} \pm s_{\bar{x}}$)	No. of biopsies examined	Positive cells/mm^2 ($\bar{x} \pm s_{\bar{x}}$)	No. of biopsies examined	Positive cells/mm^2 ($\bar{x} \pm s_{\bar{x}}$)
Esophagus						
Normal	18	144 ± 16	16	145 ± 20	3	112 ± 56
BCH	136	338 ± 21[b]	96	308 ± 25[b]	19	103 ± 9
DYS	21	928 ± 157[b]	15	773 ± 229[b]	1	425
Gastric cardia						
Normal	9	290 ± 43	6	180 ± 41	-[1]	-
CSG	17	356 ± 78	12	195 ± 32	-	-
CAG	11	382 ± 85	6	376 ± 134[a]	-	-
DYS	4	633 ± 111	2	620 ± 143[a]	-	-

[a]Significantly different from adjacent low-grade lesions; $P < 0.05$ by ANOVA test. [b]Significantly different from adjacent low-grade lesions; $P < 0.001$ by ANOVA test. [1]Not applied for BudR incorporation. PCNA: Proliferating cell nuclear antigen; BCH: Basal cell hyperplasia; DYS: Dysplasia; CSG: Chronic superficial gastritis; CAG: Chronic atrophic gastritis.

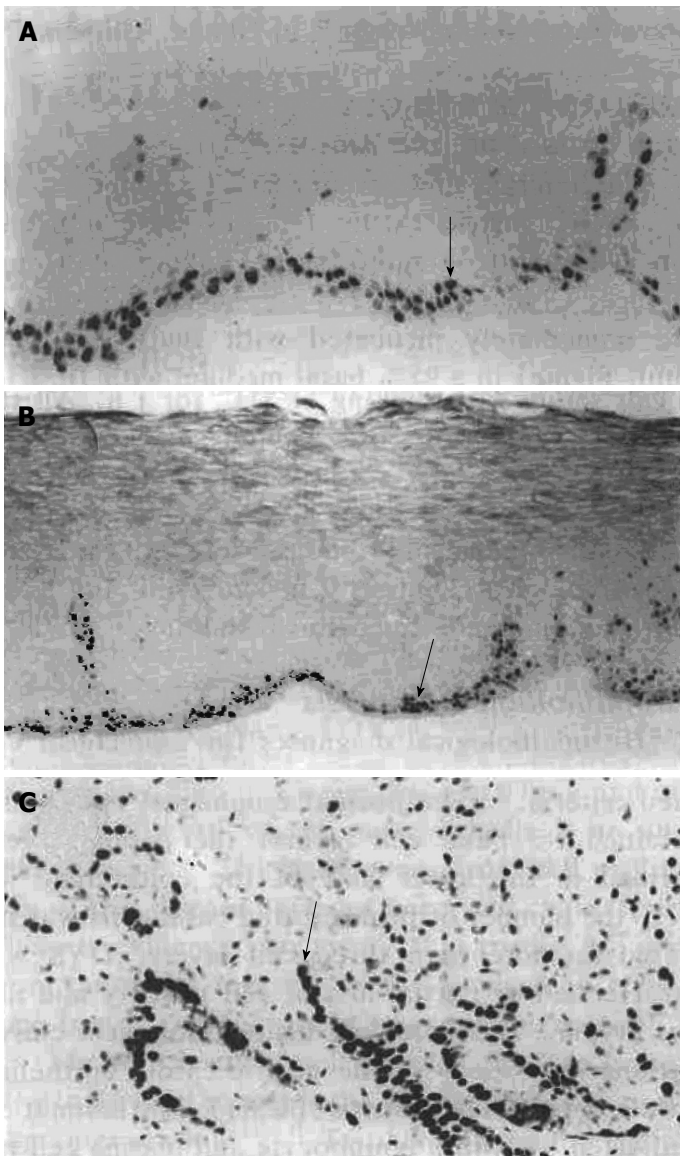

Figure 2 Immunostaining of Ki-67 in biopsied samples of esophageal and gastric cardia epithelia. Immunoreactivity is located in the nuclei of basal cells in the papillary region of the normal epithelia of esophagus (A, arrows) and the positive cells expand in the upper region of DYS (B). C: Ki-67 staining in gastric cardia epithelium with CSG (× 100). Immunoreactivity is located in the nuclei of cells at the basal cells of the crypts and at the deep glands (arrows). DYS: Dysplasia.

the normal tissues and slightly increased as the epithelia progressed from normal to CSG, CAG and DYS (Table 1, Figure 1C), but the difference was not significant. In normal gastric cardia epithelium, Ki-67 immunoreactivity was observed mostly in the basal cells of the crypt (Figure 2). As the gastric cardia tissue progressed from normal to CSG, CAG and DYS, the Ki-67 positive immunostaining cells increased significantly in number ($P < 0.05$) and expanded to the upper part of the crypt (Figure 2).

DISCUSSION

In the present study, the staining patterns for PCNA and Ki-67

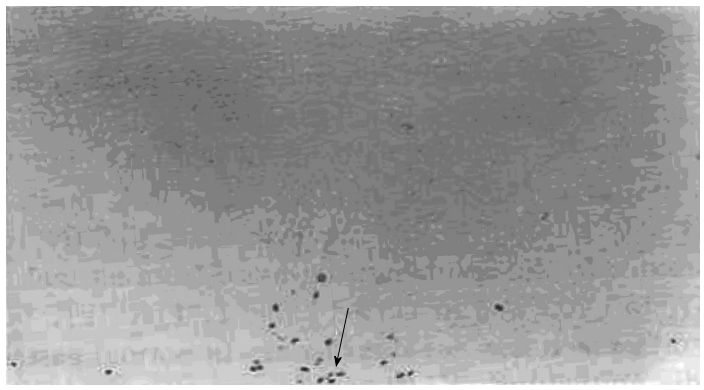

Figure 3 Immunohistochemical studies of BudR-labeled cells in the esophageal epithelia of basal cell hyperplasia (with methyl green counterstaining). Immunoreactivity is located in the cell nuclei (× 200, arrows).

were correlated with the histopathology of esophagus and gastric cardia and may represent useful additional parameters for screening subjects at high risk for esophageal and gastric cardia cancers and monitoring the effect of chemoprevention. The proliferation pattern of esophageal epithelia measured by PCNA was similar to that measured by tritiated thymidine incorporation[1]. In terms of DNA precursor and antigen unmasking pretreatment, PCNA seems to be relatively easy to analyze in a large scale study of humans. This is of special importance in view of the increasing numbers of studies on dietary intervention and chemoprevention. Quantitative analysis of the immunostaining results showed that the positive cell numbers of PCNA and Ki-67 were almost 3.5-times larger than that of BudR in the same category of BCH lesions. It is possible that the present method is not stable enough to detect BudR-labeled cells. This possibility remains to be investigated.

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Significance of microvessel count in gastric carcinoma

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Abstract

AIM: To investigate the correlation between microvessel count and various clinicopathologic factors and prognosis of gastric carcinoma using immunohistochemical staining with anti-factor-VIII-related antigen (F-RAg) antibody.

METHODS: A total of 128 specimens resected from patients with gastric carcinoma were investigated by immunohistochemical staining with a monoclonal antibody against F-RAg. Correlations between the microvessel count (the mean number of microvessel in the five areas of highest vascular density at 200 × magnification) and various clinicopathologic factors and prognosis were studied for 86 cases with complete follow-up data.

RESULTS: The mean microvessel count of all patients was 16.5 ± 8.5; the microvessel count increased with histological stage and was significantly higher in patients with lymph node metastasis than those without such metastasis (18.3 ± 8.7 *vs* 13.8 ± 7.4, $P < 0.01$). In addition, the prognosis of 86 patients who were followed up for at least 5 years after surgery was significantly worse for patients who had a tumor with a high microvessel count (≥ 16) than for those with a low microvessel count (< 16), and the 5-year survival rates were 42.5% and 58.7% respectively ($P < 0.05$).

CONCLUSION: Microvessel count may be a useful prognostic indicator in patients with gastric carcinoma.

Key words: Prognosis; Immunohistochemistry; Stomach neoplasms/pathology; Capillaries

Tao HQ, Qing LF, Lin YZ, Wang RN. Significance of microvessel count in gastric carcinoma. *World J Gastroenterol* 1996; 2(2): 86-88 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i2/86.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i2.86

INTRODUCTION

It has been shown by many studies that tumor growth depends on neovascularization and that there is correlation between high vascular density and the malignant potential as well as the presence of tumor metastasis[1,2]. Recently, increasing evidence has been presented to support that the intratumor microvessel count (MVC) is closely related to the clinical prognosis. Weidner *et al*[3] have reported a statistically significant correlation between the incidence of metastasis and MVC in histologic sections of primary breast carcinoma, with both relapse-free and overall survival rates decreasing with increasing MVC. In these studies, microvessels were usually highlighted by staining for the factor-VIII-related antigen (F-RAg).

In this study, we investigate the correlation between MVC and various clinicopathologic factors in and prognosis of gastric carcinoma by using an immunohistochemical technique with an anti F-RAg monoclonal antibody.

MATERIALS AND METHODS

Clinical materials

Resected specimens obtained from 128 patients with gastric carcinoma who underwent gastrectomy at Ruijin Hospital were studied. The patients ranged in age from 38 to 78 years-old (average age, 58.7 years-old). There were 86 males and 42 females. No patient had received chemotherapy or radiation therapy before surgery. The Rules of National Gastric Cancer Association were used for the pathologic diagnosis and classification of variables. The histologic stage was defined according to the TNM staging method (Table 1). Tumors were divided into the following two histologic subgroups: differentiated type, which included papillary and tubular adenocarcinomas; undifferentiated type, which included poorly differentiated adenocarcinomas, signet ring cell carcinomas and mucinous adenocarcinomas. A total of 109 patients underwent curative resection and 19 patients received non-curative surgical procedures, among them 86 patients were followed-up for at least 5 years after surgery.

All specimens were fixed in a 10% formaldehyde solution and embedded in paraffin, after which 4 μm thick sections were cut and mounted on glass slides.

Antibodies and reagents

Mouse monoclonal antibodies F8/86 to RAg (which recognizes F-RAg), normal mouse immunoglobulin G (IgG) and LSAB kits were purchased from Dako Inc. Diaminobenzidine was purchased from Fluka Inc.

Table 1 Correlation between clinicopathologic factors and microvessel count

Variables	*n*	MVC	*P* value
Histologic type			
Differentiated	50	15.7 ± 7.7	> 0.05
Undifferentiated	78	17.1 ± 9.1	
Serosal inversion			
Negative	60	15.7 ± 7.6	> 0.05
Positive	68	16.9 ± 9.1	
Growth pattern			
Expanding	50	15.9 ± 7.6	> 0.05
Infiltrative	78	17.0 ± 9.0	
Lymph node metastasis			
Negative	50	13.8 ± 7.4	> 0.01
Positive	78	18.3 ± 8.7	

MVC: Microvessel count.

Table 2 Correlation between microvessel count and histologic stage

Histologic stage	*n*	MVC	*P* value
Ⅰ	39	14.5 ± 7.6	
Ⅱ	27	16.4 ± 8.3	
Ⅲ	48	17.4 ± 8.4	
Ⅳ	14	21.3 ± 10.1	< 0.01[b]

[b]*vs* stage 1. MVC: Microvessel count.

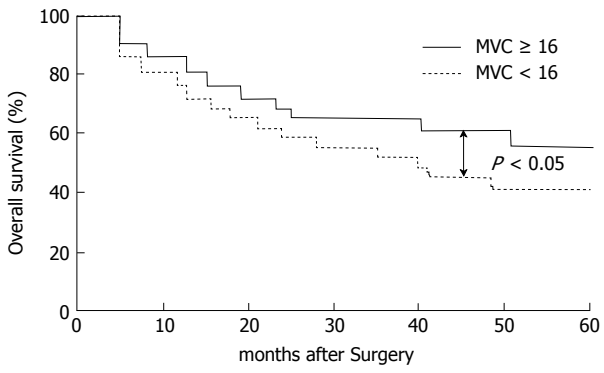

Figure 1 Association of microvessel count with survival rate for 86 patients with gastric carcinoma. MVC: Microvessel count.

Immunohistochemical staining

Monoclonal antibodies F8/86 to RAg were used at a dilution of 1:100 for the LSAB technique. The secondary antibody used was biotinylated rabbit anti-mouse IgGs at a dilution of 1:400. The experiment was carried out according to the instructions provided with the LSAB kits. Normal mouse lgG was substituted for primary antibody for the negative control.

Microvessel counting

Slides were interpreted for antigen expression by two investigators without knowledge of the corresponding clinicopathologic data. Any single brown-stained cell or cluster of cells, clearly distinguishable from background was counted as a vessel. Branching structures were counted as a single vessel, unless there was a break in the continuity of the structure. MVC was obtained in five areas of the highest vascular density within the tumor at 200 × magnification (× 20 objective and × 10 ocular, 0.739 mm^2 per field), and the mean number in these areas was calculated.

Statistical analysis

All values were expressed as $\bar{x} \pm s$. The relationship between MVC and clinicopathologic factors was examined by the Wilcoxon test. Survival rates were calculated using the Kaplan-Meier method. Statistical significance was defined as $P < 0.05$.

RESULTS

Distribution and MVC

Microvessels can be distributed anywhere within the tumor, but those in the sclerotic areas and areas adjacent to benign tissues and non-tumor tissues were not counted. Individual tumors demonstrated considerable heterogeneity in MVC. MVCs of the 128 patients with gastric carcinoma ranged from 3 to 50, with a mean of 16.5 ± 8.5. Table 2 shows the correlations between MVC and various clinicopathologic factors. There was no statistically significant difference found between MVC and histological type, growth pattern or depth of invasion. However, the MVC in patients with lymph node metastases was significantly higher ($P < 0.01$) than in those without lymph node metastases.

Correlation between MVC and histologic tumor stage

Table 1 shows the correlation between MVC and histologic tumor stage. MVC tended to increase with histologic stage. The MVC of patients with stage IV disease was significantly higher than those of patients with all other stages ($P < 0.01$).

Relationship between MVC and prognosis

Among the 86 patients who underwent curative resection and were followed-up for at least 5 years, 42 died of recurrence; the mean MVC of these patients was 15.9 ± 8.2. To evaluate the relationship between MVC and the overall survival, tumors were separated on the basis of the mean values for MVC (16) as follows: hypervascular group having MVC ≥ 16 and hypovascular group having MVC < 16. As shown in Figure 1, the 5-year survival rate was 58.7% (27/46) for the patients with low MVC, but only 42.5% (17/40) for the patients with high MVC; the difference was significant between the two groups ($P < 0.05$).

DISCUSSION

It has been demonstrated by much experimental data that malignant tumors depend on neovascularization for their growth, invasiveness and metastasis[4]; in addition, it has been shown that tumors produce several angiogenic factors, such as bFGF, VEGF, *etc*[5]. Recently, it has been suggested that the degree of tumor angiogenesis plays an important role in clinical outcome, indicating that angiogenic properties may be correlated with tumor aggressiveness[2]. In our study, there were no significant correlations found between MVC and histological type, depth of invasion, or growth pattern. However, MVC increased with histologic stage and was significantly higher in patients with lymph node metastases than in those without lymph node metastases. In other tumors, some studies have shown a significant correlation between MVC and the presence of metastatic disease in invasive breast cancer. All these investigations suggested that angiogenesis reflects an increased malignancy.

Both relapse-free and overall survival rates decrease with increasing MVC, and MVC is an independent significant prognostic factor in breast carcinoma[3,6]. Our study of gastric carcinomas found a significantly poorer prognosis in the group with higher MVC than in the group with lower MVC. This result is similar to the data reported by Maeda *et al*[7] In addition, Maeda *et al*[7] showed that in regard to the recurrence of gastric carcinoma after resection, hepatic metastases were significantly more common in the group with higher MVC, whereas the group with lower MVC showed a tendency for peritoneal recurrence; we did not observe this correlation. However, our results have demonstrated that intratumor MVC and conventional clinicopathologic factors are prognostic indicators, and MVC is a useful predictor of recurrence in patients with gastric carcinoma.

Chemotherapy, radiation therapy and other adjuvant therapies have been given to patients with advanced gastric cancer to prevent recurrence after surgery. However, Miwa[8] reported that 50% of the patients with advanced gastric cancer survived after curative resection without need for any post-operative treatment. Therefore, patients who will need adjuvant therapies should be selected by some indicators reflecting the probability of recurrence. Although little is known about the strong indicators for recurrence of gastric carcinoma, we think that MVC in resected specimens may be a better index for identifying patients who will need adjuvant therapy post-operatively.

In summary, this retrospective study showed that intratumor MVC may be one of the useful prognostic indicators of patients with gastric carcinoma. If this result was further confirmed in larger samples, it might be possible to add along with other

prognostic factors to the strategy for identifying patients at high risk of metastasis and tumor recurrence and to guide decisions on application of adjuvant therapy after operation.

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Biological significance of serum soluble tumor necrosis factor receptor I in hepatoma patients

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Abstract

AIM: In order to elucidate the biological significance of soluble tumor necrosis factor receptor (sTNF-R) in hepatomas, we observed the differential profiles of serum sTNF-R levels in 83 hepatoma patients and 61 healthy controls.

METHODS: The serum levels of soluble sTNF-R were measured with sandwich enzyme immunoassay.

RESULTS: The mean serum sTNF-R levels were significantly higher in the hepatoma patients than in the controls (2.69 ± 0.79 μg/L *vs* 0.93 ± 0.29 μg/L, $P < 0.001$). The increment correlated well with the stage of the disease, *i.e.* the serum levels of soluble sTNF-RI in the patients with stages III-IV were greater than in those with stages I-II (2.97 ± 0.43 μg/L *vs* 1.74 ± 0.41 μg/L, $P < 0.001$). Additionally, we found that increased sTNF-R level correlated positively with serum alkaline phosphatase ($r = 0.59$), white cell count ($r = 0.43$) and serum globulin ($r = 0.32$), and correlated negatively with serum albumin ($r = -0.71$). Among the hepatoma patients, the frequency of increased serum sTNF-RI level (89.16%) greatly exceeded that of serum AFP (54.22%). Moreover, comparison of 25 patients before and after chemotherapy indicated that patients with a rise in sTNF-R over the therapy course had decreased clinical response (3.39 ± 0.43 μg/L *vs* 2.67 ± 0.34 μg/L, $P < 0.001$).

CONCLUSION: In patients with hepatoma, serum soluble sTNF-R levels correlate well with disease stage and response to chemotherapy. It is reasonable to postulate that this determination can serve as an aid for the detection of these cancers, for follow-up studies, and for assessments of prognosis.

Key words: Liver neoplasms/drug therapy; Receptors, tumor necrosis factor/blood

Wang YF, Wu XN, Wu Q, Zhang XQ, Chen XF, Zhou XH, Wen WQ, Chen MY. Biological significance of serum soluble tumor necrosis factor receptor I in hepatoma patients. *World J Gastroenterol* 1996; 2(2): 89-91 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i2/89.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i2.89

INTRODUCTION

Tumor necrosis factors (TNFs) are pleiotrophic cytokines produced primarily by monocytic phagocytes, acting as the primary modifiers of body immune response and initiating multiple effects on cell function[1] by binding to specific, high-affinity cell surface receptors. There are two forms of TNFs, known as TNFα (cachetin) and TNFβ (lymphotoxin). In addition, there are two molecular species of TNF receptors (TNF-Rs)[2]. The cell surface form of TNF-Rs mediates the intracellular signaling of the cellular response to TNF[3]. Each species possesses not only a cell surface form but also two soluble TNF receptor forms, which act to neutralize the biological activities of TNFα- and TNFβ, and are respectively designated as sTNF-RII and sTNF-RI[2]. It has been demonstrated that these two types of TNF-Rs represent the soluble molecules detached from the cell surface receptors.

The physiological effects of sTNF-Rs remain to be elucidated. Both types of receptors are able to bind to TNF *in vitro* and inhibit its biological activity by competing with other cell surface receptors for TNF. It is suggested that shedding of a soluble receptor in response to release of TNF serves as a mechanism for binding, meanwhile inhibiting the immediate binding of the released TNF to surface receptors. This process is also considered as capable of providing protective effects to other cells[4]. It has been reported that low concentrations of TNF binding to soluble receptors can stabilize TNF and augment its activity[5]. In the present study, we measured the circulating level of sTNF-RI in patients with hepatoma and assessed its function and its relationship with the clinical stage of disease and the patients' responses to chemotherapy.

MATERIALS AND METHODS

Subjects

This study included 83 patients with hepatoma (PLC), hospitalized for treatment between October 1994 and July 1995. The mean age was 47.38 ± 16.14 years-old (range, 29-64 years-old). The ratio of males:females was 58:21. All cases were diagnosed by findings from CT scan and/or pathologic biopsy. No patient was

Table 1 Comparison of frequency of increase of sTNF-RI and alpha-fetoprotein

Hepatoma stage	*n*	$\bar{x} \pm s$	Percentage of increased sTNF-RI (*n*)	Percentage of increased AFP (*n*)
Ⅰ-Ⅳ	83	2.62 ± 0.79	89.16% (74)	54.22% (45)
Ⅰ-Ⅱ	24	1.74 ± 0.41	66.67% (16)	45.83% (11)
Ⅲ-Ⅳ	59	2.97 ± 0.43	98.30% (58)	57.63% (34)
Control	61	0.93 ± 0.29		

AFP: Alpha-fetoprotein.

Table 2 Correlations between sTNF-RI and other parameters

	r
Hemoglobin	-0.11
Total white blood cell count	0.43[b]
Albumin	-0.71[b]
Globulin	0.32[a]
Alkaline phosphatase	0.59[b]
Gamma-glutamyltransferase	0.05

[a]$P < 0.05$, [b]$P < 0.01$

Table 3 Mean serum levels of sTNF-RI in 25 hepatoma patients before and after chemotherapy and their relationship to clinical response

Patient no.	Stage	Chemotherapy	Clinical response	sTNF-RI	
				Before	After
1	Ⅳ	A	PR	3.25	3.07
2	Ⅳ	A	PR	3.38	3.11
3	Ⅳ	A	PR	3.15	2.83
4	Ⅳ	V	D	2.95	3.36
5	Ⅲ	A	D	2.33	2.91
6	Ⅲ	A	D	2.97	3.38
7	Ⅳ	A	PR	2.65	2.37
8	Ⅲ	A	D	2.36	2.84
9	Ⅲ	A	D	2.38	2.74
10	Ⅳ	A	D	2.79	3.16
11	Ⅲ	A	PR	2.62	2.53
12	Ⅳ	A	D	3.18	3.46
13	Ⅳ	A	D	3.07	3.23
14	Ⅳ	A	D	3.52	3.93
15	Ⅳ	V	D	3.44	3.51
16	Ⅳ	A	D	3.27	3.67
17	Ⅳ	A	PR	2.96	2.73
18	Ⅲ	A	CR	2.78	2.11
19	Ⅲ	A	PR	2.74	2.53
20	Ⅳ	A	CR	3.25	2.87
21	Ⅳ	V	D	2.79	3.43
22	Ⅳ	V	D	3.54	4.18
23	Ⅲ	V	D	2.62	3.11
24	Ⅳ	V	D	2.88	3.22
25	Ⅳ	V	D	3.85	4.63
	$\bar{x} \pm s$	Patients in remission ($n = 9$)		2.98 ± 0.29	2.67 ± 0.34
		Patients showing deterioration ($n = 16$)		2.99 ± 0.53	3.39 ± 0.43

A: FAPM transcatheter arterial chemoembolization with 5-Fu 1 g, ADM 50 mg, CDDP 100 mg, MMC 10 mg; V: FAM chemotherapy *via* peripheral vein with 5-Fu 500 mg, ADM 40 mg, MMC 8 mg. CR: Complete remission; PR: Partial remission; D: Deterioration.

given steroids or opioids during the investigation. In order to avoid possible interference, the patients with concomitant inflammatory diseases were excluded.

All patients had a detailed medical history and underwent a thorough physical examination. Laboratory tests included complete blood counts, hepatic and renal function tests, and serum alpha-fetoprotein (AFP) measurement. Staging of the hepatomas was done in accordance with the International TNM classification system[6].

The control group consisted of 61 consecutive healthy subjects seen in our outpatient department for a routine check-up. The mean age was 44.24 ± 12.31 years-old (range, 20-70 year-old) and the ratio of males:females was 28:33.

In addition, 25 patients scheduled to undergo chemotherapy were enrolled in the study for evaluation of their data before chemotherapy or 20 d after delivery of the last chemotherapeutic dose.

Methods

Venous blood samples were drawn in the morning, immediately centrifuged and stored at -20 ℃. The serum levels of sTNF-RI were measured using a sandwich enzyme immunoassay kit (Amersham). The measured sTNF-RI concentration was expressed in μg/L units and the sensitivity was 0.01 μg/L. The intra- and inter-assay coefficients of variation were less than 6.85% and 8.73%, respectively.

Serum AFP levels were measured using a double antibody ^{125}I-radioimmunoassay kit (China Institute of Atomic Science), for which 20 μg/L was taken as the normal upper limit in our laboratory.

Statistical analysis

Data were analyzed with the Student's *t*-test. The correlation coefficients were calculated and regression equation analyses were derived appropriately.

RESULTS

The serum concentrations of sTNF-RI in the 83 hepatoma patients and the 61 healthy controls are listed in Table 1.

Among the healthy subjects, the mean concentration ($\bar{x} \pm s$) of sTNF-RI was 0.93 ± 0.29 μg/L. The mean value in the hepatoma patients was 2.97 ± 0.43 μg/L, which was significantly higher than that in the healthy subjects ($P < 0.001$).

The hepatoma patients showed significantly higher serum levels of sTNF-RI. The increment in serum sTNF-RI concentration correlated well with the staging of the cancers, being higher in more advanced cases.

A positive correlation was found between increasing serum sTNF-RI level and alkaline phosphatase level ($r = 0.59$, $P < 0.01$). The increase in sTNF-RI also correlated positively with the white cell counts and the serum globulin concentration, but correlated negatively with the serum albumin concentration. No correlation was found between sTNF-RI and hemoglobin or gamma-glutamyltransferase (Table 2).

Among the cancer patients, only partial correlation was observed between the increase in the serum sTNF-RI and AFP. AFP was elevated (> 20 μg/L) in 45 patients only (40.96%) (Table 2). All the other patients had elevation of the sTNF-RI level; however, this increase was also observed in the other patients with normal serum AFP. Altogether, 74 cancer patients had an increase in sTNF-RI (89.15%). In the chemotherapy group, the sTNF-RI levels measured at the end of the chemotherapeutic schemes were significantly higher (3.39 ± 0.43 μg/L *vs* 2.99±0.53 μg/L, $P < 0.01$) in patients who showed deterioration than in those who showed good responses to the therapies (2.67 ± 0.34 μg/L *vs* 2.98 ± 0.29 μg/L); no difference was seen before the use of chemotherapy (see Table 3).

DISCUSSION

The sTNF-RI level is reportedly increased significantly in a large proportion of patients with hepatoma, and the increment correlates well with the staging of the disease, but the mechanism involved as well as its functional implication remained unknown[7]. We had previously found elevation of the soluble IL-2 receptor in cancer patients[8,9] and speculated that the observed excess of TNF receptors in the serum of cancer patients were, at least in part, produced by the tumor cells. Some authors[10] reported that various tumor-derived cell lines could produce the soluble TNF receptors spontaneously in culture. This finding supports our speculation. But, there is still much diversity among the ideas related to the presumption that the receptors could be produced by a normal cell population in response to the tumor process, which represents an alternative possibility. Furthermore, this suggests that the two possibilities are not mutually exclusive. The excess of soluble receptors reflects an over-production, both by the malignant cells and by the normal cells, and this process may be related to the immune system. There is evidence from *in vitro* studies that indicates the tumor cells have a greater tendency than the normal cells to shed the soluble form of their cell surface proteins, apparently as a result of enhancing the cleavages of the cell surface

molecules[7]. This process is similar to that seen in cases of sIL-2R[8].

It has been shown that sTNF-R can compete with the cell surface receptor for TNF, thereby interfering with its cytokine function[7]. TNF can have a destructive effect on tumor cells, as shown in some experimental cancer models[11]; as such, the elevated levels of sTNF-R in cancer patients may represent a tumor "escape" mechanism from the suppressive effect of sTNF-R. It is possible that the increased binding of receptors to other cytokines in the sera of cancer patients may contribute to the general suppression of certain immunologic functions that has been observed in advanced cancer patients.

An increase in the level of serum sTNF-RI is certainly not a distinctive feature of cancer, since it also occurs in a variety of other inflammatory and autoimmune diseases[12]. Nevertheless, sTNF-RI might have a diagnostic value, since after exclusion of an inflammatory or autoimmune disease elevated levels of soluble TNF receptor may be suggestive of the presence of a malignant process. In our study, increased serum sTNF-RI was found in a cancer condition involving the gastrointestinal tract, other types of these cancers include gastric, pancreatic and colorectal. Such increased serum sTNF-RI has also been reported in cases of breast cancer[7]. The frequency of increased serum sTNF-RI in our current hepatoma patient population (90%) is similar to that of sIL-2R (85%) and also higher than that of serum AFP (54%), which is a common tumor marker of the hepatoma.

The present investigation seems to suggest that the clinical response to chemotherapy may be associated with normalization of the serum sTNF-RI level, whereas further increase would indicate deterioration. However, the patient series examined is as yet not large enough to draw a proper conclusion. Further investigation will be required to delineate the biological and prognostic significance for hepatoma patients. So far, there is no evidence that the type of therapy and the type of histologic pattern may have any impact on sTNF-RI release.

Our results suggest, however, that the serum sTNF-RI concentration in patients with hepatoma correlates well with the staging of the disease and also may serve as an aid for the detection of the cancers, for follow-up studies, and for assessments of prognoses.

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Design and performance of laparoscopic video biliary operations

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Abstract

AIM: To explore and enlarge the application range of laparoscopic video technology in biliary surgery.

METHODS: Since November 1992, 45 cases were treated with laparoscopic video choledocholithotomy T-tube drainage (LCTD) and 5 cases were treated with laparoscopic video choledochojejunostomy (LCJS). The indications and main technical procedures for these two technologies are discussed and presented here.

RESULTS: The LCTD procedure was successfully applied in all 45 cases. One patient with sustained biliary leakage and one patient with sustained residual choledocholith were completely cured. The LCJS procedure was also successfully applied to all 5 cases, and all the patients recovered in a short period without any complications.

CONCLUSION: The results show that LCTD and LCJS are characterized by minor trauma, less sufferings, fast postoperative recovery and good curative effect; these procedures are worth popularizing and extending their application. The laparoscopic video biliary operations that were designed and performed in this study are not only in conformity with the biliary surgical therapeutic principle, but also easy to conduct, economically beneficial, practical and convenient.

Key words: Laparoscopy; Drainage; Anastomosis, Roux-en-Y; Choledochostomy

Xu HB, Xiao YQ, Li WM, Li HC, Tu XQ. Design and performance of laparoscopic video biliary operations. *World J Gastroenterol* 1996; 2(2): 92-94 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i2/92.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i2.92

INTRODUCTION

Since November 1992, the application range of laparoscopic video technology in biliary surgery has been further explored and enlarged on the basis of successful application of the laparoscopic video cholecystectomy (LC). In our hospital, laparoscopic video biliary operations have been successfully applied, including laparoscopic video choledocholithotomy T-tube drainage (LCTD)[1] for 45 cases and laparoscopic video choledochojejunostomy (LCJS)[2] for 5 cases. The results, for all cases, have been satisfactory. Here, we present these cases and discuss the indications and main technical parameters of LCTD and LCJS.

MATERIALS AND METHODS

Surgical instruments

The instruments used for LCTD included a 25° wide-angle laparoscope (WOLF Co., Germany), an Olympus P-20 choledochofiberscope (Japan), and self-made common additional biliary appliances. The instruments used for LCJS included an Endo GIA, Endo Gauge, Endo Babcock, Endo Retractor and Endo Clip (ETHICON Co., United States), and LCTD instruments.

Anesthesia, posture and puncture

Under general anesthesia, the patient was placed in the semi-supination position at 20°, inclining to the left at 10°-15° and with a slight elevation of the right lumbar region. After the artificial pneumoperitoneum was established, four trocars were inserted into the abdomen. The viewing incision was located in the lower margin of the umbilicus, and the main operational incision was located at 3 cm under the xiphoid and 3 cm to the right of midline; the two trocars were 1 cm in diameter each. The other two additional incisions were located in the right intercostal midclavicular line and the anterior axillary line; the two trocars were 0.5 cm in diameter each.

For LCJS, the anesthesia, posture and the first four incisions were the same as those described above for LCTD, but the diameter was 1 cm for all. In addition, an insertion incision for Endo GIA was added, located in the intermediate part of the straight muscle of the right upper abdomen, and this trocar had a diameter of 1.2 cm.

Methods

The cystic duct was first dissected and clipped with a titanium clip. The common bile duct (CBD) was exposed and the junction of the cystic duct and common hepatic duct (CHD) was confirmed by a puncture, after which it was incised (2 cm in length) using a sickle-type surgical knife. The choledochofiberscope was inserted into

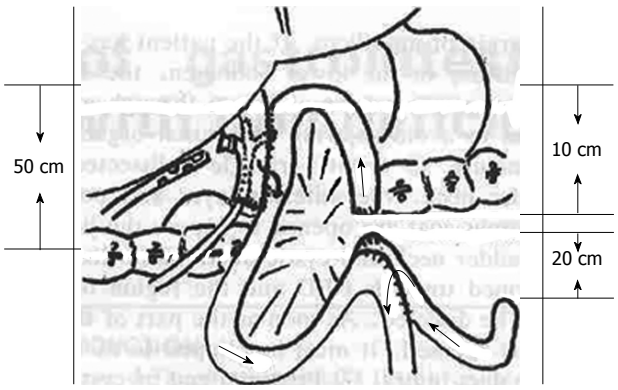

Figure 1 Choledochojejunostomy.

the CBD and the gallstones are removed with gallstone forceps and scoops; if the number of gallstones was < 3 (small and free), they were able to be removed with the lithotomy net of the choledochofiberscope. Next, No. 3-6 biliary tract bougies were passed through Oddi's muscle and a urinary catheter was inserted into the CBD for douching, after which the choledochofiberscope was re-inserted into the CBD to confirm the extraction of all gallstones. The two short arms of the T-tube were put into the CBD, the incision was closed with 1-3 stitches, and a tight knot was tied with a short thread or both the suture roots were clipped to suture the incision. The gallbladder itself was excised and removed through the umbilical incision. Finally, the long arm of the T-tube was pulled out through the incision in the midclavicular line, and a drainage tube was placed in Winslow's foramen and pulled out through the incision in the anterior axillary line. In case of biliogenic pancreatitis, the tube (used as a drainage tube or a perfusion tube) was inserted into the omental bursa through Winslow's foramen, after which the gastrocolic ligament was opened and the head of the other emulsion tube was placed in the tail region of the pancreas and the tail of the tube was pulled out through the other incision that had entered into the left upper abdomen for the postoperative lavage of the pancreas. According to the patient's condition, decompression gastrostomy and nutritional jejunostomy were performed under the video laparoscope guidance.

The anterior layer of the hepatoduodenal ligament was striped so that the duodenal posterior segment of the CBD was fully exposed and its inferior extremity was incised by 1 cm to remove the stones. The opposite mesangial margin of the jejunum, which was 30 cm from Treitz's ligament, was transected by 1 cm in length. An Endo GIA 30 was inserted upwards through the two small incisions in the CBD and jejunum in order to facilitate the precolonic choledochojejunostomy (CJS) at 3 cm; the two short arms of the T-tube were put into the CHD through the small incision made beneath the anastomotic stoma. The small incision was closed with a continuous whole layer inversion suture and an interrupted sero-muscular suture. A jejunal afferent loop, at 20 cm from Treitz' s ligament, was made by a 1 cm small incision, and the efferent loop, at 50 cm from the CJS, was also made by a 1 cm small incision. An Endo GIA 30 was inserted downwards through the two small incisions of the loops for the jejunal afferent-efferent loop anastomosis at 3 cm, near the anastomotic stoma. The afferent loop was severed with an Endo GIA 30 and, simultaneously, both severed ends were closed. The small incision was closed in the above-mentioned way. Like in LCTD, the gallbladder was excised and removed, with the T-tube and drainage tube being pulled out. The LCJS procedure is diagramed in Figure 1.

RESULTS

The LCTD procedure was successfully applied to all 45 cases. One patient with sustained biliary leakage and one patient with sustained residual choledocholith were cured completely. The LCJS procedure was successfully applied to all 5 cases, and all the patients recovered without any complications. Usually, gastrointestinal functions were restored within 24 h after the LCTD and LCJS procedures, at which time the patients could take food. The T-tube was extracted at 2-3 wk after the LCTD or LCJS procedure if the T-tube cholangiography did not show any abnormal results.

DISCUSSION

Surgery indications

LCTD: LCTD is suitable for patients with gallstones that are complicated with secondary choledocholith, especially for patients who have excess fat, are aged, or are complicated with diabetes (*i.e.* these patients usually cannot tolerate a major surgical operation), or for patients with primary or secondary choledocholith that is complicated with acute cholangitis or biliogenic pancreatitis (*i.e.* these patients are considered high risk and cannot tolerate major operations but are operated on under emergency conditions). Simple choledocholithotomy with T-tube drainage for primary choledocholith usually yields a poor result, since biliary stones can be easily missed and leading to high risk of recurrence; these patients should be treated with LCJS. However, in cases of severe cholangitis or acute hemorrhagic necrotic pancreatitis, if patients present with shock and diffuse peritonitis, they should be treated with routine surgical operations.

LCJS: LCJS is suitable for patients with primary choledocholith or hepatolith, but who present without stricture of the intrahepatic duct and with a CBD diameter > 1.5 cm, for patients with benign distal obstruction of CBD with co-existence of pancreatitis, cholangitis or duodentitis, or for patients with malignant obstruction and late stage cancer who are in need of palliative treatment of jaundice. However, in cases of stricture of intrahepatic duct or high-level malignant biliary obstruction, there is no indication for LCJS.

Main points for operations

LCTD: First, the viewing incision is located in the lower margin of the umbilicus. If the patient has an operative history involving the lower abdomen, the trocars must be inserted into the abdomen through an incision so as to avoid injuring abdominal organs by a blind puncture. Second, Calot's triangle is dissected from the gallbladder neck, after which the anterior layer and posterior layer of serous coat are opened to expose the junction of the gallbladder neck and cystic duct and blunt dissection is performed towards the CBD; the CBD region must not be dissected. As soon as the part of the cystic duct is exposed, it must be clipped so as to prevent bile duct injury. Third, the proximal end of the cystic duct is clipped with two titanium clips, the dissection of the cystic artery is not "skeletal-changed" so that the chances of creating cystic duct stump leakage and bleeding are decreased. Fourth, the CBD can be seen clearly after the lumbar region is raised. Fifth, the incision under the xiphoid is at the right midline, facilitating easy performance of LCTD. Sixth, if the gallstones have passed to the CBD during the operation, the cystic duct is first dissected and clipped with a titanium clip. For the time being, the cystic duct is not amputated and the gallbladder is not dissected either, so that traction of the gallbladder could push the hepatic margin up simultaneously and the gallbladder bed could be protected from early denuding with bleeding that would otherwise blur the operative field. Seventh, the sickle-type surgical knife blade must be used to incise the CBD, which can not only help to make the incised margins regular but also avoid injuring the posterior wall of the CBD. Eighth, lithotomy relies mainly on the common lithotomic apparatus, making the fiberscope subsidiary. When it is impossible to tie the two sutures of the incision into a tight knot, both of the suture roots should be clipped to close the incision; therefore, the operation is simple and economical, and will be easy to adopt more widely. Ninth, a thicker cystic duct is amputated by repeated clipping and cutting. Tenth, if the stones in the CBD are numerous, necessitating a longer lithotomy time, the lithotomy can be stopped and a T-tube can be put in. Three weeks after the LCTD, then, the lithotomy can be performed again with the fiberscope passing through the T-tube sinus. Eleventh, in cases of biliogenic pancreatitis, decompression gastrostomy and nutritional jejunostomy can be performed under the video laparoscope.

LCJS: First, the Endo GIA is inserted through the intermediate part of the straight muscle of the right upper abdomen, so that the LCJS could be performed easily. Second, at first the gallbladder is not excised, so that the traction of the gallbladder can help to

expose the CBD more clearly. Third, the CBD is dissected until the duodenal posterior segment and inferior extremity are incised, to gain access for removal of the stones. Fourth, precolonic ansiform CJS is much easier and simpler than the postcolonic Roux-en-Y CJS. Fifth, the anastomotic stoma of CJS is 30 cm from the Treitz' s ligament, and a T-tube is inserted into the CBD through the small incision beneath the anastomotic stoma. Sixth, the jejunal afferent-efferent loop anastomosis is performed. Located above the anastomotic stoma, the afferent loop is 10 cm and the efferent loop is 50 cm; after the afferent loop is severed, the ansiform CJS is nearly changed into a Roux-en-Y CJS, and development of infection can be avoided. Seventh, near the afferent-efferent loop anastomotic stoma, the afferent loop is severed so that a cecum cannot survive, thereby helping to avoid a secondary infection.

Laparoscopic video biliary operations which we have developed and performed are not only in conformity with the biliary surgical therapeutic principle, but also easy to operate, inexpensive, practical and convenient for performance. Our results show that LCTD and LCJS have the benefits of producing minimal trauma and less suffering, while providing a quick postoperative recovery and good curative effect. Therefore, we suggest that LCTD and LCJS be popularized and more widely applied in medical practice.

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Endoscopic evaluation of gastrointestinal tract lesions in patients with iron-deficiency anemia

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Abstract

AIM: To prospectively evaluate the relation of gastrointestinal lesions and iron-deficiency anemia (IDA) caused by gastrointestinal tract bleeding.

METHODS: Sixty-one patients with IDA caused by gastrointestinal tract bleeding were included in our study. Lesions were detected by esophagogastroduodenoscopy (EGD) and/or colonoscopy. Histories of upper gastrointestinal tract symptoms, including pyrosis, dysphagia, upper abdominal pain, dyspepsia, nausea and vomiting, and lower gastrointestinal tract symptoms such as changes in bowel habits, constipation, diarrhea and lower abdominal pain, were obtained from each patient. History of NSAID drugs intake was also obtained. Each patient underwent complete physical examination and fecal occult blood test (FOBT) of three spontaneously passed stools or of stool samples obtained by digital rectal examination. All the patients completed testing for complete blood count, total iron-binding capacity, and serum ferritin level. Some patients underwent bone marrow aspiration to examine the iron stores.

RESULTS: The 61 patients included 35 men and 26 women, with mean age of 53.6 years (range of 18-67 years). The mean hemoglobin level was 82-19 g/L and the mean ferritin level was 8.9 ± 4.8 μmol/L. In the 10 patients diagnosed with concomitant inflammatory conditions, the ferritin levels were lower, ranging between 1.8 μmol/L and 4.2 μmol/L, and the patients showed an elevated white blood cell count. The mean transferritin saturation was 6.8% ± 4.2%. Bone marrow aspiration was performed on 5 patients to confirm IDA. The upper endoscopic findings of 43 patients with IDA included gastric erosions (n = 10), esophagitis (n = 8), gastric cancer (n = 6), gastric ulcer (n = 8), duodenal ulcer (n = 9), gastric polyps (n = 1) and esophageal cancer (n = 1). Colonoscopic findings of 18 patients with IDA included colon cancer (n = 2), colon polyp (n = 6), ulcerative colitis (n = 9), and Crohn's colitis (n = 1). Five of the total 61 (8.2%) patients had lesions in both the upper and lower gastrointestinal tract that met our criteria for potentially causing IDA.

CONCLUSION: Combined application of EGD and colonoscopy is able to identify potential bleeding sources in most patients with IDA.

Key words: Anemia, iron-deficiency; Gastrointestinal hemorrhage; Endoscopy

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INTRODUCTION

Iron-deficiency anemia (IDA) caused by gastrointestinal tract bleeding very commonly occurs in 3.5%-5.3% of adult men and postmenopausal women[1], and in 7% of hospitalized geriatric in-patients[2]. Among patients over 65 years old with anemia, 36%-70% have IDA[3]. Gastrointestinal tract pathology has proven that IDA patients account for 27%-95%[1,4]. Studies that have overestimated the prevalence of gastrointestinal tract pathologies causing IDA have reported lesions such as esophageal varices, prior gastric surgery, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) without documented gastropathy, all of which cannot actually cause IDA[1,4-6]. In contrast, other studies may have underestimated the prevalence according to use of findings from primarily radiologic evaluation or incomplete upper and lower gastrointestinal tract examinations[1,4]. Furthermore, still other studies have evaluated patients with positive fecal occult blood test (FOBT) with or without IDA[2] or have primarily evaluated the upper gastrointestinal tract lesions in 21%-59% of patients with all types of anemia including IDA, reporting incidence of upper gastrointestinal tract lesions in 21%-59% of the patients[2,7-9]. In a recent prospective study by Rockey and Cello[6], all 100 patients with IDA showed negative endoscopic results; however, since the authors did not obtain small bowel biopsies they were unable to exclude celiac disease.

Therefore, the aim of our study was to prospectively evaluate the gastrointestinal tract pathology in patients with IDA using

Table 1 Upper GI endoscopic findings of 43 patients with iron-deficiency anemia

Lesion	Patients, *n*
Gastric erosions	10
Esophagitis	8
Gastric cancer	6
Gastric ulcer	8
Duodenal ulcer	9
Gastric polyps	1
Esophageal cancer	1
Total lesions	43

Table 2 Colonoscopic findings of 18 patients with iron-deficiency anemia

Lesion	Patients, *n*
Colon cancer	2
Colon polyp	6
Ulcerative colitis	9
Crohn's colitis	1
Total lesions	18

Table 3 Findings of 5 patients with both upper and lower gastrointestinal tract lesions and iron-deficiency anemia

Patient	Upper gastrointestinal tract lesion	Lower gastrointestinal tract lesion
1	Esophagitis	Ulcerative colitis
2	Esophagitis	Cancer
3	Duodenal ulcer	Colon polyp
4	Duodenal ulcer	Cancer
5	Gastric cancer	Colon polyp

a combination of esophagogastroduodenoscopy (EGD) and colonoscopy.

MATERIALS AND METHODS

All of the patients included in this study had a history of upper gastrointestinal tract symptoms, including pyrosis, dysphagia, upper abdominal pain, dyspepsia, nausea and vomiting, and/or lower gastrointestinal tract symptoms such as changes in bowel habits, constipation, diarrhea, and lower abdominal pain. History taking included use of NSAIDs. Each study participant underwent complete physical examination and FOBT of three spontaneously passed stools or of a stool sample obtained by digital rectal examination. All the patients underwent testing to determine complete blood cell counts, total iron-binding capacity, and serum ferritin level. Some patients underwent bone marrow aspiration to examine iron stores.

Anemia was defined as hemoglobin (Hb) levels of < 120 g/L in men and < 110 g/L in women, according to the World Health Organization criteria combined with the general conditions (affecting anemia) particular to populations of the Lanzhou city region. Specifically, IDA was diagnosed according to absence of detectable (stainable) iron in the bone marrow aspirates or a combination of concentration of serum ferritin at < 13 μmol/L (below the normal range of 13-31 μmol/L), of serum iron at < 45 μmol/L (below the normal range of 45-73 μmol/L) and of transferritin saturation at < 20% (below the normal range of 20%-55%). Ferritin is an accepted marker of iron stores, with levels of 1.8-3.6 μmol/L indicating iron deficiency[3,7]. Patients with IDA and inflammatory conditions present with serum ferritin levels elevated to 8.7-10.4 μmol/L[7]. Therefore, in this study, in patients with inflammatory conditions, serum ferritin < 8.7 μmol/L in association with red blood cell mean corpuscular volume of < 80% and transferritin saturation of < 10% was considered diagnostic of IDA.

Patients were excluded from our study based upon the following: previous evaluation for IDA; women with symptoms of premenopause; current diagnosis of gastrointestinal cancer, hemoglobinopathy or renal failure; recent experience of surgery, trauma or acute gastrointestinal bleeding; any previous gastric surgery. For this study, the following lesions were considered as potential causes of IDA: EGA-detected lesions of esophagitis with multiple linear erosions and/or ulcers in the distal 2 cm or more of the esophagus with or without active bleeding, gastritic erosions defined as breaks in mucosa covered with thick exudates of 2 mm and 5 mm, gastric ulcer or duodenal ulcer > 10 mm, esophageal cancer, gastric cancer, polyps > 1 cm; colonoscopy-detected lesions of colorectal cancer, polyps > 1 cm, bleeding hemorrhoids defined by 15-30 mL of blood loss for an average of three times a week for over 6 mo.

From 1991 to 1994, 61 of a total of 73 patients with IDA who underwent EGD and/or colonoscopy met the above-mentioned criteria for study inclusion. The IDA treatment administered (endoscopy-based) was safe and effective for most of these patients. The treating hospital was dependent on EGD or colonoscopy for management of IDA patients for the study years based upon the demonstrated safety and cost benefits.

RESULTS

A total of 73 patients showed gastrointestinal tract lesions under endoscopy during our study period. Sixty-one (83.6%) of those 73 patients showed lesions endoscopically that were considered responsible for their IDA according to our criteria described above. However, 12 (16.4%) of the total 73 patients showed endoscopic lesions that did not meet our criteria, resulting in those lesions not being considered as causative of the patient's IDA. All the 61 patients (35 men and 26 women) with gastrointestinal tract bleeding associated with IDA were included in the study. The mean age was 53.6 years, with a range encompassed by 18.67 years. The mean Hb was 82 ± 19 g/L ($\bar{x}$ ± s). The mean ferritin was 8.9 ± 4.8 μmol/L. In the 10 patients who presented with inflammatory conditions, the ferritin was lower, ranging from 1.8-4.2 μmol/L, and they showed increased white blood cell count and decreased mean corpuscular volume. The mean transferritin saturation was 6.8% ± 4.2%. Bone marrow aspiration was performed to confirm IDA in 5 patients.

EGD showed potentially bleeding lesions in 43 of the total 61 (70.5%) study participants (Table 1). Gastric erosions were the most common lesions found in the upper gastrointestinal tract, accounting for 10 of 43 (23.5%) of the detected lesions, followed by erosive and/or peptic ulcers, which were found in 17 of the 43 (39.5%) lesions. Only one patient with Crohn's disease showed signs of terminal ileal Crohn's disease by colonoscopy.

Colonoscopy showed potentially bleeding lesions in 18 of the 61 (29.5%) study participants (Table 2). Ulcerative colitis was the most common lesion. The location of cancers was rectum for 2 patients (both, Duke's stage B1). Three patients with internal hemorrhoids were bleeding more than 60 mL of blood over three times a week for more than 6 mo. Five (8.2%) of the total 61 patients had lesions in both upper and lower gastrointestinal tracts that met our criteria for potentially causing IDA. In these patients, either the upper or the lower gastrointestinal tract lesions, or both, were considered as having the ability to lead to IDA-causative bleeding. The endoscopic findings of these patients are summarized in Table 3.

Symptomology among the 61 study participants included upper gastrointestinal tract symptoms (n = 30), lower gastrointestinal tract symptoms (n = 14), and combined upper and lower symptoms (n = 5). All of the patients with upper and/or lower gastrointestinal tract symptoms showed upper and/or lower gastrointestinal tract lesions upon examination, but only 27 (44.3%) had positive results on FOBT [the res, 34 (55.7%), had negative results on FOBT]. There was no significant difference between the FOBT-positive group of patients and the FOBT-negative group of patients in regard to the location of the detected IDA-causative lesions (P > 0.05). The sensitivity and specificity of FOBT for detecting a, IDA-causative lesion was 49.8% and 61.2% respectively, and the positive predictive value was 75.2%. The sensitivity, specificity and positive predictive value of FOBT for detecting colorectal neoplasms (polyps as well as cancers) were 61.6%, 61.6% and 56.6% respectively. FOBT gave positive results for 6 of the 9 patients with gastrointestinal tract cancers. FOBT gave positive results for 4 patients with colon cancer, but negative results for 3 patients with gastric cancer.

Fourteen (22.9%) patients took NSAIDs on a regular basis, and

13 patients had upper or lower gastrointestinal tract lesions that were potentially caused by the NSAID use and included esophagitis, gastric erosions, gastric ulcer, duodenal ulcer, and colon lesions. Forty-seven (77%) patients were not taking NSAIDs and had lesions associated with IDA. Forty-six patients participated in follow-up study (15 patients did not participate in follow-up), and IDA was cured in 29 of the 46 followed-up patients.

DISCUSSION

IDA is believed to result from chronic gastrointestinal tract blood loss. The reported prevalence of gastrointestinal tract lesions in patients with IDA has varied significantly from study to study. This general discrepancy may be due to incomplete evaluation of both upper and lower gastrointestinal tracts, the retrospective nature of the studies, inclusion of radiologic evaluation instead of endoscopic evaluation, a heterogenous patient population and their distinctive (but un-analyzed) characteristics, and/or lack of precise definition of the IDA-causative lesions[4,9]. In this study, we included lesions that have been well-described in the literature as resulting in chronic or intermittent blood loss that is causative of IDA[6]. We included mucosal lesions that lead to bleeding, such as esophagitis, gastritis, gastric erosions, and gastric and duodenal ulcers[6]; the latter were the most common IDA-causing lesions detected in our patients by both EGD and colonoscopy, similar to the findings by Rockey and Collo[6]. IDA, however, may be the initial presentation of gastrointestinal tract cancer[10]. In our study, diagnosis of cancer (by the gastrointestinal endoscopic evaluations) was made in 8 of the patients, representing a percentage (13.1%) of the study population that is similar to that reported previously (6%-28%)[1]. In particular, Rockey and Cello[6] reported finding 12 cancers in a prospective evaluation of 100 patients with IDA.

Zoli *et al*[11] investigated whether serum transferrin receptors (sTfRs) were affected by rheumatoid arthritis (RA), in order to verify a possible relationship with the degree of anemia and with the severity of the inflammatory disease. The authors reported that, in patients with RA, sTfR levels were significantly higher than those in the normal control group but lower than those in iron-deficient anemic patients, and that this feature correlated positively with erythrocyte sedimentation rate and IL-1beta and negatively with Hb. Further analysis was conducted with anemic patients with RA divided into two groups, with group A (56%) having a possible iron deficiency (total serum iron < 2.7 and ferritin < 8.7 μmol/L) and group B having no observable iron deficiency (total serum iron > 16 and ferritin > 8.7 μmol/L). No significant difference in sTfR was observed between the two groups. Thus, sTfR was elevated and related to the degree of anemia and to the inflammatory process in RA. The authors concluded that reduced sTfR levels in patients with RA, as compared with patients with iron-deficiency anemia, may indicate a reduced erythropoietic activity in RA. Indeed, it has been reported that the bioavailability of iron from a habitually consumed diet can affect this process; specifically, a study compared the diets of pre-adolescent children of low socio-economic status with supplementation of an additional 8 g of protein supplied by 34 g of legumes (Green gram and Moth bean ratio of 1:1) and of anemic rats with supplementation of 195 mL of buffalo's milk. In the latter, the bioavailability of iron for hemoglobin regeneration relative to $FeSO_4$ was 69.5%, as compared with 47.5% in the former; the legume supplementation showed no improvements for the pre-adolescent children of low socio-economic status[12].

Anemia is usually difficult to correct in kidney transplant recipients, likely because of an iron deficient or inadequate erythropoietin (Epo) production status post-transplantation. Moore *et al*[13] evaluated the effects of iron (Fe) availability on correction of anemia in renal transplant recipients and sought to characterize patterns of early Epo production by transplanted kidneys as related to peri-transplant factors. In a prospective randomized trial, 51 consecutive renal transplant patients were followed-up for 6 mo. Epo was measured on days 0, 3, 14, 48 and 168 post-transplantation. Fe status was monitored on days 14, 48 and 168. Patients were randomized on day 14 based on Fe status. Iron-deficient (FeD) patients (n = 24) were randomized to receive daily Fe supplementation (FeDs; n = 12) or no supplementation (FeDns; n = 12). Those with normal Fe status (FeN; n = 27) were followed as controls. No differences were found between groups on day 0 for hematocrit, creatine, Epo, age, dialysis history, or type of donor. On day 3, the creatine and hematocrit levels were similar among the groups, while only Epo was significantly higher in the FeD group (*vs* FeN, $P < 0.004$); this higher level persisted to the end of the study. Although the hematocrit improved in each patient over the study period, most of the FeDs and FeN group members were anemic and Fe deficient at the end of 6 mo; all FEDs patients, however, had corrected their anemia ($P < 0.009$) and Fe status by study end. Four FeDs patients developed polycythemia. Epo production correlated inversely to cold ischemia time in cadaver renal allografts ($P < 0.008$). One-hundred-and-fourteen female pediatric patients (aged 11-14 years) from Wembley, Middlesex, were assessed for Fe status (*via* Hb), packed cell volume, mean corpuscular Hb concentration, height, weight, eating habits and ethnic origin, and undertook a step test to assess physical performance. Twenty-percent of the total of girls had Hb < 120 g/L, ranging from 11% in girls of white ethnicity to 22%-25% in girls of Asian origin. Prevalence of low Hb was 20% in vegetarians, being higher in white vegetarians compared with non-vegetarians (23% *vs* 4%) but lower in Indian vegetarians compared with non-vegetarians (17% *vs* 32%). Low Hb was present in 25% of girls who had tried to lose weight in previous years, and was more common in girls from manual laborer social class status than non-manual laborer status (24% *vs* 10%). At the start of the step test, 23 girls with low Hb had heart rates similar to those with normal Hb, but heart rates in the low Hb group were significantly elevated immediately after the step test, and the rate was still significantly elevated 1 min later. The results from the present study agree with the findings of this previous study in girls of white ethnicity, and suggest that physical performance may be compromised at mild levels of anemia[14].

Gastrointestinal bleeding is believed to cause IDA. Kepezyk *et al*[5] reported on 70 patients with IDA that underwent EGD and colonoscopy; patients with unremarkable findings by these two methods then underwent small bowel biopsy by EGD to determine the presence of celiac disease. Enteroclysis was performed if this further evaluation was negative. Endoscopic results showed that at least one lesion potentially accounted for the IDA in 50 (71%) of the patients. Colonoscopic results showed that 21 (30%) of the patients had 22 lesions (including 4 cases of colon cancer, 7 of adenoma > 1 cm, 6 of vascular malformation, 4 of severely bleeding hemorrhoids, and 1 of ileal Crohn's disease). At EGD, 39 (56%) of the patients were found to have 43 lesions, including 11 of gastric erosion, 10 of esophagitis, 4 of vascular malformation, 4 of celiac disease, 3 of gastric cancer, 3 of gastric ulcer, 3 of duodenal ulcer, 2 of gastric polyp > 1 cm, 1 of duodenal lymphoma, 1 of esophageal cancer, and 1 of duodenal Crohn's disease. Twelve (17%) of the patients had both upper and lower gastrointestinal tract lesions. Twenty-four (75%) of the 32 patients with positive FOBT results had potentially bleeding lesions, as compared with 24 (63%) of the 38 patients with negative FOBT results ($P > 0.05$). Six of the 9 patients with malignancy had positive FOBT. Twenty patients with normal endoscopic findings and who underwent small bowel biopsy had normal enteroclysis results. It is thus concluded that the combination of colonoscopy and EGD identifies potential bleeding sources in most patients with IDA. In the absence of a potentially bleeding lesion, small bowel biopsy during EDG is essential to diagnose celiac disease.

Aspirin or NSAIDs can cause esophagogastroduodenopathy, which itself has been associated with an increased frequency of IDA. However, our study shows that the prevalence of endoscopic findings causing IDA is not similar among patients who have taken NSAIDs and those who have not (22.9% *vs* 77.1%). In contrast, Rockey and Cello[6] found no correlation between the use of NSAIDs and lesions in the upper gastrointestinal tract. In our study, some of the patients with NSAIDs-related upper gastrointestinal tract lesions also had colonic lesions that were determined to be the actual IDA-causing lesions. These findings indicate that evaluation of IDA patients taking NSAIDs should not be limited to the upper gastrointestinal tract only. It is possible that many patients who

have ingested NSAIDs may not voluntarily supply the information concerning their history of taking NSAIDs. This possibility was clearly shown to be true in a recent study by Lanas *et al*[15], wherein the objective evidence of platelet cyclooxygenase inhibition revealed a large number of patients with acute upper and lower gastrointestinal bleeding who failed to report use of aspirin or NSAIDs. It is also possible that in some of the patients, the NSAID-unrelated lesions in elderly patients, either in the upper gastrointestinal tract or the lower gastrointestinal tract, may bleed acutely or chronically due to antiplatelet aggregation properties of the NSAIDs or aspirin[5,16]. Finally, some patients with IDA may have small bowel lesions induced by NSAIDs that may lead to bleeding chronically.

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Experience in diagnosis and treatment of 119 patients with traumatic visceral rupture

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Abstract

AIM: To summarize our experience in diagnosis and treatment of 119 patients with traumatic visceral rupture.

METHODS: One-hundred-and-twenty-two patients (21 women and 101 men, with an average age of 44.75 years) were studied. The causes of injury included bullet wound, stabbing wound, falling from building, traffic accident, *etc*. First, more than 2 intravenous transfusion pathways were set up immediately, and pre-operative preparations were made as quickly as possible. The patient was put immediately under monitoring in the ICU. Then, emergency explorative surgery was carried out to stop the bleeding and repair the ruptured viscera. In 20 patients with severe illness, the combined therapy of "Four High Doses in Large Volume and One Support" (FHDOS) was used, consisting of a short delivery period of high doses of anisodaminum in large volumes, a short delivery period of high doses of dexamethosone in large volumes, high doses of antibiotics in large volumes, high doses of abdominal cavity washing liquid in large volumes, and one nutritional support.

RESULTS: One-hundred-and-nineteen patients recovered after the active treatment and 3 patients died. The mortality rate was 2.5%.

CONCLUSION: Diagnosis and treatment should be quick, decisive, correct and rational. It is imperative to keep the respiratory tract unobstructed and to treat shock. Abdominal explorative surgery should be carried out to stop bleeding and repair ruptured viscera simultaneously. Efforts should be made to avoid misdiagnosis and ICU monitoring provides significant benefit to the patient. The new treatment method of FHDOS is simple, practical and effective for critically ill patients, and it plays a key role in treating multiple organ failure.

Key words: Traumatic visceral rupture/diagnosis; Shock; Traumatic visceral rupture/therapy

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INTRODUCTION

Visceral rupture following trauma is one of the most common acute abdominal illnesses encountered in general surgery, with most patients being in a complicated and critical condition and near death. Therefore, it is necessary to choose a simple and reliable method of examination that will be completed in a short period of time and provide a clear diagnosis; it is also necessary to choose the optimum time for surgery and to apply a rational operative approach as well as the right postoperative measures for recovery. The following is a report on the diagnosis and treatment of 122 patients with visceral rupture following trauma that were treated in our department over the past 10 years. Of these patients, 119 were cured by the treatment and 3 died. The mortality rate was 2.5%.

MATERIALS AND METHODS

Patients

The patient population included 99 men and 20 women, ranging in age from 42 years-old to 75 years-old. The causes of injury included bullet wound, stabbing wound, falling from building, traffic accident, *etc*. The cases of ruptured viscera involved liver (n = 21), spleen (n = 50), small intestine (n = 19), colon (n = 9), stomach (n = 10), duodenum (n = 3), pancreas (n = 7), and multiple organs (n = 51).

For the operation time and anesthetic methods used, 79 patients were operated upon within 30 min of the cause of injury, 29 between 30 min and 3 h, and 11 patients at 3 h later. Fifty-nine patients were treated under general anesthesia with trachea incubation, and all others were treated under epidural anesthesia.

For the complications, 3 patients developed infection at the incision site, 1 patient had an intestinal obstruction, 1 patient experienced a disruption at the incision site, and 1 patient had multiple intraperitoneal abscesses.

Methods

In each case, we first opened at least two intravenous passages immediately, one of which was in the upper limb. Preoperative preparations were made quickly, while the patients were under ICU monitoring[1]. The emergency abdominal explorative surgery was performed with the primary aims of stopping the bleeding and

repairing the ruptured viscera.

For patients classified as being in very severe condition, especially those with multiple organ dysfunction (MOD), we applied the combined therapy of "four high doses in large volume and one support" (FHDOS). This treatment was carried out as follows. First, a short period of high dose anisodaminum was delivered in large volumes as an intravenous injection of 40 mg once, followed by another 40 mg at 30 min later according to patient's condition; the total amount was allowed to reach 120-240 mg a day or 40 mg by intravenous injection every 15 min until the patient's condition was under control. Second, a short period of high dose dexamethasone was delivered in large volumes as an intravenous injection of 100-200 mg once, followed by similar or decreasing doses every day for 1-3 d accordingly. Third, high doses of antibiotics were delivered in a large volume; we selected and used antibiotics according to the clinical condition and the type of possible bacterial pathogens, which was then adjusted according to the drug sensitivity tests for the bacteria. In addition, we always paid attention to the prevention and treatment of dual or mixed infections. Fourth, high doses of abdominal cavity washing liquid were delivered in large volume; in general, we cleaned the abdominal cavity with 6000-8000 mL of normal saline. Fifth, the "one support" portion of the treatment was applied as full nutritional support (TPN and TEN)[2].

RESULTS

One-hundred-and-nineteen patients were cured successfully, and 3 patients died. The mortality rate was 2.5%.

DISCUSSION

In this study group, 92 patients (77.3%) were in shock on admission. Abdominal punctures were carried out in 94 of the patients (97.9%) and positive results were obtained for 92. Of the 81 patients who underwent B-ultrasound examination, 79 showed positive results.

Conditions of such patients are usually desperate and critical. The following emergency management procedures should be organized and applied with all-out effort of the critical care team. First, the surgeon should immediately open more than 2 venous passages, one of which should be in the upper limb. Second, the team should make pre-operative preparations as quickly as possible and immediately put the patients under ICU monitoring. Third, an emergency explorative laparotomy should be conducted to stop bleeding and repair the ruptured viscera. Fourth, for patients with co-existing craniocerebral injury (such as the 9 in our study), the emergency abdominal explorative surgery should be carried out first. However, it is necessary to simultaneously open the cranium for decompression and evacuation of intracranial hematoma, if present. Fifth, bleeding from a rupture of the large intracelial or external blood vessels and viscera rupture endangers the patient' s life most; the amount of this type of bleeding in the 39 patients in our study was 2000 mL to 5100 mL.

ICU monitoring is of significance for patients suffering from viscera rupture with mixed injuries.

Ruptures of the gastrointestinal tract occurred in 42 patients in our study population. Intra-abdominal infection and surgical wound infection should be prevented by washing with a large volume of saline.

In summary, the diagnosis and treatment of these cases should be quick, decisive, correct and rational. It is necessary to keep the respiratory tract unobstructed and to combat shock while simultaneously performing abdominal explorative surgery to stop the bleeding and repair the ruptured viscera. It is critical to avoid misdiagnosis, and patients in critical condition will benefit from ICU monitoring and treatment by FHDOS[3]. Ultimately, by intensifying the level care and correcting the shock condition, it is possible to prevent and rationally treat complications and life-threatening multiple organ failure.

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Diagnosis and therapy of esophageal perforation in the aged

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Abstract

AIM: To study the diagnosis and therapy of esophageal perforation in the aged.

METHODS: Three-hundred-and-thirty-five elderly patients undergoing endoscopy or therapeutic esophageal dilation in our hospital between July 1988 and August 1995 were studied retrospectively. Of these patients, 31 had esophageal perforation, 17 of whom were treated nonsurgically (nasogastric drainage, antibiotics, and intravenous alimentation), 8 underwent total esophagectomy, and 6 received surgical drainage.

RESULTS: Sixteen of 31 patients with esophageal perforation were cured. Of the patients in whom the perforation healed, 9 had persistent dysphagia while swallowing solid foods. Six patients died. The cure rate was 51.6% and the mortality rate was 19.4%.

CONCLUSION: Aged people are subject to esophageal perforation. The main typical symptoms of esophageal perforation are chest pain, shortness of breath and dysphagia; It is not difficult to make a correct diagnosis, but early diagnosis and treatment are crucial to the saving the patient's life.

Key words: Esophageal perforation/etiology; Esophageal perforation/surgery; Esophageal perforation/diagnosis; Esophageal perforation/therapy

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INTRODUCTION

Esophageal perforation is a serious complication of instrumental examination of the esophagus and also occurs with increased luminal pressure (barogenic perforation) that usually results from vomiting (Boerhaave's syndrome)[1]. With advances in antibiotic therapy and a heightened awareness of this complication, patients receiving early treatment may survive and many of them can be cured.

MATERIALS AND METHODS

Patients

Thirty-one patients (23 men and 8 women; mean age: 68±4 years, range: 60-79 years) underwent endoscopy or therapeutic esophageal dilation in our hospital between July 1988 and August 1995.

Etiology

In 24 patients, esophageal perforation occurred after instrumental examination, including 14 patients undergoing dilation for benign esophageal stricture (13 chronic strictures caused by reflux esophagitis and 1 radiative stricture). In the 14 patients, the perforation occurred in 6 after dilation with Savary dilators performed under fluoroscopic guidance, and in 6 after dilation with Maloney dilators that was completed without fluoroscopy. Among the latter 6 cases, 1 perforation occurred and enlarged after endoscopic balloon dilation for benign esophageal stricture, 1 occurred after pneumatic dilation for achalasia, 1 occurred as a complication of sclerotherapy, and 3 were caused by attempts of intubation (1 by a rigid esophagoscope, 1 by endotracheal intubation, and 1 by endoscopic retrograde cholangiopancreatography with a flexible endoscope for a Zenker's diverticulum). The last one was the only case of perforation by diagnostic flexible endoscope during this period. Perforation occurred in 6 patients after emergency gastroscopy for stomachache, in 5 after vomiting (barogenic perforation), in 1 after blunt trauma to the chest and in 1 without any definite causes (spontaneous perforation).

Symptoms

The main clinical symptoms in esophageal perforation were chest pain, dysphagia (unable to take food *via* mouth) and shortness of breath. Chest pain was the most frequent symptom, and was noted in 28 of the 31 cases (90.3%). Twelve patients (38.7%) had dysphagia and 6 (19.4%) complained of shortness of breath.

Diagnosis

Chest radiography was performed in 22 of the 31 patients and revealed mediastinal emphysema in 2; twenty cases were diagnosed as perforation of the esophagus by contrast study. Results of barium swallow studies were initially normal in 2 cases but a repeated

Table 1 Causes of perforation and means of treatment

Cause	*n*	Nonsurgical therapy	Surgical drainage	Esophagectomy
Esophageal dilation	14	10	2	2
	3	1	1	1
Sclerotherapy	1	0	1	0
Gastroscopy	6	3	1	2
Blunt trauma	1	1	0	0
Barogenic	5	1	1	3
Spontaneous	1	1	0	0

Table 2 Means and results of therapy

Result	Nonsurgical therapy	Surgical drainage	Esophagectomy
Effective	9	2	5
Improved	6	3	0
Ineffective	2	1	3

examination several hours later showed the esophageal perforation. Another patient with a normal barium swallow showed mediastinal emphysema on the chest radiograph and did not undergo the contrast study. Three patients were diagnosed as having perforation of the esophagus by gastroscopy, and 1 perforation was recognized during the examination with rigid endoscopy. Of the 31 perforations, 21 occurred in the upper esophagus, 6 in the midesophagus, and 4 in the lower esophagus.

Criteria for treatment of perforation

The choice of therapy for perforation was based on clinical criteria. Patients were treated nonsurgically if they met the following criteria described by Cameron *et al*[2]: recently-developed perforation (within 24 h); no food intake after the perforation; perforation not proximal to a high-grade stenosis; minor symptoms (pain or dysphagia) without clinical signs of sepsis or hemodynamic compromise; perforation contained within the mediastinum without contamination of adjacent body cavities (*e.g.* pleural space); contrast studies showing a small perforation with good drainage of contrast material back into the esophagus.

Esophagectomy should be performed if longstanding suppuration exists at the periphery of the perforation or underlying diseases (*e.g.* mega-esophagus, neoplasm and chronic refractory stricture) lead to dysfunction of the esophagus, or even to closure of perforation. The operation must be decided based on the general condition of the patient. The diagnosis of esophageal perforation was established within 24 h in 25 patients. All of the patients with suspected perforation were initially treated with nasogastric aspiration and intravenous antibiotics. Seventeen patients were treated nonsurgically (nasogastric drainage, antibiotics, and intravenous alimentation) using the criteria mentioned above. Six patients underwent surgical drainage, and 8 patients underwent total esophagectomy (Table 1). Serious contaminations of the pleural space and mediastinitis were noted in 1 patient. Because of purulent mediastinitis, the exact location of the perforation could not be identified in 1 case. One patient could not be thoroughly treated owing to a distal esophageal stricture and a small ulcer at the top. Hyperalimentation therapy or feeding through a jejunostomy tube inserted during operation was performed in all the surgically treated patients.

RESULTS

The therapeutic efficiency was judged on the basis of the following clinical criteria. (1) Effective: the perforation healed without dysphagia. (2) Improved: the perforation closed with persistent dysphagia while swallowing solid food. (3) Ineffective: the patient died after therapy. Means and results of the therapy are shown in Table 2.

Sixteen patients were cured, among whom 9 received nonsurgical therapy, 2 surgical drainage, and 5 esophagectomy. Nine patients had closure of the perforation that was accompanied by persistent dysphagia while swallowing solid food; among these patients, 6 received nonsurgical therapy and 3 received surgical drainage. Six patients died in total, with 2 from massive hemorrhage from the large collateral vessels near the gastroesophageal junction during operation, 1 from postoperative sepsis at 2 wk after the esophagectomy, 1 from suffocation resulting from enlargement of the perforation, 1 from infection, and 1 from arrythmia during surgical drainage. The cure rate was 51.6% (16/31) and the mortality rate was 19.4% (6/31).

DISCUSSION

Esophageal perforation is an important complication of esophageal endoscopy and dilation, which commonly occurs in the aged. It has been reported that esophageal dilation is the most frequent cause of esophageal perforation[3-5], and that the most frequent site of perforation is the thoracic esophagus. In our study, however, perforation caused by fiberoptic endoscopy was seen in only 1 case, in which a high cervical perforation of a Zenker's diverticulum occurred during blind intubation for endoscopic retrograde cholangiopancreatography. The esophageal perforation rate due to diverticulum has been reported as 2% to 15%[6,7]. We performed endoscopy for 335 patients, and esophageal perforation only occurred in 1.8% (6/335). Ninety-seven patients were treated by esophageal dilation for benign stricture in our study, and the perforation rate was 14.4% (14/97). Barogenic perforation refers to esophageal disruption caused by an abrupt rise in esophageal pressure. In our study, acute vomiting was the most common cause of barogenic perforation in some healthy patients. One patient had non-barogenic spontaneous perforation. Pain of the chest, dysphagia, and shortness of breath were the most frequent symptoms. However, it is noteworthy that even in the cases of acute perforation after dilation, some patients did not have pain, and instead they complained of dysphagia on swallowing. It is important to recognize that perforation may occur within a short time after examination.

The temporary asymptomatic condition, perhaps, is due to edema at the site of a small perforation. Therefore, the patients should be presumptively treated only if a perforation is suspected. Foley *et al*[8] reported on 6 patients in whom water-soluble contrast media failure to display the perforation and subsequently received barium examination. Our study also demonstrated that perforation of the esophagus can be displayed with barium. Some experts have recommended that the initial contrast examination be performed with a water-soluble contrast agent, because it is thought to cause less irritation of the mediastinum. If the result is negative, barium meal examination should be followed[8]. Others recommended the use of thin barium as a selective agent[9]. There were two patients who each had an esophageal perforation that was confirmed by roentgenograms, but the results of the initial examination were negative. Thus, a repeated examination after several hours is important. Esophagoscopy can be used to identify the site of the perforation[3], but it is quite difficult to find the site of the perforation if a contrast agent is not applied before the endoscopic examination. In addition, insufflation of air may increase contamination of the mediastinum. Furthermore, some perforations may appear small, but they may again become open perforations requiring surgical treatment if medical treatment is given only.

Generally, the symptoms of perforation are very typical. The low mortality in our series of patients was in part related to early investigation of the patients with dysphagia, chest pain, or shortness of breath after dilation. The endoscopist often stands for conservative treatment for esophageal perforation caused by endoscopy, which may bungle the chance of surgical treatment. Delayed treatment of more than 24 h may cause more dangerous complications and led to a higher mortality rate. In our study, surgical treatment was immediately performed in the patients who did not meet the criteria for nonsurgical management (within 24 h in the majority of the cases), and no patient initially treated with

medical treatment required subsequent surgery.

The rates of mortality from esophageal perforation ranged from 7% to 47%[4,10]. In a collective review of 450 cases, Jones and Ginsberg[9] reported that the mortality of patients with iatrogenic or instrumental perforation was 19%, whereas those with spontaneous perforations had a mortality of 39%. Two other studies[1,12], however, showed no difference between spontaneous and instrumental perforations. In our study, the higher mortality in the surgical group was attributable to serious contamination of the mediastinum in those patients with large perforations, delayed diagnosis and life-threatening complication, which should have manifested symptoms in the course of medical treatment.

The primary closure of esophageal perforation has a poor outcome, particularly in patients with a distal esophageal stricture, and some surgeons recommended more definitive surgery for the stricture in early or late stage[3]. The second operation on patients with dysphagia must be determined according to the condition of each patient. Among the two patients undergoing primary closure in our hospital in 1987, perforation recurred in 1 patient and another had aggravated dysphagia (unable to take liquid); both patients underwent another esophagectomy. In the follow-up of our patients, we found that some patients refused surgery despite their persistent dysphagia, and a small number of the patients preferred surgical treatment to dilation. As a matter of fact, esophageal dilation is liable to lead to a perforation in the aged because of degenerative changes in the tissues of the esophagus. Early diagnosis is not difficult because of its typical symptoms, but esophageal dilation should be improved.

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Prevalence of *Helicobacter pylori* in cirrhotic patients with portal hypertensive gastropathy

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Abstract

AIM: To determine the relation between *Helicobacter pylori* infection and portal hypertensive gastropathy.

METHODS: Thirty-four patients diagnosed with portal hypertensive gastropathy according to the McCormack criteria were studied, and 136 age- and sex-matched individuals with chronic superficial gastritis served as controls. *H. pylori* infection was assessed by the rapid urease test.

RESULTS: *H. pylori* infection was confirmed in 19 of 34 (55.9%) patients and in 74 of 136 (54.4%) controls. There was no significant difference in *H. pylori* infection rates of the patients and controls ($P < 0.05$). The findings show that the presence of *H. pylori* was not closely associated with portal hypertensive gastropathy. In addition, there was no significant difference in *H. pylori* infection rates in patients sub-grouped by severity of gastropathy, duration of the disease and Child-Pugh classification of liver function ($P < 0.05$). The results suggest that factors other than *H. pylori* infection may be important in the pathogenesis of endoscopic changes.

CONCLUSION: The prevalence of *H. pylori* infection was not higher in portal hypertensive gastropathy patients than in controls with chronic superficial gastritis who were matched by sex and age. *H. pylori* infection is unrelated to the severity of endoscopic changes, duration of the disease and Child-Pugh classification of liver function. *H. pylori* infection is not the only essential factor for the development of portal hypertensive gastropathy.

Key words: *Helicobacter pylori*; Hypertension, portal; Stomach diseases; Helicobacter infections

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INTRODUCTION

Congestive gastropathy is a frequent endoscopic finding in patients with portal hypertension and is believed to be responsible for approximately 20% of episodes of bleeding in these patients. The pathogenesis of the disorder is, however, controversial. It has been reported that there is a high prevalence of *Helicobacter pylori* colonization in cases of portal hypertension, particularly in patients with gastric erosions[1]. In contrast, some other reports have shown no relation between *H. pylori* infection and portal hypertensive gastropathy[2]. The purpose of this study was to determine whether *H. pylori* infection is a necessary factor for the development of portal hypertensive gastropathy in Chinese patients and to further investigate the relationship between *H. pylori* infection and the severity of endoscopic changes, the disease duration and the Child-Pugh classification of cirrhosis.

MATERIALS AND METHODS

Patients

Thirty-four patients with portal hypertensive gastropathy were included in this study. These patients were represented by 31 males and 3 females, and their mean age was 47.0 ± 6.7 years-old (range: 25-67 years-old). The diagnosis was confirmed by endoscopy, according to the McCormack criteria. Liver function was graded according to the Child-Pugh criteria[3], and 12 patients were grade A, 11 were grade B, and 10 were grade C. The duration of the cirrhosis was < 4 years for 22 patients and > 4 years for 12 patients. The severity of endoscopic changes was graded according to the McCormack criteria, with 15 patients graded as mild (mosaic or snake skin appearance), 10 as moderate (erythema), and 9 as severe (erosion or hemorrhagic lesion).

Controls

Since, most epidemiological studies to date have shown an increasing prevalence of *H. pylori* with age, to eliminate the influence of age, we used case-control methods to choose patients with chronic superficial gastritis as controls. All controls were matched by age and sex.

Detection of H. pylori

All patients and controls underwent upper gastrointestinal endoscopy. Two antral biopsied specimens were taken for each case

Table 1 Factors associated with *H. pylori* infection in portal hypertensive gastropathy *n* (%)

Factor	*n*	*H. pylori* infection
Severity of endoscopic changes		
Mild	15	9 (60.0)
Moderate	10	6 (60.0)
Severe	9	4 (44.4)
Duration of the disease (a)		
≤ 4	22	12 (54.5)
> 4	12	7 (58.3)
Child-Pugh classification		
A	12	6 (50.0)
B	11	7 (63.6)
C	10	6 (60.0)

and assessed *via* the rapid urease test (CLO test; Shanghai Institute of Digestive Diseases). *H. pylori* infection was diagnosed if two specimens tested positive for *H. pylori*.

Data analysis and statistical methods

The prevalence of *H. pylori* infection was compared between patients and controls. The difference in the severity of endoscopic changes, disease duration and the Child-Pugh classification of cirrhosis was statistically analyzed between the *H. pylori*-positive and -negative patients by using the χ^2 test. Significance was set at $P < 0.05$.

RESULTS

H. pylori infection was confirmed by the rapid urease test for 19 of the 34 (55.9%) patients and for 74 of the 136 (54.4%) controls. There was no significant difference in *H. pylori* infection rate between patients and controls ($P > 0.05$), indicating that the presence of *H. pylori* was not closely associated with portal hypertension.

The relationship between *H. pylori* infection and the severity of endoscopic changes, duration of the disease and Child-Pugh liver function classification of cirrhosis is shown in Table 1. Similarly, there was no significant difference in the rate of *H. pylori* infection between the different grades of severity of gastropathy, duration of the disease and Child-Pugh liver function classification ($P > 0.05$). The findings further suggest that factors other than *H. pylori* may be important in the pathogenesis of the endoscopic changes.

DISCUSSION

There is a strong correlation between *H. pylori* infection and histologically-confirmed gastritis, duodenitis and duodenal ulceration; infection with the organism may also contribute to an increased risk of gastric carcinoma. Although the endoscopic appearance of portal hypertensive gastropathy is usually more noticeable in the fundus of the stomach[4], we choose antral biopsy specimens for this study so as to avoid bleeding and increase the detection rate of *H. pylori* colonization. We initially suspected that the colonization of *H. pylori* might be related to the endoscopic change. Our results do not, however, support this hypothesis, as we found no relation between *H. pylori* infection and portal hypertensive gastropathy. Our findings are similar to McCormack's[2]. The question of whether *H. pylori* infection should be treated when identified in patients with portal hypertensive gastropathy has not been evaluated. Since the presence of *H. pylori* is unrelated to the endoscopic appearance, we only treat the patients to improve their dyspeptic symptoms.

We feel that the most likely explanation for the pathogenesis of the endoscopic change in portal hypertensive gastropathy is that mucosal congestion may render the mucosa more prone to injury or reduce its capacity to repair injury. Animal experiments showed an increased susceptibility of the portal hypertensive mucosa to injury induced by agents such as bile acid, aspirin, alcohol, or *H. pylori*[5]. However, whether the results from this animal model can be generalized to explain human portal hypertensive gastropathy awaits further studies.

In summary, this study shows that the prevalence of *H. pylori* infection is not higher in portal hypertensive gastropathy patients than that in chronic superficial gastritis patients who were matched by age and that the *H. pylori* infection is unrelated to the severity of endoscopic changes, the duration of the disease and the Child-Pugh classification of liver function. *H. pylori* infection is not the only necessary factor for the development of portal hypertensive gastropathy.

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P53 and PCNA expression in glandular dilatation of gastric mucosa

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Abstract

AIM: To study the expression of p53 and PCNA in relation to gastric mucosa lesions.

METHODS: The S-P immunohistochemical method was used to observe 92 samples of glandular dilatation of gastric mucosa and 30 cases of adenocarcinoma of the stomach.

RESULTS: P53 showed no expression in simple glandular dilatation, but positive expression in a small number of cells in atypical glandular dilatation (< 8.6%, 5/58) as well as strong expression in gastric glandular cancer (< 46.7%, 14/30). There were a small number of PCNA positive cells in simple glandular dilatation, and a significant increase of positive cells in atypical glandular dilatation ($P < 0.01$); the positive cells were widely distributed and deeply stained in the cases of gastric cancer.

CONCLUSION: P53 and PCNA may be jointly used as an important criterion for diagnosing, classifying and treating the precancerous lesions of gastric mucosa.

Key words: Gastric mucosa; Precancerous conditions; Stomach neoplasms; Adenocarcinoma; Genes, P53

Wang YK, Ji XL, Gu YG, Zhang SC, Xiao JH. P53 and PCNA expression in glandular dilatation of gastric mucosa. *World J Gastroenterol* 1996; 2(2): 106-108 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i2/106.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i2.106

INTRODUCTION

In recent years, there has been much research attention paid to the glandular dilatation of gastric mucosa. It was proposed that some morphological forms of gastric glandular dilatation are related to gastric cancer, but other authors suggested that the cystic dilatation of various degrees in gastric and pyloric glands is only a kind of alteration that occurs in the late stage of atrophic gastritis[1-7]. The glandular dilatation of gastric mucosa is commonly seen in clinical pathology, so it is necessary to distinguish the types of glandular dilatation and determine the pathologic forms that are involved in the cancerous lesions. In this study, the S-P immunohistochemical method was used to perform a comparison between 92 samples of different histologic types of glandular dilatation and 30 cases of adenocarcinoma of the stomach. The results from assessment of p53 and PCNA expression will contribute to the clinical treatment and research of precancerous lesions.

MATERIALS AND METHODS

In total, 122 samples of gastric mucosa biopsy were observed. Among them, 92 were gastric mucosa glandular dilatation and 30 were adenocarcinoma of the stomach. The patients ranged in age from 25 to 74 years old, with an average age of 49.3 years. There were 72 males and 50 females. The samples were fixed in 10% formalin, embedded in paraffin, and sectioned to 5 μm in thickness. After standard hematoxylin-eosin (HE) staining, cell morphology and tissue structure were observed. Immunohistochemical staining was carried out by linking the streptomyces avidin and peroxidase agents. Anti-p53 monoclonal-antibodies (DO-7, reactive with both wild type and mutant proteins), anti-PCNA and instant-type reagent boxes were all the products of the Maixi Biochemical Technology Company. DAB coloration and hematoxylin staining were used, with PBS serving as the first antibody to make negative comparison. The positive reactions were graded according to the number of brown particles appearing in the cell nucleus: Ⅰ, 0%-25%; Ⅱ, 26%-50%; Ⅲ, 51%-75%; Ⅳ, 76%-100%.

The simple and heterotype dilatations were distinguishable; the latter included the cystadenomatoid and adenomatoid atypical hyperplasia, in accordance with the degree, form and number of the glandular dilatation, and the amount of mucus in the lumen of gland, the presence of atypical epithelioglandular cells, as well as the accompanying glandular atrophy and/or intestinal metaplasia, *etc*.

RESULTS

Histopathology

Simple glandular dilatation was found in a small number of cases with mild degree of focal or isolated glandular dilatation, showing an increased amount of mucus in the lumen of the gland. The epithelioglandular cells appeared as monolayer pavement or columnar in shape; there were no atypical alterations of the

Table 1 P53 and PCNA expression in 122 cases of gastric mucosa biopsy

Type	Number of cases	P53 expression					PCNA expression				
		-	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Positive cases	Ⅳ	Ⅰ	Ⅱ	Ⅲ
Simple dilatation	34	0	0	0	0	0	31 (91.2)	3 (8.8)	0	0	
Heterotype dilatation	27	25	2	0	0	0	2 (7.4)	4 (14.8)	14 (51.9)	9 (333)	0
Cystadenomatoid Adenomatoid	31	28	3	0	0	0	3 (9.7)	1 (3.2)	13 (41.9)	15 (48.4)	2 (6.5)
Glandular cancer	30	16	1	3	5	7	14 (46.7)	9	2 (6.7)	4 (13.3)	21 (70.0)

epithelium and no accompanying glandular atrophy or intestinal metaplasia. Atypical glandular dilatation showed cystadeno- and adenomatoid atypical hyperplasia.

The cystadeno-atypical proliferation showed extensive glandular hyperplasia in cystic dilatation, of various degrees and forms, with possibly papilla-like or branch-like structures, irregularly dilated glands, and large diameters of several- or dozens-times that of the ones with smaller diameters. Epithelial cells showed a monolayer or multilayer arrangement. Most atypical hyperplasia showed a decrease in amount of mucus in the lumen of the gland, accompanied by some degree of glandular atrophy in most cases and/or with intestinal metaplasia.

The adenomatoid atypical hyperplasia showed glands that were dilated to a moderate degree, 4- to 6-times that of the normal size, clustered in groups, and distributed in a back-to-back or conjugated pattern. There was a possible presence of intraglandular cannula[7]. Decreased interstitial and epithelioglandular cells in monolayer or stratified arrangement were noted, as was decreased lumen of the gland; there was also possibly accompanying glandular atrophy to various degrees, with or without intestinal metaplasia.

Immunohistochemical staining showed no p53 expression in simple glandular dilatation but positive expression (8.6%, 5/58) in heterotypical glandular dilatation. The pattern of positive expression demonstrated a focal expression or expression in some cells of some dilated glandular ducts, but no positive expression in many glandular ducts located in the same area; the staining density was weak grade Ⅰ positive expression. For the detected expression in adenocarcinoma, the positive expression was strong, widely distributed and with deep staining, pertaining to grade Ⅲ or grade Ⅳ. The PCNA positive expression in simple glandular dilatation was less extensive, mostly corresponding to grade Ⅰ, while that in heterotype dilatation involved obviously more cells, mostly corresponding to grade Ⅱ or grade Ⅲ, presenting a positive expression of many glandular ducts in one area. The positive expression of PCNA in adenocarcinoma was widely distributed, with rather deep staining, mostly corresponding to an expression of grade Ⅲ and grade Ⅳ (Table 1).

DISCUSSION

Glandular dilatation of gastric mucosa may be due to many diseases, such as chronic atrophic gastritis[8], gastric polyp[3], chronic ulcer[9], chronic superficial gastritis and the presence of cancerous gland[10], all of which may be observed characteristically in gastric mucosa biopsy. While it is not a primary disease itself, it presents with different degrees, forms and numbers. Hence, it has been suggested that the cystic dilatation of the gastric gland and pyloric gland in deep mucosa covered by the pavement of epithelial cells should be a sign of alteration of a denatured or atrophic gastritis in the advanced stage, and also that the cystic dilatations occurring in 95% of the early stage gastric cancers could be closely associated with the cancerous formation. Some reports have dealt with the naming of terms, such as cystadeno dilatation and gastric adenocyst[4,11]. We believe that in a case simply involving the cystic dilatation of glands and inflammatory infiltration, the dilated glands are small in number and mild in degree, the possible definition may be simple glandular dilatation caused by an inflammation or a compression in the ducts, not belonging to a precancerous lesion. However, in a case of cystic dilatation, inflammatory infiltration, and dilated glands with great numbers and severe degrees of various forms, with epithelioglandular cells in monolayer or stratified arrangement, and nuclei of various sizes, this kind of dilatation would be called the cystadeno-atypical hyperplasia because of its cystadeno-structure and atypical epithelium; this form is recognized as an important precancerous lesion. As for adenomatoid atypical hyperplasia, on which there had been few reports, the dilated glands are 4- to 6-times that of normal glands in number, cluster in groups, and show interstitial decrease, back-to-back or conjugation position, and epithelioglandular cells displaying atypical hyperplasia accompanied by glandular atrophy or intestinal metaplasia to various degrees. Because of its adenomatoid structure and epithelium atypical hyperplasia, this form is called adenomatoid atypical hyperplasia, another important feature of the precancerous lesion.

The wild type *p53* gene encodes a tumor suppressor and plays a role in control of cell differentiation and prevention of abnormal growth of cells. Because of its short and unstable half-life, and its low concentration in cells, it can not be detected by immunohistochemical examinations. The product of the mutant *p53* gene, however, is a more stable protein that accumulates in cells; its loss of normal function includes extension of its half-life, which may inactivate the wild type p53. The immunohistochemical assessment of mutant p53 has thus become a standard tool to research genetic alteration of a tumor. People who present with excessive p53 protein expression in benign lesions accompanied by severe degree, non-typical epithelium hyperplasia, have been subsequently proven to have a cancerous lesion, suggesting that there should be alteration of cells in some precancerous lesions, which can be expressed (and detectable) at the protein level[12]. The expression detected in samples of gastric mucosa biopsy of our study is consistent with that in previous reports. Five cases of atypical glandular dilatation of gastric mucosa showed positive expression, but the positive cells were focal in distribution; the simple dilatation displayed no expression. The comparison between atypical glandular dilatation and adenocarcinoma of stomach shows a significant difference ($P < 0.01$), indicating that p53 occurs in a great number of cells only after occurrence of malignant alteration of the cells and a transformation from gastric mucosa to cancer. Thus, this event plays a role of great significance in formation of early cancer.

PCNA is a kind of 36 Mr nucleoprotein, related to the synthesis of DNA in the cell cycle. It seems that cells can remain in an active proliferation state, which at present has been used as a criterion for assessment of malignant potential of a tumor in the precancerous stage and of the dynamics of tumor cells. In this series of 92 samples of biopsy, the expression in glandular dilatation of gastric mucosa was found to be increased along with the rise in the active level of cell proliferation. The comparison between the simple glandular dilatation and the atypical one showed significant difference ($P < 0.01$). In gastric cancer, the positive expression is widely distributed and deep in tinction. PCNA is an important criterion for the diagnosis, classification and treatment of precancerous lesion.

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Synergic effect of erythromycin and CoAA on gallbladder contraction in patients with cholelithiasis

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Abstract

AIM: To study the effect of combined use of compound amino acids (CoAA) and erythromycin in the promotion of gallbladder emptying and their mechanism of action.

METHODS: Sucralfate-NS, sucralfate-CoAA, proglumide-CoAA or erythromycin-CoAA was administered to 36 patients with cholelithiasis, and their gallbladder emptying rates (PGER) were compared by ultrasonographic measurement of gallbladder volume.

RESULTS: The PGER of the CoAA group was 40.52% ± 6.84%, which was significantly increased. The gallbladder contractive effect of CoAA could be blocked partially by the cholecystokinin (CCK) receptor blocker Proglumide. The PGER of combined erythromycin and CoAA was 54.17% ± 5.35%, being much higher than that in the group treated with CoAA alone ($P < 0.05$).

CONCLUSION: Rapid infusion of CoAA in patients with cholelithiasis can increase PGER, *via* a mechanism that is related to the release of CCK. When erythromycin is administrated at the same time, the PGER increases more significantly and the peak effect appears earlier. This finding suggests that combined use of the two has a synergic effect on gallbladder contraction, and that it is more effective than CoAA alone; thus, combined treatment might be useful in correcting gallbladder emptying impairment and preventing bile retention.

Key words: Erythromycin; Cholelithiasis; Gallbladder; Amino acids; Sucralfate

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INTRODUCTION

Gallbladder contraction impairment and bile retention are two important factors involved in gallstone formation. So far, there have been few drugs that can promote gallbladder emptying. Recent studies showed that rapid pulsatile intravenous infusion of compound amino acids (CoAA) elevated the plasma amino acids up to the level equivalent to that of a Sustagen-supplemented diet, which is able to cause gallbladder emptying and release of cholecystokinin (CCK)[1]. In contrast, erythromycin stimulates motilin activity[2]. We studied the effects of the two drugs on fasting gallbladder contraction in patients with gallstones and a brief appraisal is presented herein.

MATERIALS AND METHODS

Subjects

Thirty-six male and female patients with gallstones, ages ranging from 18 years to 65 years, were studied. Those with thickened gallbladder wall, gallstones more than one-half of the volume of the gallbladder, atrophied gallbladder with adhesions, cardiac insufficiency, active peptic ulcer, and long-term dietary restriction of greasy food were excluded. No drugs were given 3 d before the test.

Methods

The study was conducted over a 2-d period. On the first day, the 36 patients were randomly divided into three groups, each consisting of 12 cases, with similar mean age and sex distribution. The fasting gallbladder volume (FGV) was measured

Table 1 Comparison of gallbladder volume and postprandial gallbladder emptying rate in three groups with gallstone disease ($\bar{x} \pm s$)

	Group	*n*	FGV, cm^3	Minimum volume after injection, cm^3	PGER, %	Peak time
	1	12	28.55 ± 4.98	26.83 ± 5.12	6.21 ± 3.56	
1st day	2	12	27.54 ± 4.47	16.28 ± 3.05	40.52 ± 6.84[a]	40th min
	3	12	33.55 ± 6.52	22.40 ± 4.83	34.47 ± 4.54[b]	
2nd day	All	36	30.49 ± 3.45	13.91 ± 1.81	54.17 ± 5.35[c]	20th min

Group 2 *vs* group 1 on the first day ([a]$P < 0.05$); Group 3 *vs* groups 1 and 2 on the first day ([b]$P < 0.01$); All cases on the second day *vs* group 2 on the first day ([c]$P < 0.05$). FGV: Fasting gallbladder volume; PGER: Postprandial gallbladder emptying rate.

in all 36 patients. Group 1 was given sucralfate powder (1.0 g), after which the gallbladder volume was measured once every 15 min and continuously for 4 times, then 250 mL of 0.9% NS was infused within 30 min; the gallbladder volume was measured once every 10 min for 6 times. Group 2 was given 250 mL of 5% CoAA, and the rate of infusion, method and times of measurements were the same as those of the group 1. Group 3 received proglumide powder (0.8 g orally), and the gallbladder volume was measured once every 15 min and continuously for 5 times; then 250 mL of 5% CoAA was given in the same manner as in group 2. The FGV was measured again the next day for all patients. Erythromycin (0.5 g) was then given orally, and the gallbladder volume was measured once every 15 min and continuously for 4 times; then 5% CoAA (250 mL) was given afterwards.

The single-blind research method was used in this investigation. The gallbladder volume was measured by ultrasonography using a Siemens-450 type probe with 35 MHz and according to periphery diameter. Each volume was detected twice, and averaged. The gallbladder emptying rate (PGER) was calculated as: (FGVs-PGVs)/FGVs × 100%, where PGVs was the post-administration gallbladder volume.

RESULTS

On the first day, the gallbladder volume contracted gradually within 30-60 min after drug administration for group 2. The peak contraction time was relatively concentrated at the 40th minute. The PGER (40.52% ± 6.84%) increased much more significantly than in group 1 (6.21% ± 3.56%) ($P < 0.05$). On the next day, after administration of both drugs, the gallbladder volume of all 36 patients reduced gradually within 10-30 min. The peak contraction time occurred at about the 20th minute. The PGER (54.17% ± 5.35%) also increased more markedly than that of group 2 on the first day ($P < 0.05$). The PGER (34.47% ± 4.54%) of group 3 on the first day was significantly different from those of group 1 and 2 ($P < 0.01$; Table 1).

DISCUSSION

CCK is involved, to a greater degree, in the postprandial gallbladder contraction phase. Recent studies showed that dose-related and rate-related gallbladder emptying in patients receiving intravenous CoAA infusion might be due to its release from CCK-containing cells stimulated either directly or indirectly. Motilin and 5-HT, as prokinetic agents, might also play some roles but the precise mechanism has not been elucidated. Nealon *et al*[1] found that the plasma amino acid levels were similar to those found following a Sustagen-supplemented diet at 30-60 min after intravenous CoAA infusion given at two intermediate rates (125 mg/kg·h and 240 mg/kg·h).

The 12 patients in group 2 with cholecystitis and cholelithiasis who received rapid CoAA infusion within 30 min showed a gradual decrease of gallbladder volume within 30-60 min, with a mean minimum gallbladder volume of 16.28 ± 3.05 cm^3, and a peak response was observed at 40 min after infusion along with the mean PGER being 40.52% ± 6.84%. The volume of the gallbladder also increased somewhat after 1 h. Only one case showed the smallest gallbladder volume at the 20th minute. It was confirmed that CoAA had a significant effect on gallbladder contraction; sucralfate is not absorbable and has no effect on gallbladder contraction, therefore serving as a placebo.

Proglumide has a structure similar to the active constituent of gastrin/CKK molecules, so that may play a role as gastrin/CKK blocker upon combining with each receptor. About 120 min after oral delivery of proglumide, its plasma concentration reached its peak, which was 20 min later than that after the oral erythromycin. Therefore, in group 3, the gallbladder volume measurement was carried out 5 times consecutively (*i.e.* once every 15 min) and CoAA was given afterwards. The mean PGER was 34.47% ± 4.54% after the infusion, and there was a significant difference between group 1 and group 2 ($P < 0.05$). This finding suggests that proglumide might have a significant effect on blocking the action of amino acid. Whether the latter is related to the promoting effect of proglumide in bile secretion deserves further study.

The cholinergic nerve in the cephalic phase also participates in the postprandial gallbladder contraction[3]. Erythromycin, as a motilin receptor agonist, can decrease the FGV in healthy men and patients with gallstone diseases. The plasma level peaked at 100 min after oral administration of the drug and maintained this level for 2 h. The postprandial gallbladder emptying was decreased in patients with cholelithiasis, but became normal after oral erythromycin; likewise, it had a significant increase in healthy persons[2] and its effect could be blocked by atropine[4]. It is suggested that the cholinergic neural humoral factors play a crucial role in gallbladder contraction, whereas motilin only acts as a trigger for provoking contraction and mediates strength of contraction.

In this study, the gallbladder contraction was seen about 10 min after infusion and maintained about 60 min in 36 patients with cholelithiasis who were given intravenous CoAA and oral erythromycin; the peak response was relatively concentrated and advanced at the 20th minute. The average smallest gallbladder volume was 13.91 ± 1.81 cm^3 and the mean PGER was 54.17% ± 5.35%. As compared with CoAA alone, there was a marked difference ($P < 0.05$). No significant adverse reactions were found after the rapid infusion of CoAA and oral erythromycin at the above dosage, except in two patients who experienced transient dizziness, nausea and gastric distress.

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Clinical significance of expression of metastasis-associated splice variants of CD44 mRNA in early primary liver cancer

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Abstract

AIM: To study the clinical significance of the expression of metastasis-associated splice variants of CD44 (CD44v) in early primary liver cancer (PLC).

METHODS: Expression of CD44v mRNA in fresh resected tumor samples from 8 patients with early PLC was detected by RT-PCR. The expression intensity of CD44v mRNA was analyzed on images of Southern hybridization using the Kontron IBAS image analysis system. Meanwhile, the pathological evidence of metastasis was studied by microscopy.

RESULTS: All samples of early PLC expressed CD44v mRNA. The expression intensities of CD44v mRNA showed positive correlation with evidence of metastasis (deficiency of tumor capsule, penetration of tumor capsule, tumor emboli in portal vein and daughter tumor nodules near main mass, *etc*.) ($P < 0.05$).

CONCLUSION: Expression of CD44v mRNA may be a useful indicator of the metastatic potential of early PLC.

Key words: Liver neoplasms; RNA, messenger; CD44; Neoplasms metastasis

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INTRODUCTION

The importance of metastasis-associated splice variants of CD44 (CD44v) in tumor metastasis has been noted recently. In this study, we detected the expression of CD44v in patients with early primary liver cancer (PLC) and explored the clinical significance of CD44v in early PLC.

MATERIALS AND METHODS

Tissue specimens

Fresh samples of tumor tissues (0.5 g each) were obtained from surgically resected specimens of 8 patients with early PLC. According to the standard made by the Chinese Cooperated Pathological Study Group of Liver Cancer, these tumors were early PLC (tumor diameter < 3 cm). Control samples were obtained from 6 patients with cavernous hemangioma and 3 patients with liver trauma. All samples were stored at -196 ℃ within 10 min of obtainment and were kept frozen until use.

RNA isolation and RT-PCR

RNA was extracted from the samples according to the method of Chomzynski and Sacchi[1]. Reverse transcription was carried out on the total RNA samples using an AMV reverse transcriptase kit (Promega Co, United States). The first strands of cDNA were synthesized at 42 ℃ for 60 min in a 20 μL solution (upstream primer: 5'-GACAGACACCTCAGTTTTTCTGGA-3'; downstream primer: 5'-TTCCTTCGTGTGTGGGTAATGAGA-3'). PCR was carried out using 35 cycles of the following conditions: 94 ℃ for 1 min, 55 ℃ for 1 min, and 72 ℃ for 1.5 min. Twenty μL of the PCR products was separated on an ethidium bromide-stained 1.5% agarose gel and transferred to a nitrocellulose filter (Amersham Co., Britain). Hybridization was carried out at 42 ℃ for 16 h with ^{32}P-labelled oligonucleotide probe (5′-TGAGATTGGGTTGAAGAAATC-3′). Primers and probe were designed according to the published human CD44v cDNA sequence[2]. The filters were exposed to Fuji X-ray film for 20 h, and images of the Southern hybridization results were semi-quantitatively analyzed with the IBAS II image analysis system (Kontron Co, Germany); the expression intensity of CD44v mRNA was indicated by gray values of these films, with the gray value of negative expression set at zero. Weak expression was classified for grey values of 0-50, moderate expressions for 51-150, and over-expression for > 150.

Pathological indices of metastasis

All specimens were studied under light microscope to evaluate know whether the tumor capsule was complete or deficient, whether it had been penetrated by tumor cells, whether tumor cells had gone into the portal vein of normal liver tissue, and whether daughter tumor nodules had appeared in normal liver tissue near the main

Table 1 Expression of CD44v mRNA and clinical and pathological details of 8 patients with early primary liver cancer

Case No.	Sex	Age, years	Diameter of Tumor, cm	Expression Intensity of CD44v mRNA	DTC	PTC	TEPV	DTN	AFP, μg/L	Time of H, years	Cirrhosis	DCC
1	M	45	3	758.23	-		+	+	< 25	4	+	Ⅲ
2	M	54	3	47.82	+	-	-	-	< 25	18	+	Ⅱ
3	M	52	2	42.08	+	-	-	-	< 25	20	+	Ⅱ
4	M	46	3	218.55	-	-	+	+	< 25	15	+	Ⅲ
5	M	32	2.2	95.06	+	+	-	+	< 25	7	+	Ⅲ
6	M	35	2.5	130.4	+	+	-	+	< 25	11	+	Ⅱ
7	M	29	2.7	210.99	+	+	-	+	< 25	16	+	Ⅱ
8	M	34	2	88.19	+	+	+	+	1000	12	+	Ⅲ

No patient showed any clinical symptom of PLC and the levels of serum AFP were normal in 7 of the 8 cases. The hepatic masses of these patients were discovered by B-ultrasound on occasion, and the diagnoses of PLC were confirmed by CT or MRI and pathological examinations. All steps of this work were standardized as much as possible. Expression intensity of CD44v mRNA was shown by gray values of images of Southern blotting results. +: yes; -: no; DTC: Deficiency of tumor capsule; PTC: Penetration of tumor capsule; TEPV: Tumor embolus in portal vein under microscope; DTN: Daughter tumor nodules under microscope; DCC: Differentiation of tumor cells; H: Hepatitis B; PLC: Primary liver cancer; AFP: Alpha-fetoprotein.

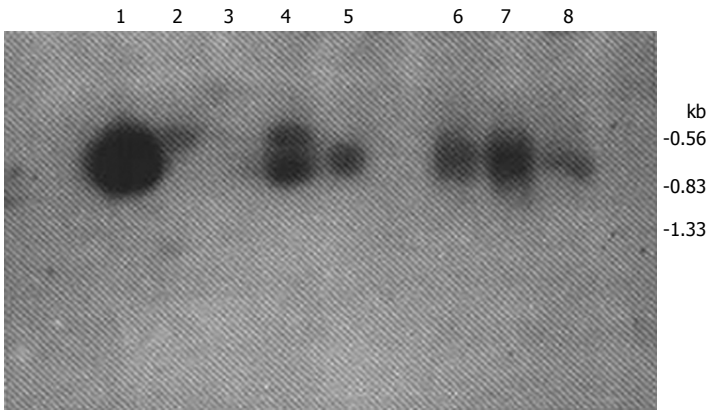

Figure 1 Expression of CD44v mRNA in eight patients with early primary liver cancer.

mass. In addition, we assessed the degree of differentiation of the tumor cells. The data for these criteria are not published. The age, sex, level of alpha-fetoprotein (AFP) and other details of the patients are shown in Table 1, Figure 1.

Statistical analysis

Statistical analysis was performed by SAS software using an AST 486DX2/66 microcomputer.

RESULTS

The expression of CD44v mRNA in the liver tissue was significantly different between the PLC and control samples ($P < 0.05$). The expression intensities of CD44v mRNA in early PLC had positive correlation with the metastatic evidence, including deficiency of tumor capsule, penetration of tumor capsule, tumor embolus in portal vein and daughter tumor nodules near main mass, *etc.*) ($P < 0.05$).

Only one control sample expressed CD44v mRNA weakly.

DISCUSSION

CD44 is a surface adhesion molecule that participates in cell-cell and cell-matrix interactions[3,4]. "Standard" CD44 can be modified by the insertion of transcripts from any, some, or all of at least five extra exons[2,5], leading to production of CD44v. A single cell may contain two or more variants of mRNA from the same gene simultaneously. CD44v may play an important role in tumor growth and metastasis. Convincing evidence for this feature has been provided previously[6,7]. Currently, a human homologue of rat metastasis-associated CD44v has been isolated from the cell lines of human large cell lung carcinoma, colon carcinoma and breast carcinoma[5]. Moreover, expression of CD44v has been detected in fresh human tumor tissues of breast carcinoma, colon carcinoma[8], gastric carcinoma[9], melanoma[10] and metastases in brain[11].

In our study, all the PLC specimens were found to express CD44v mRNA. By statistical analysis we found that the expression intensities of CD44v mRNA in early PLC had positive correlation with the metastatic evidence. Thus, the expression of CD44v mRNA may be a useful indicator for evaluating the metastatic potential of early PLC.

No clinical symptoms of PLC had occurred at the time of diagnosis for any of the 8 cases evaluated. The levels of serum AFP were normal in 7 of the 8 cases after the patients were hospitalized. It is indicated that when treating patients with early PLC not to ignore the possibility of the presence of metastases, regardless of an absence of clinical symptoms of PLC and low level of serum AFP. The whole condition of the patient should be paid attention, including the expression of CD44v.

All of the 8 patients with early PLC were associated with hepatitis of 4 years to 20 years duration, as well as pathological evidence of cirrhosis. Even though we cannot be sure whether there is an exact correlation between the expression of CD44v mRNA and hepatitis or/and cirrhosis in early PLC, we believe that the expression of CD44v mRNA may indicate the metastatic potential in PLC patients with hepatitis and cirrhosis.

The degree of differentiation of PLC cells was poorer in patients with moderate and intense expression of CD44v mRNA than in those with weak expression. The reason for this, however, needs to be further studied.

One control sample expressed CD44v mRNA weakly, and we do not know whether this is a false-positive result or it has some other significance.

CONCLUSION

Metastatic potential of early PLC may be related to the expression of CD44v mRNA. The latter may be a useful indicator in evaluating metastasis. In addition, anti-CD44v treatment may be an important method for increasing the survival rate of patients with early PLC.

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Relation between viremia level and liver disease in patients with chronic HCV infection

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Abstract

AIM: To explore the relation between level of hepatitis C virus (HCV) viremia and HCV-related chronic liver disease.

METHODS: Serum HCV RNA was measured by competitive reverse transcription polymerase chain reaction (CRT-PCR) in 27 patients with chronic HCV infection.

RESULTS: Levels of serum HCV RNA were low (10^2-10^6 copies/50 μL serum) in patients with chronic HCV infection. Patients with chronic active hepatitis ($10^{5.739 \pm 0.25}$ copies/50 μL serum) and with cirrhosis ($10^{5.803 \pm 0.76}$ copies/50 μL serum) had higher levels of serum HCV RNA than patients with chronic persistent hepatitis ($10^{5.068 \pm 1.04}$ copies/50 μL serum) ($P < 0.05$). There was a positive relation between levels of serum HCV RNA and alanine aminotransferase.

CONCLUSION: All of these results suggest that viremia level is low in chronic HCV infection. HCV itself plays an important role in progress of chronic liver disease, and HCV replication is related to liver damage.

Key words: Hepatitis C virus; RNA, viral; Polymerase chain reaction; Hepatitis C

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INTRODUCTION

The pathogenesis of hepatitis C virus (HCV) infection is complex. Replication of HCV as well as host immune responses are involved in the liver cell damage that manifests in HCV infected individuals. Some studies have demonstrated that during the late stage of chronic liver disease, HCV still replicates actively[1]. We developed a competitive reverse transcription-polymerase chain reaction (CRT-PCR) for quantitative analysis of HCV RNA and investigated 27 patients with chronic HCV infection to explore the relation between HCV viremia level and chronic liver disease.

MATERIALS AND METHODS

Subjects

Twenty-seven patients (17 males and 10 females, aged 28-60 years) were hospitalized in our Department of Infectious Disease during the period from June 1993 to July 1995. All of these patients tested positive for anti-HCV and serum HCV RNA, and had shown abnormal levels of alanine aminotransferase (ALT) for at least 6 months. Thirteen of the cases were diagnosed as chronic persistent hepatitis (CPH), 10 as chronic active hepatitis (CAH) and 4 as cirrhosis, according to the criteria made at the National Conference on Viral Hepatitis, Shanghai, 1990. All 27 patients tested negative for hepatitis B surface antigen (HBsAg) and antibodies to the hepatitis A, D an E viruses (anti-HAV-IgM, anti-HDV IgM and anti-HEV IgM respectively). Serum samples were taken upon the first finding of HCV RNA positivity and stored at -20 ℃ until use.

Methods

RNA was extracted from 50 μL of each serum sample by using the acid quanidium thiocyanate phenol-chloroform method. HCV cDNA was synthesized from each extract by reverse transcription using primer 2 (Table 1). A competitive DNA plate was constructed by recombinant PCR. The restriction enzyme target site recognized by *BamH* I (GGATCC) was inserted into the HCV cDNA products by using primers 1 and 5, and primers 2 and 6 (Figure 1)[2,3]. Quantitative detection of serum HCV RNA was then carried out. The HCV cDNA from each serum sample was added to the competitive plate DNA ($10^{3\text{-}5}$ copies), respectively, and amplified by nested PCR using primers 1 and 2, and primers 3 and 4. The DNA product was then cut with endonuclease *BamH* I, electrophoresed through a 1.5% agarose gel, and stained with ethidium bromide; the staining pattern included three bands of 286 bp, 208 bp and 78 bp. The DNA was isolated from the 286-bp and 208-bp sized bands, respectively, by electro-osmosis and the OD_{260} value was detected. The number of HCV RNA copies in the 50 μL serum sample was calculated according to the formula: C1 = OD1/OD2 × C2 × 1.2, where C1 was the number of HCV RNA copies in 50 μL serum sample, C2 was the number of competitive plate copies, OD1 was the OD_{260} value of

Table 1 Sequences of primers located in the 5'-NC region of the hepatitis C virus genome

Primer NO.	Nuclear acid position	Polarity	Sequence (5'-3')
1	1-20	+	CGAGGCGACACTCCACCATAGAT
2	323-303	-	GAGGTGCACGGTCTACGAGACCT
3	10-32	+	CCACCATAGACTTCTCCCCTGT
4	296-270	-	CACTCTCGAGCACCCTCTCAGGCAGT
5	78*-60	-	CATGGATCCACTAACGCCATGGCTAGA
6	78*-96	+	ATGGGATCCATGATGGTCGTGCAGCCT

*The 10th base is the 78th nucleic acid in the sequence of the HCV genome. "GGATCC" is the site recognized by *BamH* I. HCV: Hepatitis C virus.

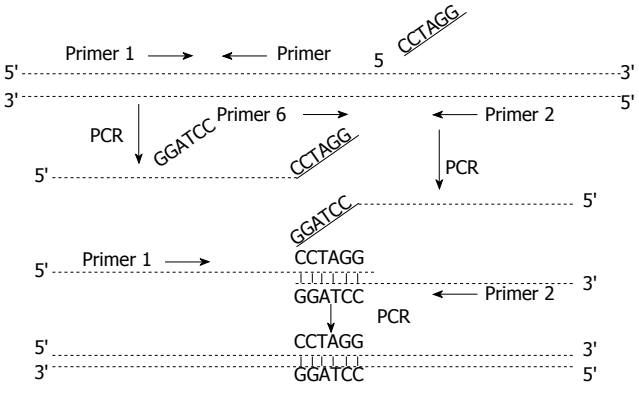

Figure 1 Competitive DNA plate construction by recombinant polymerase chain reaction.

Table 2 Comparison of serum hepatitis C virus RNA levels

Clinical classification	Number of cases	HCV RNA, copies/50 μL serum
CPH	13	$10^{5.068 \pm 1.04}$
CAH	10	$10^{5.739 \pm 0.25a}$
Cirrhosis	4	$10^{5.803 \pm 0.76}$

[a]$P < 0.05$ *vs* cirrhosis. HCV: Hepatitis C virus; CPH: Chronic persistent hepatitis; CAH: Chronic active hepatitis.

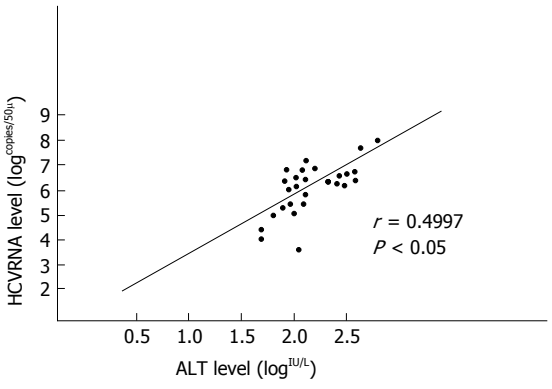

Figure 2 Relation between hepatitis C virus RNA and alanine aminotransferase.

DNA from the 286 bp band, OD2 was the OD_{260} value of DNA from the 208 bp band, and 1.2 was the transformation coefficient.

Anti-HCV was detected by second generation radioimmunoassay (RIA-2). HBsAg and IgM anti-HAV were detected by standard RIA. Anti-HDV and anti-HEV were detected by enzyme-linked immunosorbent assay.

RESULTS

HCV RNA level in serum

The serum levels of HCV RNA in patients with chronic HCV infection were low (10^2-10^6 copies/50 μL serum).

Serum level of HCV RNA in patients with different clinical classifications

The HCV RNA levels in patients with CAH and cirrhosis were much higher than those in patients with CPH ($P < 0.05$; Table 2).

Relation of HCV RNA level to ALT level

There was a positive correlation between levels of serum HCV RNA and ALT ($r = 0.4997$, $P < 0.05$; Figure 2).

DISCUSSION

In chronic HCV infection the viremia level is low. Bradley[4] conducted a chimpanzee infection experiment and found that serum titer of HCV was 10^2-10^4 CID/mL. A CID/mL of $10^{6.5}$ equates to 4 × 10^8 copies of HCV RNA/mL in serum[4]. Ulrich *et al*[1] used end-point dilution PCR to show that serum HCV RNA levels in patients with chronic hepatitis C were $10^{2\text{-}5}$ × 10^7 copies/mL. Hagiwara *et al*[5] used CRT-PCR to demonstrate that the serum HCV RNA was 10^4-10^9copies/mL. We obtained similar results in this study, detecting 2 × 10^3-2 × 10^7 copies/mL by the CRT-PCR technique. However, Jenison *et al*[6] demonstrated that the serum HBV DNA level in patients with chronic hepatitis B was 5 × 10^8-3 × 10^{10} copies/mL by dot blot hybridization, which is much less sensitive than PCR. It can be postulated that the viremia level in chronic HCV infection is significantly lower than that in HBV infection.

Replication of HCV is related to the progress of chronic liver disease. Our results show that serum HCV RNA levels in patients with CAH and cirrhosis are much higher than that in patients with CPH ($P < 0.01$). A similar result was reported by Kato *et al*[7], from a study in which they used CRT-PCR to detect HCV RNA in 36 patients with chronic liver diseases; specifically, the serum levels in patients with CAH ($10^{5.6 \pm 1.6}$ copies/50 μL) and with cirrhosis ($10^{6.0 \pm 1.6}$ copies/50 μL) were higher than that in patients with CPH ($10^{3.3 \pm 1.9}$copies/50 μL) ($P < 0.01$)[7]. Gordon *et al*[8] demonstrated that viremia level is positively related to liver histological score in patients with chronic HCV infection. Tsutsumi *et al*[9] studied the expression of NS5 antigen related to RNA-dependent RNA polymerase in liver, and found that the detection rate of NS5 antigen in patients with cirrhosis was higher than that in patients with CAH and CPH. Collectively, these data suggest that HCV itself plays an important role in the progress of HCV-related chronic liver disease.

HCV is known to replicate actively during the course of chronic HCV infection, and to replicate at high level even in the late stage of the disease. Moreover, HCV replication is well recognized as closely related to liver damage. It was demonstrated in this study that there is a positive correlation between the levels of serum HCV RNA and ALT in patients with chronic HCV infection ($r = 0.4997$, $P < 0.05$). Kurasaki *et al*[10] detected serum HCV RNA in 41 patients with chronic HCV infection by using PCR. Subgroup analysis of the 41 patients, by high-level HCV RNA (showing positive test results on the first amplification) and low-level HCV RNA (showing positive test results on the second amplification), indicated that the ALT in the high-level group was much higher than that in the low-level group (191.7 ± 103.4 μkat/L *vs* 98.4 ± 66.7 μkat/L, $P < 0.05$); additionally, all the patients showing ALT > 166.7 μkat/L belonged to the high-level HCV RNA group[10], suggesting that HCV may have a cytopathologenic effect.

In conclusion, the results of the current study demonstrate that the viremia level is low in chronic HCV infection, indicating that HCV replication is related to the progress of chronic liver disease and supporting the notion that HCV may have a cytopathologenic effect.

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BRIEF REPORT

Clinical study on external counterpulsation in treatment of viral hepatitis

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INTRODUCTION

External counterpulsation (ECP) is an artificial blood circulation apparatus, and has been widely used for treatment of some ischemic diseases. Since June 1988, we have been observing the therapeutic effects of ECP in treatment of viral hepatitis; in general, this therapy has attained excellent results, which are presented here.

MATERIALS AND METHODS

Patients

All the patients in our study were admitted to our hospital in 1990. All the cases were diagnosed with viral hepatitis according to the criteria formulated by the National Virus Hepatic Conference in Shanghai, China (1990). There were 1004 patients in the ECP group and 783 patients in the control group; the groups were comparable in sex, age and etiological diagnosis (Table 1). The methods of ELISA and PCR were used to detect hepatitis B virus serological markers, including HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe, anti-HBcIgM, anti-HAVIgM, *etc*, in all the hospitalized patients.

Methods

The ECP group and the control group were treated with similar general therapy, which included administration of vitamins C and B orally; for all, when serum bilirubin was > 85.6 μmol/L, potassium magnesium aspartate was administered (40 mL in 10% glucose 500 mL, once a day at the same time each day). The ECP group received the addition of ECP (SKB-Ⅱ), delivered for 45 min, one time every day for 14 d until the end of one therapeutic course. The pressure of the air pocket wrapped around the patient's limbs ranged from 0 kg/cm to 5 kg/cm (fat: 0.55 kg/cm). Complaints accompanying the ECP treatment included nausea, anorexia, sleeplessness, and acratia. Levels of SB, ALT, TTT, *etc*. were detected in all patients and compared for the ECP group and the control group.

Criteria used to determine therapeutic effect were as follows. 'Notable efficacy' was indicated by extinction of symptoms and normalized levels of SB and ALT within 2 wk or normalized levels of SB, ALT and TTT within 4 wk. 'Effectiveness' was indicated by disappearance of symptoms and levels of SB at < 34.2 μmol/L and ALT at < 1000.2 nmol/s within 2-4 wk. 'Improvement' was indicated by patients general improvement within 6 wk, with levels of SB at < 34.2 μmol/L and ALT at 1016.87-1667 nmol/s. 'No efficacy' was indicated by persistence of symptoms after 6 wk, with SB at > 34.2 μmol/L and ALT at > 1667 nmol/s, and/or further deteriorated liver function.

RESULTS

The therapeutic effects, *i.e.* disappearance of four major symptoms (nausea, sleeplessness, acratia, anorexia), are shown in Table 2 ($P < 0.05$). After 4 wk of treatment, the effective rates in the ECP group are much higher than those of the control group, which are shown in Table 3 ($P < 0.01$).

DISCUSSION

ECP is a non-traumatic therapy and has been used in treatment of ischemic diseases (heart, brain, kidney) for about 10 years[1]. In 1988, we began to apply ECP as treatment for viral hepatitis. Clinical observations indicated that ECP can accelerate elimination of jaundice and relieve clinical symptoms as well as promote recovery of liver function[2]. The underlying mechanism of its therapeutic efficacy may involve the following features. First, it may increase hepatic artery perfusion. It is known that hepatic cell trauma induced by viral hepatitis is caused by a series of immunoreactive responses. Each type of viral hepatitis produces a varying degree of microcirculatory disturbance[3], and ECP may prompt blood flow from the four limbs to the visceral artery system in the ventricular diastole period, which is advantageous to cell regeneration. Second, when ECP is used, more nutrients, such as oxygen, can be carried to the hepatic tissues and the noxious substances in cells can be taken away, more so by the increased hepatic blood flow. ECP may raise PaO2, reduce platelet aggregation in circulation, improve the quantity and quality of blood that flows into the liver, abate the microcirculatory disturbance and stimulate cell regeneration[4]. Third, it has been reported that the patients with hepatitis show a positive correlation between the digestive tract symptoms and concentration of cortisol in the body; the higher the cortisol concentration,

Table 1 General data

Type	ECP Group				Control Group			
	n	Age	m	f	*n*	Age	m	f
IHA	309	30.4 ± 8.5	200	109	205	30.9 ± 8.7	129	76
IHB	270	30.0 ± 6.6	164	106	187	31.2 ± 8.1	104	83
NIHA	97	31.8 ± 8.9	59	38	102	29.9 ± 9.1	57	45
NIHB	98	29.5 ± 6.2	62	36	104	30.9 ± 7.0	64	40
CPH	190	30.6 ± 6.2	135	55	147	33.4 ± 10.1	103	40
CHA	40	32.1 ± 5.7	30	10	38	37.4 ± 12.4	27	11
TOTAL	1004	30.7 ± 7.0	650	354	783	32.3 ± 9.2	484	299

I: Ictero, NI: No ictero, n: Number, m: Male, f: Female.

Table 2 Extinction of symptoms after 2 weeks of external counterpulsation treatment

Type	Acratia	Anorexia	Nausea	Sleeplessness
ECP group	82.93 (%)[a]	86.04 (%)[a]	82.58 (%)[a]	94.50 (%)[a]
Control group	53.12 (%)	67.43 (%)	62.25 (%)	25.78 (%)

[a]$P < 0.05$ *vs* control group. ECP: External counterpulsation.

Table 3 Effective rate comparison among group

	ECP Group									Control Group								
	N	Notably		Effective		Improved		Ineffective		N	Notably		Effective		Improved		Ineffective	
		n	%	*n*	%	*n*	%	*n*	%		*n*	%	*n*	%	*n*	%	*n*	%
IHA	300	182	58.8	96	31.1	25	8.1	6	1.9	205	30	14.6	73	35.7	65	31.7	37	18.0
IHB	270	129	47.8	103	38.1	23	8.5	15	5.6	137	24	12.8	75	40.1	57	30.5	31	16.6
NIHA	96	51	52.6	34	35.1	8	8.2	4	4.1	102	26	25.5	32	31.4	31	30.1	13	12.7
NIHB	98	37	37.8	39	39.8	12	12.2	10	10.2	104	25	24.0	35	33.7	28	26.9	16	15.4
CPH	190	76	40.0	70	36.8	36	18.9	8	4.3	147	36	24.5	43	29.3	49	33.3	19	12.9
CAH	40	4	10.0	19	47.5	10	25.0	7	17.5	38	6	15.8	8	21.1	15	39.5	9	26.7
TPTAL	1004	479	47.7	361	35.9	14	11.4	5	5.1	783	147	18.8	266	34.0	245	31.3	125	16.0

[b]$P < 0.01$ *vs* control group. I: Ictero; NI: No ictero; ECP: External counterpulsation.

the more serious the symptoms serious. Approximately 75.5% of patients with acute hepatitis reportedly have higher cortisol concentration[5]. ECP may improve perfusion of the digestive tract mucosa and accelerate the inactivation of cortisol. Fourth, the patients in the ECP in this study had better curative effect than that of the control group, in regard to disappearance of jaundice. This finding could be due to improvement in the liver microcirculation and enhancement in the enzymatic activity (Na^+-K^+ ATPase), both of which are advantageous to bile excretion; moreover, it is possible that hepatic cell protein intake, and bilirubin, may be improved as the renal blood flow is increased during the course of ECP and that the amount of urine becomes increased also. Fifth, the liver cells repair and regenerate in response to ECP, as a result of the therapy improving tissue perfusion, ameliorating microcirculation, decreasing blood viscosity, increasing the scavenging of free radicals, and abating endothelial cell injury[6]. For the reasons outlined above, ECP may be a useful accessory treatment of viral hepatitis.

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BRIEF REPORT

Retrospective analysis of 186 cases of upper gastrointestinal bleeding in recent 5 years

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INTRODUCTION

We have reviewed 186 cases of patients with upper gastrointestinal bleeding (UGIB) who were admitted to our hospital between 1991 and 1995 (the data of 1991 is not complete). The causes of UGIB, in order of frequency, were peptic ulcer (PU), liver cirrhosis (LC), acute lesions of the gastric mucosa (ALGM), tumor, unclear pathogeny, and others. According to the volume and frequency of bleeding, the cases of UGIB, severe gastrointestinal hemorrhage (GIH) and rebleeding were analyzed.

MATERIALS AND METHODS

Data were collected for in-patients who had undergone endoscopic examination, and included the time, volume, frequency and location of bleeding. PU cases were divided into subgroups according to duodenal ulcer (DU), gastric ulcer (GU) and special type ulcer (STU). The STU cases included pyloric ulcer and complex ulcer. A total of 186 cases were examined, including 147 men with a mean age of 41.6 years (12.83 years) and 39 women with a mean age of 48.6 years (21-78 years). The mean ages at the first episode of bleeding for DU, GU, STU, LC, tumor, ALGM and unclear pathogeny were 47.8, 58.3, 51.9, 51.6, 53.6, 46.7 and 42 years, respectively. The age distributions for the severe GIH and rebleeding cases are shown in Table 1 and Figure 1.

Seasonal distribution (Table 2)

There was no obvious difference among the morbidities from 1991 to 1995. The annual numbers of hospitalized patients from 1991 to 1995 were 36, 40, 33, 44 and 33, respectively. The morbidity was higher in January, March, May and December. However, cases of bleeding in PU was most common from December to March and less common from August to October. The seasonal fluctuation affected the bleeding more in DU and STU than in GU. The incidence of severe GIH was slightly higher from December to March and that of rebleeding was higher from January to March.

Pathogeny

Of the 186 patients, there were 87 cases of PU (46.7%), including DU (28.5%), GU (8.6%), and STU (9.6%); 44 cases of LC (23.7%), including bursting of esophageal, gastric varices; 26 cases of tumor (14%), including esophageal cancer (7), gastric cancer (17), gastric adenoma (1), and biliary tract cancer (1); 19 cases of ALGM (10.2%); and 10 cases of unclear pathogeny (5.4%). The sites of UGIB are: esophagus in 36 cases (19.4%), stomach in 75 cases (40.3%), duodenum in 62 cases (33.3%), pylorus in 12 cases (6.5%), and biliary tract in 1 case (0.5%).

Volume and frequency of UGIB

There were 139 cases of UGIB in our series and 48 cases of rebleeding (25.8%), consisting of 42 men and 6 women (M/F ratio of 7:1). Among these patients, 25 suffered from PU (52.1%), including 15 cases of DU, 6 of GU, and 4 of STU; 12 suffered from LC, 8 from ALGM, 2 from unclear pathogeny, and 1 from tumor. The highest rebleeding frequency of LC was 8 times, and that of DU was 5 times. On the other hand, according to the volume of bleeding, 49 cases (26.3%) were severe GIH (bleeding volume > 800 mL per day (24 h)[1]), 57 cases (31%) were moderate GIH, and 80 cases (43.7%) were of slight bleeding (with melena and Hb of > 60 g/L and 90 g/L, respectively). Forty-one males and 8 females had severe GIH (M/F ratio of 5.1:1). According to the pathogeny of severe GIH, the numbers of LC, PU (including DU), ALGM, tumor, and unclear pathogeny were 24 (48.9%), 17 (34.2%), 4 (8.2%), 3 (6.1%) and 1 (2%), respectively (Table 1). Rebleeding occurred in 18 cases of severe GIH (36.2%), including 10 of LC, 7 of PU and 1 of ALGM.

RESULTS

A total of 38 patients were treated surgically, among which 14 (16.1%) had PU, 7 (15.9%) had bursting of esophageal and gastric varices, and lesion of esophageal and gastric mucosa, and 17 (65.4%) had tumor. The PU cases included 9 DU, 1 of which

Table 1 Distribution of age among different causes of upper gastrointestinal bleeding, severe gastrointestinal hemorrhage and rebleeding

	First episode of bleeding		Severe GIH		Rebleeding	
	Mean age (range)	Cases	Mean age (range)	Cases	Mean age (range)	Cases
DU	47.8 (17-30)	53	47.6 (24-62)	12	46.9 (18-57)	15
GU	56.3 (43-78)	16	58.3 (52-67)	3	57.0 (45-69)	6
STU	51.9 (38-68)	18	54.0 (52-56)	2	53.0 (51-55)	4
LC	51.6 (36-64)	44	52.1 (36-60)	24	55.8 (40-57)	12
ALGM	46.7 (12-60)	19	52.1 (39-60)	4	52.0 (49-60)	8
Tumor	53.6 (41-83)	26	61.2 (45-78)	3	62.2	1
Unclear pathogeny	420 (23-74)	10	23	1	50.5 (49-52)	2
Total	49.9	87	49.6	49	53.9	48

DU: Duodenal ulcer; GU: Gastric ulcer; STU: Special type ulcer; LC: Liver cirrhosis; ALGM: Acute lesions of the gastric mucosa; GIH: Gastrointestinal hemorrhage.

Table 2 Seasonal distribution of upper gastrointestinal bleeding, severe gastrointestinal hemorrhage and rebleeding

Pathogeny	Winter			Spring			Summer			Autumn			Total (%)
	12	1	2	3	4	5	6	7	8	9	10	11	
PU	11	11	7	15	7	10	6	7	2	3	3	5	87 (46.7)
LC	4	4	4	8	2	3	3	3	6	3	2	3	44 (23.7)
ALGM	2	3	3	2	2	3	0	0	1	2	1	0	19 (10.2)
Tumor	2	3	1	5	2	3	2	2	3	1	1	1	26 (14.0)
Unclear pathogeny	2	2	0	1	2	1	0	1	0	0	1	0	10 (5.4)
Total	21	23	15	31	15	20	10	13	12	9	8	9	186 (100)
Severe GIH	7	4	8	7	3	3	3	3	4	3	2	2	49 (26.3)
Rebleeding	2	4	5	5	1	4	5	2	2	1	3	1	48 (25.8)

LC: Liver cirrhosis; ALGM: Acute lesions of the gastric mucosa; PU: Peptic ulcer.

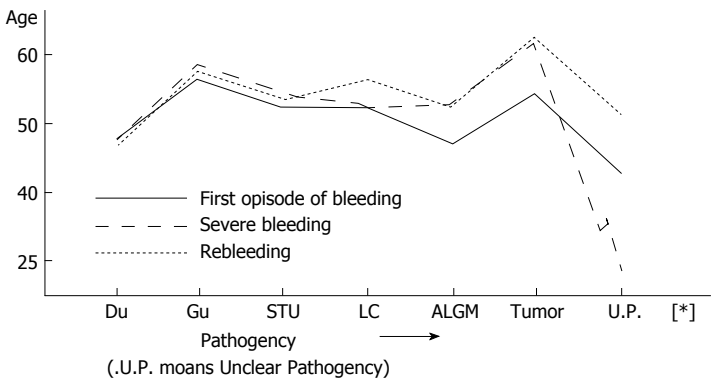

Figure 1 Curves of pathogeny versus age for the first episode of bleeding, severe bleeding and rebleeding. DU: Duodenal ulcer; GU: Gastric ulcer; STU: Special type ulcer; ALGM: Acute lesions of the gastric mucosa.

perforated and hemorrhaged, 5 GU, and 8 patients who suffered from rebleeding after surgery at the anastomotic stoma. Five of the 7 LC patients had rebleeding after surgery. There were 13 deaths, including 8 patients with severe GIH of LC, 3 with tumor and bleeding, and 2 with severe GIH from DIC with gastric mucosa hemorrhage. The surgical and mortality rates of non-tumor UGIB were 15.1% and 7.2%, respectively. The principal treatment for UGIB was antacid agents, with 120 of 186 patients having taken H_2-receptor blockers and 65 having taken Losec. All of these patients showed improvement to varying degrees. The recovery rate of UGIB was 93.1%, and the improvement rate was 6.9% (all of the 6 patients were discharged from the hospital for different causes). The recovery rate of LC-GIH was 95%. The other UGIB patients, excepting those with tumors, recovered.

DISCUSSION

Mino *et al*[2] reported that the major causes of UGIB are PU (54.13%), bursting of esophageal and gastric varices (10.73%), and ALGM (6.72%); the etiology of hemorrhage could not be established in 8% of cases. Our data are in agreement with these. Another study showed that bleeding of PU has a tendency to increase year by year, the age of the hospitalized patients suffering from PU bleeding was between 40-60 years, with the morbidity increasing gradually after 60 years old[3]. This is also consistent with our results. PU bleeding and rebleeding are characterized by some seasonal distributions, with the highest incidence of bleeding being observed during winter and early spring, accounting for 50.6% of all PU bleeding cases and 52.1% of rebleeding cases. These results are similar to those reported by some scholars[3,4]. The reasons why the cold climate is associated with more frequent DU bleeding may be related to an enhanced aggressive factor and a weakened defending factor during that period. Another reason is that patients take nonsteroidal anti-inflammatory drugs (commonly known as NSAIDs) more often in these seasons.

The causes of LC hemorrhage include bursting of esophageal and gastric varices, ulcer, lesions of gastric mucosa, and so on. Because this condition is more severe and dangerous, we should pay more attention to it. Staritz[5] has reported that the mortality rate of patients with LC from esophageal variceal bleeding can reach up to 30% after the first bleeding episode. Meanwhile, there is an obvious relationship between liver function and severe GIH. The liver function of the 8 patients who died in our case series suffered from severe GIH of LC and showed Child grade C. The criteria gives a better evaluation of risk of hemorrhage. However, this kind of UGIB and rebleeding occurs more often in cold climate, suggesting that the related factors that produce harmful effects on the blood vessels may increase due to the cold climate.

According to the number of cases, the features of bleeding of tumor, ALGM and unclear pathogeny ranked the third, fourth, and fifth, respectively. Since the cause of tumor bleeding is always indistinct, the prognosis is better after the pathogeny and site are determined since an appropriate therapy may be identified and applied. Of the 26 patients, only 17 were indicated for surgery. For ALGM, the site is diffuse and presents acute changes visible by endoscopy. Four of the 19 patients with severe hemorrhage were aged > 55 years. The function of the mucosal barrier is easily damaged, inducing severe hemorrhage. Because the examination was not made in time and the technique itself was limited, the underlying causes of 10 cases of hemorrhage were unknown. These patients were the youngest, as represented by the mean age (only 1 who had rebleeding was aged 74 years), and without complications of other organs; thus, their gastric mucosa regenerated rapidly and they recovered well after bleeding. It is possible that these patients may have had a lesion of gastric mucosa, but there are likely other pathogenies, such as Dieulafoy lesion.

Age is another important factor for UGIB, severe GIH and rebleeding. Our data show that ages at the first episode of bleeding for GU and tumor are the oldest in our study population (56.3 and 53.5 years respectively); the next coincided with STU, LC and DU (51.9, 51.6 and 47.8 years respectively), and the youngest were

for ALGM and unclear pathogeny (46.7 and 42 years respectively) (Figure 1). The distribution curve of age and etiology of bleeding gives the explanation for the epidemiological tendency of UGIB showing different pathogeny according to age, which is consistent with Nishida's view[6].

Our data also demonstrated that age distribution curves for both severe GIH and rebleeding are higher (26.3% and 25.8%) than that for the first episode of bleeding (Figure 1). The rebleeding rate was also high (36.2%) after severe GIH. These findings may be related to the poor contractility of bleeding vessels and the general poor response to drug treatment in older patients with arteriosclerosis. It has also been demonstrated that severe hemorrhage is more common in patients older than 60 years, and reportedly associated with a death rate of 23.2%[7]. The etiology of severe hemorrhage varies in different areas of our country. In the northeast, the order of frequency reported is LC, PU and ALGM[1], similar to our results. It has also been reported that in this kind of hemorrhage, 20.15% and 29.25% of cases are persistent and self-limiting[2]. However, we think this notion is contradictory to the bursting of esophageal and gastric varices. Cases of severe GIH caused by ALGM have increased in recent years, from 3.6% in the 1970s to 10% in the most current report, and this condition has emerged as a common pathogeny of UGIB[1]; so, greater importance should be attached to it for future research studies.

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CASE REPORTS

Menetrier's disease with lymphocytic gastritis: Report of two cases

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CASE REPORT

Case 1

An 18-year-old male patient was admitted to our hospital with complaints of a 3-year history of intermittent epigastric pain with vomiting and a 1-year history of peripheral edema and anorexia. Physical examination revealed pale face, edema of eyelids and legs, and epigastric pain when pressed. Laboratory tests indicated that his hemoglobin (Hb) was 95 g/L, total serum protein was 30 g/L, and albumin:globulin ratio was 0.8:2.2. Radiologic study showed disordered and thickened gastric folds and slow wriggling movement of the gastric wall. Nodular defects were observed from 0.8-3.0 cm and appeared well circumscribed. Clinical diagnoses included carcinoma of stomach, secondary anemia, an hypoproteinemia. After surgical treatment of subtotal gastrectomy, the patient recovered quickly and the level of serum albumin decreased after 2 wk.

Pathologic findings

The specimen was characterized by enlarged gastric mucosal folds, involving both length and width, which produced the observed thickened and convoluted radiologic appearance of the mucosal membrane and which was reminiscent of the appearance of cerebral convolution. The width of the folds ranged from 1.5 cm to 2.5 cm with deep grooves in between and non-uniform in pattern. It was noted that the enlarged gastric region showed sharply defined margins. The mucosal folds were not present in the pars pylorica. The nodules located in the body of the stomach corresponded to the diffuse form of the varioliform gastritis condition (Figure 1). Evaluation of cross-sections showed that the thickness of the mucosal membrane was 0.7 cm. The histologic changes essentially involved the mucosal folds of the body of the stomach. Most epithelial surfaces were covered with mucus. The gastric pits showed marked elongation and tortuosity, increased number and depth, and with some dilation. The foveolar mucosa of the epithelium generally consisted of active mucus-secreting cells, and the upper part of the mucosal membrane had been replaced by these cells. The mucosal lamina propria contained moderately excessive lymphocytes, plasma cells, eosinophils and neutrophils, which were localized to the upper half of the mucosa, mimicking chronic superficial gastritis. In addition, numerous intraepithelial lymphocytes were observed on the surface and foveolar epithelium, and were present as single cells rather than in clusters, with most being surrounded by a clear "halo" (Figure 2). Abscesses, involving the pits and crypt cells, were observed (Figure 3). The muscularis mucosa showed irregular hyperplasia and hypertrophy, with bundles of smooth muscle fibers extending up into the lamina propria of the mucosa. The ratio of intraepithelial lymphocyte counts to total lymphocyte counts ranged from 31% to 49%, with an average of 43%. The normal mucosa, atrophic gastritis, and superficial gastritis accounted for 1.6%, 4.4% and 2.9% of the area respectively; for each, the depths of the lamina propria mucosa was 12.7 mm, 0.7 mm and 2.1 mm respectively. Pathologic diagnosis was Menetrier's disease with lymphocytic gastritis.

Case 2

A 57-year-old female patient was admitted to our hospital with complaints of a 4-year history of epigastric pain with poor appetite, vomiting and intermittent diarrhea. Physical examination revealed weight loss and epigastric pain on pressure, but no palpable mass. Ascites sign was doubtful, but edema was present in the legs. Laboratory findings indicated that Hb was 110 g/L and total serum protein was 42 g/L. Radiologic study of the upper alimentary tract showed thickened mucosal folds and slow wriggling movements of the stomach. There were some nodular defects, of various sizes. The maximum diameter was 3.5 cm. Clinical diagnosis was multiple polyposis, and the entire stomach was resected.

Pathologic findings

Pathologic changes were found throughout the entire stomach. Changes were similar to those of case 1, with the exceptions of more thickened mucosal folds (macroscopically) and more foveolar epithelium lining with mucus-secreting cells (microscopically) (Figure 4). Mucosal intraepithelial lymphocytes of the surface and foveolar epithelium accounted for 40.5% and the depth of lamina propria was 13.9 mm. Findings from the pathologic examination indicated a diagnosis of Menetrier's disease with lymphocytic gastritis.

Figure 1 Mucosal folds of the body of the stomach with appearance reminiscent of cerebral convolution.

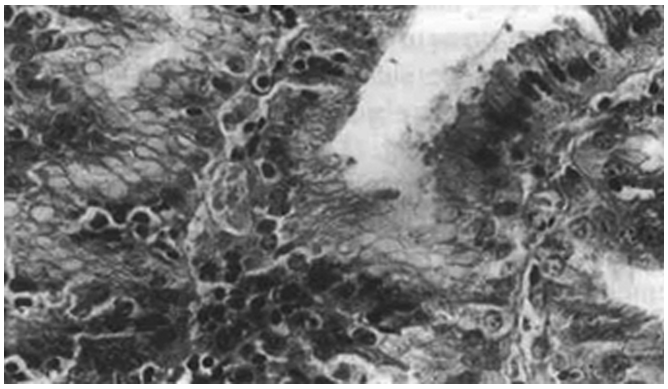

Figure 2 Numorous intraepithelial lymphocytic infiltration of surface and epithelial pits. HE staining, magnification × 132.

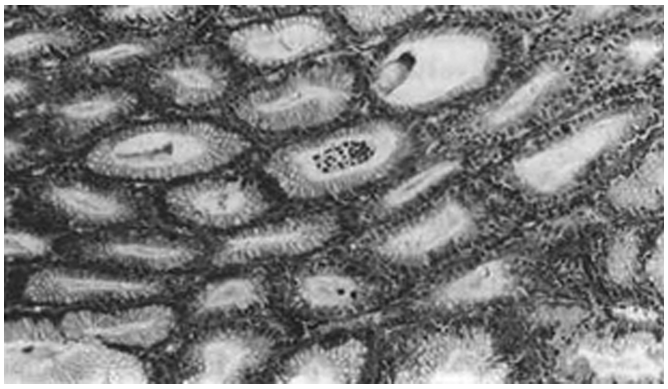

Figure 3 Crypts lined with mucus-secreting cells and containing lymphocytes and neutrophils, with formation of crypt abscesses. HE staining, magnification × 66.

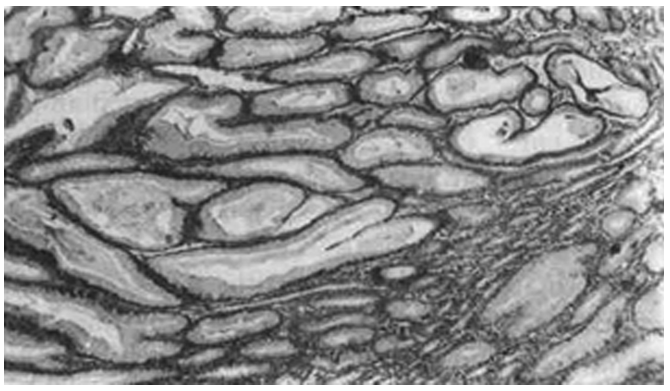

Figure 4 Gland normally containing parietal and chief cells that were replaced by the active mucus-secreting cells. HE staining, magnification × 66.

DISCUSSION

Gastric rugal hypertrophy is a morphologically descriptive name, and can encompass many disease conditions. Fieber *et al*[1] reported that a variety of names used for this condition, including at least 37 synonyms, such as Menetrier's disease (MD), Zollinger-Ellison syndrome, hypertrophic hypersecretory gastropathy, diffusely infiltrative adenocarcinoma, malignant lymphoma, and certain forms of gastric polyposis (*e.g*. Cronkhite—Canada syndrome). The cases reported above were diagnosed as Menetrier's disease with lymphocytic gastritis.

Macroscopically, Menetrier's disease may be categorized as one of three types: 1. localized, essentially involving the body of the stomach; 2. diffuse, involvement of the whole stomach; 3. associated with other gastropathy, such as gastric adenocarcinoma, multiple ulcers, varioliform gastritis, *etc*. Microscopically, the foveolar epithelium shows marked hyperplasia and metaplasia; the glands containing the parietal and chief cells are replaced by mucus-secreting cells. Finally, chronic inflammatory cell infiltration occurs in the lamina propria. A few of cases in the literature have been reported with associated atypical hyperplasia and carcinomatous changes.

Lymphocytic gastritis (LG) is characterized by the presence of numerous intraepithelial lymphocytes associated with the surface and foveolar epithelium, with some of these cells infiltrating the dilated pits and forming crypt abscesses. In our cases, a variety of inflammatory cells were found to have infiltrated the lamina propria. A case reported by Dixon *et al*[4] had lymphocytic counts of surface and foveolar epithelium in LG with an average of 57%, and with normal gastric mucosa and chronic atrophic gastritis accounting for 2.5% and 3.4% respectively; immunohistochemical study verified that these lymphocytes were T lymphocytes.

The above cases presented the unusual combination of Menetrier's disease intermingled with LG. The intraepithelial lymphocytes of the surface and foveolar epithelium were found not only in the stomach body's mucosal folds affected by Menetrier's disease but also in the inter-rugal body mucosa as well as in the uninvolved antral mucosa. It remains unclear whether the presence of LG was a purely coincidental event, or if the LG significantly contributed to the pathogenesis or the course of Menetrier's disease. Therefore, researchers should continue to study the etiology, pathogenesis and natural history of Menetrier's disease with LG.

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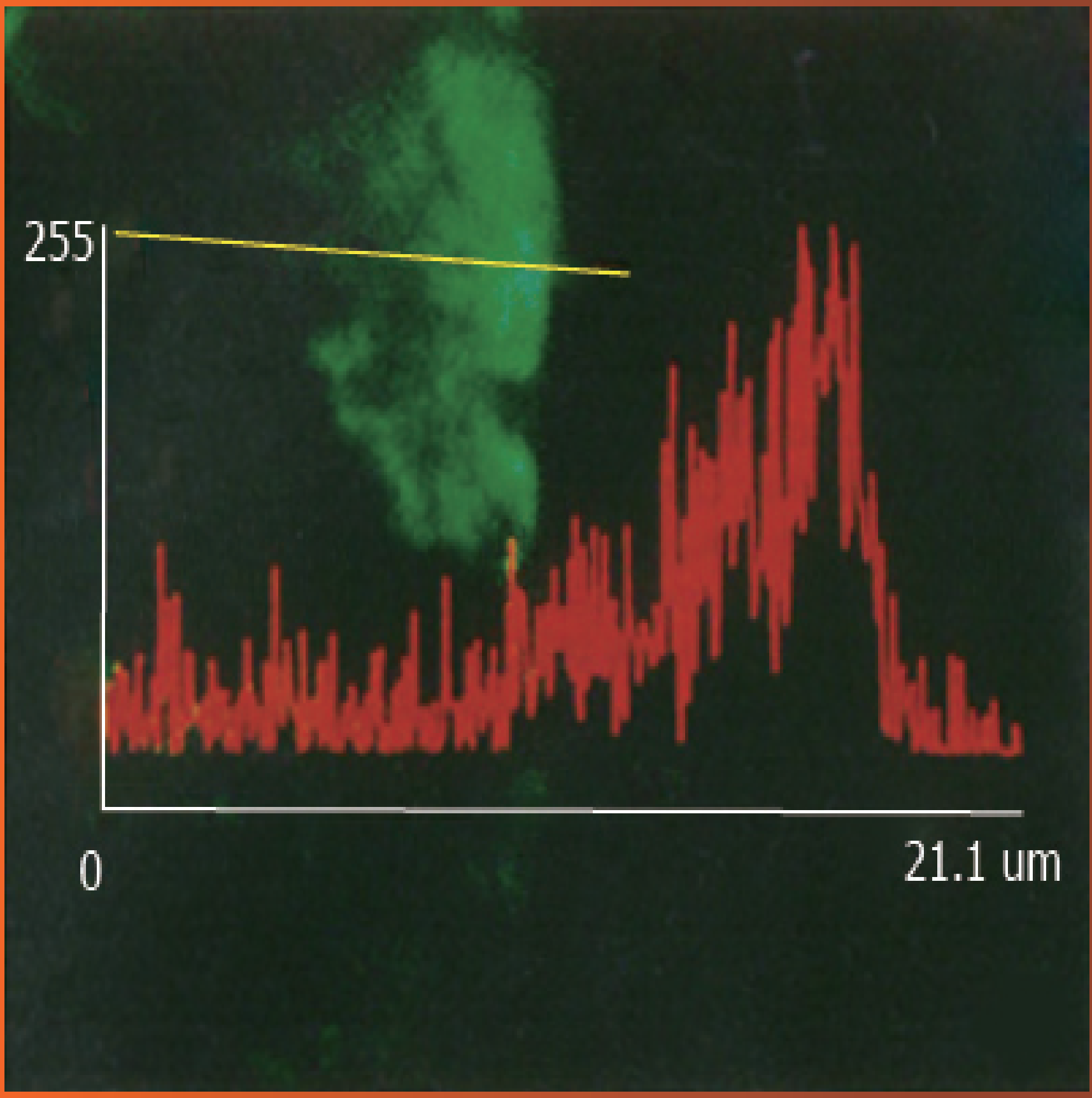

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Mucosal permeability to lipopolysaccharides in the colon in chronic alcoholic rats

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Abstract

AIM: To evaluate the effects of chronic alcohol abuse on the mucosal permeability to lipopolysaccharide in the colon in rats.

METHODS: *Escherichia coli* lipopolysaccharide (LPS, 20 μg/mL) was injected into the colon of chronic alcoholic rats (n = 10) and the rats were supplied with Lieber diets every other day for 6 weeks. Before LPS injection and 5, 10, 20, 30 min after injection, blood samples from the portal vein were obtained and contents of LPS in the blood were measured. The distribution of LPS in the colon tissues was observed with a confocal laser scanning microscope by immunofluorescent technique using a monoclonal antibody specific to the lipid A region of LPS. Normal rats were used as controls (n = 6).

RESULTS: Before LPS injection into the colon, LPS levels in the blood of portal vein of chronic alcoholic rats were significantly higher than those of normal controls (3.56 ± 0.67 pg/mLaa, *vs* 2.45 ± 0.15 pg/mLaa, $P < 0.01$). At 5, 10, 20, 30 min after injection of LPS, LPS contents were significantly higher than those before LPS injection (173.56 ± 23.45 pg/mLaa, 154.78 ± 20.57 pg/mLaa, 43.89 pg/mLaa ± 8.67 pg/mLaa, 45.38 ± 7.89 pg/mLaa *vs* 3.56 ± 0.67 pg/mLaa, $P < 0.01$ respectively). Most mucosal cells showed strong positive reactions to LPS in the rats of chronic alcohol abuse, but no significant changes of LPS contents in blood from the portal vein and fluorescentreactions to LPS in mucosal cells of normal rats were found after LPS injection.

CONCLUSION: Chronic alcohol abuse resulted in a significant increase of permeability to LPS in colon mucosal cells in rats.

Key words: Colon; Alcohol; Ethyl polysaccharides; Bacterial; *Escherichia coli*

Chen XM, Xu RL, Ma XH, Zhou YC, Han DW. Mucosal permeability to lipopolysaccharides in the colon in chronic alcoholic rats. *World J Gastroenterol* 1996; 2(3): 125-127 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/125.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.125

INTRODUCTION

Endotoxins are potent biological moieties from the outer membrane of Gram-negative bacteria and mainly composed of lipopolysaccharides (LPS) and proteins[1]. LPS causes all the toxic actions endotoxin inflicts[2]. Endotoxemia is a common complication in patients with alcoholic hepatitis, and the liver injury was associated with the development of endotoxemia. Normally, a lot of endotoxins present in the gut and nonpathogenic amounts of this toxic macromolecule material are absorbed through the intestinal wall, reaching the liver[3]. The sinusoidal cell is the key to the uptake and detoxication of endotoxins in the liver[4] Under pathological conditions, such as alcoholic hepatitis, endotoxins in the portal vein may enter the peripheral blood (known as endogenous endotoxemia) as a result of depression of Kupffer cells, or portal systemic circulation[5,6]. However, there is little information concerning the permeability mechanism of LPS in the gut under those pathological conditions. In the present study, by using a monoclonal antibody specific to the lipid A region of endotoxin, the distribution and localization of endotoxins in the colon in rats of chronic alcohol abuse was observed with confocal laser scanning microscopy. LPS concentrations in the portal vein were measured.

MATERIALS AND METHODS

Animals

Thirty male Wistar rats were divided into two groups: chronic alcoholic group and control group. Rats were fed on a complete liquid diet with the Lieber protocol[7] in chronic alcoholic group. Diets were supplied every other day with homogenation and administered through Richter feeding tubes for 6 wk. Rats in control group (n = 6) were fed ad libitum with ordinary rat chow. Under the anaesthesia

Table 1 lipopolysaccharides contents in blood from the portal vein (pg/mL, $\bar{x} \pm s$)

	n	Before LPS injection	Time points after LPS injection			
			5	10	20	30
Normal control	6	2.45 ± 0.15	3.04 ± 0.98	3.56 ± 1.65	3.05 ± 0.97	2.37 ± 0.56
Alcoholic group	10	3.56 ± 0.67[b]	173.56 ± 23.45[b]	154.78 ± 20.57[b]	43.89 ± 8.67[b]	45.38 ± 7.89[b]

[b]$P < 0.01$, *vs* normal control at the same time point.

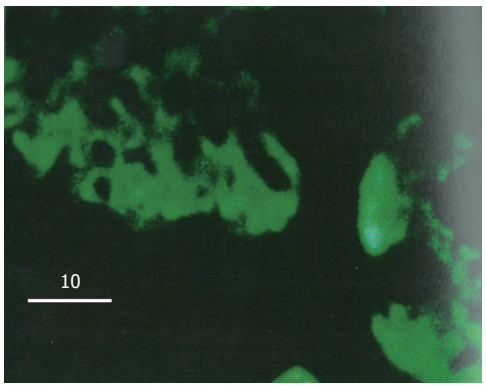

Figure 1 Fluorescent reactions to LPS in the colon mucosa in chronic alcoholic rats 30 min after LPS injection. Those positive reactions were found in the cytoplasm of mucosal cells, tissues under the basement membrane, but not found in the neclei of the mucosal cells.

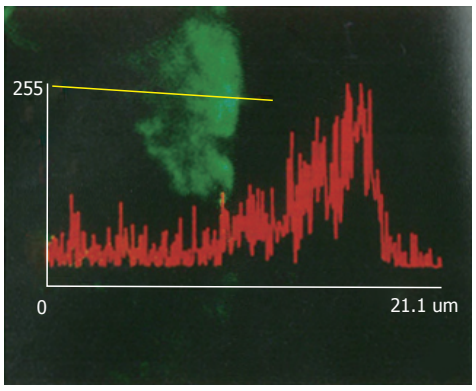

Figure 2 Fluorescent reactions to LPS in the colon mucosa in chronic alcoholic rats 30 min after LPS injection. No significant increases of fluorescent reactions were found between the membrane of those mucosal cells.

by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), abdomens of rats of both groups were opened. The colons about 1.5 cm-2.0 cm long were ligated at two ends without interruption to their blood circulation. After being washed with sterile saline, the colon was injected *Escherichia coli* LPS. LPS was diluted with sterile saline to 20 μg/mL and the amount of the injected LPS corresponded to the pressure in the colon which was maintained to 10 cm/H_2O. About 0.5 mL-1.0 mL of LPS was injected in to each rat. Before LPS injection and 5, 10, 20, 30 min after the injection of LPS into the colon, blood samples about 0.5 mL were obtained from the portal vein for the determination of LPS in the blood. At 30 min after LPS injection, the colon was taken and washed. Tissue specimens were fixed in buffered formalin, embedded in paraffin and 4 μm sections were cut. The sections were then performed for immunofluorescent staining.

Immunofluorescence staining

As for location of LPS in tissues and cells, the indirect immunofluorescence staining technique was used. Briefly, sections (4 μm thick) were dewaxed with xylene and washed successively with 100%, 96%, 70% aqueous ethanol. After being washed with phosphate buffered saline (PBS), each section was treated with 1% normal goat serum (Vectastain, Vector Laboratories, Inc. Burlingame) for 30 min to cover possible nonspecific sites. Then, all the sections were washed and incubated with the monoclonal antibody against LPS (a gift from Dr. Noguchi) for 60 min at 37 ℃. After washing with PBS for three times, fluorescein isocthiocyanate (FITC) conjugated goat anti mouse IgM (H&L) F(ab')2 fragments (1:30, O.E.M. Concepts Inc.) was added for 60 min at 37 ℃. Excess conjugate was removed by washing with PBS. Then the sections were mounted with VECTASHIEID mounting medium (Vector Laboratories, North Chicago) for fluorescence under coverslip and ready for observation with Confocal Laser Scanning Microscopy. For the sake of negative controls, sections were incubated with PBS omitting the first antibody or FITC conjugated secondary antibody.

Confocal laser scanning microscopy

Sections were observed within 2 h after immunofluorescence staining. With a confocal laser scanning microscope (LSM-GB200, Olympus, Japan), cellular localization and distribution of LPS in the mucosal cells of the colon were observed.

Statistical analysis

All data were expressed as $\bar{x} \pm s$, and were analyzed with the Stat View Statistical Program. Student's *t* and Anoval *F* test were employed where appropriated.

RESULTS

Changes of LPS concentration in portal vein blood

In rat of chronic alcohol abuse for 6 weeks, LPS concentration in portal vein blood was significantly higher than that of control even before LPS injection into the colon. At 5 min after the injection of LPS, LPS level in the portal vein blood increased markedly and reached the peak point. At 10 min after LPS injection, LPS level in the blood decreased a little but even at 30 min after LPS injection, the LPS level was still significantly higher than that of the normal control. For the rats of normal control, LPS levels in the portal vein blood increased a little after LPS injection, but no significant difference was found in comparison with that before LPS injection (Table 1).

Observation by confocal laser scanning microscopy

In normal control rats after LPS injection, colon mucosal cells showed no obvious FITC fluorescence reaction to LPS except membrane cells on the cavity side of the mucosa which showed slight fluorescence. In the rats of chronic alcoholic abuse, nearly all the mucosal cells showed strong FITC fluorescent reactions to LPS 30 min after LPS injection. The fluorescent reactions were found in the cytoplasm of mucosal cells, tissues under the basement membrane, but not found in the nuclei of the mucosal cells (Figure 1). Fluorescent reactions did not significantly increase between the membrane of the mucosal cells (Figure 2). The positive fluorescent reactions to LPS were evenly scattered, not in spot or particulate form. No fluorescence was detected in negative control tissues using only the monoclonal antibody or FITC labelled secondary antibody.

DISCUSSION

Confocal laser scanning microscopy can scan tissues or cells at thickness as thin as 1 μm, and can actually reflect the FITC fluorescent distribution in tissues and cells. For the location and distribution of LPS in tissues and cells, the monoclonal antibody specific to the lipid A region of LPS is more specific than antibody against the polysaccharide chain of LPS by the immunohistochemical technique, because the antigen is characterized by the fact that the polysaccharide chain of LPS might be changed or separated from lipid A part after LPS enter cells[8]. Most of the toxic effects of LPS, if not all, reside in the core lipid A part[9]. No fluorescence was detected in negative controls using only the monoclonal antibody or FITC labelled secondary antibody, and it was reasonable to estimate that the fluorescence reaction detected was specific for the location

and distribution of LPS in tissues and cells.

Endotoxins are mainly composed of lipopolysaccharides and proteins[1]. There is good evidence that portal vein endotoxemia is in a normal state[3]. It is generally known that the liver, mainly by Kupffer cells and hepatocytes, is responsible for the clearance of endotoxins in the portal vein blood[4]. In patients with alcoholic hepatitis, endotoxemia is a common complication[5,6] and the endotoxins are affirmed primarily from the gut by the portal vei[10] and the lymphatic vessels[11] in intestine. The liver injury due to alcohol and its clinical manifestation were confirmed to be associated with the development of endotoxemia[12], suggesting that endotoxin might play a role in hepatic injury induced by alcohol. In the rats of chronic alcohol abuse with Lieber diets for 6 wk, we found that endotoxin concentration in the portal vein blood was markedly higher than that of the control, and the level increased progressively at various time point after LPS injection, suggesting that the permeability to LPS in the intestine increased after the stimulation by alcohol abuse, a phenomenon which might be critical to the development of endotoxemia in alcoholic hepatitis.

Two different mechanisms, namely specific and non-specific, are suggested to be involved in the initial interaction of LPS with cells[13]. Specific interactions result from the binding of LPS to a specific receptor on the plasma membrane, and, on the other hand, non-specific interactions result from the binding of LPS macromolecule to any membrane constituents other than the receptors. Both mechanisms are involved in the uptake procedure of LPS by cells[14,15]. A number of receptors specific to different regions of LPS were recently reported. By using a LPS derivative as a probe to define LPS specific binding structures, Lei MG *et al*[16] identified a 73 kDa membrane-localized protein which existed in almost all the mammalian cell subpopulations and was lipid A specific. LPS was found in the cytoplasm of macrophages in spot and particulate form after phagocytized by those cells[17]. In the present study, the positive reactions to LPS were found in nearly all the mucosal cells in chronic alcoholic rats, indicating that there was no cellular specificity to the permeability of LPS in the colon. There were no positive reactions to LPS among those mucosal cells membranes also suggested that the permeability to LPS in the colon may be through the cells, not through the injection between cells. The positive fluorescent reactions to LPS in the cytoplasm of mucosal cells were evenly distributed, not in spot or particulate form. This phenomenon suggested that the uptake mechanism of LPS by those mucosal cells might not be through phagocytic procedure.

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Expression of vascular endothelial growth factor and its prognostic significance in gastric carcinoma

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Abstract

AIM: The aim was to investigate the clinical significance of vascular endothelial growth factor (VEGF) expression in gastric carcinoma.

METHODS: The expression of VEGF in 128 gastric carcinomas was investigated by immunohistochemical staining with an anti-VEGF polyclonal antibody. Correlations between VEGF expression and various clinicopathological factors and prognosis were studied.

RESULTS: The overall VEGF-rich expression rate was 64.1% in gastric carcinoma tissue, and was significantly higher in patients with stage Ⅲ and Ⅳ disease than in those with stage Ⅰ disease ($P < 0.05$). Significant differences in expression rate were related to growth pattern, serosal invasion, and lymph node metastasis. VEGF-rich expression was much higher in tumors with an expanding growth pattern (71.8%) or serosal invasion (73.5%) than in those with an infiltrative growth pattern (52.0%) or nonserosal invasion (53.3%) ($P < 0.025$, respectively). Expression was also significantly higher in patients with lymph node metastases (75.0%) than in those without such metastases (50.0%, $P < 0.05$). A postoperative survey of 86 patients who had been followed for at least 5 years found that the 5-year survival rate of patients with VEGF-rich tumors was significantly lower than that of patients with VEGF-poor tumors ($P < 0.05$).

CONCLUSION: VEGF expression may be associated with invasion and metastasis and may also be a useful indicator of gastric carcinoma prognosis.

Key words: Stomach neoplasms/pathology; Endothelial growth factors; Prognosis

Tao HQ, Qin LF, Lin YZ, Wang RN. Expression of vascular endothelial growth factor and its prognostic significance in gastric carcinoma. *World J Gastroenterol* 1996; 2(3): 128-130 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/125.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.128

INTRODUCTION

Solid tumors require neovascularization for growth and metastasis. Experimental evidence shows that endothelial growth factors, such as basic fibroblastic growth factor (bFGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF) secreted by tumor cells play a crucial role in tumor angiogenesis[1]. VEGF regulates microvasculature permeability and is an endothelial cell-specific mitogen[2,3], and is likely to play an important role in both tumor angiogenesis and generation of tumor stroma[4]. Results obtained with monoclonal neutralizing antibodies to VEGF have produced strong evidence that VEGF contributes to the progression and metastasis of solid tumors by promoting angiogenesis[5,6].We used immunohistochemical staining of gastric carcinoma tissue with an anti-VEGF polyclonal antibody to investigate the correlations between VEGF expression, various clinicopathological factors, and prognosis.

MATERIALS AND METHODS

Clinical material

Resected specimens from 128 patients with gastric carcinoma who underwent gastrectomy at Ruijin Hospital were studied. The patients were 38 to 78 years of age (mean: 58.7 years), 86 were men, and 42 were women. No patient had received chemotherapy or radiation therapy before surgery. The Guidelines of National Gastric Cancer Association were used for pathologic diagnosis and classification of variables, and histologic staging was determined according to the TNM criteria (Table 1). In this study, tumors were divided into two histologic subgroups, a differentiated type including of papillary and tubular adenocarcinomas, and an undifferentiated type including poorly differentiated adenocarcinomas, signet ring cell carcinomas, and mucinous adenocarcinomas. Curative resection was performed in 109 patients, 86 of whom were followed for at least 5 years after surgery; 19 patients underwent noncurative surgical procedures.

For pathological evaluation and immunohistochemistry, tissue specimens were fixed in a 10% formaldehyde solution, embedded in paraffin, sectioned at 4 μm, and mounted on glass slides.

Antibodies and reagents

Rabbit polyclonal antibody to VEGF was purchased from Santa Cruz Inc. Recombinant human VEGF was a kind gift from Genentech Ltd. (CA). Labeled streptavidin-biotin (LSAB) staining kits were produced by DAKO Inc.

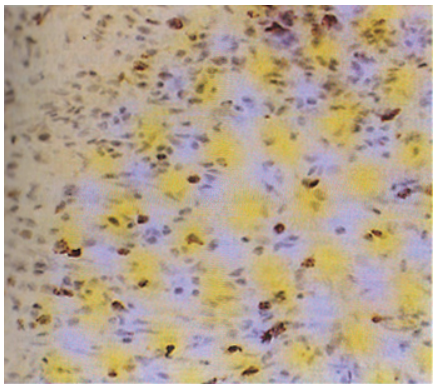

Figure 1 Poorly differentiated adenocarcinoma tissue with VEGF++ staining. Cytoplasmic VEGF staining was seen in tumor cells and diffuse distribution of VEGF-positive tumor cells is apparent. (original magnification × 200)

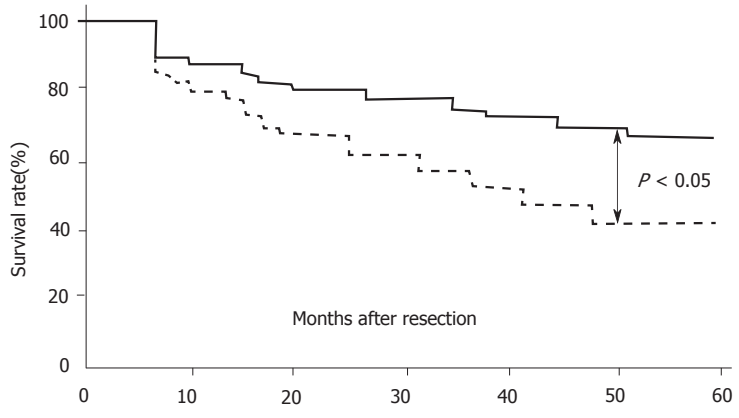

Figure 2 Survival rate after curative resection of VEGF-poor (*n* = 30) and VEGF-rich tumors (*n* = 56)

Immunohistochemical study

Immunohistochemical staining was performed by the immunoperoxidase technique following predigestion and trypsinization. Anti-VEGF polyclonal antibodies were used at a 1:20 dilution. Normal rabbit immunoglobulin G was substituted for primary antibody as the negative control. Immunoreactivity was graded as (-), (±), (+) and (++) by the staining intensity. Tumors graded as (+) or (++) were designated as VEGF-rich and those with (-) or (±) staining were VEGF-poor. Tumors including both VEGF-positive and VEGF-negative subpopulations were graded as (+). The evaluations were done by an observer completely blinded to the patient characteristics.

Statistical methods

The significance of relationships between VEGF expression and clinicopathological factors was tested by the chi-square method. Survival curves were calculated using the Kaplan–Meier method and analyzed by the log rank test. Statistical significance was defined as $P < 0.05$.

RESULTS

VEGF Expression

The expression of VEGF was observed mainly in the cytoplasm of tumor cells. A representative case of VEGF (++) staining is shown in Figure 1. VEGF staining was blocked by pretreatment with an antibody to recombinant human VEGF (data not shown). Weakly positive VEGF staining was seen in endothelial cells, no direct correlation was found between the staining intensity of tumor cells and that of endothelial cells. A heterogeneous distribution of VEGF-stained tumor cells was seen in tissue from several of the tumors. Of 128 tumors, 82 (64.1%) were VEGF-rich; faint staining was seen in the cells of most VEGF-poor tumors.

Correlation between VEGF expression and tumor histologic stage

Table 1 shows the correlation between VEGF expression and tumor histologic stage. The VEGF-rich expression rates in patients with stage Ⅲ and Ⅳ disease was significantly higher than that in patients with stage Ⅰ disease.

Table 1 Correlation between vascular endothelial growth factor expression and TNM stage

TNM stage	*n*	VEGF-poor rate (%)	VEGF-rich rate (%)
I	39	48.7	51.3
II	27	37.0	63.0
III	48	27.1	72.9[a]
IV	14	28.6	71.4[a]

[a]$P < 0.05$, compared with stage 1.

Table 2 Correlation between clinicopathologic factors and vascular endothelial growth factor expression

Variable	Patients (*n*)	VEGF-poor rate (%)	VEGF-rich rate (%)	*P* value
Histologic type				
Differentiated	50	36.0	64.0	> 0.05
Undifferentiated	78	35.9	64.1	
Serosal invasion				
Positive	68	26.5	73.5	< 0.025
Negative	60	46.7	53.3	
Growth pattern				
Expanding	50	48.0	52.0	< 0.025
Infiltrative	78	28.2	71.8	
Lymph node metastasis				
Negative	50	25.6	74.4	< 0.05
Positive	78	52.0	48.0	

Relationship between VEGF expression and clinicopathological factors

Table 2 shows the relationships of VEGF expression with various clinicopathologic factors. There was a statistically significant association between VEGF-rich expression and growth pattern, depth of invasion, and lymph node metastasis. However, expression rate was not significantly correlated with histologic type.

Survival

Of the 86 patients who underwent curative resection and were followed for at least 5 years, 42 died following tumor recurrence. Postoperative analysis demonstrated that the 5-year survival rate of patients with VEGF-rich tumors (42.8%, 24/56) was significantly lower than that of patients with VEGF-poor tumors (66.7%, 20/30; (Figure 2, $P < 0.05$, log–rank test).

DISCUSSION

VEGF is an endothelial cell-specific mitogen and an *in vivo* inducer of tumor angiogenesis. It has also been purified and shown to act independently as a tumor-derived vascular permeability factor promoting the extravasation of plasma proteins, including fibrinogen[2,3]. VEGF thus has important biological significance for the generation of vascularized tumor stroma. VEGF is known to be expressed in a variety of tumor cell types and in tumor tissues that have are characterized clinically by their neovascularization. In this study, we investigated the expression of VEGF in gastric cancer and observed a significant correlation between VEGF expression and tumor growth pattern, invasion depth, and lymph node metastasis. Melnyk *et al*[8] investigated the effect of VEGF inhibition on growth of primary tumors and on micrometastasis in experimental animals, and found that anti-VEGF antibodies not only inhibited growth of primary tumors but also suppressed metastasis to distant sites. This suggests that VEGF may have a clinically important effect on tumor growth and distant metastasis. We noted that the VEGF expression rate in patients with stage Ⅲ and stage Ⅳ gastric carcinoma was significantly higher than that in patients with stage Ⅰ disease, which supports the view that VEGF has a direct effect on tumor growth and metastasis. Many studies have suggested that VEGF contributes to tumor growth and invasion by promoting angiogenesis, which increases the tumor blood supply. Moreover, newly formed tumor capillaries have fragmented basement membranes and leak, making it easier for tumor cells to enter the circulation and form metastases.

VEGF expression level may thus predict the biological behavior of gastric carcinoma.

Although angiogenesis is seen as in increase in blood vessel formation, some studies have found that it is also correlated with increased lymph node metastasis. Smith and Basu demonstrated that neovascularization of rabbit corneas following injection of India ink led to the the appearance of ink particles in ipsilateral lymph nodes. These findings indicate that lymphocapillary anastomoses are present and/or that angiogenesis correlates with the formation of new lymphatic vessels. Therefore, we consider that VEGF not only promotes angiogenesis but also induces the formation of new lymphatic vessels, increasing the opportunity for tumor cells to enter lymphatic vessels and form lymph node metastases.

With regard to prognosis, Toi *et al*[7] have shown that VEGF expression is closely associated with early relapse after surgery for primary breast cancer. The relapse-free survival rate of patients with VEGF-rich tumors was significantly lower than that of patients with VEGF-poor tumors, and VEGF status was found to be an independent prognostic indicator. Our study confirmed these findings in gastric carcinoma. We observed a significantly worse prognosis in patients with VEGF-rich compared with VEGF-poor tumors. This result suggests that VEGF expression status is a useful prognostic indicator in gastric carcinoma. Recently, it was reported that administration of a neutralizing monoclonal antibody against human VEGF inhibited the growth and metastasis of human tumor xenografts in nude mouse[5,6]. Millauer *et al*[9] reported that growth of C6 rat glioblastoma cells in nude mice was suppressed after local administration of retrovirus expressing a dominant negative mutant of flk-1, the receptor of VEGF. These results provided a scientific rationale for studying the effect of VEGF on human tumorigenesis, invasion and metastasis, and for adopting treatment strategies targeting VEGF.

Gastric carcinoma is one of the most common human malignant tumors. Our study showed that 64.1% of patients had tumors with VEGF-rich expression, and that the prognosis of patients with VEGF-rich tumors was significantly worse than that of patients with VEGF-poor tumors. We conclude that assay of VEGF expression in gastric carcinoma, and treating patients with VEGF-rich tumors with anti-VEGF antibodies may help prevent postoperative recurrence and metastasis. If this proposed treatment is applied in combination with other adjuvant therapies, the postoperative overall survival rate of patients with gastric cancer would increase.

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EXPERIMENTAL PAPERS

Structure-activity relationship of pituitary adenylate cyclase activating polypeptide

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Abstract

AIM: To investigate the structure-activity relationship of pituitary adenylate cyclase activating polypeptide (PACAP) in guinea pig gallbladder using a synthetic PACAP/vasoactive intestinal peptide (VIP) hybrid.

METHODS: We synthesized PACAP–VIP hybrid peptides using the Fmoc strategy and a simultaneous multiple solid-phase peptide synthesizer. The peptides were tested in isolated guinea pig gallbladders using an improved horizontal type organ bath.

RESULTS: VIP induced relaxation of gallbladder smooth muscle strips, while PACAP27 contracted them. Amino acids at positions 4, 5, 9, and 24–26 were replaced without significant loss of activity. [Leu^{13}]–PACAP27, a substitution in the α-helix domain, also had no significant loss in activity ($P < 0.05$). It was more potent than [Gly^{8}]– and [DAsp^{8}]–PACAP27 and could substitute peptides at position 21. Des–[His^{1}] and [Ala^{6}]–PACAP27 had no activity at 10^{-7} mol/L. [Gly^{8}]–, [DAsp^{8}]–, [Phe^{21}]– and [Pro^{21}]–PACAP27 at 10^{-7} mol/L had about 25% of the activity of PACAP27 at 10^{-7} mol/L ($P < 0.05$).

CONCLUSION: The N-terminal disordered region is more important than other regions for determining the physiological activity of PACAP in the guinea pig gallbladder.

Key words: Gallbladder; Vasoactive intestinal peptide; Pituitary adenylate cyclase activating polypeptide; Amino acids

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INTRODUCTION

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide structurally related to vasoactive intestinal polypeptide (VIP)[1]. It exists in the ovine hypothalamus in two molecular forms, PACAP38 and PACAP27. The terminal amino acid 27 residue of PACAP38 (PACAP27) has high sequence homology (68%) with VIP. Many biological actions of PACAP are very similar to those of VIP[1–3]; but we recently found that PACAP contracts the guinea pig gallbladder, while VIP relaxes it[4]. The guinea pig gallbladder is a good animal model system to study the structure-activity relationship of PACAP and VIP. In this investigation, we synthesized PACAP–VIP hybrid peptides using the Fmoc strategy and a simultaneous multiple solid phase peptide synthesizer, and we compared the effects of the peptides on gallbladder smooth muscle *in vitro*.

MATERIALS AND METHODS

Peptide Synthesis

Considering the sequence homology of VIP–PACAP, NMR data, and computer-aided molecular graphics[5], positions 4, 5, 6, 8, 9, 10, 21 and 24–26 were selected as mutation points in this study (Figure 1). Peptides were synthesized using the Fmoc strategy and a simultaneous multiple solid phase peptide synthesizer (Model PSSM 8M, Shimadzu Corp.). After simultaneous cleavage with a trifluororacetic acid (TFA) cocktail, the desired crude peptides were easily purified by single step reversed phase-high performance liquid chromatography (RP-HPLC), and then characterized and shown to be homogeneous by HPLC, sequencing, amino acid analysis and liquid secondary ion mass spectrometry (LSIMS).

Biological Methods

The peptides were tested in isolated guinea pig gallbladders using an improved horizontal type organ bath. Thirty male Hartley

VIP: HSDAVFTDNY TRLRKQMAVK KYLNSILN-NH2
PACAO27: HSDGIFTDSY SRYRKQMAVK KYLAACL-NH2
Des–[His^{1}] PACAO27: SDGIFTDSY SRYRKQMAVK KYLAACL-NH2
[Ala^{4}]–PACAP27: HSDAIFTDSY SRYRKQMAVK KYLAACL-NH2
[Val^{5}]–PACAP27: HSDGVFTDSY SRYRKQMAVK KYLAACL-NH2
[Ala^{6}]–PACAP27: HSDGIATDSY SRYRKQMAVK KYLAACL-NH2
[Gly^{6}]–PACAP27: HSDGIFTGSY SRYRKQMAVK KYLAACL-NH2
[$DAsp^{8}$]–PACAP27: HSDGIFTDSY SRYRKQMAVK KYLAACL-NH2
[Asn^{9}]–PACAP27: HSDGIFTDNY SRYRKQMAVK KYLAACL-NH2
[Leu^{13}]–PACAP27: HSDGIFTDSY SRYRKQMAVK KYLAACL-NH2
[Ala^{21}]–PACAP27: HSDGIFTDSY SRYRKQMAVK AYLAACL-NH2
[Phe^{21}]–PACAP27: HSDGIFTDSY SRYRKQMAVK FYLAACL-NH2
[Pro^{21}]–PACAP27: HSDGIFTDSY SRYRKQMAVK PYLAACL-NH2
[Asn^{24}, Ser^{25}, Ile^{26}]–PACAP27: HSDGIFTDSY SRYRKQMAVK KYLNSIL-NH2

Figure 1 Amino acid sequences of the synthetic PACAP–VIP peptides

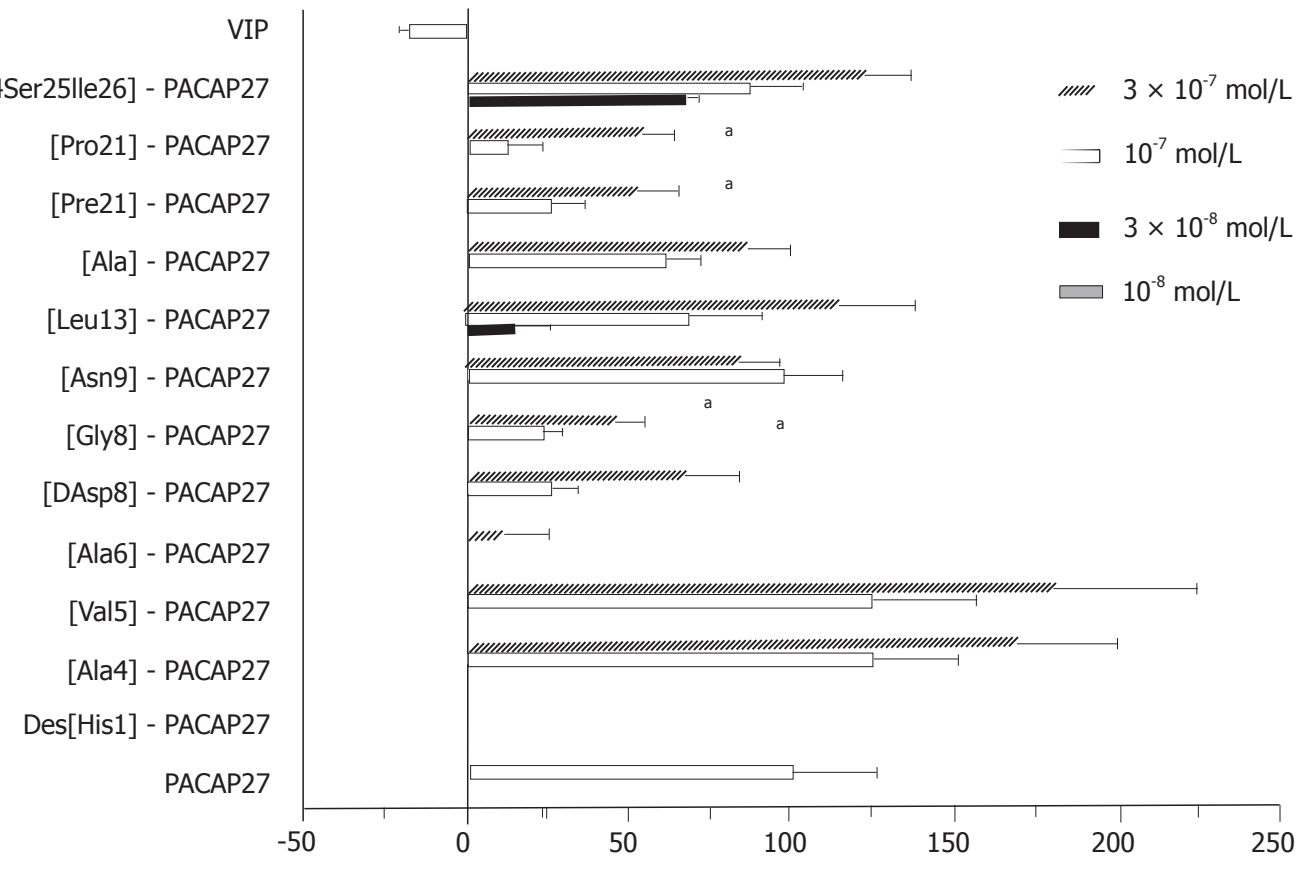

Figure 2 The effect of PACAP–VIP analogues on isolated guinea pig gallbladder. Data are shown as $\bar{x} \pm s$ ($n = 5$). $^{a}P < 0.05$ *vs* PACAP27 at 10^{-7} mol/L.

strain guinea pigs (450–580 g) were killed by exanguination. The gallbladders were excised and four 1.2 mm × 10 mm smooth muscle strips were prepared with the aid of a binocular microscope. The strips were suspended in an organ bath containing (4 mL) Krebs–Ringer solution (pH 7.4), maintained at 37 ± 5 ℃, aerated with 95% O_2 and 5% CO_2, and connected to an isotonic displacement transducer. After setting the initial tension at 500 mg, the muscle strips were allowed to equilibrate for 60 min. The length of the strips was recorded using a pen recorder and a computer-controlled data acquisition system. An acetylcholine (Sigma, St. Louis.) control (10^{-8}–10^{-4} mol/L), synthetic PACAP27, VIP, or the hybrid peptides were added to the organ bath at a concentration of 10^{-8}, 3 × 10^{-8}, 10^{-7}, or 3 × 10^{-7}mol/L. The gallbladder response to the peptides and control solution were standardized to the PACAP27 response. The maximal contraction induced by PACAP27 (10^{-7} mol/L) was taken as 100% contraction and the resting length as 0%.

Statistical analysis

The data (Figure 2) were expressed as means ± standard deviation ($\bar{x} \pm s$). One way factorial analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) multiple comparison test were used to compare the peak responses after adding the peptides. $P < 0.05$ was taken as the level of significance with n equal to the number of animals.

RESULTS

VIP induced relaxation of gallbladder smooth muscle strips, while PACAP27 contracted them. Positions 4, 5, 9, and 24–26 could be replaced without significant loss in activity. [Leu^{13}]–PACAP27, a substitution in the α-helix domain, also had no significant loss in activity ($P > 0.05$), was more potent than [Gly^{8}]– or [$DAsp^{8}$]–PACAP27, and could substitute peptides at position 21. Des–[His^{1}]– and [Ala^{6}]–PACAP27 had no activity at 10^{-7} mol/L. [Gly^{8}]– [$DAsp^{8}$]–, [Phe^{21}]–, and [Pro^{21}]–PACAP27 at 10^{-7} mol/L had about 25% of the activity of PACAP27 at 10^{-7} mol/L ($P < 0.05$).

DISCUSSION

The N-terminus of PACAP has a high sequence homology with VIP.

At least two types of PACAP binding sites have been demonstrated in rat tissues. One is a type 1 site that is the dominant site in the hypothalamus, and is highly specific for PACAP. The other is a type 2 site that is the dominant site in the lungs and liver, and has almost equal affinities for both PACAP and VIP[6,7]. The action of PACAP on guinea pig gallbladder smooth muscle seemed to be directly via the activation of type 1 PACAP receptors because tetrodotoxin, atropine, and CCK antagonists failed to block it.

In our previous studies, the C-terminus (positions 9–26) of PACAP27, similar to VIP, had a propensity for α-helix formation in a hydrophobic environment, but the N-terminus from positions 1 to 8 did not have a well defined helical or strand structure[5]. PACAP substitutions in this region had a greater loss in activity than was seen with substitutions in other regions. These data suggest that the disordered region from positions 1 to 8 is very important for the recognition of PACAP by its receptor. Position 21 is also important, although it showed no significant loss in activity at a higher dose (3×10^{-7} mol/L).

In conclusion, the N-terminal disordered region is more important than other regions for the physiological activity of PACAP in guinea pig gallbladder.

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Prevention of liver metastasis by intraperitoneal 5-FU chemotherapy in nude mice inoculated with human colon cancer cells

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Abstract

AIM: To prevent hepatic metastasis by regional adjuvant chemotherapy after radical surgery for colon cancer.

METHODS: A nude mouse model of human colon cancer (HCC) was used to evaluate the prevention of metastasis of HCC cells following the application of early postoperative intraperitoneal (IP) high-dose 5-fluorouracil chemotherapy.

RESULTS: The incidence of liver metastasis was decreased by 40%, and the mean number of metastatic liver nodules was reduced by 50.89%. Compared with controls, 5-FU 40 mg in NS 40 mL/kg IP for 2 consecutive days prolonged mean survival by 48.21%.

CONCLUSION: IP is a promising and effective novel regional adjuvant chemotherapy for the prevention of liver metastasis of HCC cells after radical surgery for colon cancer.

Key words: Colonic neoplasms/surgery; Liver neoplasms/drug therapy; Fluorouracil/therapeutic use; Liver neoplasms/secondary

Feng GG, Zhou XG, Yu BM. Prevention of liver metastasis by intraperitoneal 5-FU chemotherapy in nude mice inoculated with human colon cancer cells. *World J Gastroenterol* 1996; 2(3): 134-135 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/134.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.134

INTRODUCTION

The incidence of liver metastasis after radical surgery for colon cancer is very high and and significantly shortens the survival of patients. Intravenous (IV) 5-FU chemotherapy is often not effective for the prevention of liver metastasis. Therefore, early postoperative intraperitoneal (IP) chemotherapy with high-dose 5-FU was evaluated for prevention of liver metastasis by human colonic cancer (HCC) cells in a nude mouse model. HCC cells were inoculated via the spleen.

MATERIALS AND METHODS

Animals

Thirty athymic BALB/c nude mice 5–6 wk of age were provided by the Shanghai Cancer Institute. During the conduct of the study procedures, mice were maintained in a laminar flow cabinet under specific pathogen free conditions.

Cell lines

Colon cancer cell lines derived from human colonic adenocarcinoma were provided by Shanghai Immunology Institute[1]. The cells were cultured and maintained with more than 95% viability in RPMI 1640 medium with 10% fetal calf serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 ℃ in a 5% CO_2 atmosphere. Aliquots of 1×10^6 cells were suspended in 0.03 mL in normal saline (NS) prior to use.

Splenic injection

Mice were anesthetized by intraperitoneal (IP) injection of phenobarbital and the spleen was exposed under sterile conditions by an incision in the left side of the animal. A 0.03 mL volume of NS containing 1×10^6 cells was slowly injected into the splenic pulp over 1 min, then the spleen was replaced in position and the incision was closed[2]. The mice were randomly assigned to three groups of 10 each. Group A was given 5-Fu 40 mg in NS 40 $mL^{-1} \cdot kg^{-1}$, for 2 consecutive days, group B was given 5-Fu 20 mg in NS 40 $mL^{-1} \cdot kg^{-1}$, for 5 consecutive days, and group C (controls) was given NS 40 mL/kg, Animals were treated 24 h after tumor inoculation and followed-up to determine survival time. The animals were killed 60 days after tumor inoculation, the liver was removed and the tumor nodules were counted.

Statistics

Results were expressed as means ± standard deviation ($\bar{x} \pm s$). Data were analyzed by analysis of variance (ANOVA) and the chi-square test . $P \leqslant 0.05$ was considered statistically significant.

RESULTS

The incidence of metastasis in groups A, B and C was 60% (6/10), 70% (7/10), and 100% (10/10), respectively. The 40% difference

Table 1 Metastatic nodules in the livers of experimental and control groups and controls ($\bar{x} \pm s_{\bar{x}}$, nodule)

Group	Liver metastatic nodules (*n*)					liver nodules ($\bar{x} \pm s$)
	0	1-20	21-40	41-60	61-80	
5-FU 40 mg[a]	4	2	1	1	2	24.80 ± 8.64
5-FU 20 mg	3	1	2	1	3	31.50 ± 8.68
Controls	0	2	1	3	4	50.50 ± 7.32

[a]$P < 0.05$, *vs* controls

Table 2 Survival of mice in experimental and control groups

Group	Survival time (d)				Mean survival (d)
	21-30	31-40	41-50	51-60	
5-FU 40 mg	3	1	0	6	45.50 ± 5.12[a]
5-FU 20 mg	3	1	2	4	43.90 ± 4.77[a]
Controls	6	2	2	0	30.70 ± 2.59

[a]$P < 0.05$, *vs* controls

between group A and 30% difference between group B and controls were not statistically significant ($P > 0.05$).The mean number of metastatic liver nodules was 24.80 ± 8.64 in group A, 31.50 ± 8.68 in group B, and 50.50 ± 7.32 in group C. The differences of 50.89% between group A and controls and 37.62% between group B and controls were both significant ($P < 0.05$, Table 1).

The mean survival times were 45.50 ± 5.12 days in group A, 43.90 ± 4.77 days in group B, and 30.70 ± 2.59 days in the control mice. Survival was prolonged by 48.21% and 43% in groups A and B, respectively ($P < 0.05$, Table 2). surgery

DISCUSSION

Previous studies reported high concentrations of antineoplastic agents in the abdominal cavity, portal vein, and liver after IP administration, but low concentrations in the systemic circulation, indicating a pharmacokinetic advantage of IP over IV chemotherapy[3-6]. We reported in our previous studies that HCC cells survived, grew, and disseminated following IP injection, and that chemically induced peritonitis, peritoneal adhesion, and nephrotoxicity were not induced by early postoperative IP chemotherapy with 5-Fu 40 mg in NS, 40 $mL^{-1} \cdot kg^{-1}$ for 2 consecutive days[7]. We used a nude mouse model of HCC cell metastasis to evaluate the effects of early postoperative IP chemotherapy with high-dose, large-volume 5-FU chemotherapy. We found a 40% reduction in the incidence of liver metastasis and a decrease in 50.89% in the mean number metastatic liver nodules. The mean survival time was prolonged by 48.21% compared with control mice. The results obtained in this experimental animal model suggest that IP chemotherapy has potential as an effective adjuvant approach in prevention of hepatic metastases in colon cancer .

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Pharmacology of novel Chinese medicines screened for treatment of severe acute pancreatitis

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Abstract

AIM: To screen the enzyme-inhibition and observe the pharmacologic effects of circulation-improving Chinese medicines on intestinal and pancreatic hemodynamics.

METHODS: *In vitro* screening of the amylase-and lipase-inhibiting effects of nine Chinese traditional medicines was carried out. Each extract was prepared following methods described by LIN Qi-Shou. Tests of the inhibition of trypsin and elastase activity were carried out by following methods described by ZHANG Tian-Min s and JIANG Chuan-Kui. A Modified Leslie method was used determine the effects of the best circulation-improving Chinese herb, Tao-Ren (Semen Persicae), and its extract (HHI-I), on intestinal hemodynamics. An electromagnetic flowmeter was used to measure intestinal blood flow and and a blood oxygen meter was used to measure oxygen consumption. A laser Doppler microcirculation dynamic analyzer was used for to measurement the effect of HHI-I on pancreatic microcirculation, and a tissue oxygen meter was used to measure changes of pancreatic oxygen partial pressure.

RESULTS: Of the nine Chinese traditional medicines, Yuan-Hu (Rhizome Corydalis) had the most potent inhibitory activity on both amylase and lipase activity, followed by Da Huang, Zhi Zi, Huang Qin, and Hang Shao. Canine experiments found that Huoxue Huayu (HHI-I) improved intestinal hemodynamics. Intestinal blood flow 20 min after infusion of HHI-I, was 225.0 ± 68.51 mL/min, which was significantly higher than that before infusion (201.34 ± 70.21 mL/min, $P < 0.05$). Blood flow increased to 245.40 ± 82.78 mL/min ($P < 0.05$) at 40 min and 252.20 ± 82.41 mL/min ($P < 0.01$). 60 min after infusion. Intestinal oxygen consumption also increased significantly, to 5.33 ± 2.57 mL/min at 60 min after infusion, compared with (2.72 ± 1.09 mL/min ($P < 0.05$) at baseline. HHI-I improved pancreatic microcirculation and tissue oxygen partial pressure. The basal microcirculatory blood flow of 20.4 ± 5.0 mL/min/100 g body weight increased to 49.0 ± 9.0 mL/min/100g body weight 20 min after administration ($P < 0.05$). Blood flow increase further to 51.0 ± 7.97 mL/min/100 g body weight ($P < 0.01$) at 40 min and 54.8 ± 15.51 mL/min/100 g body weight ($P < 0.01$) at 60 min. Pancreatic oxygen partial pressure was 6.21 ± 0.94 kPa at baseline, rose to 7.55 ± 1.40 kPa at 20 min ($P < 0.01$), 7.65 ± 1.76 kPa ($P < 0.05$) at 40 min, and 7.67 ± 1.64 kPa ($P < 0.01$) at 60 min after HHI-I administration. In the control group, saline administration had no significant effect of the indices.

CONCLUSION: Yuan-Hu and Tao-Ren used together have the potential to become novel, effective medicines

Key words: Pancreatitis treatment; Yuan hu; Tao ren

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INTRODUCTION

The mortality of severe acute pancreatitis (SAP) is high, and there are no specific treatments. The development of effective SAP therapy is a worldwide priority. Inhibition of pancreatic enzymes and improving pancreatic blood circulation are are potential treatment approach. There have been many reports of successful treatment of SAP with Chinese medicines. Treating SAP with the most effective enzyme-inhibiting and circulation-improving herbs may result in decreased in SAP mortality. In this study, we screened nine enzyme-inhibiting herbs and evaluated the hemodynamic effects of the most effective circulation-improving Chinese medicinal herbs.

MATERIALS AND METHODS

Inhibitory effect on trypsin activity

Method Extracts of each herb were prepared following methods previously described by Lin Qi-Shou[1]. The anti-trypsin inhibitory activity of 300 μL samples of each extract were tested and compared with control reactions that proceeded without inhibitors[2].

Results All nine herbal extracts inhibited trypsin activity, but they

Table 1 Effect of nine of Chinese herbal extracts on trypsin activity

Herbs	1	2	3	4	5	6	7	8	9
Activity change (± %)	-0.3	-6	-26	-12	-25	-17	-11	-5.1	-77

1: Chai Hu (Radix Bupleuri), 2: Bin Lang (Semen Arecae), 3: Da Huang, 4: Hu Lian (Rhizoma Picrorhizae), 5: Zhi Zi, 6:Huang Qin (Radix Scutellariae), 7: Hang Shao (Radix paeoniae Alba), 8: Mu Xiang (Radix Auxklandiae), 9: Yuan Hu

Table 2 Effect of nine of Chinese herbal extracts on elastase activity

Herbs	1	2	3	4	5	6	7	8	9
Activity change (± %)	-6	-6	13	5	-19	-26	-25	19	-90

Table 3 Effect of HHI-I on intestinal hymodynamics

	Before infusion	After infusion (min)		
		20	40	60
BF (mL/min)	201.34 ± 70.21	225.00 ± 68.51[a]	245.40 ± 82.78[b]	252.20 ± 82.41[b]
OC (mL/min)	2.72 ± 1.09	4.92 ± 3.39	5.03 ± 2.55	5.33 ± 2.57[a]
(A-V)O_2 (mL/100 mL)	1.47 ± 0.68	1.21 ± 1.34	2.16 ± 1.11[a]	2.29 ± 1.31
VR (kPa/mL/min)	0.10 ± 0.04	0.09 ± 0.04	0.08 ± 0.03[a]	0.08 ± 0.03a
HCT (%)	46.85 ± 10.58	47.73 ± 11.10	46.25 ± 10.47	45.20 ± 12.30

Five animals were tested. Data are expressed as ($\bar{x} \pm s_{\bar{x}}$); [a]$P < 0.05$, [b]$P \leqslant 0.01$ *vs*. controls.

Table 4 Effect of HHI-I on pancreatic oxygen tension and microcirculation

	Group	Before HHI-I	After HHI-I (min)		
			20	40	60
Microcirculation	HHI-I	20.4 ± 5.0	49.0 ± 9.0[a]	51.0 ± 7.97[b]	54.8 ± 15.51[b]
(mL/min/100 g)	Control	32.4 ± 6.35	34.6 ± 3.65	34.2 ± 4.02	31.2 ± 5.71
Oxygen tension	HHI-I	6.21 ± 0.94	7.55 ± 1.40[b]	7.65 ± 1.76[a]	7.67 ± 1.64[b]
(kPa)	Control	5.60 ± 0.97	5.76 ± 1.07	5.49 ± 0.93	5.52 ± 0.79

Data are expressed as ($\bar{x} \pm s_{\bar{x}}$); [a]$P < 0.05$; [b]$P < 0.01$ *vs* controls

differed in effectiveness (Table 1), which may have been related to differences in the concentrations of active substance in each herb. Yuan Hu was the most effective followed by Da Huang (Radix et Rhizoma Rhei), and Zhi Zi (Fructus Gardeniae) (Table 1).

Inhibitory effect on elastase activity

Method The *in vitro* test followed a method previously described by Chuan-Rui Jiang[3]. Anti-elastase activity was tested in 200 μL sample of each extract.

Results Most of the nine extracts inhibited elastase activity, but a few had an activating effect (Table 2). Yuan Hu was the most effective inhibitor, followed by Huang Qin and Hang Shao.

Effect of HHI I on intestinal hemodynamics in dogs

Method Intestinal hemodynamics were evaluated following a method previously described by Leslie[5]. Blood flow was measured in the superior mesenteric artery with an electromagnetic flowmeter. Blood samples were obtained from femoral artery and superior mesenteric vein, and the oxygen consumption of intestine was calculated. Five dogs with mean body weight of 19.2 ± 3.96 kg were used. A solution of Huoxue Huayu (HHI-I) was infused intravenously at a dose of 2.0 mL/kg of body weight.

Results HHI-I increased intestinal blood flow and oxygen consumption (Table 3, Figure 1). It also increased oxygen extraction (A–V)O_2 and decreased vascular resistance. No effect of HHI-I on hematocrit was observed. No effects were observed in a control group given an intravenous infusion of saline.

Effect of HHI I on microcirculation and pancreatic tissue oxygenation in dogs

Experimental methods Five healthy male and female dogs weighing 18.9 ± 2.61 kg were anesthetized, and the pancreas was exposed. An oxygen meter (POG-5000, Japan) electrode was inserted into the pancreatic tissue to measure tissue oxygen tension,

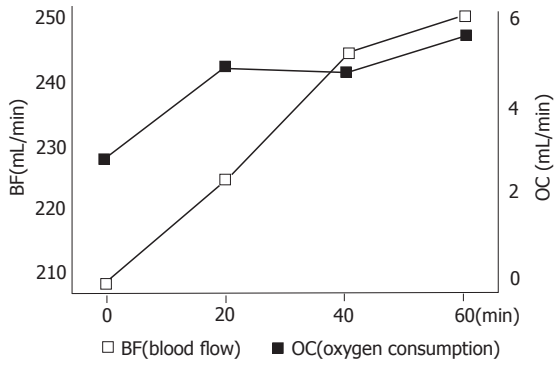

Figure 1 Effect of HHI-I on intestinal hymodynamics

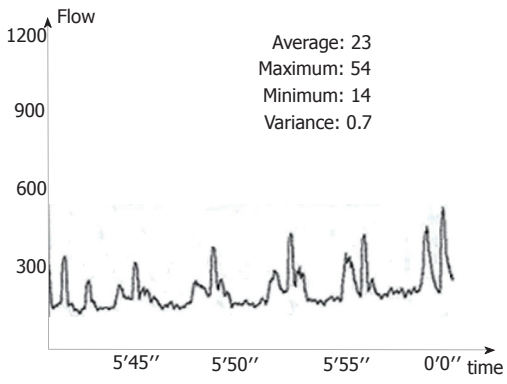

Figure 2 Effect of HHI-I on pancreatic microcirculation before administration

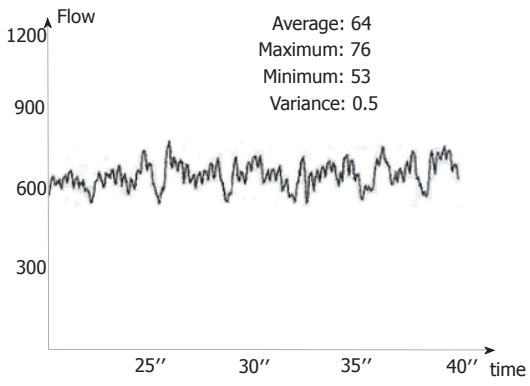

Figure 3 Effect of HHI-I on pancreatic microcirculation at 40 min

and pancreatic microcirculation was measured by a dynamic laser Doppler microcirculation analyzer (JI-200, Tianjin). The dose of HHI I used in this experiment was the same as that used to evaluate the effect on intestinal hemodynamics.

Results Blood microcirculation in pancreatic tissue significantly increased 10 min after HHI-I infusion, and the increase was maintained during the whole period of infusion. The oxygen tension of pancreas increased at the same time. The two indices did not change in control animals (Table 4, Figures 2 and 3).

DISCUSSION

Even though aprotinin has shown disappointing results in experimental and clinical treatment of SAP, inhibition of pancreatic enzymes should not be ignored as a possible treatment. In addition to trypsin, gabexate mesilate (417 Daltons) also inhibits phospholipase A2 and has shown satisfactory therapeutic effects in the preclinical studies and clinical trials. Camostate (FOY-305, 494.5 Daltons) having a more extensive spectrum of enzyme inhibition, and has markedly increased SAP survival rates in animal models[6,7]. Because it contains agglutinin and native antiproteases, human plasma can significantly improve survival time in rats with SAP caused by 5% sodium taurocholate compared with control animals. In a rat SAP model caused by caerulein infusion (5 μg/kg of body weight per hour), urinastatin, a protease inhibitor extracted from human urine, had protective

effects on serum amylase, pancreatic water content, amylase content of pancreatic tissue, lysosome distribution, and fragility of acinar cells and lysosomes[8]. ON03307, a synthetic antiprotease with low molecular weight and high activity, was effective against SAP when administered with allopurinol[9]. Combination treatment increased release of amylase and cathepsin by pancreatic juice and increased the proportion of zymogen-containing lysosomes. Combined administration of a protease inhibitor and a xanthine oxygenase inhibitor thus improved SAP treatment. In the pathogenesis of SAP, elastase digests elastin in blood vessel walls playing a pivotal role in the conversion of mild into severe hemorrhagic and necrotic pancreatitis. A synthetic, low molecular weight inhibitor of pancreatic elastase would be of great value, and glutaryl-trialanin-ethylamide a competitive inhibitor of pancreatic elastase, has been shown to slow the progress of SAP in a rat model[10].

There have been many reports of the enzyme inhibitory activity of Chinese herbs, including Ban Xia (Rhizoma Pinelliae), Da Huang, Huang Qin, Hu Lian, and Bai Shao[11]. This study found Yuan Hu to be the strongest inhibitor of trypsin and elastase; and in our laboratory, the injectable form is called YHI. It has also been found that combined treatment with YHI and HHI-I reduces the severity of experimental SAP rabbits (unpublished data).

Many experiment al studies and clinical trials have found that pancreatic ischemia in the early stage of AP is an important step in the development of necrosis[12,13]. In dogs, occlusion of small pancreatic arterioles by 8–20 μm microsphere induced hemorrhagic and necrotic pancreatitis; pancreatic edema superimposed on pancreatic ischemia can lead to necrotic pancreatitis. The effectiveness of low molecular weight dextran for prevention and treatment of pancreatic ischemia has been reported in experimental animal models of AP. Dextran has specific effects on pancreatic microcirculation, and isovolemic hemodilution with dextran 60 at a dose of 10 ± 1.3 mL/kg body weight reduced vacuolization of acinar cells and parenchymal edema compared with controls[14]. In a clinical trial including 13 SAP patients (mean Ranson score of 4.6). The average time from symptom appearance to dextran hemodilution was 38 h (range 19–90 h). Hematocrit decreased from 50% ± 6% to 34% ± 3% in the first hour an 31% ± 4% in the second hour. The volume exchanged was 750–1700 mL, and the mortality rate was only 7.7%[15]. HHI-I, Tao Ren injection, is the most effective herb selected from the HuoXue HuaYu decoction used in our hospital for improving blood circulation and increasing oxygen consumption[4]. This study provides additional evidence of the positive effect of HHI-I on intestinal hemodynamics and pancreatic oxygen supply, and microcirculation in this dog model. HHI-I has potential as an effective medicine to improving blood circulation and to treat SAP.

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Effects of basic fibroblast growth factor on ischemic gut and liver injuries

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Abstract

AIM: To explore the effects of basic fibroblast growth factor (bFGF) on ischemic gut and liver injuries following trauma.

METHODS: An animal model of superior mesenteric artery (SMA) occlusion was used. Seventy-two male Wistar rats were randomized equally to three treatment groups of 24 each. Group 1 was injected with 4 μg of bFGF in 0.15 mL of normal saline solution containing 0.1% (w/v) heparin through the jugular vein at the onset of reperfusion. Group 2 received normal saline without bFGF. Group 3 was treated was given a sham operation without SMA occlusion. Liver function, serum tumor necrosis factor (TNF)α, bacterial cultures, and pathological evaluations were carried out.

RESULTS: In group 1, alanine transaminase (ALT), aspartate transaminase (AST), and serum TNFα were significantly reduced at 6, 24 and 48 h compared with group 2. Bacterial cultures showed that the bacterial translocation from gut to liver, spleen, and mesenteric lymph nodes (MLN) was significantly lower in group 1 than in group 2. Pathological evaluation was consistent with a significant protective effect of bFGF.

CONCLUSION: Venous administration of bFGF may help reduce gut and liver ischemia/reperfusion injury through its mitogenic and nonmitogenic hormone-like effects.

Key words: Intestine; Liver; Fibroblast growth factor; Mesenteric arteries

Fu XB, Sheng ZY, Wang YP, Ye YX, Sun TZ, Ma NS, Chang GY, Xu MH, Zhou BT. Effects of basic fibroblast growth factor on ischemic gut and liver injuries. *World J Gastroenterol* 1996; 2(3): 139-140 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/139.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.139

INTRODUCTION

Gut ischemia/reperfusion injury after serious trauma is an important problem in the clinic. Damage to gut barrier function may lead to translocation of bacteria from the gut to internal organs, resulting in organ impairment. Preservation of the gut barrier is thus a key step in preventing multiple organ failure after trauma[1]. The involvement of growth factors and their effects on ischemic injury to internal organs is an area of active research[2]. We evaluated systemic administration of basic fibroblast growth factor (bFGF) for prevention of serious gut and liver injury after occlusion of the superior mesenteric artery (SMA) and investigated its mechanism of action.

MATERIALS AND METHODS

Animal model

Seventy-two male Wistar rats weighing 200–250 grams were housed in the laboratory for 1 wk prior to use in the study, and had free access to food and water. Occlusion of the superior mesenteric artery (SMA) was done under intravenous anesthesia with sodium pentothal (40 mg/kg). A midabdominal incision was made, and the SMA was identified and isolated by blunt dissection. Blood flow was completely blocked by a microvascular clamp placed on the root of SMA. The clamp was removed after 45 min, and return of blood flow to the gut was confirmed visually. The incision was then closed in two layers, animals were allowed to recover spontaneously and were observed for 3 d. All operations were performed under aseptic conditions.

Animals were randomly allocated to three groups of 24 each. At the onset of reperfusion, rats in Group 1 were given 4 μg of bFGF in 0.15 mL of normal saline containing 0.1% (w/v) heparin intravenously through the jugular vein. Animals in Group 2 were given the same volume of normal saline plus heparin, but not bFGF. Animals in Group 3 were given sham-operation consisting of a laparotomy without SMA occlusion and reperfusion.

Liver function tests and cytokine measurement

Serum alanine transaminase (ALT) and aspartate aminotransferase (AST) were assayed with an automatic biochemistry analyzer (Monarch, United States) before ischemia and 6, 24 and 48 h respectively after ischemia. Serum tumor necrosis factor α (TNFα) was determined with a radioassay kit (Hun Qun Co., Beijing) following the same schedule.

Bacterial cultures

A midabdominal incision was made under sterile conditions at 6, 24, and 48 h after ischemia, and swabs of the peritoneal cavity were obtained for aerobic culture. The mesenteric lymph nodes

Table 1 Changes in alanine transaminase in the three study groups

Group	ALT (nmol/L)			
	0 h	6 h	24 h	48 h
1	792.7 ± 97.0	850.2 ± 498.1[b]	786.3 ± 308.6[a]	622.3 ± 134.9
2	925.2 ± 68.8	4777.6 ± 710.6	2771.2 ± 646.6	969.0 ± 105.7
3	1100.2 ± 148.5	858.5 ± 257.4	919.4 ± 170.0	923.5 ± 116.0

Data are expressed as ($\bar{x}$ ± s). [a]$P < 0.05$; [b]$P < 0.01$ *vs* Group 2

Table 2 Changes of aspartate transaminase in the three study groups

Group	ALT (nmol/L)			
	0 h	6 h	24 h	48 h
1	2130.9 ± 217.9	3453.5 ± 1532.1[b]	2155.9 ± 946.0[a]	2125.4 ± 437.6
2	2352.6 ± 281.2	7988.3 ± 1087.6	5845.7 ± 1274.8	3627.4 ± 695.3
3	3386.3 ± 587.5	3717.4 ± 1147.9	6079.0 ± 1464.0	2353.8 ± 242.9

Data are expressed as ($\bar{x}$ ± s). [a]$P < 0.05$, [b]$P < 0.01$, *vs* Group 2.

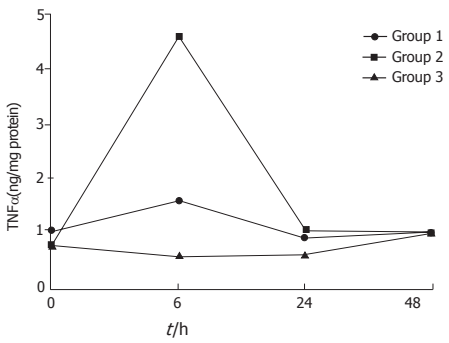

Figure 1 Changes of plasma TNFα after ischemia.

(MLN), liver, and spleen were removed, weighed, and homogenized in sterile grinding tubes. The samples were plated for quantitative culture and incubated at 37 ℃ for 48 h before reading.

Histopathological examination

Specimens of gut and liver tissue were taken for examination by light microscopy at 6, 24 and 48 h after ischemia.

Statistical analysis

Student's-*t* test was used to determine the significance of changes before and after ischemia and the significance of between-group differences. Statistical significance was defined as $P < 0.05$. Data were expressed as means ± standard deviation ($\bar{x}$ ± s).

RESULTS

Organ function and serum TNFα

In group 2, serum ALT and AST were increased at 6, 24, and 48 h, both were significantly higher than before injury ($P < 0.05$) and were higher than in group 1 ($P < 0.01$). In group 1, all values were increased at 6 h, but were not significantly different than preinjury values or the group 3 values (Tables 1 and 2).

In groups 1 and 2, serum TNFα concentrations rose and fell, with a peak at 6 h, but declined at 24 and 48 h. No significant changes in serum TN were found at all times (Figure 1).

Bacterial study and pathologic examination

At 6 h after ischemia, MLN, liver, and spleen tissue samples in groups 1 and 2 had positive bacterial cultures, but the proportion of positive cultures was lower in group 1 than in group 2. In group 3, positive bacterial cultures were obtained only from MLN. The calculated bacteria per gram of tissue in the three groups differed (Tables 3 and 4).

Pathologic examination found that the proportion of intestine and liver tissue samples with subepithelial edema, hemorrhage, erosion, or neutrophilic and lymphocytic infiltration was much larger in saline-treated than in bFGF-treated rats. No changes in tissue structure were observed in sham operated rats.

DISCUSSION

Mucosal injuries that lead to translocation of bacteria or endotoxins from the gut to other tissues and organs are the primary factor in the pathogenesis of multiple organ injury or failure after serious trauma[1,2].

Table 3 Positive bacterial cultures from organs in the three study groups

Group	Positive bacterial cultures (%)		
	Liver	Spleen	MLN
1	12.5[a] (1/8)	12.5[a] (1/8)	75.0 (6/8)
2	62.5 (5/8)	v57.1 (4/7)	62.5 (5/8)
3	0.0 (0/8)	0.0 (0/8)	50.0 (4/8)

[a]$P < 0.05$ *vs* Group 2.

Table 4 Bacteria cultured from organ samples

Group	Organs sampled (*n*)	Positive (*n*)	Bacteria[1] (log CFU/g tissue)
1	33	30	3.70 ± 0.91
2	9	8	4.06 ± 1.19
3	18	7	3.42 ± 0.50

[1]Data expressed as $\bar{x}$ ± s. CFU, colony forming unit

Resuscitation and anti-oxidation treatment are effective in reducing multiple organ injury and mortality[3], but the traditional methods can only block the cycle of tissue injury. They do not accelerate the repair of damaged tissues. From what is known of the pathogenesis of multiple organ failure, we considered that the mitogenic effects and nonmitogenic hormone-like activity of bFGF, might be protective against ischemic organ injuries[4–6]. The study data showed that systemic administration of bFGF at the onset of reperfusion reduced the functional and morphological changes of the gut and liver and reduced bacterial translocation from the gut to the liver and spleen. Moreover, inflammatory factors such as TNFα were reduced by bFGF treatment. In a model of stomach ischemia, Ishikawa *et al*[7] observed that the administration of epidermal growth factor (EGF) reduced mucosal edema and necrosis. It follows that growth factors may be useful in promoting internal organ repair after trauma.

Possible mechanisms of bFGF's protection against ischemic injury may its vasodilator activity, which may help open microvascular beds and ameliorate the "no reflow" phenomenon that occurs after ischemia[8]. It has been found that the longer ischemia persists, the more serious the gut and liver damage and the higher the mortality. Increased intracellular calcium and redistribution of intracellular calcium pools may result in cell injury and contribute to cell death. Because bFGF can trigger the influx and intracellular release of Ca^{2+}, it may have a role in regulating extracellular and intracellular Ca^{2+} balance and modulate transduction of transmembrane signals to inhibit Ca^{2+}-dependent ATPase and other mitochondrial enzyme activity associated with ischemia/reperfusion[9] Finally, signal transduction by bFGF and its interactions with other enzymes might also be involved in the protective effect of bFGF on ischemia/reperfusion injury. Further study is needed.

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Study on traditional Chinese Medicine Syndrome-typing of chronic ulcerative colitis

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Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To study the relationship between the modern clinical and pathohistological classification and the traditional Chinese Medicine (TCM) Syndrome-typing of chronic ulcerative colitis (CUC).

METHODS: In total, 452 patients with CUC were classified according to the standards of the TCM Syndrome-typing set up by the Conference of the Combination of the Chinese-Western Medicine on Digestive Diseases in Linfen. The relevant changes between both classifications were analyzed and compared through the colonofiberscopic and pathohistological examination.

RESULTS: The type of retention of interior damp-heat is more commonly seen at the initial onset of disease ($P < 0.01$). No significant differences among other TCM Syndrome-typing groups in patients with persistent disease and with recurrent disease ($P > 0.05$) were observed. The congestion, edema, reduction of goblet cells and the infiltration of neutrophils are pathologically common to all TCM Syndrome-typing groups. Mucosal ulcers were dominant in damp-heat syndrome while crypt ulcers were dominant in spleen-stomach asthenia and spleen-kidney Yang deficiency ($P < 0.01$).

CONCLUSION: There appeared to be a certain relationship between the TCM syndrome-typing and pathohistological changes of the colon mucosa of CUC.

Key words: Ulcerative colitis/pathology; Zheng differentiation classification

Chen ZS, Zhou CM, Lu Y, Nie ZW, Sun QL, Wang YX, Chi Y. Study on traditional Chinese Medicine Syndrome-typing of chronic ulcerative colitis. *World J Gastroenterol* 1996; 2(3): 141-143 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/141.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.143

INTRODUCTION

In recent years, both Chinese and Western specialists have been studied the pathogenesis and treatment methods of chronic ulcerative colitis (CUC). However, the relationships between traditional Chinese Medicine (TCM) Syndrome-typing and pathohistological changes of western medicine have not yet been extensively reported. In this study of 452 patients with CUC, we analyzed this relationship and report the results.

MATERIALS AND METHODS

Clinical materials

In total, 452 patients, including 263 males and 189 females were observed in our study. The age range was from 17 years to 65 years, with an average age of 37.8 ± 9.1. The duration of illness varied between 5 months to 23 years, with an average time of 5.6 ± 4.1 years. The rectum and sigmoid were affected in 215 patients. The left half of the colon was affected in 179 patients. The right half of the colon was affected in 17 patients. The whole colon was affected in 41 patients. The number of patients with mild, moderate and severe disease was 179, 205 and 68, respectively.

Diagnosis

All patients were diagnosed in accordance with the criteria set up in the 1987 Conference of Digestive Diseases in Hangzhou[1]. The standards of the TCM Syndrome-typing were the same as those set up in the 1992 Conference of Combination of Chinese Western Medicine on Digestive Diseases in Linfen[2].

Methods

Colonofiberscope, biopsy and the relevant pathohistological observations were carried out in all patients. Two doctors classified the TCM Syndrome-typing. The χ^2 test was used to analyze the data statistically.

RESULTS

The relationship between the TCM Syndrome-typing and the duration of CUC is shown in Table 1. Patients who have had CUC for less than a year were more likely to have damp-heat syndrome. Patients who have had CUC for one year to ten years were more

Table 1 The relationship between the traditional Chinese Medicine Syndrome-typing and the duration of chronic ulcerative colitis *n* (%)

TCM Syndrome-typing	Number	Duration (yr)			
		< 1	1-5	6-10	> 10
Spleen-stomach asthenia	185	23 (12.4)[1]	81 (43.8)	46 (24.9)	35 (18.9)
Spleen-kidney Yang deficiency	81	3 (3.7)	19 (23.4)	26 (32.1)	33 (40.7)
Yin and blood asthenia	46	9 (19.6)	13 (28.3)	14 (30.4)	10 (21.7)
Liver stagnation and spleen deficiency	55	15 (27.3)	17 (30.9)	18 (32.7)	5 (9.1)
Vital energy stagnation and blood stasis	33	6 (18.2)	8 (24.2)	10 (30.3)	9 (27.3)
Damp-heat	52	27 (51.9)	18 (34.6)	5 (9.6)	2 (3.8)

[1]Data in parentheses denotes the percentage.

Table 2 Relationship between the traditional Chinese Medicine Syndrome-typing and clinical types *n* (%)

TCM Syndrome-typing	Number	Clinical types			
		Initial	Persistent	Recurrent	Onset
Spleen-stomach asthenia	185	12 (6.5)[1]	59 (31.9)	114 (61.6)	
Spleen-kidney Yang deficiency	81	2 (2.5)	37 (45.7)	41 (50.6)	1 (1.2)
Yin and blood asthenia	46	1 (2.2)	25 (54.3)	20 (43.5)	
Liver stagnation and spleen deficiency	55	8 (14.5)	17 (30.9)	30 (54.5)	
Vital energy stagnation and blood stasis	33	2 (6.1)	15 (45.5)	16 (48.5)	
Damp-heat retention	52	39 (75.0)	3 (5.8)	8 (15.4)	2 (3.8)

[1]Data in parentheses denotes the percentage.

Table 3 Relationship between the traditional Chinese Medicine Syndrome-typing and mucosal changes *n* (%)

Mucosal changes	Number	Spleen stomach deficiency (*n* = 185)	Spleen-kidney deficiency (*n* = 81)	Yin-blood deficiency (*n* = 46)	Liver stagnation spleen deficiency (*n* = 55)	Vital energy stagnation and blood stasis (*n* = 33)	Damp-heat ratention (*n* = 52)	*p* value
Mild congestion and edema	166	71 (38.4)[1]	12 (14.8)	35 (76.1)	37 (67.3)	9 (27.3)	2 (3.8)	< 0.01
Moderate congestion and edema	184	83 (44.9)	30 (37.0)	11 (23.9)	18 (32.7)	21 (63.6)	21 (40.4)	< 0.01
Severe congestion and edema	102	31 (16.7)	39 (48.1)			3 (9.1)	29 (55.8)	< 0.01
Erosion	304	152 (82.2)	75 (92.6)	7 (15.2)	9 (16.4)	12 (36.4)	49 (94.2)	< 0.01
Ulcer	383	163 (88.1)	79 (97.5)	31 (67.4)	36 (65.5)	23 (69.7)	51 (98.1)	< 0.01
Bleeding	147	61 (33.0)	45 (55.6)	2 (4.3)	3 (5.5)	7 (21.2)	29 (55.8)	< 0.01
Granulation	179	77 (41.6)	47 (58.0)	19 (41.3)	5 (9.1)	29 (87.8)	2 (3.8)	< 0.01
Atrophy	55	11 (5.9)	9 (11.1)	27 (58.7)	1 (1.8)	7 (21.2)		< 0.01
Polyp proliferation	67	27 (14.6)	19 (23.5)	1 (2.2)	2 (3.6)	17 (51.5)	1 (1.9)	< 0.01

[1]Data in parentheses denotes the percentage.

Table 4 Relationship between the traditional Chinese Medicine Syndrome-typing and pathohistological changes *n* (%)

Pathological change	Number	Spleen stomach deficiency (*n* = 185)	Spleen kidney Yang deficiency (*n* = 81)	Yin blood deficiency (*n* = 46)	Liver stagnation spleen deficiency (*n* = 55)	Vital energy stagnation, blood stasis (*n* = 33)	Damp-heat retention (*n* = 52)	*p* value
Goblet cell reduction	444	182 (98.4)[1]	81 (100.0)	44 (95.6)	53 (96.4)	32 (97.0)	52 (100.0)	> 0.05
Neutrophil infiltration	421	171 (92.4)	79 (97.5)	41 (89.1)	47 (85.5)	31 (93.9)	52 (100.0)	< 0.05
Lymphocyte infiltration	248	127 (68.6)	52 (64.2)	15 (32.6)	41 (74.5)	10 (30.0)	3 (5.7)	< 0.01
Angiitis of small vessels	295	121 (65.4)	54 (66.7)	23 (50.0)	36 (65.5)	24 (72.7)	37 (71.2)	> 0.05
Crypt abscess	395	161 (87.0)	72 (88.9)	40 (87.0)	44 (80.0)	29 (87.9)	49 (94.2)	> 0.05
Mucosal ulcer	188	68 (38.8)	32 (39.5)	11 (23.9)	13 (23.6)	10 (30.0)	34 (65.4)	< 0.01
Crypt ulcer	311	142 (76.8)	59 (72.8)	24 (52.2)	28 (50.9)	21 (63.6)	37 (71.2)	< 0.01
Abnormal epitheliosis	63	27 (14.6)	19 (23.5)	3 (6.5)	4 (7.3)	9 (27.3)	1 (1.9)	< 0.01

[1]Data in parentheses denotes the percentage.

likely to have the spleen-stomach asthenia syndrome. Patients who have had CUC for more than ten years were more likely to have the spleen-kidney Yang deficiency. The other three TCM types (asthenia of Yin, asthenia of blood, liver stagnation and spleen deficiency, and stagnation of vital energy and blood stasis) had no definite relationship with the duration of the disease.

The relationship between the TCM Syndrome-typing and the clinical classification of CUC is shown in Table 2. The occurrence of damp-heat was more common in the initially affected patients ($p < 0.01$) and less common in the persistent and recurrent cases. There is no significant difference among the other TCM Syndrome-typing groups in the persistent and recurrent patients ($p > 0.05$).

The relationship between the TCM Syndrome-typing and the changes of the colon mucosa is shown in Table 3. The congestion and edema found in all of the TCM Syndrome-typing groups had different severity levels in the different groups. Damp-heat, spleen-kidney Yang deficiency, and spleen-stomach asthenia syndrome were characterized by erosion, ulcers and bleeding. Stagnation of vital energy and blood stasis were characterized by granulation and polyp proliferation. Both Yin and blood deficiency were characterized by atrophy.

The relationship between the TCM Syndrome-typing and the pathohistological changes of the colon mucosa is shown in Table 4. The reduction of goblet cells and the infiltration of neutrophils are pathological features common to all groups. However, neutrophil infiltration was prevalent in damp-heat syndrome, while lymphocyte infiltration was prevalent in spleen-stomach asthenia, spleen-kidney Yang deficiency, and stagnation of liver and deficiency of spleen. Mucosal ulcers were prevalent in damp-heat, while crypt ulcers were prevalent in spleen-stomach asthenia and spleen-kidney Yang deficiency. Abnormal epithelial proliferation was prevalent both in stagnation of vital energy and blood, and in spleen-kidney Yang deficiency.

DISCUSSION

Chronic and non-specific ulcerative colitis has been under investigation for more than 100 years. The etiology and pathogenesis, according to modern medicine are associated with

many factors such as the immune system, heredity, intestinal infection, mental stress, food sensitivity, and intestinal bacteriolysis. The etiology and pathogenesis, according to TCM, are associated with many factors including mental injury, disorder of diet rhythms, invasion of outside pathogenic evils, and spleen-kidney asthenia. In this series of 452 patients, 40.9% of cases were spleen-stomach asthenia, 17.9% of cases were spleen kidney Yang deficiency, 12.1% of cases were stagnation of liver and deficiency of spleen, 11.5% of cases were for damp-heat retention, 10.1% of cases were deficiency of both Yin and blood, and 7.3% of cases were stagnation of vital energy and blood. The cases involving spleen-kidney asthenia account for more than 50% and the cases involving sthenia syndromes (damp-heat and stagnation of vital energy and blood stasis) account for less than 20%. We theorized that the spleen kidney asthenia could be the prevalent syndromes in CUC, while the damp-heat is superficial. Though the lesion is located in the large intestine, the effects of the lesion could extend to the spleen, kidney and liver.

In order to investigate some objective rules of the TCM Syndrome-typing, we carried out systematic observations through clinical, pathological and laboratory examinations. After analysis of the data from 452 patients, there is a clear relationship between the TCM Syndrome-typing and the duration of CUC. The sequence of progression tended to begin with damp-heat, then to spleen asthenia, and further to kidney asthenia. This indicated that damp-heat was more common in cases with a shorter duration. The pathogenic damp may stay in the spleen as the duration of illness was prolonged. This can lead to injury of the organ and its vital energy. The disease may further progress to spleen-stomach asthenia syndrome. Moreover, the kidney could be affected by the spleen distress. Therefore, the spleen kidney Yang asthenia results.

Currently, there are no studies investigating the relationship between the TCM Syndrome-typing and the pathological change of the colon mucosa. Dai *et al*[3] hypothesized that the body of the tongue had significant influence on an endoscopy diagnosis. This alludes to a relationship between Chinese glossoscopy and intestinal diseases. Our study elucidated the relationship between the TCM Syndrome-typing and pathological changes of the colon mucosa, as observed by the naked eye or by micro-pathohistological examination. For example, mild congestion and edema are prevalent in cases of asthenia of both Yin and blood, and the stagnation of liver and deficiency of spleen. Moderate congestion and edema are prevalent in cases of spleen-stomach asthenia, and stagnation of vital energy and stasis of blood. Severe congestion and edema are prevalent in cases of damp-heat retention and spleen-kidney Yang deficiency.

Although cellular infiltration and ulcerative changes of the colon mucosa are features common to all of the TCM Syndrome-typings, the types of infiltrating cells and the sites of the ulcers are different. The nature of spleen-kidney Yang deficiency is opposite to that of damp-heat. The former belongs to the syndrome of Yin, deficiency and cold, while the latter belongs to the syndrome of Yang, sthenic and heat. All of the TCM Syndrome-typings have the same notable congestion, edema, and ulcers. Therefore, further studies are required to investigate the pathogenesis. Notably, we observed several differences. In the cases of spleen-kidney Yang deficiency, the mucosal edema was more apparent than congestion, there was no marked swelling around the ulcer, and a white secretion covered the surface. In the cases of damp-heat, the mucosal edema was more apparent than congestion, there was marked swelling around the ulcer, and a yellow purulent secretion covered the surface. Taken together, there are internal relationships between the TCM Syndrome-typing and pathological changes in CUC.

Our study is only a preliminary investigation of the relationships between the TCM Syndrome-typing and the pathological, pathohistological, and clinical classification of modern medicine. Further study is required for understanding the pathogenesis of CUC and establishing objective parameters of the TCM Syndrome-typing.

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Radiological diagnosis of inflammatory ulcerative diseases of the small bowel

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Abstract

AIM: To analyze the radiological features of ulcerative diseases of the small bowel.

METHODS: Thirty-five patients (20 men and 15 women) with inflammatory ulcerative bowel diseases were studied by radiography (barium meal and/or double contrast study). Patient diseases included eleven cases of tuberculosis (TB), thirteen cases of Crohn' s disease, seven cases of bowel Behcet disease, two cases of simple ulcers, and two cases of ischemic bowel disease. Diagnosis was established pathologically in 33 cases and by clinical observation after therapy in two cases.

RESULTS: The lesions were located in the ileum of 82% of TB cases, 77% of Crohn's disease cases, 71% of bowel Behcet disease cases, 50% of simple ulcer cases, and 100% of ischemic bowel disease cases. Ulceration was always present with variable appearances. Longitudinal ulcers, and fissures were noted in Crohn's disease only. There were five cases of large and deep ulcers, three of which were bowel Behcet disease. Superficial and irregular ulcers were present in ten TB cases, and , and transverse ulcers were identified in two TB cases.

CONCLUSION: The morphological appearances of the ulcer, surrounding mucosal alterations, and bowel deformation were the basis for the radiological diagnosis. Correct diagnosis was dependent on optimal X-ray examination techniques and proper interpretation of the morphological changes.

Key words: Small intestine; Gastrointestinal tuberculosis; Crohn's disease/radiography

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INTRODUCTION

Intestinal ulcers are often found in cases of small intestine inflammatory disease. They are accompanied by a high mortality rate.

It is difficult to make differential diagnoses by radiographic examination[1,2]. In this study, we reviewed 35 cases of small intestine disease, and analyzed the radiographic characteristics of the disease. Serial changes in radiographic examination were observed.

MATERIALS AND METHODS

We performed radiography (barium meal and/or double contrast study) in 35 patients (20 men and 15 women). All patients were diagnosed with small intestine inflammation with an intestinal ulcer. Diagnosis was established by pathology in 33 cases and by clinical observation after therapy in two cases. The age range of the patients was 14 years to 78 years. Most patients had gastrointestinal symptoms such as abdominal pain, diarrhea, and bloody stool. The inflammatory ulcerative bowel diseases included eleven cases of tuberculosis (TB), thirteen cases of Crohn's disease, seven cases of bowel Behcet disease, two cases of simple ulcers, and two cases of ischemic bowel disease.

RESULT

The lesions were located in the ileum of 82% of TB cases, of 77% of Crohn's disease cases, of 71% of bowel Behcet disease cases, of 50% of simple ulcer cases, and of 100% of ischemic bowel disease cases. Ulceration was always present with variable appearances. Longitudinal ulcers and fissures were noted in Crohn's disease only. There were 5 cases of large and deep ulcers, three of which occurred in bowel Behcet disease. Superficial and irregular ulcers were present in ten TB cases, and transverse ulcers were observed in two TB cases (Figures 1 and 2).

DISCUSSION

Ulceration is present in the majority of small bowel inflammatory diseases including TB, Crohn's disease, bowel Behcet disease, simple ulcers, and ischemic bowel disease. The morphological appearances of the ulcer, surrounding mucosal alterations and bowel deformation were the basis for radiological diagnosis[3-9]. TB was located in the terminal ileum. Ulcerations had variable appearances, but no longitudinal ulcers and fissures were noted. In Crohn's disease, the above characteristics were noted. In addition, cobblestone and fistulas

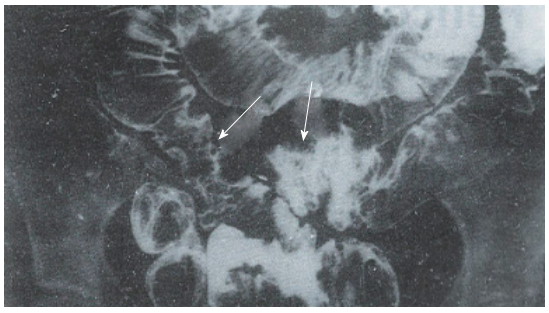

Figure 1 The mucosa is irregular and superficial ulcers (arrows) were present in several segments of the terminal ileum of TB cases.

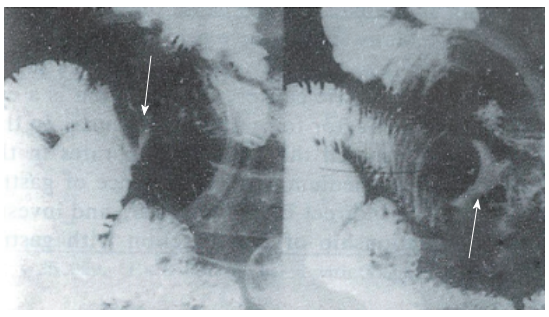

Figure 2 After compression, a transverse ulcer (arrow) is observed in a TB case.

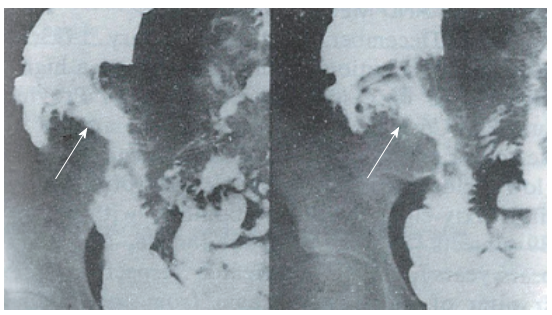

Figure 3 Triangular ulcers measuring 4 cm^2 in size at the terminal ileum (arrow) were present Crohn's disease cases. The surrounding mucosal pattern exhibited a cobblestone appearance.

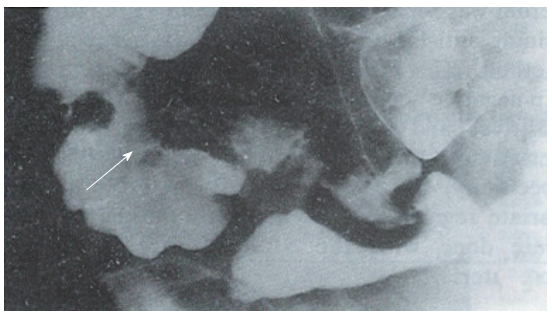

Figure 4 A round ulcer (arrow), with surrounding mild mucosal edema in the ileocecal region was present in a Crohn's disease case.

occurred frequently (Figures 3 and 4). The radiographic findings of bowel Behcet disease and simple ulcer were similar. However, the ulcers in bowel Behcet disease tended to be larger and deeper with surrounding mucosal edema (Figures 5 and 6). All patients had mucocutaneous ocular symptoms. Ulcers in ischemic bowel disease had no characteristics (Figure 7). Correct diagnosis was dependent on optimal X-ray examination techniques and proper interpretation of the morphological changes. Enteroclysis, a controlled infusion method offering a double contrast and highly detailed examination of the small bowel, is relatively easy to perform. Despite the introduction of enteroclysis, the peroral small bowel examination remains the predominant radiographic method of imaging the small intestine. This report emphasizes the technical factors needed to produce excellent peroral small bowel examinations.

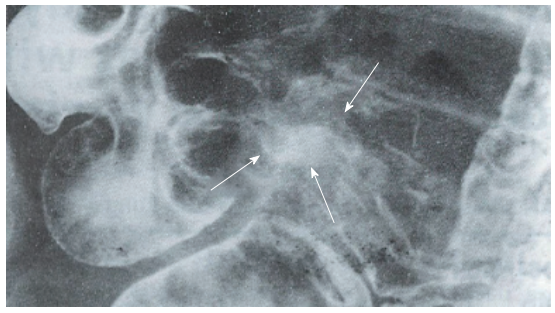

Figure 5 A large and deep irregular ulcer (4 × 3 cm^2 in size) at the terminal ileum near the ileocecal valve (double arrow) was present in a bowel Behcet disease case. The surrounding mucosal edema (arrow) is shown.

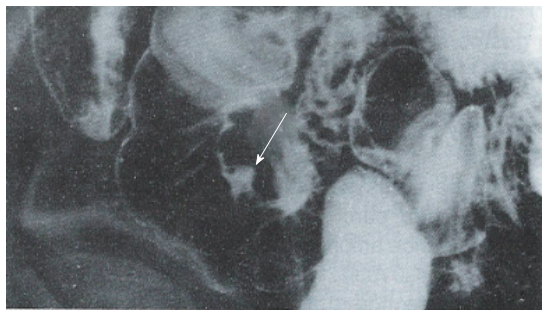

Figure 6 A small ulcer (0.3 × 0.4 cm^2 in size) at the ileum (arrow) was present in a bowel Behcet disease case. The surrounding mucosal edema (arrow) is shown.

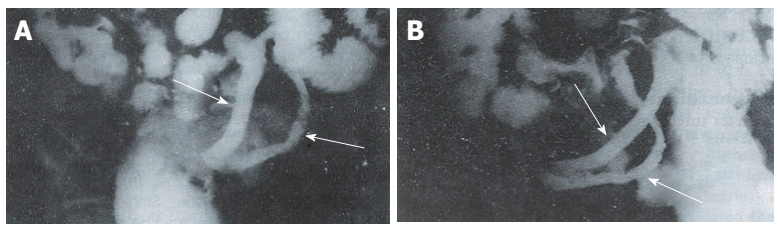

Figure 7 A and B: Several long, segmented, contracted, narrow, and rigid loops of the bowel in the ileum (arrow), with superficial ulcers were present in ischemic bowel disease. No mucosal abnormalities were observed.

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An epidemiologic study of *Helicobacter pylori* infection in three areas with high, moderate or low incidences of gastric carcinoma

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Abstract

AIM: To study the relationship between the *Helicobacter pylori* (Hp) infection rate and the incidence and mortality of gastric cancer.

METHODS: The Hp infection rates of the natural population in three areas were detected by measuring the specific IgG antibody to Hp using the indirect ELISA method.

RESULTS: The Hp positive rates were 59.4%, 55.9% and 34.5% in the areas with high, moderate and low incidences of gastric carcinoma, respectively. The differences in incidence among the areas were significant (χ^2 = 25.029, P < 0.05). The Hp infection rate was the highest in the high incidence area of gastric cancer in people younger than 40 years. The Hp infection rate was 50% in children younger than 5 years in the high incidence area. The Hp infection rates were not different among the three areas in the people older than 40 years. The average levels of anti-Hp IgG in the high, moderate and low incidence areas were 2.3 ± 0.49, 2.04 ± 0.47 and 1.84 ± 0.46, respectively. Multivariate regression analysis showed that the Hp infection was related to bad hygienic habits, low income, frequent use of antibiotics, and mental depression. Univariate analysis showed that Hp infection might also be associated with raising animals in the home.

CONCLUSION: Gastric cancer is closely related to the incidence of Hp infection.

Key words: Stomach neoplasms/etiology; *Helicobacter* infections/epidemiology

Zhang WD, Wu Y, Liu GL, Yang HT, Zhou DY. An epidemiologic study of *Helicobacter pylori* infection in three areas with high, moderate or low incidences of gastric carcinoma. *World J Gastroenterol* 1996; 2(3): 146-148 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/146.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.148

INTRODUCTION

Gastric carcinoma is the most common malignant tumor in China. However, the cause of the disease remains unclear. Many studies have proved that environmental factors may play a very important role in the pathogenesis of gastric cancer. Hp infection is an environmental risk factor for gastric carcinoma. In this study, we determined the Hp infection rates in areas with high, moderate or low incidences of gastric cancer using an indirect ELISA method. We then investigated the relationship of the Hp infection with the gastric cancer incidence.

MATERIALS AND METHODS

From December 1992 to February 1993, we collected samples from people in Changle County, a high incidence area of gastric cancer of Fujian Province, Huaiyuan County, a moderate incidence area of gastric cancer of Anhui Province, and Conghua County, a low incidence area of gastric cancer of Guangzhou City. A total of 639 subjects from 68 families in the high incidence area, 64 families in the moderate incidence area, and 63 families in the low incidence area participated. There were 358 males and 281 females, with ages ranging from 1 year old to 65 years old. Two milliliters of blood was drawn from each subject. Serum was separated and stored at -20 ℃ until the samples were processed for the anti-Hp IgG antibody by an indirect ELISA method. If the ratio of the OD of the subject to the OD of the Hp-negative control was ⩾ 2.0, then the sample was considered Hp-positive. If the ratio of the OD of the subject to the OD of the Hp-negative control was < 2.0, then the sample was considered Hp-negative. The sensitivity of this method was 93.9%, and the specificity was 86.8%. Statistical analysis and multivariate regression analysis of all the data obtained were done with the SAS software on an AST-486 computer.

RESULTS

The overall positive rate of the anti-Hp IgG antibody was 50.5% (329/639) in these subjects. The Hp infection rates of the populations in the high, moderate and low incidence areas are shown in Table 1. The Hp infection rate in the high incidence area

Table 1 ***Helicobacter pylori*** **infection rates of subjects from areas with low, moderate, and high incidence rates of gastric cancer**

Age (yr)	Low (Conghua County)		Moderate (Huaiyuan County)		High (Changle County)	
	n	Positive rate (%)	*n*	Positive rate (%)	*n*	Positive rate (%)
1-4	13	15.4	14	35.7	14	50.0
5-9	43	30.2	17	41.2	42	54.8
10-19	32	37.5	23	43.5	32	53.1
20-29	27	33.3	66	63.6	44	72.7
30-39	30	33.3	34	64.7	40	67.5
40-49	23	43.5	28	53.6	19	47.4
50 +	29	41.4	31	58.7	38	55.3
Total	197	34.5	213	55.9[a]	229	59.4[a]

$\chi^2 = 25.029$, [a]$P < 0.05$, compared with the area of low incidence rate (Conghua County)

Table 2 **Anti-*Helicobacter pylori* IgG levels in subjects from areas with low, moderate, and high incidence rates of gastric cancer**

	n	(S/N)
Low (Conghua County)	197	1.84 ± 0.46[b]
Moderate (Huaiyuan County)	213	2.04 ± 0.47[b]
High (Changle County)	229	2.30 ± 0.49

[b]$P < 0.01$, compared with the area of high incidence rate (Changle County)

Table 3 **Multivariate regression analysis of *Helicobacter pylori* related variables**

N (Xi)	Variable	*r*	*F*	*P* value
X3	Drinking natural water	0.052	4.34	0.038
X11	Eating red pepper	0.079	8.57	0.003
X18	Poor dietary habits	-0.110	11.98	0.001
X25	Frequent use of antibiotics	-0.110	12.79	0.000
X39	Low income	0.087	21.14	0.000
X51	Mental depression	-0.108	4.23	0.040

Table 4 **Univariate analysis of *Helicobacter pylori* related factors**

N (Xi)	Variable	x^2M-H	*P* value
X11	Eating red pepper	10.53	0.001
X25	Frequent use of antibiotics	6.39	0.011
X29	Raising animals in the home	3.86	0.049
X32	Abdominal symptoms	5.82	0.016
X39	Low income	9.99	0.002
X51	Mental depression	5.26	0.022

was higher than that in the moderate incidence area, and much higher than that in the low incidence area in people younger than 40 years. Also, the Hp infection rate in children under 5 years of age in the high incidence area was 50%, which was significantly higher than those in the other two areas ($P < 0.05$). However, the Hp infection rates were not different among the three areas in the subjects older than 40 years. The Anti-Hp IgG level in the subjects of the high incidence area was significantly higher than that of the other two areas ($P < 0.01$) (Table 2). A Mantel-Haenszel analysis showed that X1, X25, X29, X32, X39 and X51 were highly associated with an Hp infection (Table 3). Multivariate regression analysis and logistic regression analysis showed that X3, X11, X18, X25, X39 and X51 were risk factors for an Hp infection (Table 4).

DISCUSSION

Gastric cancer is the most common malignant tumor in China. The average yearly mortality from gastric cancer between 1975 and 1978 was 15.41 people out of 1000000 people. Interestingly, mortality rates varied by geographic location[1]. Changle County is located on the southeast coast and has the highest incidence and mortality rates from gastric carcinoma (120.47 people out of 1000000 people) in China. Conghua County of Guangzhou City has a low incidence rate of gastric carcinoma in China, with a mortality rate of one person out of 100000. Huaiyuan County of Anhui Province has a moderate incidence of gastric cancer. The mortality rate of gastric cancer approaches the average level in China[2-4].

In the present study, each family was taken as an observation unit for determining the Hp infection rates in Changle (high incidence), Huaiyuan (moderate incidence) and Conghua (low incidence) counties. The overall Hp infection rate was 50.55%. The Hp infection rate and anti-Hp IgG antibody levels in the subjects of the high incidence area were higher than those of the other two areas, especially in children younger than 5 years. However, the Hp infection rates were not different among the three areas in subjects older than 40 years.

Many studies have shown that atrophic gastritis incidence is associated with an Hp infection. The Hp infection rate was 60% to 90% in patients with atrophic gastritis and 92% in patients with active atrophic gastritis in China[5]. In other countries the Hp infection rate was 43.5% to 89.5% in patients with atrophic gastritis[6]. Atrophic gastritis is considered to be a risk factor for gastric cancer. The incidence of gastric cancer in high incidence areas is seven times higher than in low incidence areas of gastric cancer, and atrophic gastritis is usually more severe in high incidence areas of gastric cancer[7]. Notably, the majority of atrophic gastritis cases and Hp infections affect the gastric antrum. Anti-Hp IgG antibody levels were related to the number of Hp and the reaction of the gastric mucosa to Hp[8]. The Hp infection rates and anti-Hp IgG levels were higher, and the average age of the infected patients were younger in the high incidence areas of gastric cancer than in the low incidence areas[9,10]. This data suggests that gastric cancer might be associated with an Hp infection, and thus an Hp infection may be one of the risk factors for gastric cancer.

Multivariate regression analysis showed that drinking natural water, poor dietary habits, low income and mental depression were all risk factors for an Hp infection. Ling *et al*[8] proved that Hp can live in natural river water for approximately 10 days, indicating that Hp infection may be transmitted by water. Inhabitants of Changle County (high incidence area) usually drink natural water. The nitrate and nitrosamine levels are higher there than the nitrate and nitrosamine in water of other areas. Hp infection or long time exposure to high levels of nitrate and nitrosamine may destroy gastric mucosa and result in gastric cancer. It is hypothesized that an Hp infection and high levels of nitrate and nitrosamine in the drinking water may be the cause of the high incidence rate of gastric cancer in Changle County.

Graham *et al*[11] observed that the Hp infection rate was 70% in black people and 34% in white people in the United States. They also found that the Hp infection rate was higher in low-income families, which coincides with our results. Univariate analysis showed that an Hp infection was also associated with raising animals in the home. Some studies have shown that both humans and animals can be infected with Hp. Experimental Hp infection of the stomachs of newborn pigs, newborn dogs, and nude mice has been successful[12]. In addition, Euler isolated an Hp-like organism from the monkey, suggesting that humans and animals may transmit Hp to one another.

In summary, we observed an association between gastric cancer and Hp infections. Hp infection rates were higher and the age of the infected patents were younger in the high incidence area compared to the rates and ages in the low incidence area. The risk factors related to gastric cancer included low income, mental depression, and poor dietary habits. Our results also suggested that the gastric

cancer incidence and mortality have positive correlations with Hp infection. In other words, Hp infection may be a risk factor for gastric cancer.

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Metal stent implantation for palliation of malignant biliary obstruction: A report of 57 cases

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Abstract

AIM: To report the first experience in China in the treatment of malignant biliary obstruction with expandable metal stent, which allows the insertion of an endoprosthesis as large as 1 cm in diameter.

METHODS: Between April 1994 and May 1996, we implanted expandable metal stents in 57 patients with incurable malignant biliary obstruction. Fifty-four patients underwent endoscopic procedure, and the other three patients received percutaneous transhepatic placement.

RESULTS: Insertion of the stent following guide wire positioning was successful in 95% of the patients. Two patients developed cholangitis after stent insertion and were successfully treated with conservative treatment. The jaundice was eliminated completely in 21 cases and markedly decreased in 23 cases within 2 wk after stent placement. However, nine patients had late cholangitis due to stent failure after a median interval of 147 d. Twenty-three cases underwent nasobiliary transient drainage, and three underwent plastic stent transient drainage prior to metal stent insertion. The advantages of transient drainage were drainage pre-assessment and infection control.

CONCLUSION: Our results show that an expandable metal stent is suitable for the unresectable malignant choledochal stenosis. It can eliminate jaundice and improve the patient's quality of life. To get the highest benefit, however, the indication should be strictly selected. To get long-term patency, the proximal and distal end of the stent preceding the tumor should be no shorter than 2 cm. In the case of hilar cancer, Bismuth classification is helpful for the selection of the drainage site.

Key words: Biliary tract obstruction/surgery; Biliary tract, neoplasms/surgery; Stents

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INTRODUCTION

Endoscopic biliary drainage with polyethylene endoprosthesis has become a well-established palliative treatment for patients with inoperable, malignant, and obstructive jaundice. The main drawback of this method is rapid incrustation or occlusion as a result of bacterial biofilm formation on the stent, and biliary sludge[1]. Drainage occlusion of the commonly used 7-12 Fr gauge plastic prosthesis occurs in 20% to 30% of cases within the first three mo and in up to 60% of cases within 6 mo[2]. Though larger stents may perform better, stents over 14 Fr have not been used in the biliary tract up to now because the maximum diameter of the stent depends upon the diameter of the working channel of the endoscope. The development of expandable metal stents allows the insertion of an endoprosthesis as large as 1 cm in diameter. Such an endoprosthesis, previously employed for vascular and urethral stricture, recently became available for biliary use.

In this paper, we report our first experience in China in the treatment of 57 patients with malignant bile duct stenosis using metal stents during the period from April 1994 to May 1996.

MATERIALS AND METHODS

Fifty-seven patients (40 male and 17 female) with a median age of 56.1 years (range: 25 years to 85 years) were treated with a total of 60 expandable stents. In all patients, the malignant diseases led to invasive or compressive occlusion of the hepatocholedochal duct (Table 1). None were operable due to advanced stage, metastasis, post-operative recurrence of the disease, old age, or accompanying disease.

Two types (several kinds) of metal stents, self-expandable stents (Wallstent, Instent and Angiomed) and balloon-mounted stents (Strecker) were used.

General diagnostic endoscopic retrograde cholangiopancreatography was first made using a side-view duodenoscope (TJF-30,

Table 1 Causes of bile duct obstruction in 57 patients

Cause of bile duct obstruction	Location of stenosis			No. of patients
	P	M	D	
Cholangiocarcinoma	30	3	1	34
Gallbladder carcinoma	3	1	0	4
Hepatocellular carcinoma	7	0	0	7
Pancreatic carcinoma	1	0	6	7
Ampullary tumor	0	0	2	2
Metastasis	3	0	0	3
Total	44	4	9	57

P: Proximal part of the common bile duct; M: Middle part of the common bile duct; D: Distal part of the common bile duct.

Table 2 Relation between the Bismuth classification and drainage sites in patients with hilar malignancy

Type	No. of patients	Drainage site				
		H	L	R	RA	RP
I	8	2	3	2	0	1
II	3	0	1	1	0	1
IIIa	5	0	2	0	1	2
IIIb	4	0	0	0	0	4
IV	24	0	6	2	4	12
Total	44	2	12	5	5	20

H: Common hepatic duct; L: Left hepatic duct; R: Right hepatic duct; RA: Right anterior intrahepatic duct; RP: Right posterior intrahepatic duct.

4.2 mm working channel, or JF-1T30, 3.2 mm working channel). When biliary stenosis was found, a 4 m long, 0.035 inch standard guide was inserted into papilla passing the strictured segment. No papillotomy was performed, but a simple dilation was made with a 10 Fr or 8.5 Fr Teflon dilator (Wilson-Cook) instead. Afterward, the delivery catheter with the constrained stent was inserted over the guide wire. The stent was released under continuous fluoroscopic and endoscopic control. If we were uncertain of the drainage effect in the patients with extensive stenosis or severe infection, a transient nasobiliary drainage was performed first. After a satisfactory drainage was achieved, or the bile duct inflammation was successfully controlled, another endoscopic procedure for the metal stent implantation was given.

In three patients, endoscopic transpapillary implantation was impossible because of previous Billroth Ⅱ gastrectomy, cannulation failure of the ampulla of Vater, or duodenal stricture. Percutaneous transhepatic route was therefore chosen. One week after an ordinary percutaneous transhepatic biliary drainage (PTBD), the drainage tract was dilated to 10 Fr. A passage through biliary stenosis was made using a guide wire, and then the metallic stent was delivered into the biliary tree and released under fluoroscopy.

All patients were followed up as long as possible. Their general condition, laboratory findings, as well as recurrence of jaundice and fever were recorded.

RESULTS

Fifty-seven patients were implanted with a total of 60 metal stents. The success rate for metal endoprosthesis insertion was 95%. In three cases, there were technical problems during implantation of the first stent. In one of the patients, a Strecker stent failed to cannulate into papilla because its distal end had already been released. In another patient, a Wallstent could not be placed due to the breakage of its outer membrane. In the final patient, a Wallstent was released by mistake inside the working channel of the duodenoscope. However, in all three cases, another stent was successfully implanted the second time.

A transient rise in cholestasis parameters and fever indicating cholangitis as an early complication were seen in two (3.5%) patients. They were rapidly and effectively treated with antibiotics. No other complications related to stent insertion were found.

During the first two weeks after the placement of the stent, cholestasis parameters returned to normal in 21 cases, decreased significantly in 23 cases, and were unchanged or even elevated in two cases. Follow-up was unavailable for the remaining nine

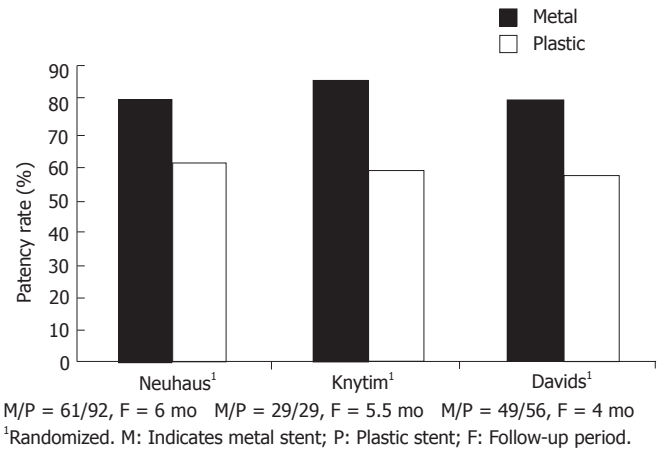

Figure 1 Overview of current comparative or randomized studies of patency rate on metal stents versus conventional plastic stents.

patients. However, during the long-term follow-up, nine patients had late cholangitis due to stent failure after a median interval of 146.7 d. The causes of stent failure were distal tumor overgrowth in four patients after a median interval of 127.5 d, tumor ingrowth through the meshes of the stent in four cases after a median of 150 d, and biliary sludge obstruction in one case after 210 d. The cholangitis was treated with a 9 Fr polyethylene stent through the metal stent in four patients, with a nasobiliary drainage in one patient, PTBD in one patient, and biliary lavage and balloon pull-through in one patient.

During the follow-up, four patients died due to non-stent related causes (renal failure in two cases 12 d and 210 d, respectively post stent insertion, esophageal variceal bleeding in one patient at 51 d post stent insertion, and ileus bleeding in one patient at 63 d post stent insertion). Two patients died of stent failure and cholangitis 90 d and 153 d after endoprosthesis, respectively. To our knowledge, eight patients with a successful stent insertion have been alive for more than 6 mo after stent implantation. The patient with the longest survival (over 18 mo) has been free from jaundice as of this publication.

DISCUSSION

Expandable intraluminal stents were initially developed to prevent early reocclusion or delayed restenosis after percutaneous transluminal coronary angioplasty (PTCA). The first biliary expandable stent was inserted in an animal study in 1985[3]. The expandable diameters of the metal stents of up to 7-10 mm, the reduced surface area for bacterial attachment, and the capacity of being epithelialized by biliary mucosal cells are the major advantages in preventing bacterial colonization and biofilm formation. Figure 1 shows the results of current comparative or randomized studies by several specialists on wire mesh endoprostheses versus conventional plastic prostheses[4-6]. Hoepffner *et al*[2] reported a long term study on Wallstent therapy for 118 malignant choledochal stenosis patients, and found the survival rate at 6 mo, 12 mo, and 24 mo was 40%, 20% and 10% respectively, with the longest survival of 1295 d, which was similar to that after palliation bypass surgery in a previous report.

There were 44 (77.2%) patients with malignant biliary obstruction due to proximal bile duct stenosis in our study. It is attributable to the high incidence of hilar tumor (Klatskin's tumor) and extraordinarily low resection rates of the cancer. According to the Bismuth classification[7], our patients were classified into type Ⅰ (8 cases, 18.2%), type Ⅱ (3 cases, 6.8%), type Ⅲa (5 cases, 11.4%), type IIIb (4 cases, 9.1%) and type Ⅳ (24 cases, 54.5%). The relationship between the typing and drainage sites is shown in Table 2. Generally, type Ⅰ patients can get satisfactory drainage, followed by type Ⅱ and type Ⅲ. Type Ⅳ patients can only achieve poor drainage. We emphasize the importance of total revelation of intrahepatic duct, upon which it is possible to select a suitable site for drainage. Normally, the wider the range of the intrahepatic tree, the better the drainage. If the drained region can reach a little more than half a liver, it is still hopeful to eliminate jaundice completely.

Table 3 Common complications of metal biliary stenting[2,9]

	Huibregtse *et al*, 1992 (103 cases)	Hoepffner *et al*, 1994 (118 cases)	Our group, 1996 (57 cases)
Early cholangitis	2	5	2
Late cholangitis	18 (125 d)[1]	17 (148 d)	9 (147 d)
Tumor ingrowth	10	4 (150 d)	4 (150 d)
Tumor overgrowth	4	5	4 (128 d)
Sludge clogging	5 (175 d)	N/A	1 (210 d)

[1]Numbers in parentheses are median days

Sharp angles should be avoided when selecting a drainage site in order to get full expansion of the stent.

The length of the stent should also be determined carefully. In our group, there were four patients with late stent failure due to tumor overgrowth after a median interval of 127.5 d. Therefore, we recommend that the two ends of the stent preceding the tumor should not be shorter than 2 cm after full expansion in order to get long-term stenting.

We also attach importance to the use of transient nasobiliary drainage prior to the stenting. Because the metal stent is very expensive, we should be more prudent in choosing cases. Nasobiliary drainage is a simple and cheap way to know the quantity of the drain, and is very effective in controlling bile duct infection. It also gives the surgeon time to discuss if the patient has any opportunity to undergo a radical operation. In our group, 23 patients had undergone nasobiliary drainage, and three patients had undergone plastic stent drainage for a mean period of 15 d before metal endoprosthesis insertion. When the bile fluid was less than 300 mL per day and the cholestasis parameters decreased significantly, another endoscopic procedure for metal stent implantation can be performed.

Though expandable metal stents provide a longer median period of patency over conventional plastic prosthesis, they are not without problems. Tumor ingrowth through the metal mesh, or overgrowth at the ends of the stents may occur several weeks to several mo later, causing occlusion of bile flow (Table 3). Many stent occlusions can be successfully treated by implantation of a second metal stent or a conventional plastic one through the metal stent. Some endoscopists also reported the method of coagulation using a unipolar electrohydral probe or bipolar electrohydrothermal probe[2,8]. In recent studies, a "coating" stent or "electrolytic" stent may solve the above-mentioned disadvantages of metal stents[10,11].

The indications of metal stent implantation remain controversial. Though some doctors have tried to use metal stents to treat benign biliary stricture, most specialists are reluctant to use this unremovable stent in patients with benign disease because the recurrent ulceration caused by sharp filaments of the metal stent can give rise to restricture proximal to the stent. The high cost of this treatment is also under discussion. Although several authors have argued that when the costs of retreatment due to plastic stent failure are included, the metal stents are cost-effective, considering the limited life span of these patients with terminal malignancies, the price of the metal stent is still a concern. "Is the expanse worth the expense?" is an interesting question raised by Cotton[12]. In our opinion, metal stent implantation is the treatment of choice for palliating jaundice in patients (1) with inoperable pancreatobiliary malignancies, (2) with adequate biliary tree to drainage, (3) without major organic exhausting, (4) who may survive for more than 3 mo, and (5) whose financial condition allows.

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Ultrastructural observation of *Helicobacter pylori* to the gastric epithelia in chronic gastritis and peptic ulcers

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Abstract

AIM: The relationship between *Helicobacter pylori* (Hp) and gastric epithelia in chronic gastritis and in peptic ulcers was studied by transmission electron microscopy (TEM).

METHODS: Seventy-five patients were screened for Hp. Gastric antral biopsy specimens were fixed in glutaraldehyde and treated with tannic acid before OsO_4 staining. Samples were routinely processed for TEM studies (at least four semi-thin sections oriented for ultrathin sections in each sample).

RESULTS: The bacilli were detected by TEM within the gastric mucosa in 53 of 55 patients infected with Hp. Ultrathin sections revealed clear glycocalyx by which the bacillus was connected to the epithelium. As the bacilli colonized, the adjacent mucous cells degenerated. They were characterized by erosion of the juxtaluminal cytoplasm, vacuolation or blebbing, and desquamation of the cell membrane. The bacilli located in the lumen attracted neutrophils, which migrated into intercellular space of the epithelia or into the lumen to begin phagocytosis of Hp.

CONCLUSION: The sensitivity and specificity of TEM diagnosis is 96% and 95%, respectively. Tannic acid is suitable for the preservation of the glycocalyx of a cell. The colonized bacilli, usually with the wide periplasm, contributed to the degeneration of epithelia, including mucous neck cells. If Hp infection persists, the degeneration and regeneration of mucous neck cells occurs alternatively. Ultimately the generative stem cells were damaged, and as a result chronic atrophic gastritis could occur.

Key words: Gastritis; Peptic ulcer; *Helicobacter pylori*

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INTRODUCTION

Ultrastructural studies have demonstrated a loss of apical microvilli and depletion of mucin granules in *Helicobacter pylori* (Hp) infected cells[1,2]. Further investigation of Hp adherence, cell penetration, and immune response of the gastric epithelia is needed to delineate the relationship between the bacilli and the epithelia or the monocytes.

MATERIALS AND METHODS

Biopsies of the gastric antrum (four biopsies), the body (one biopsy), and serum from 75 patients with chronic gastritis or peptic ulcers were individually tested for Hp by culture, histopathology, cytology of smear, Hp rapid analytical chemistry urease kit (Hp-RACU), Hp enzyme linked immunosorbent analysis kit (Hp-EIA), and Hp polymerase chain reaction kit (Hp-PCR) (Cancer Research Center, Xiamen University). Positivity in more than three methods was considered the standard for detection of Hp infection. One of antral biopsies was also examined by a JEM 100CX/II transmission electron microscope (TEM). Biopsies were prefixed in 2.5% glutaraldehyde, treated for 4 h in 1% tannic acid (except for ten samples), then post-fixed and stained in a 1% osmium tetroxide and a 1% potassium ferricyanide mixture. Ultrathin sections (90 nm) were selected from the oriented semi-thin sections (at least four blocks/sample), and were examined by TEM for the relationship between the bacilli and the gastric epithelia.

RESULTS

Hp appeared dense and opaque under the microscope. An electron-lucent zone in the mucin pool usually surrounded curved or spiral bodies. They were also located near the microvilli of epithelial cells, typically in the gastric neck region or adjacent to a depression in the plasma membrane that resulted from a lost microvillus (Figure 1) or apocrine secretion. Hp bacilli were found disrupting the cellular junction of rolled microvilli and fragmentated cells, and were found to be insinuated deep in between the epithelial cells (Figure 2). Hp infection was confirmed in 55 (30 chronic gastritis and 25 peptic ulcer) patients out of 75 patients. Curved or spiral organisms were detected in 53 patients (with one false positive) by TEM in the gastric mucosa (96%). Specificity of TEM was 95%, with 19 true negative and one false negative. After tannic acid staining, the glycocalyx was observed at the surface of the organisms, especially on the tip of the microvilli (Figure 3).

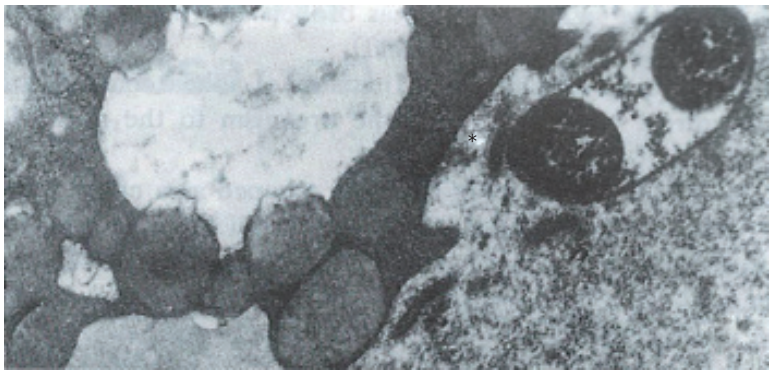

Figure 1 The bacillus abuts upon the depression of the plasma membrane (*) of the vacuolated cell. (× 21000)

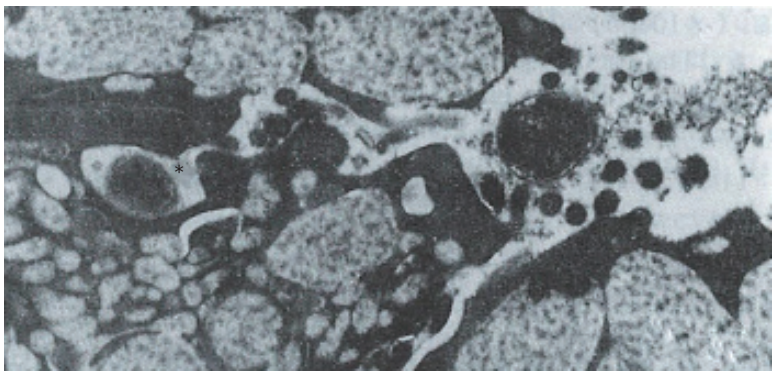

Figure 2 The bacilli (*) penetrating deep in between the mucous cells. The tight junction was broken and the intercellular space was dilated. (× 21000)

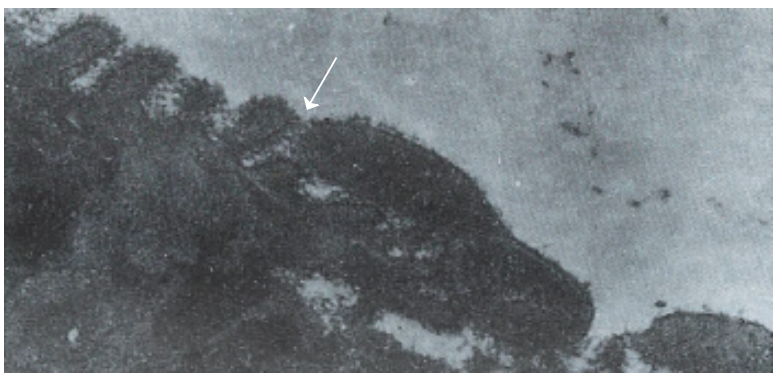

Figure 3 The bacillus shows the first step of adherence initiated by the direct contact of the organism to the microvilla glycocalyx. (× 28500)

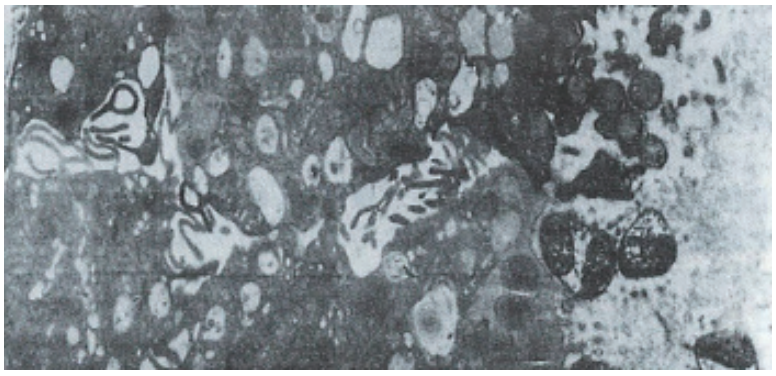

Figure 4 A great number of bacteria grouped in a colony shown with periplasmic pools (s). The bacilli abutting upon the depression of the plasma membrane of mucous neck cells lost microvilli and vacuolated. (× 8700)

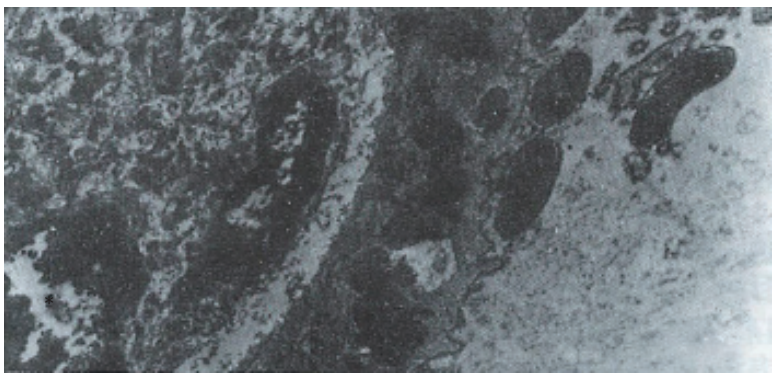

Figure 5 Neutrophils (*) penetrated into the epithelia and migrated to the bacilli located in the apical region of mucous neck cells. (× 10800)

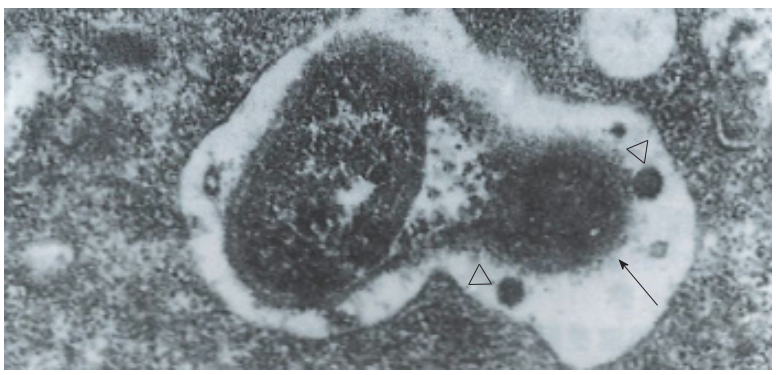

Figure 6 The bacillus is phagocytized by neutrophils located in the lumen. In the phagosome, two lysosomes approach the bacillus, of which the cell wall was lysed and discontinuous. (× 43500)

Where the bacilli colonized, the adjacent mucous cells degenerated and were characterized by vacuolation (Figure 4), accumulation of lysosomes, and the appearance of mucous apocrine or blebs displaying a myeline figure. The bacilli located in the lumen attracted polymorphonuclear leukocytes (PMNL) that migrated into the intercellular space of the epithelia (Figure 5), or into the lumen to exert the effect of Hp phagocytosis (Figure 6).

DISCUSSION

In order to preserve delicate structures typically destroyed by osmium tetroxide, we fixed biopsies in a tannic acid solution. We found that some fragile glycocalyx on the bacilli and the epithelia remained intact (Figure 3). Based on our observation, where the bacilli colonized, mitotic figures and vacuolation of adjacent epithelial cells were present. A similar observation was noted by Caselli *et al*[3]. The bacilli firmly attached to the epithelium or hiding in the niche of injured cells may have released cytotoxins or vacuolotoxin to impair the cells[4]. As the organisms colonized, ammonia was released in order for the bacilli to escape the microbicidal effect for a long period of time.

Hp can be cleared by neutrophils from the site of infection most effectively through the opsonophagocytotic process *in vivo* and *in vitro*. Caselli *et al*[5] demonstrated the specific IgG antibody-promoted complement-dependent phagocytosis and killing of Hp by PMNL *in vitro*. Until now, the evidence of Hp phagocytosis by PMNL in antrum *in vivo* was scant. It is known that a certain protein or chemotactic factor of Hp[6] can attract neutrophils to migrate into the epithelia or the gland and begin phagocytosis. Degranulation of proteolytic enzymes from neutrophils may also contribute to gastric mucosa damage, especially in the cells of the generative mucous neck region. As a result of persistent Hp infection, the degeneration and regeneration of the mucous neck region alternatively occurs and gives rise to atrophic gastritis. Regeneration of the stem cells of the mucous neck region would ultimately promote gene instability, which accounts for genetic mutation in neoplastic transformation in gastric stem cells.

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Quantitative ultrastructure analysis of neuroendocrine cells of gastric mucosa in normal and pathological conditions

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Abstract

AIM: To study the quantitative ultrastructure of neuroendocrine cells of the gastric mucosa in normal and pathological conditions, including the duodenal ulcer (DU) and Zollinger-Ellison syndrome (ZES).

METHODS: The neuroendocrine cells of the gastric mucosa of eight normal subjects, six patients with DU, and five patients with ZES were quantitatively investigated with by electron microscopy and ultrastructure image analysis.

RESULTS: The volume density of neuroendocrine (NE) cells in the DU was 1.3% and 0.8% (*vs* 1.6% and 0.9%, $P < 0.05$) in gastric antrum and corpus, respectively. In the antrum, G cells were 65% ($P < 0.05$), D cells decreased in cell density (3% *vs* 9.5%) and in number per unit area ($P < 0.01$). In the corpus, the cell density of enterochromaffin-like (ECL) cells increased (49% *vs* 30%, $P < 0.05$); D cells and enterochromaffin (EC) cells decreased (2%, $P < 0.01$ and 4%, $P < 0.05$, respectively), and the number of D cells per unit area markedly decreased. In ZES, D cells in the corpus decreased in cell density (4% *vs* 22%, $P < 0.01$), and P cells also decreased (11% *vs* 24%, $P < 0.05$). The density of ECL cells increased (65% *vs* 30%, $P < 0.01$).

CONCLUSION: In DU and ZES, both the number and type of NE cells presented some changes. Increased gastrin in DU and ZES patients may be caused by the decrease of D cells and somatostatin secretion.

Key words: Gastric mucosa/pathology; Neuroendocrine cells; Duodenal ulcer; Zollinger-Ellison syndrome

Yu JY, Adda T. Quantitative ultrastructure analysis of neuroendocrine cells of gastric mucosa in normal and pathological conditions. *World J Gastroenterol* 1996; 2(3): 155-157 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/155.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.155

INTRODUCTION

Quantitation has increasingly become a fundamental supplement of investigations on gastric endocrine cells[1]. In humans, up to six cell types [enterochromaffin-like (ECL), enterochromaffin (EC), D, P, D1 and X] in oxyntic mucosa and four endocrine cell types (G, D, P and EC)[2] in antrum mucosa have been identified. However, the secretory product of some cell types is either unknown or requires non-conventional tissue processing, thus preventing the use of immunohistochemistry. We have developed an ultrastructural morphometric procedure for quantitative assessment of endocrine cells of human gastric mucosa in normal and pathological conditions.

MATERIALS AND METHODS

The material was obtained from eight healthy volunteers equally divided by sex, aged 19 years to 34 years, on whom the study of normal gastric endocrine cells was performed. Endoscopic biopsies were performed in the central area of the posterior wall of the gastric corpus and antrum on six patients with duodenal ulcer (DU) aged 31 years to 57 years, and five patients with Zollinger-Ellison syndrome (ZES) aged 36 years to 62 years. The samples were fixed in a glutaraldehyde-paraformaldehyde mixture and cut with a razor blade to obtain slices of mucosa sections perpendicular to the gastric surface. These fragments were post fixed in osmium tetroxide, dehydrated in acetone and embedded in Araldite. Four to five blocks from different biopsy specimens including the whole thickness of the mucosa were selected at random. The blocks were trimmed to examine the entire thickness of the mucosa. Thin sections were collected with formvar-coated Robertson multiple slot grids, stained and examined with the electron microscope. Measurements were made under low power (× 760) electron micrographs mounted to cover the entire thickness of the mucosa between two bars of the grid, and under high power (× 13700) electron micrographs to cover all endocrine cell profiles in the same area. By using an ultrastructural quantitative analysis (GmbH German Str), the relative volumes of the epithelial component and the lamina propria were estimated with low power micrographs whereas morphometric analysis of endocrine cells was performed with high power micrographs.

RESULTS

The volume densities of different endocrine cells depended on the amount of the measured lamina propria, which was 26% in our estimation in normal subjects, but 33% in the patients with ZES ($p < 0.01$) and 32% in the patients with DU ($p < 0.05$). Such

Table 1 Quantitative ultrastructure analysis of neuroendocrine cells of gastric mucosa in normal subjects

Cell type	Volume fraction (%)	Profiles/unit area (n/m^2)	Cytoplasm composition (%)	Granule constituents (%)
Antrum				
NE	1.6 ± 0.5			
G	65.0 ± 2.0	2.4 ± 0.8	75.0 ± 3.0	16.0 ± 9.0
D	9.5 ± 0.6	0.3 ± 0.8	81.0 ± 4.0	35.0 ± 6.0
EC	8.6 ± 2.9	0.3 ± 0.7	76.0 ± 12.0	18.0 ± 7.0
Remaining cells	16.9 ± 3.2	0.6 ± 0.6	77.0 ± 8.0	3.8 ± 2.9
Corpus				
NE	0.9 ± 0.4			
ECL	30.0 ± 9.0	1.6 ± 0.7	70.0 ± 2.0	8.0 ± 2.0
EC	7.0 ± 5.0	0.3 ± 0.2	72.0 ± 13.0	16.0 ± 8.0
D	22.0 ± 4.0	1.5 ± 0.8	81.0 ± 7.0	31.0 ± 5.0
P	24.0 ± 7.0	0.8 ± 0.3	72.0 ± 7.0	6.0 ± 2.0
Remaining cells	17.6 ± 8.0	0.9 ± 0.5	68.0 ± 19.0	3.2 ± 2.6

Table 2 Quantitative ultrastructure analysis of neuroendocrine cells of the corpus mucosa in Zollinger-Ellison syndrome patients

Cell type	Volume fraction (%)	Profiles/unit area (n/m^2)	Cytoplasm composition (%)	Granule constituents (%)
Antrum				
NE	3.2 ± 1.1			
ECL	65.0 ± 15.0[d]	5.2 ± 2.4[c]	82.0 ± 2.0[d]	5.0 ± 2.0[c]
EC	5.0 ± 2.0	0.7 ± 0.5	80.0 ± 13.0	6.0 ± 9.0[a]
D	4.0 ± 4.0[d]	0.6 ± 0.4	75.0 ± 14.0	18.0 ± 5.0[d]
P	11.0 ± 8.0[a]	1.1 ± 0.4	76.0 ± 7.0	3.0 ± 0.5[b]
Remaining cells	10.0 ± 3.0	2.8 ± 1.5	78.0 ± 9.0	1.6 ± 0.8

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.05$; [d]$P < 0.01$

Table 3 Quantitative ultrastructure analysis of neuroendocrine cells of gastric mucosa in duodenal ulcer patients

Cell type	Volume fraction (%)	Profiles/unit area (n/m^2)	Cytoplasm composition (%)	Granule constituents (%)
Antrum				
NE	1.3 ± 0.2			
G	46.0 ± 2.0	1.8 ± 0.7	71.0 ± 3.0	16.0 ± 9.0
D	3.0 ± 1.6[a]	0.1 ± 0.8[b]	87.0 ± 4.0[a]	28.0 ± 12.0
EC	16.0 ± 6.5	0.6 ± 0.7[b]	77.0 ± 12.0	12.0 ± 5.0
Remaining cells	35.0 ± 13.0	1.2 ± 1.5	81.0 ± 8.0	3.8 ± 2.9
Corpus				
NE	0.8 ± 0.3			
ECL	49.0 ± 16.0[a]	1.7 ± 1.0	70.0 ± 5.0	5.0 ± 2.0[a]
EC	4.0 ± 2.0	0.3 ± 0.2	72.0 ± 24.0	8.0 ± 8.0
D	2.0 ± 4.0[a]	0.3 ± 0.3[c]	93.0 ± 6.0[a]	33.0 ± 4.0
P	25.0 ± 4.0	1.7 ± 1.0	67.0 ± 5.0	3.0 ± 1.0
Remaining cells	20.0 ± 12.0	1.9 ± 1.5	81.0 ± 9.0	3.2 ± 2.8

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.05$

differences, which were statistically significant, were related to inflammatory changes in the upper region of the lamina propria occurring in pathological conditions. For this reason, the epithelial compartment alone has to be kept as the reference volume for endocrine cell densities in comparative studies. Ultrastructural characteristics[3] of secretory granules in different types of gastric endocrine cells including G, D, P, EC and ECL cells are shown in Figures 1-5.

Table 1 shows the quantitative ultrastructural data of the mucosa in the antrum and corpus from normal male subjects. The volume density of endocrine cells in corpus mucosa in patients with ZES was 3.2% of the mucosal epithelial component, a 168% increase ($p < 0.01$) over the value found in normal subjects (Table 2). This result is evidence for a net increase in the total volume of endocrine cells of corpus mucosa augmented in ZES. In all the cases, the ECL cells composed more than 50% of the whole endocrine cell mass. As shown in Table 2, the mean volume fraction of this cell type was 65%, a 119% increase over the normal value. In contrast, other cell types of endocrine cells showed a mean volume fraction lower than that found in normal subjects, a difference statistically significant in the case of D and P cells.

In this series of DU patients (Table 3), the mean volume density

Figure 1 G cell of the antrum containing both vesicular and compact granules with a floccular content.

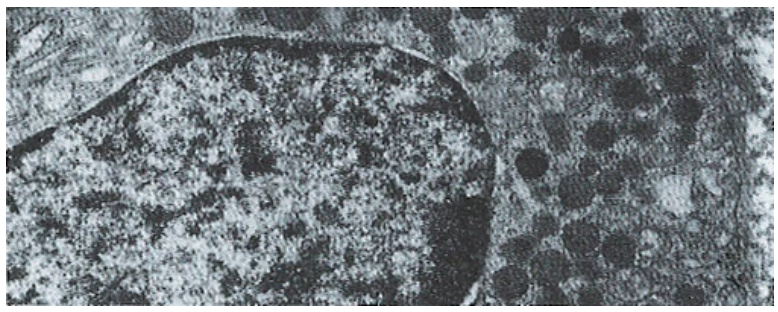

Figure 2 D cell granules are usually rounded, homogeneous, of low electron density and with closely applied membranes.

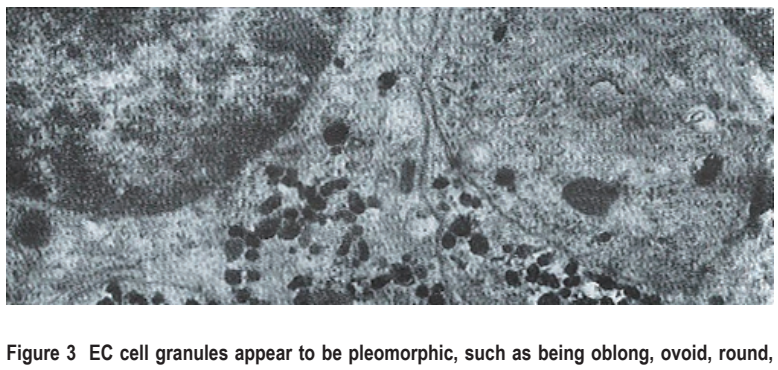

Figure 3 EC cell granules appear to be pleomorphic, such as being oblong, ovoid, round, *etc*., with high density.

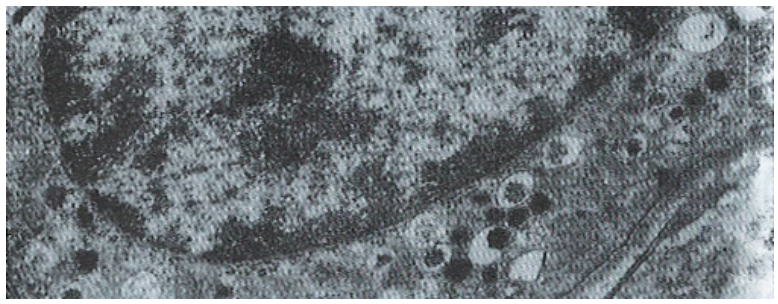

Figure 4 ECL cells show the characteristic of vesicular-type granules and thin haloed granules with an irregular argyrophilic core eccentrically located in wide space.

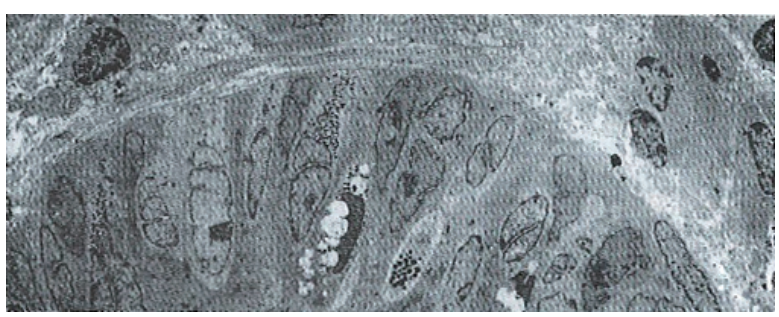

Figure 5 Electron micrograph of contiguous gastric glands shows cross sections belonging to different endocrine cells.

of the whole endocrine cell population was 1.6% and 0.8% in the gastric antrum and corpus, respectively, which did not differ significantly from that of healthy subjects. In the antrum, D cells decreased in cell density and in number per unit area, as well as in

the corpus. The mean volume fraction of ECL cells was found to be increased 49%.

DISCUSSION

From the morphometric point of view, absolute counts of cells, usually restricted to nucleated sections, have commonly been used. The frames of reference for these counts, however, include visual fields, unit length of mucosa, unit area of tissue section, unit area of mucosa surface, gland crypt, nucleus-containing gland cells, and whole antrum or whole stomach. Such heterogeneity makes comparisons between different studies difficult, and occasionally opposite results would be achieved. Direct counts of endocrine cells from pelleted cell suspensions of gastric mucosa have also been proposed[4]. With this background in our laboratory, we have developed an ultrastructural morphometric procedure for quantitative assessment of endocrine cells of human gastric mucosa in normal and pathological conditions. In ECL cells of ZES patients, the number of cell profiles per unit area, the cross section area and the cytoplasmic values were tested. These data supported the previous observations that both hyperplasia and hypertrophy occurred in the oxyntic endocrine cells in the pathological condition. Secretory granules were not restricted to ECL cells, but were shared by the other cell types, which were significant in the case of P, D, and EC cells[5]. On the basis of experimental evidence obtained from the rat, reduction of the granule volume density in corpus endocrine cells could be regarded as a sign of gastrin induction increase in functional activity, which was associated with activation of the specific enzyme, histidine decarboxylase[6]. Therefore, our studies supported a similar result about the functional activity of gastrin in humans involving cell types that were not influenced by the stimulus of gastrin.

In this series of DU patients, the mean volume density of the endocrine cells of gastric mucosa did not differ significantly from that of normal subjects. In contrast, the mean volume fraction of ECL cells was increased. The volume fraction of D cells markedly decreased. Although age influences cannot be ruled out, such changes are possibly relevant to the pathophysiology of the DU disease[7]. In this regard, it is pertinent to note that the gastric mucosa of DU patients releases significantly lower amounts of somatostatin[8]. Our results, therefore, provide a cellular basis for this functional deficiency and suggest a reduced paracrine control of gastric secretion in this pathological condition.

From the above discussions, it can be concluded that ultrastructural morphometry is a useful tool for investigating endocrine cells of human gastric mucosa. Indeed, it allows identification of all cell types composing the gastric endocrine cell population. Recently developed stereological procedures, such as the selector or the nucleator, may efficiently supplement the data provided here with unbiased estimates of absolute structural quantities, including cell volume, thus opening new dimensions to the ultrastructural morphometry of gastric endocrine cells.

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Comparative study on the effects of hepatic arterial embolization with *Bletilla striata* or Gelfoam in treatment of primary hepatic carcinoma

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Abstract

AIM: To study the safety and efficacy of hepatic arterial embolization (HAE) with Bletilla striata powders containing traditional Chinese herbs in the treatment of primary hepatic carcinoma (PHC).

METHODS: From May 1990 to September 1993, 106 patients with PHC were treated by HAE with different types of *Bletilla striata* powders (n = 56) or Gelfoam powders (n = 50) under clearly specified conditions. We analyzed the effects and complications associated with these two types of treatment.

RESULTS: The *Bletilla striata* powders produced extensive and permanent proximal embolization of the hepatic artery, and stimulated the formation of collateral circulation. Treatment could be stopped for as long as 6-12 mo, and there was obvious evidence of tumor necrosis and shrinkage. The patient survival rates at 1, 2, and 3 years were 81.9%, 44.9%, and 33.6%, respectively, and the mean survival time without a serious complication was 19.8 mo. Patients in the *Bletilla striata* group displayed better clinical effects from their treatment when compared with patients in the Gelfoam group.

CONCLUSION: *Bletilla striata* powders are superior to Gelfoam powders when used for angioembolus in patients with hepatic carcinoma.

Key words: Liver neoplasms/therapy; Embolization; Therapeutic *Bletilla striata*

Feng GS, Kramann B, Zheng CS, Zhou RM, Liang B, Zhang YF. Comparative study on the effects of hepatic arterial embolization with *Bletilla striata* or gelfoam in treatment of primary hepatic carcinoma. *World J Gastroenterol* 1996; 2(3): 158-160 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/158.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.158

INTRODUCTION

From May 1990 to September 1993, 106 patients with primary hepatic carcinoma (PHC) were treated by hepatic arterial embolization (HAE). We objectively compared the effects of permanent HAE produced with *Bletilla striata* with the effects of temporary HAE produced with Gelfoam.

MATERIALS AND METHODS

This study enrolled 166 patients (93 males and 13 females; mean age = 46 years) who were clinically diagnosed as PHC. The patients were randomly assigned to a *Bletill striata* group (BS group) and a Gelfoam group (GF group). The general medical conditions of the patients and their respective methods of treatment are shown in Table 1. Both groups of patients received hepatic arterial chemotherapy with Adriamycin (ADM; 50 mg) and carboplatin (CAP; 500 mg). A peripheral angioembolus was created by administering an emulsion consisting of 40% Lipiodol (LP) and 10 mg mitomycin C (MMC). The femoral artery was percutaneously punctured using Seldinger's method, and a catheter was inserted into the segmental or lobar artery of the hepatic tumor. Following transcatheter intra-arterial infusion of the chemotherapeutic agents and the LP-MMC emulsion, 2-3 mL of mixed *Bletilla striata* powders (300 mg) and 5 mL of 76% meglumini diatrizoatis composita were slowly infused to produce proximal HAE. If the normal hepatic segmental and lobar arteries were not available for catheterization, proximal HAE was performed using Gelfoam powder.

RESULTS

Vascular recanalization and revascularization of the tumor

At three mo after HAE, 92% of patients in the Gelfoam group showed recanalization of the embolized artery and formation of collateral circulation (Figures 1 and 2). In contrast, none of the patients in the *Bletilla striata* group showed recanalization, even at 3 yr after embolization. Collateral circulation was rarely formed, and when it did, it required > 6 mo to develop. Additionally, there was no collateral blood supply to the tumor in ~ 50% of the patients at one year after their embolization procedure (Figures 3 and 4).

Changes in tumor size and pathohistological characteristics

In the *Bletilla striata* group, 36.2%, 55.8%, and 71.4% of patients showed signs of tumor shrinkage at 3, 6, and 12 mo, respectively, after embolization. The corresponding rates in the Gelform group were 25.0%, 46.4%, and 64.3%, respectively. In the *Bletilla striata*

Table 1 General conditions and methods of treatment

Subjects	GF group	BS group
Number	50	56
Clinical stage; cases (%)		
I	3 (6.0)	4 (7.1)
II	42 (84.0)	46 (82.2)
III	5 (10.0)	6 (10.7)
Pathologic type; cases (%)		
Massive	38 (76.0)	43 (76.8)
Multinodular	12 (24.0)	13 (23.2)
Proximal angioembolus	GF powders	BS powders
Total number of treatments	203	151
Mean number of treatments	4.06	2.7
Mean intermission between treatments (mo)	3-6	6-12

Table 2 Survival rates and survival times for patients in the two groups (Life-table method: *U*-test

Group	*n*	Survival rates (%)				Mean survival time (mo)
		6 m	12 m	24 m	36 m	
BS	56	92.9	81.9	44.9	33.6	19.8
GF	50	80.0[a]	48.9[b]	31.1[c]	16.0[d]	15.7

[a]$P > 0.05$; [b,d]$P < 0.01$; [c]$P < 0.05$.

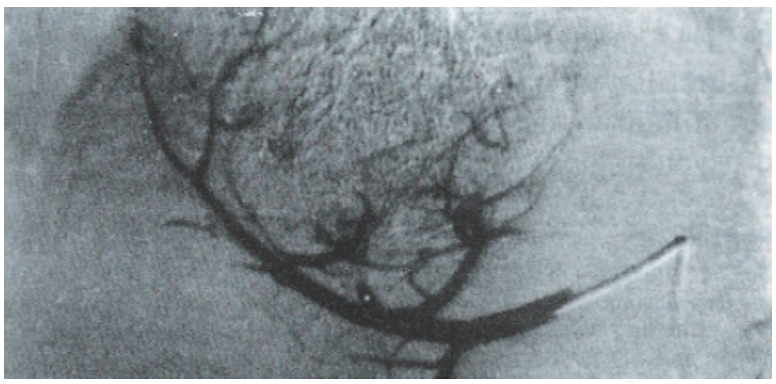

Figure 1 PHC treated by HAE with Lp MMC and GF powders.

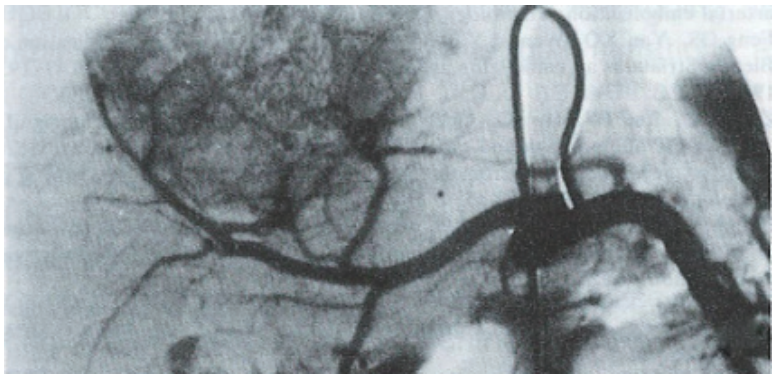

Figure 2 Three months after HAE, angiography of this patient showed tumor staining and slight shrinkage.

group, tumor necrosis was most obvious at 3 mo; at which time, the hepatic tumor tissue had sunken due to obvious fibrosis and lipiodol retention in the sites (Figures 5 and 6). In some patients, the tumor became calcified one year later.

Specimens obtained during second surgical resections showed no surviving tumor cells in 2 of 3 cases in the *Bletilla striata* group, while 2 cases in the Gelform group had surviving tumor cells.

Syndrome after HAE and changes in hepatic function

After HAE, hepatic pain, fever, and damage to hepatic function were all more severe in the *Blatilla striata* group when compared to the Gelfoam group. In both groups, pain lasted for 2-5 d and fever for 3-20 d, and the differences between groups were not statistically significant.

Survival rate and time

The survival rates and survival times in the two groups of patients

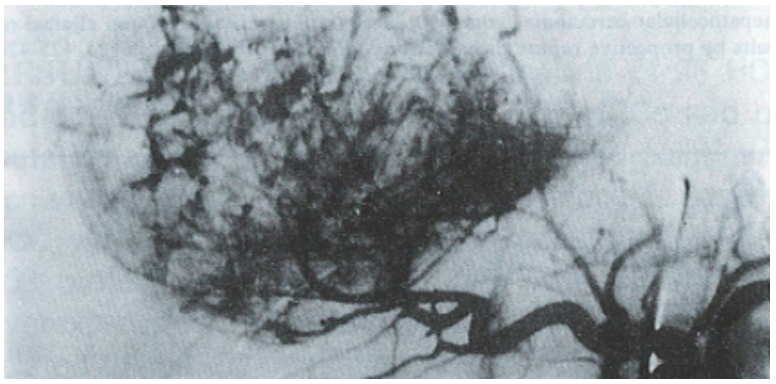

Figure 3 The right lobar massive PHC treated by HAE with Lp-MMC and BS powders.

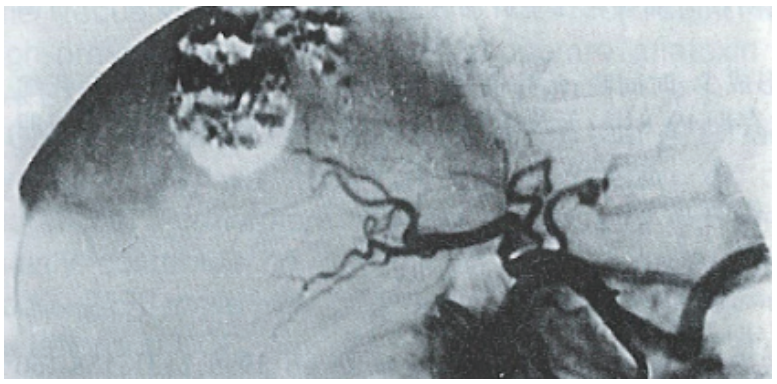

Figure 4 Ten months after HAE, angiography of this patient showed obvious tumor shrinkage, and no tumor vessels or collateral circulation.

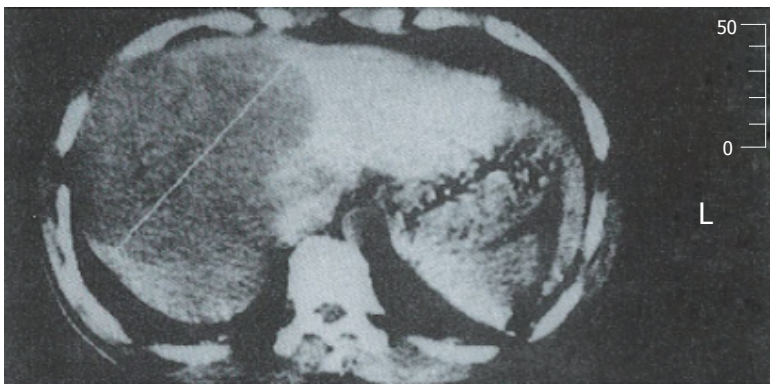

Figure 5 Prior to HAE with Lp MMC and BS powders, a CT scan of the massive PHC showed local low density in the right hepatic lobe.

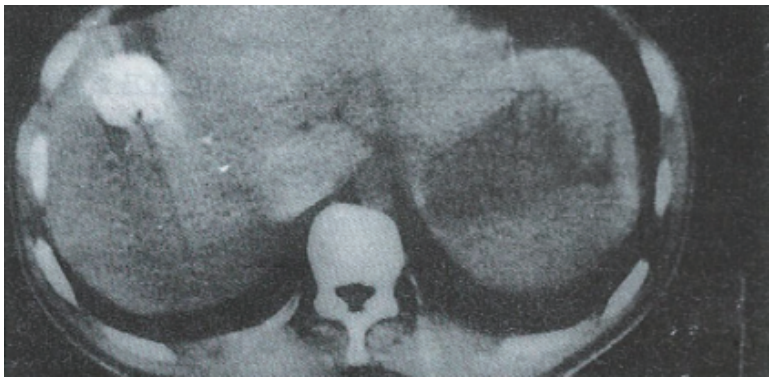

Figure 6 Nine months after HAE, a CT scan of this patient showed obvious tumor shrinkage and Lipiodol retention in the tumor area.

are shown in Table 2.

DISCUSSION

The use of HAE in treatment of PHC has attracted a great deal of attention as a novel methodology[1,2]. *Bletilla striata* powders display the following characteristics when used in treatment of PHC: (1) They produce permanent proximal embolization of the hepatic artery. Besides mechanically blocking blood vessels, they

also induce extensive coagulation[3]. Embolization occurs several seconds after the powder's application, and a vascular cast appears. (2) *Bletilla striata* powders can be custom produced at an appropriate concentration for use in combination with a contrast medium, and to meet the requirements of a particular embolization procedure. *Bletilla striata* powders not only embolize the main trunk of an artery, but also block tumor vessel beds that are not completely filled with Lipiodol. As a result, they effectively prevent the establishment of collateral circulation. However, this viewpoint is not consistent with some research conclusions which state that extensive embolization of a hepatic carcinoma artery may induce the formation of collateral circulation[4,5]. The reason that tumors become revascularized and develop collateral circulation after HAE with Gelfoam powders may be that Lipiodol and Gelfoam powders only block small tumor vessels and blood supply arteries respectively, while mid-sized vessels that are not embolized allow the tumor to establish collateral circulation with surrounding hepatic segmental or lobar arteries. As a result, HAE with Gelfoam does not produce complete necrosis of the tumor, and these patients need to be re-embolized after a short time period[6]. (3) Both the retention and elimination times of Lipiodol in the tumor area were better in the *Bletilla striata* group when compared with those parameters in the Gelfoam group. This may be because the arterial blood flow washes out the intratumor Lipiodol as intra-arterial Gelfoam powders are being absorbed[7]. And (4) When performing HAE with *Bletilla striata* powders, the catheter must be inserted into the peripheral branch of the hepatic artery to avoid penetrating the normal hepatic parenchyma. If this is not possible, Gelfoam powders must be used when creating a proximal angioembolus. Additionally, any backflow of the angioembolus must be avoided in order to prevent a misembolization.

In view of the characteristics of *Bletilla striata*, we believe that a permanent HAE produced with *Bletilla strata* powders in treatment of PHC not only produces a better clinical effect, but also reduces the economic burden and psychological pressure experienced by patients.

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Overexpression and mutations of tumor suppressor gene *p53* in hepatocellular carcinoma

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Abstract

AIM: To examine the prevalence of p53 mutations in cases of hepatocellular carcinoma (HCC) in the Chongqing area of China, and the relationship between *p53* mutations and the clinicopathological features of HCC, as well as its risk factors.

METHODS: The overexpression and point mutations of tumor suppressor gene *p53* in 38 cases of HCC were detected by a sensitive antigen retrieval fluid (ARF) immunohistochemical method, polymerase chain reaction (PCR) a2 restriction fragment length polymorphism (RFLP) analysis, and a single strand conformation polymorphism (SSCP) a2silver staining analysis.

RESULTS: The results showed that 16 of 38 HCCs were positive for P53 protein (42.1%). Seven HCCs (18.4%) had a *p53* mutation at codon 249, and 2 other HCCs had point a mutation within exon 7 but not at codon 249. Among 9 cases of HCC which showed genetic mutations, 8 cases (88.9%) were positive for p53 protein. Both *p53* overexpression and mutation were significantly related to the degree of HCC tissue differentiation and the presence or absence of metastases. The frequency of *p53* mutations was consistent with the high prevalence of HBV infection and moderate aflatoxin B1 (AFB1) exposure in the Chongqing area.

CONCLUSION: Our results suggest that AFB1 acts synergistically with HBV in the generation of *p53* mutations. Furthermore, dietary exposure to AFB1 may mainly contribute to the tumor specific mutation at codon 249, while HBV may account for other scattered mutations found in cases of HCC.

Key words: Liver neoplasms; Genes; Suppressor; Tumor protein *p53* point mutation

Wang D, Shi JQ. Overexpression and mutations of tumor suppressor gene *p53* in hepatocellular carcinoma. *World J Gastroenterol* 1996; 2(3): 161-164 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i3/161.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i3.161

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in China. A striking correlation exists between the development of HCC and infection with the hepatitis B virus (HBV), as well as exposure to aflatoxin B1 (AFB1). However, the molecular mechanisms underlying these correlations are largely unknown. Tumor suppressor gene *p53,* located on the short arm of human chromosome 17 (17 p 13.1), is one of the most important and broad spectrum anti-oncogenes, and researchers have documented > 51 types of human tumors that have *p53* mutations. Since Bressac[1] and Hsu *et al*[2] first reported in 1991 that ~ 50% of resected HCCs in China and South Africa had *p53* mutations, increasing numbers of similar reports have been published by researchers in various areas of the world. It is very interesting that the presence of p53 mutations in HCC appears to vary geographically[3]. The city of Chongqing is located in southwestern China, an area with a high prevalence of HBV infections, and a moderate exposure to dietary AFB1. However, to date, there has been no report concerning the HCC incidence in that geographic region.

In this study, we used antigen retrieval fluid (ARF) immunohistochemistry, PCR, and PCR-SSCP silver staining methods to detect the gene overexpressions and mutations that existed in 38 cases of HCC. Our goal was to demonstrate the prevalence of *p53* mutations in cases of HCC in the Chongqing region of China, and investigate the relationship between the *p53* alterations and the clinicopathological features of HCC, as well as HCC risk factors.

MATERIALS AND METHODS

Tissue samples

Between 1992 and 1993, 38 samples of resected HCC tissue (one sample per case) were obtained from the Southwest Hospital affiliated with our college. Thirty-six of the samples were fresh tissue. The tissue specimens were fixed in 10% formalin and embedded in paraffin; after which, 5 μm thick sections were cut for use in our study. When based on their WHO classification, the tissue specimens represented the following types of HCC: trabecular (n = 15), pseudoglandular (n = 16), and compact (n = 7). Histological grading of the specimens based on Edmondson and Steiner's standard showed: grade Ⅰ, 3 cases; grade, 7 cases; grade Ⅲ, 15 cases; grade Ⅳ, 13 cases. The tumors could be divided into the following three groups depending on their diameter: 3 cases, ⩽ 3 cm; 5 cases, 3 cm-5 cm; 30 cases, ⩾ 5 cm. Fourteen cases had

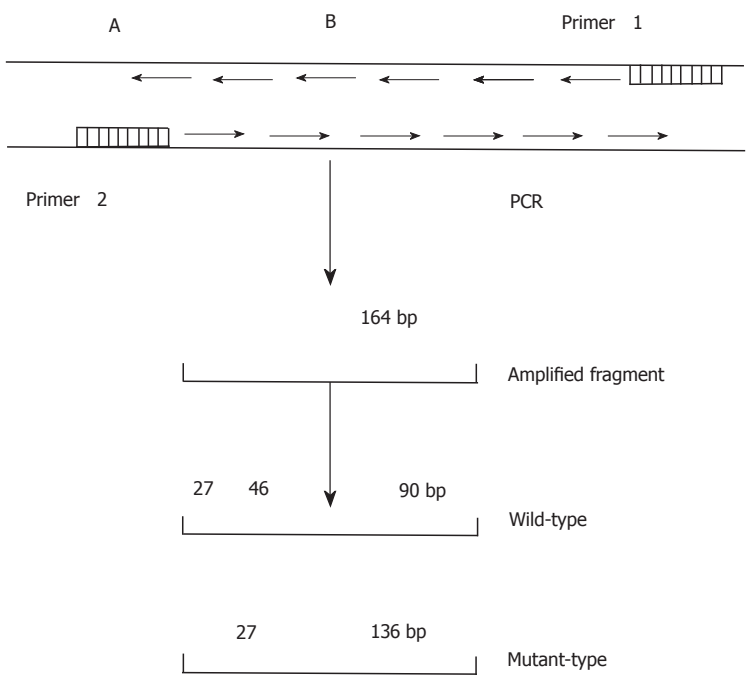

Figure 1 Schematic diagram of the p53 codon 249. A: control cleavage site of the restriction endonuclease, Hae Ⅲ. B: Hae Ⅲ cleavage site in codon 249.

intrahepatic metastases at the time of operation.

DNA extraction

High-molecular weight DNA was prepared from 36 fresh tissue samples, 2 paraffin-embedded blocks, and 2 samples of normal lymphocytes using promase K and phenol-chloroform methods as described in "Molecular Cloning: A laboratory Manual."

ARF-immunohistochemical staining

Monoclonal antibody Pab1801 donated by Dr. L. Crawford of ICRF, United Kingdom, and polyclonal antiserum CM-1, donated by Dr. D.P. Lane, Dundee, United Kingdom, were used to detect p53 protein expression. An ABC kit was purchased from the Dako Company (Demark). Immunohistochemical staining with enhancement was performed using the method described by Vendenberg *et al*[4,5].

Detection of mutated codon 249

The DNA sequence of *p53* exon 7 was amplified by PCR (Figure 1) using primers with the following nucleotide sequences:

Primer 1: 5′CCCAAGGCGCACTGACCTCA3′;

Primer 2: 5′GCTCCTGACCTGGAGTCTTC3′.

PCR was performed according to a standard protocol. Briefly, each PCR reaction was performed in a final volume of 40 μL, which contained 1.8 μL of target DNA, 28 μL ddH_2O, 3.2 μL of 2.5 mmol/L dNTP, 2 μL of 20 pmol/L primers, and 4 μL of 10 × Taq DNA polymerase buffer. The samples and reagents were heated for 8 min at 93 ℃ to denature the DNA. Next, 1 μL (2.2 U) of Taq DNA polymerase was added to each sample, which was then heated for 35 s at 94 ℃ for denaturation, followed by 40 s at 54 ℃ for annealing, and 60 s at 72 ℃ for extension. After a total of 35 rounds, each sample was maintained at 72 ℃ for 10 min. The final DNA product was purified with phenol/chloroform, precipitated with ethanol, dissolved with TE buffer, and then digested with Hae Ⅲ for 12-24 h. The digests were separated by electrophoresis on an 8% polyacrylamide gel.

Single-strand conformation polymorphism-silver staining

A 5 μL sample DNA was mixed with a loading solution containing 950 mM deionized formamide, 10 mmol/L EDTA, 0.05% bromophenol blue, and xylene cyanol. After denaturation at 85 ℃ for 5 min, 20 μL of the mixture was applied to a 12% polyacrylamide gel (39:1 acrylamide:bisacrylamide ratio) containing 1 × TBE buffer and 50 mL/L glycerol. The size of the gel was 10 cm × 9 cm × 0.1 cm (thickness). Electrophoresis was then performed for 4.5 h at 200 V using a buffer system containing 25 mM Tris and 192 mM glycine. The buffer temperature was maintained in a range of 16 ℃-23 ℃.

After electrophoresis, the gel was fixed for 30 min in 100 mL of a mixture consisting of 100 mL/L ethanol and 50 mL/L acetic acid, and then washed twice in distilled water. The gel was stained in 1 mL/L $AgNO_3$ for 30 min, and then washed three times in distilled water. After washing, the gel was submerged in a mixture of 25 g/L Na_2CO_3 and 0.4 mL/L formalin, and then allowed to develop for 20 min-30 min until bands became visible. The reaction was stopped by transferring the stained gel into 100 mL/L acetic acid solution.

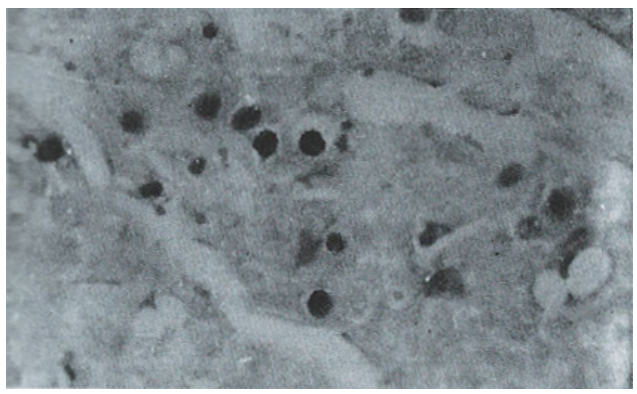

Figure 2 A well differentiated HCC with nuclear staining in carcinomatous cells in a single pattern. Some cells also show cytoplasmic staining. Immunohistochemistry with Pab1801. ABC, × 400

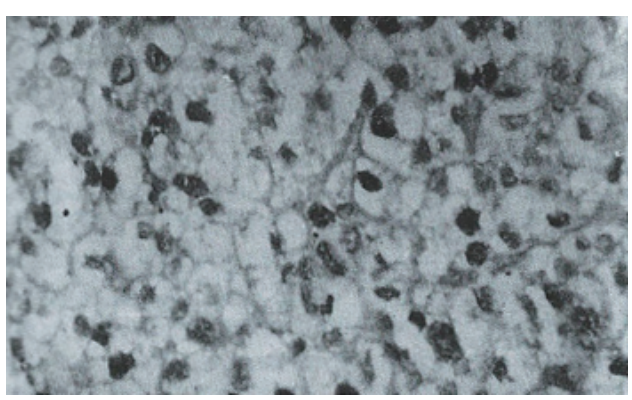

Figure 3 Moderately differentiated HCC with nuclear staining in carcinomatous cells in a diffuse pattern. Immunohistochemistry with CM-1; ABC, × 400.

RESULTS

P53 Protein expression in HCC specimens

Sixteen of 38 HCC specimens were positive for P53 protein (42.1%), while the corresponding noncancerous tissues were all negative. The majority of malignant cells in all of the P53-positive specimens displayed a stained nucleus, and some also showed cytoplasmic staining. The distribution of p53-positive cells showed a single, focal or diffuse pattern (Figures 2 and 3).

Mutations of p53 exon 7

After digesting the PCR product with Hae Ⅲ, seven (18.4%) of the 38 tissue samples were identified as mutant-types (136 bp and 27 bp fragments) (Figure 4). No mutations were found in 38 corresponding noncancerous tissue samples and 22 samples of normal lymphocytes. When the remaining 31 pairs of wild type HCC specimens were analyzed by PCR-SSCP-silver staining, two showed a mobility shift (Figure 5), indicating the presence of mutations in exon 7, rather than codon 249. Thus the mutation incidence in *p53* exon 7 was 23.7% (9/38 samples). Among 9 mutant-type HCC specimens 8 specimens (88.9%) tested positive for P53 protein.

Correlation of p53 overexpression with the clinicopathological features of HCCs (Table 1).

Our results showed that overexpression of p53 was more frequent in cases of metastasized grade Ⅳ HCC. No significant relationship was found between p53 overexpression and either tumor size or histological classification.

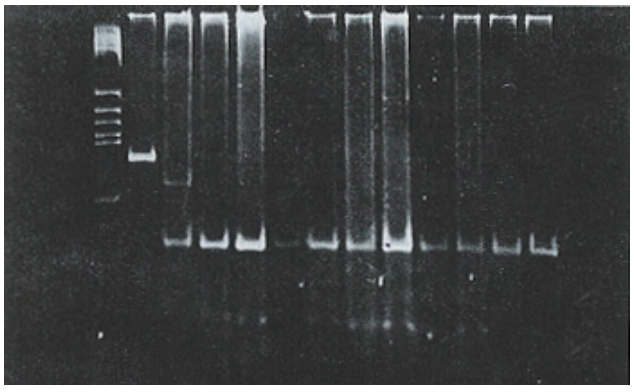

Figure 4 PCR analysis of p53 codon 249 in hepatocellular carcinoma. M: DNA size marker (PBR322 digest of Hae Ⅲ); P: Product of PCR (164 bp); N: Surrounding noncancerous tissue; T: HCC tumor tissue; T1: Mutation of codon 249; L: Normal lymphocyte.

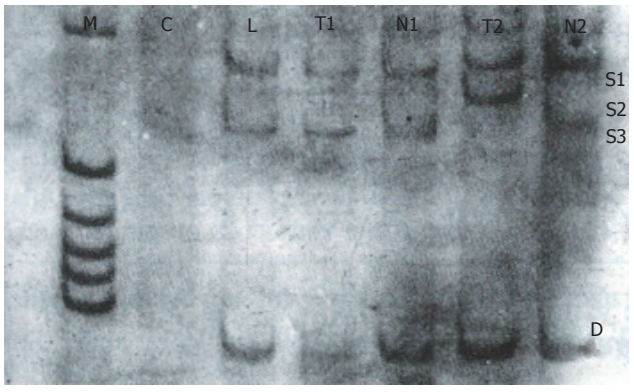

Figure 5 Analysis of p53 exon 7 by PCR-SSCP-silver staining in hepatocellular carcinoma. C: Negative control; L: Normal lymphocyte; N: Surrounding noncancerous tissue; T: HCC tumor tissue; T2: Mobility shift; S1 S3: Single strand DNA; D: Double strand DNA

Table 1 Correlations between p53 overexpression and clinicopathological features of Hepatocellular carcinoma

Clinicopathological features	Number of cases	p53 expression (+)	p53 expression (-)	Significance
Tumor size (cm)				
< 3	3	1	2	
3-5	5	2	3	a
> 5	30	13	17	
Classification				
Trabecular	15	5	10	
Pseudoglandular	16	8	8	b
Compact	7	3	4	
Grade				
Ⅰ	3	1	2	
Ⅱ	7	2	5	c
Ⅲ	15	5	10	
Ⅳ	13	8	5	
Metastasis				
Positive	14	10	4	
Negative	24	6	8	d

a and b: Significant; c and d: $P < 0.05$.

Table 2 Correlations between p53 mutations and the clinicopathological features of Hepatocellular carcinoma

Clinicopathological features	Number of cases	p53 expression (+)	p53 expression (-)	Significance
Tumor size (cm)				
< 5	8	0	8	
> 5	30	9	21	
Classification				
Trabecular	15	4	11	
Pseudoglandular	16	3	13	b
Compact	7	2	7	
Grade				
Ⅰ	3	0	3	
Ⅱ	7	1	6	c
Ⅲ	15	2	13	
Ⅳ	13	6	7	
Metastasis				
Positive	14	6	8	
Negative	24	6	21	d

b: Not significant; a, c and d: $P < 0.05$.

Correlation of p53 mutations with the clinicopathological features of HCCs (Table 2).

In this study, *p53* mutations were found more frequently in HCCs having a large size, a grade Ⅳ classification, and which had metastasized.

DISCUSSION

P53 protein has a very short half life in normal cells, and is thus undetectable by standard immunocytochemical methods. In contrast, *p53* gene mutations in tumor cells usually result in the stabilization and accumulation of p53 protein in the nuclei, at levels which can be detected by immunohistochemistry techniques. Therefore, p53 overexpression detected by immunohistochemistry strongly suggests the presence of a *p53* gene mutation[6]. Among the 9 mutant-type HCC specimens in this study, 8 specimens (88.9%) were positive for P53 protein, which confirms the above hypothesis. Because the location of a *p53* point mutation can vary, DNA sequencing is essential to reveal this alteration of the gene. However, immunohistochemical techniques are more simple to perform, and especially useful when screening large numbers of specimens for the presence of mutations.

The presence of *p53* mutations in HCC appears to vary with geographic location. In regions with a high risk for exposure to HBV and aflatoxins, (*e.g.*, China[2] and South Africa[1]), *p53* mutations have been reported in ~ 50% of HCC cases, and the majority of those mutations were found at codon 249. In Japan[7], where HBV infections are relatively common, but dietary aflatoxin intake is low, *p53* mutations have been reported in 29% of HCC cases. In countries where neither HBV nor dietary aflatoxin are prevalent (*e.g.*, France[8], Germany[9], and Great Britain[10]), mutated *p53* is rarely found in HCCs, and there have been few or no mutations reported at codon 249 in the latter two countries[3,11]. While the results of molecular epidemiological studies have suggested that HBV may act synergistically with aflatoxin in the generation of *p53* mutations in HCC, its mechanism of action remains obscure.

In this study, we divided *p53* mutations into two groups: (1) a tumor-specific mutation at codon 249, and (2) various scattered mutations at codons other than codon 249. Among 38 cases of HCC, 7 cases (18.4%) were found to have specific mutations by PCR-RFLP analysis. This 18.4% mutation rate was much lower than the 50% rate reported in Qidong[12], an area with a high exposure to aflatoxin, and much higher than the 2.2%rate reported in northern China[13], an area with low exposure to aflatoxin. Meanwhile, immunohistochemistry and PCR-SSCP analyses indentified 10 cases of HCC (26.3%) which showed scattered mutations. The overall frequency of *p53* mutations in our geographic area was 44.7% (17/38 HCC cases), and these results are in agreement with the high prevalence of HBV and moderate exposure to aflatoxin in our area. We therefore conclude that HBV acts in conjunction with aflatoxin to generate *p53* gene mutations in HCCs. Furthermore, dietary exposure to AFB1 may mainly contribute to the tumor-specific mutation, while HBV infection may account for other *p53* gene mutations. As the most common tumor suppressor gene, *p53* contributes to malignant transformation and the aggressiveness shown by several types of cancers. It was previously reported that *p53* overexpression is correlated with the aggressiveness and metastasis of HCC[14]. Because P53 protein is not abnormally expressed in peri-cancerous liver tissue, the *p53* gene probably becomes mutated after the hepatocellular carcinogenesis process has already been initiated. Both *p53* overexpression and *p53* mutations were more frequent in poorly-differentiated HCCs and cases of metastasized HCC, suggesting that the number of *p53* alterations had increased as the HCC progressed. These findings indicate that *p53* mutations represent one of the most important genetic changes in the development of HCC, and can serve as a molecular marker for high grade and metastatic HCC.

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Clinical use of hepatic carcinoma associated membrane protein antigen (HAg18-1) for detection of primary hepatocellular carcinoma

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Abstract

AIM: To evaluate the sensitivity, specificity, and clinical value of hepatic carcinoma associated membrane protein antigen (HAg18a21) as a reagent in a serum quick enzyme linked immunosorbent assay (ELISA) for detection of primary hepatocellular carcinoma (PHCC).

METHODS: A serum quick enzyme linked immunosorbent assay (ELISA) which uses HAg18a21 as a reagent, was prepared from monoclonal antibodies against human hepatic carcinoma. We assayed blood serum obtained from 100 cases of primary hepatocellular carcinoma (PHCC), 5 cases of hepatic biliary carcinoma (HBC), 10 cases of metastatic hepatic carcinoma (MHC), 20 cases of hepatitis B (HB), 20 cases of liver cirrhosis (LC), 20 cases of malignant gastrointestinal tumors, and 20 cases of inflammatory gastrointestinal diseases (including ulcers). Alpha 2 fetoprotein (AFP) was concurrently detected for each case. Twenty samples of blood bank serum were tested as controls.

RESULTS: The respective positive rates for the HAg18a21 ELISA assay and AFP detection assay were 81% and 68% in PHCC, 20% and 40% in HBC, 19% and 20% in MHC, 10% and 20% in BH, 10% and 20% in LC, 10% and 15% in malignant gastrointestinal tumors, and 5% and 10% in inflammatory gastrointestinal diseases. Neither assay showed a positive result for any tested blood bank sample.

CONCLUSION: The HAg18a21 ELISA demonstrated high sensitivity and specificity in the detection of PHCC. HAg18a21 ELISA and AFP detection, if used together, may compliment each other in the diagnosis of PHCC.

Key words: Liver neoplasms/diagnosis; Carcinoma; Hepatocellular diagnosis; Antigens; Neoplasm

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INTRODUCTION

Hepatic carcinoma is the third leading cause of death in China, and accounts for 15.08% of all cancer-related deaths[1]. Currently, hepatocellular carcinoma (PHCC) is primarily diagnosed by imaging studies and serological tests. While alpha-fetoprotein (AFP) is an internationally accepted specific biological marker for PHCC, and can be detected in 68.5%-76.5% of all cases[2], serum AFP levels can vary with the degree of tumor differentiation and the size of the tumor mass[3]. Also, AFP may be absent or present at only low concentrations in a certain proportion of PHCC cases, whereas cases of metastatic hepatic carcinoma and benign hepatic diseases may have elevated AFP levels[4]. Therefore, there is an urgent need to identify more sensitive and specific markers of hepatic carcinoma.

MATERIALS AND METHODS

Source of materials

All 195 serum samples used in this study were collected from January 1990 to December 1993 from patients at the Chinese PLA 165th Hospital, The First Affiliated Hospital of Hengyang Medical University, Hengyang Municipal Central Hospital, and The 415th Hospital of the Ministry of Nuclear Industry. The samples were obtained from 100 patients with PHCC, 5 patients with HBC, 10 patients with MHC, 20 patients with HB, 20 patients with LC, 20 patients with gastrointestinal malignant tumors, and 20 patients with various inflammatory gastrointestinal diseases (including ulcers). Twenty samples of blood bank serum served as control samples.

Methods

HAg18-1 qualitative assays were performed using a HAg18-1 serum quick ELISA Kit (Chinese PLA Fourth Medical University and Zhengzhou Bo-sai Biological Reagent Laboratory, China), and methods described in the kit's instructions. Quantitative HAg18-1 assays were performed as follows: a known Hag18-1-positive sample (blue in color) was diluted with 1 mL of 0.5 N H_2SO_4 to terminate the reaction; after which, the sample's OD at 450 nm was measured using a STAT FAX 303 plus photometer system (Awareness Technology; Technology, Palm City, FL, United States). A positive assay sample was defined as one having an OD > 0.15.

Table 1 The results of HAg18-1 Eenzyme linked immunosorbent assays and alpha-fetoprotein radioimmunoassays

Disease	No. of cases	No. of positive cases		Positive rate (%)	
		HAg18-1	AFP	HAg18-1	AFP
PHCC	100	81	68	81	68
HBC	5	1	2	20	40
MHC	10	1	2	10	20
HB	20	2	4	10	20
LC	20	2	4	10	20
GI malignant tumor	20	2	3	10	15
GI inflammatory diseases	20	1	2	5	10
Bank blood[1]	20	0	0	0	0

[1]Controls

AFP was detected with an AFP radioimmunoassay (RIA) Kit (Shanghai Biological Product Institute; Shanghai, China), using the procedure described in the instruction manual. AFP antibody was supplied by Fuzhou MaiXin Biological Technique Development Co. (China), and the "SP" method was adopted.

RESULTS

Relationship between HAg18- 1 serum assay results and AFP detection in 100 cases of PHCC (Table 1)

HAg18-1 serum assays and AFP detection assays both showed positive results for 61% (61/100) of all the cases. AFP detection assays showed positive results for 86.4% (70/81) of the HAg18-1 positive cases, and among the 19 HAg18-1 negative cases, 7 cases (36.8%) were AFP positive. The HAg18-1 serum assay showed positive results for 88.23% (62/68) of the AFP positive (> 400 ng/mL) cases, and among the 32 AFP negative cases (< 400 ng/mL), 24 cases (75%) were positive with the HAg18-1 serum assay.

Four HAg18-1 serum assay positive cases (2 clinically diagnosed cases of HB and 2 cases of LC) had been followed up for 3 years. One of the LC cases was later proven to be PHCC by autopsy after death.

The AFP test showed 100% positive results when the "SP" method was used to test sections of paraffin-embedded liver tissue from all 100 patients with PHCC.

DISCUSSION

There are numerous biological markers for human hepatic carcinoma, and substantial advances have been made in both the theories and technology involved in this area of research. Several new techniques that were previously used only in experimental studies are now used in the clinic. In summary, four types of assays are now used to detect PHCC, and these involve detecting proteins, enzymes, hormones, and metabolites, respectively[5]. Because these assays vary greatly in their sensitivity and specificity, there remains an urgent need to identify new a biological marker for hepatic carcinoma.

Practical value of the HAg18-1 assay

In this study, the HAg18-1 assay was able to detect 81% of the PHCC cases, which was markedly or slightly higher than the detection rates reported from studies conducted in Nanjing (65.9%)[6], Xi'an (74%)[4], Chengdu (79.63%)[7], and Lanzhou (80%)[8]. The assay showed a < 10% positive rate when used to detect other types of malignant tumors or inflammatory diseases. The reasons that HAg18-1 is present at certain concentrations in different diseases remain unclear. In our study, the HAg18-1 assay was 90% specific when used to detect PHCC. This result was comparable to the 89.52% specificity the same assay showed in a study conducted in Chengdu. In that study, HAg18-1 was shown to be a tumor-associated antigen (TAA) closely linked with hepatic carcinoma. In our study, the HAg18-1 assay displayed higher sensitivity for detecting PHCC, when compared with the AFP assay (68% detection rate). The HAg18-1 assay showed positive results in 75% (24/32) of the 32 PHCC cases with a serum AFP level < 400 ng/mL, suggesting that it may be especially useful for detecting PHCC cases which are AFP negative or in which the patient has a low serum AFP concentration. On the other hand, among the 19 PHCC cases that tested negative with the HAg18-1 assay, 36.8% (7/19) were detected by the AFP assay. These results indicate that the two assays are complementary to each other in their clinical use. Four HAg18-1 assay positive cases (2 clinically diagnosed HB cases and 2 LC cases) had been followed up for 3 years. Among them, one clinically diagnosed LC case was proven to be PHCC by autopsy after death. Therefore, it is reasonable to believe that the HAg18-1 assay might be helpful in the early diagnosis of PHCC. While the HAg18-1 assay showed positive results in a certain proportion of non-PHCC tumors and inflammatory diseases, these types of results occurred far less often than when using the AFP assay, and had little influence on the final diagnosis of PHCC.

In recent years, researchers have suggested that a combined detection protocol with multiple markers would be superior to detecting only a single marker in the diagnosis of PHCC, and especially when attempting to diagnose patients who are AFP negative or have a low serum AFP concentration. The serum quick enzyme-linked immunosorbent assay with HAg18-1 is easy to perform, provides consistent results, and requires no special instruments. It is a simple technique that is especially suitable for surveying high-risk populations, and can be used in general practice. If combined with AFP detection, it should greatly increase the PHCC detection rate, and thus has great potential for widespread clinical use.

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Magnetic resonance imaging of portal vein invasion in hepatocellular carcinoma: Results from 25 cases

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Abstract

AIM: To pre-operatively assess tumor thromboses associated with hepatocellular carcinoma in the portal vein.

METHODS: Twenty-five patients diagnosed with a thrombus due to hepatocellular carcinoma were included in the study. MR imaging was performed with a 1.0T superconducting magnetic imaging system. Both T1 and T2 weighed images as well as FLASH sequences were obtained in the transverse plane, and additional FLASH images were obtained in the coronal plane.

RESULTS: Thromboses located in the portal vein had signal intensities similar to those of the main tumor. An intrinsic portal vein thrombus was found in 16 patients, and six thromboses were occlusive. Thromboses were found in the diffuse narrow portal branches of 3 patients. The portal venous thromboses displayed an area of signal intensity that replaced the normal flow void of the portal vein. The affected portal veins displayed a stumpy appearance, had irregular areas of stenosis, and showed formation of a vascular net.

CONCLUSION: MRI was more sensitive, specific, and noninvasive for detecting a portal tumor thrombus, and can be used jointly with spin echo (SE) and gradient echo (GRE) imaging techniques.

Key words: Magnetic resonance imaging; Liver neoplasms/diagnosis

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INTRODUCTION

Primary hepatocellular carcinoma (HCC) is pathologically characterized by its propensity to invade the portal venous system. A pre-operative assessment should be preformed when a tumor thrombus is present in the main portal vein. If the results confirm that the tumor has invaded the portal branches of one lobe, resection may be indicated. MRI is the least invasive method by which the scanning plane can be easily changed to allow the use of angiography for examining the vascular structure. Here, we summarized the results of pre-operative MR assessments of portal venous thromboses that had been confirmed by sonography, enhanced CT or arterial portography in 25 patients with HCC.

MATERIALS AND METHODS

Materials

Twenty-five male patients (age range 33-64 years, mean age = 54 years) with HCC were enrolled in this study. The patients had tumors that ranged from 3 cm to 10 cm in diameter. Fifteen of the patients had been diagnosed as HCC by a histologic examination of tissue biopsy, and ten were diagnosed based on having a serum alpha fetoprotein (AFP) level that was persistently > 500 μg/L.

Methods

A Magneton Impact 1.0T superconducting magnetic resonance system (Siemens; Munich, Germany) was used to obtain T1 and T2 weighed images with a TR of 500 ms and an echo time (TE) of 15 ms (TR/TE = 500.660/15). Additionally, long TR/short TE and long TR/TE (1800-2150/22-120) images, as well as spin echo (SE) images in the transverse plane were obtained by using a multisection imaging technique [10 mm section thickness; 200 × 256 matrix; 380 mm × 400 mm field of view (FOV)]. Gradient echo (GRE) sequences were obtained for all patients using the sequential section technique with the following parameters: TR of 100 ms, TE of 6 ms, flip angle of 30 degrees, an 8 mm section thickness, a 128 × 256 matrix, and a 400 mm × 400 mm field of view. Additional FLASH images were obtained in the coronal plane so as to observe possible tumor thromboses located in the main portal vein or its branches, as well as the inferior vena cava (IVC). All patients were asked to momentarily suspend their respiration during GRE measurements.

RESULTS

A thrombosis was diagnosed in 25 patients enrolled in one or more studies which used contrast material enhanced CT alone, *n* = 5; color sonography alone, *n* = 9; contrast material enhanced CT plus color sonography, *n* = 6; angiography alone, *n* = 5, respectively. Tumor thromboses displayed signal intensities similar to those of the

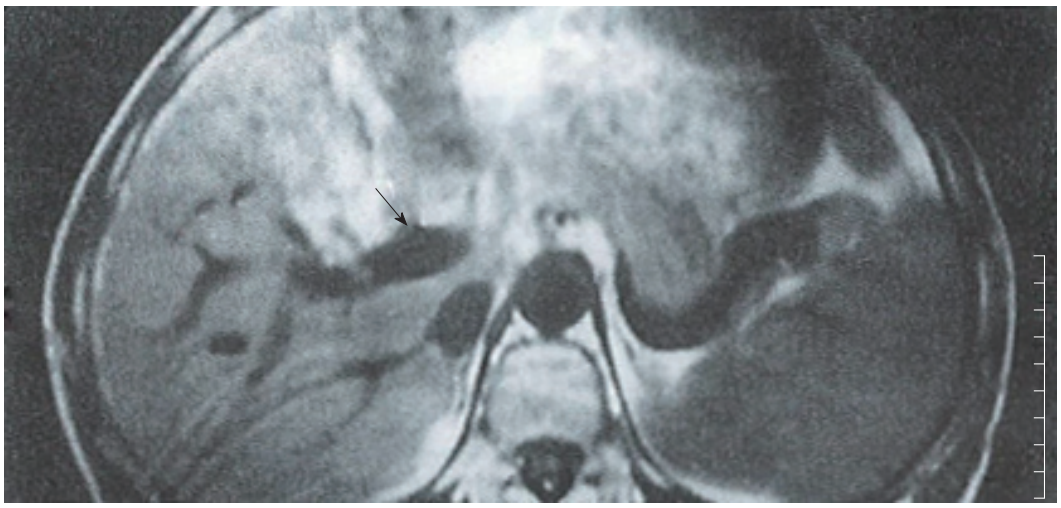

Figure 1 Patient with HCC. T1 weighed (500/15) MR images of the upper abdomen showed the portal tumor thrombus as a clot of iso-signal intensity (arrow).

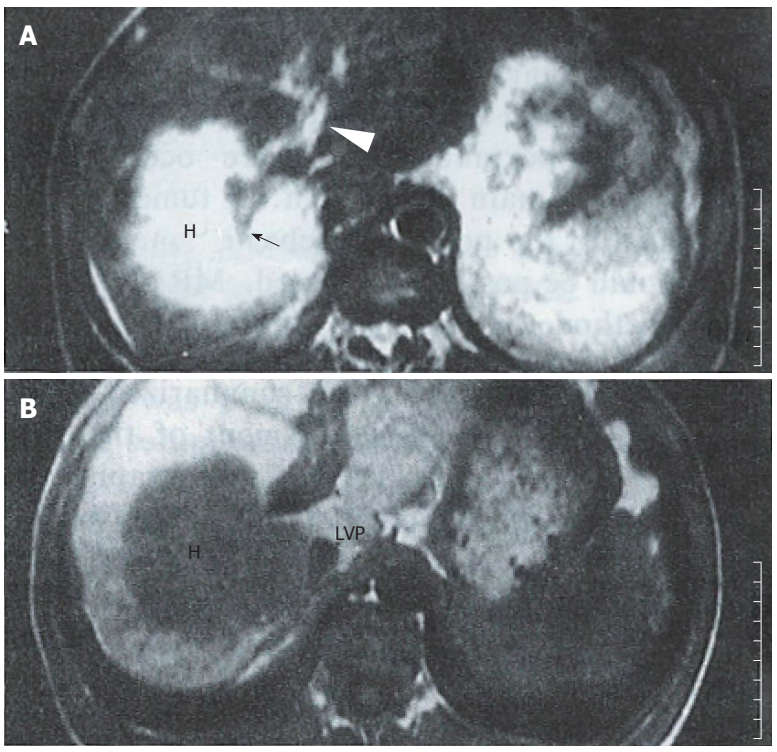

Figure 2 Patient with HCC enclosing the right branch. A: T2 weighed (2000/80) images showed the tumor (H) having a higher signal intensity than the parenchyma. The right branch of the portal vein in the tumor ended abruptly, and appeared as a stump (arrow). Some irregular hyperintensity was seen in the dilated main and left portal vein (arrowhead). B: FLASH (100/6) imaging showed main and left portal vein dilation with heterogeneous intensity more clearly than did SE imaging. The IVP was compressed by the tumor.

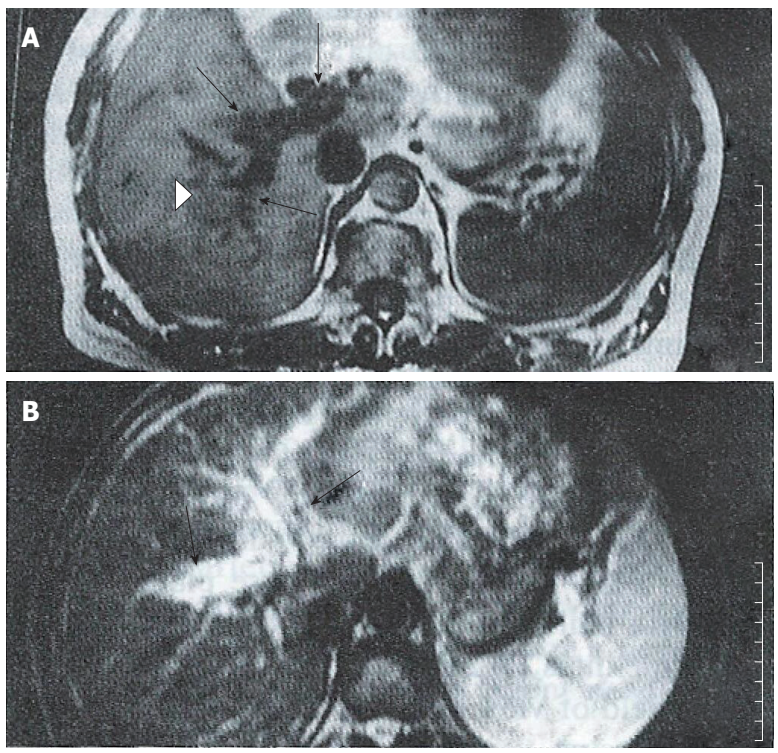

Figure 3 Patient with HCC. A: T1 weighed (500/15) images showed diffuse, irregular narrow right branches with numerous nodules (arrow); the distal portal vein was poorly delineated (arrowhead). Several small flowing voids representing collateral vessels were seen. B: T2 weighed (2000/80) MR images showed the left portal venous wall as slightly hyperintense, indicating invasion by the tumor (arrow).

main tumor masses. An intrinsic portal venous thrombus (PVT) was found in 16 patients, and appeared as an area of iso-signal intensity that replaced the normal flow void in the portal vein on SE images (Figure 1). On GRE images, the intrinsic thromboses appeared as areas of decreased signal intensity in bright blood vessels. In six cases with complete postal vein occlusion, the thrombus was located either laterally or in the surrounding portal branches. SE images showed a stumpy portal vein with dilated proximal branches; the main section of the portal vein and its peripheral branches could not be identified. On long TR images, the signal intensity was slightly higher than that of the hepatic parenchyma, and similar to that of the main tumor; additionally, occluded portal branches could be seen in the tumor (Figure 2). In the GRE images, the bright portal vein appeared to end abruptly due to poor contrast between the thrombus and adjacent stationary tissue. In 3 cases with diffuse narrow portal branches, T1 weighted images showed the portal branches had irregular stenoses with a flowing void area. T2 images showed some slight hyperintensity along the vascular wall caused by invasion of the tumor (Figure 3). Collateral vessels had developed in 3 cases. Numerous periportal collateral voids were seen on T1 weighed images. GRE images displayed features similar to those shown on SE images, but with stronger signal intensities. GRE images conclusively demonstrated the presence of collateral vessels and showed more extensive collateralization than the standard SE images. Ten patients showed evidence of extrinsic compression of the main or proximal right or left portal vein or IVC by the tumor. The SE and GRE images showed similar findings; however, when compared with the SE images, the GRE images less clearly showed that the tumor was responsible for venous compression. This was due to poor contrast between the tumor and surrounding normal tissue on the GRE images.

DISCUSSION

Direct portography is the most accurate pre-operative method for assessing portal venous invasion by HCC[1]. While arterial portography is the standard pre-operative method used in many hospitals, these examinations increase the risk of bleeding in patients with impaired clotting function. Noninvasive imaging of the portal venous system has been successfully accomplished with a variety of imaging techniques, including US, CT, and MRI. Color sonography has proved to be highly accurate in determining the presence, direction, and velocity of portal blood flow, but it

is operatively dependent[2]. Although contrast-enhanced CT with dynamic scanning is highly sensitive for identifying a thrombus in the main portal vein or its large branches, the suboptimal opacity of portal vessels and allergic reactions limit the use of this technique.

Identification of a normal portal vein

The spin-echo (SE) technique provided anatomic information similar to that provided by CT, but did not require the IV injection of a liver contrast agent. Our standard assessment of the portal venous system (PVS) by use of SE imaging produced a typical signal void, "black blood" phenomenon, and the hepatic venous signal intensity was similar to that of the portal vein. While the portal and hepatic veins could be distinguished based on their direction, they were difficult to distinguish on the peripheral parenchyma without adding an additional plane. FLASH techniques[3] generally offer two advantages that improve depiction of the hepatic vasculature: (1) high signal intensity within blood vessels due to flow enhancement; and (2) a reduction in motion artifacts, because the patients must hold their breath. The portal vein and its branches appeared to be clear when examined by FLASH with presaturation in additional coronal or sagittal planes. The high intensity signal produced by flowing blood clearly distinguished the intra-abdominal vasculature from the surrounding tissue. Additionally, the image acquisition time was significantly reduced when using GRE imaging rather than conventional SE imaging.

Appearance of PVT on MR images

Thrombus of the portal vein is usually clinically associated with hepatocellular carcinoma, and pathologically associated with its intra-hepatic metastasis. A tumor thrombus may extend into the portal trunk, even when a hepatocellular carcinoma is still small[4]. There is a poor correlation between tumor size and the presence of a tumor thrombus in the portal vein. The MR findings of PVT were classified based on their location in the portal vein.

Intrinsic clot

An intra-luminal thrombus could be present at any location in the portal vein. On spin-echo MR images, a PVT usually appeared as an area with an abnormal signal within the lumen of the portal vein. Intrinsic PVTs appeared as an isointense signal that replaced the normal portal flow void on T1 weighed images (Figure 1). Additionally, intrinsic PVTs typically showed high signal intensities on T2 weighed images, and which were similar to that of the main tumor. Some spurious signals were difficult to distinguish from a thrombus on SE images; while, GRE images showed the portal vein as an area with a high signal and a reduced number of respiratory artifacts. A PVT usually appeared as an area having a diminished intravascular signal with an intensity similar to that shown by adjacent stationary soft tissue on GRE imaging. While GRE more clearly showed the PVT, it provided less anatomic resolution and live-lesion contrast.

Portal stump

Occlusion usually occurred at the base of the narrow portal vein. The stenosed portal vein was occluded by compression caused by the extrinsic tumor. This was also true for the tumor enclosed portal vein. In SE images, the portal vein or its branches were completely replaced by tumor tissue. T1 weighed images showed the branches as stumps, the flow void of the distal segment of the portal vein as being obliterated, and the proximal branches being dilated. A stumpy appearing portal vein could be seen in the tumor, which grew around the invaded branches (Figure 2). Secondary ischemic changes caused by the PVT in the HCC could be readily distinguished from the tumor. The use of MRI was advantageous in that it showed the patchy perfusion of the occluded vein. Dynamic MR imaging showed a fan-shaped area, and also high signal intensity areas with early and prolonged enhancement in segments that were affected by the portal vein thrombus[5].GRE images only showed a high signal portal stump, because the intensity of the thrombus was similar to that of the adjacent stationary tissues. Collateral veins and varices were also visible.

Diffusely narrow portal vein

The portal vein was diffusely stenosed in one or more of its branches. Extensive stenosis is usually associated with cirrhosis. Sections of venous wall that had been penetrated by a growing tumor had an irregular appearance and displayed several small nodules. While the signal void of the portal vein was present, the small vessels were poorly delineated on the T1 weighed images. T2 weighted images showed hyperintense regions along portal branches which had been invaded by the tumor (Figure 3). The bright portal vein was poorly delineated on GRE images.

Cavernous changes

Collateral vessels developed and effectively replaced the obliterated portal vein. These vessels formed a major hepatoportal venous conduit when the portal vein was severely stenosed or occluded. The collateral vessels usually appeared in the same area as the affected vessels, and a severely stenosed or occluded portal vein showed evidence of collateralization. Small dilated and expanded collateral veins near the edge of the portal vein showed numerous areas of periportal hypointensity on SE images. GRE images showed findings similar to those on SE images, but with hyperintensity. Collateral vessels and varices were seen clearly, and appeared to be more extensive on MR images.

Comparison of the different imaging sequences

SE images were slightly more specific. Motion or flow-related artifacts can sometimes produce a spurious signal in the portal vein that mimics the signal produced by a thrombus. This is due to the markedly variable signal intensity of flowing blood. Therefore, SE and GRE should be used in combination when attempting to diagnosis a thrombus. GRE images are more sensitive than SE images, because a thrombus is usually well differentiated from flowing blood. Also, GRE images can be obtained more rapidly due to the shorter examination time. The accuracy of a thrombosis diagnosis can be significantly improved by using a combination of SE and GRE imaging techniques[6].

In our study, transverse plane images were obtained with both SE and GRE sequences for all patients, and additional corneal plane images were obtained with GRE sequences. The coronal plane provided wide coverage and was particularly useful when imaging tortuous vessels. Coronal GRE images acquired immediately after injection of Gd DTPA were similar to those acquired by X-ray angiography[7]. MR angiography was used as a non-invasive method for displaying vasculature. The MIP algorithm provided extremely high levels of contrast between blood vessels and the surrounding tissues, and was particularly useful for displaying tortuous vessels. Johnson *et al*[8] showed that MR angiography was more sensitive than conventional angiography in detecting varices. However, the joint use of SE and GRE images still had some disadvantages. The major limitations are suboptimal spatial resolution and the production of artifacts. Also, the presence of a thrombus in small vessels can be extremely difficult to delineate by MR.

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Evaluation of the IL-2/IL-2R system in patients with liver cirrhosis or carcinoma

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Abstract

AIM: To evaluate the interleukin-2/interleukin-2 receptor (IL-2/IL-2R) system in patients with liver cirrhosis or carcinoma, and compare the immune function in those patients. The clinical significance of our results is also discussed.

METHODS: Fifty patients with liver cirrhosis (LC), 50 patients with hepatocellular carcinoma (HCC), and 30 normal control subjects were studied. Cellular expression of the interleukin-2 receptor (mIL-2R) was examined by immunofluorescence, and the serum levels of IL-2 and soluble interleukin-2 receptor (sIL-2R) were measured by ELISA.

RESULTS: The levels of IL-2 and mIL-2R expression in carcinoma patients were significantly lower than those in both patients with cirrhosis ($P < 0.01$) and control subjects ($P < 0.01$). The serum levels of IL-2 and the expression of mIL-2R in patients with cirrhosis were also lower than those in normal control subjects ($P < 0.05$). The serum levels of sIL-2R in carcinoma patients were significantly higher than those in both cirrhosis patients ($P < 0.05$) and control subjects ($P < 0.01$), and the sIL-2R levels in cirrhosis patients were higher than those in control subjects ($P < 0.05$).

CONCLUSION: Patients with liver cirrhosis or carcinoma both have decreased immune function; however, this decrease is more pronounced in carcinoma patients. Such similarities in immune disturbances may be an important factor affecting the development of carcinoma in a cirrhotic liver.

Key words: Liver cirrhosis; Liver neoplasms; Interleukin-2/analysis; Receptors interleukin/analysis

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INTRODUCTION

Both liver cirrhosis (LC) and hepatocellular carcinoma (HCC) are common diseases in China. HCC and LC are closely associated with each other in the majority of carcinoma patients (60%-80%), and 10%-30% of patients with cirrhosis will eventually develop HCC. While the various etiologies of HCC are complicated, LC appears to be a major risk factor for the eventual development of carcinoma. In the present study, we determined the serum levels of interleukin-2 (IL-2) and soluble interleukin-2 receptor (sIL-2R), as well as the expression of mIL-2R in patients with liver cirrhosis or carcinoma. The clinical significance of our findings is also discussed.

MATERIALS AND METHODS

Patients and controls

This study enrolled 50 patients with LC, 50 patients with HCC, and 30 normal control subjects. HCC and LC had been diagnosed either clinically or based on a histologic evaluation. None of the patients were taking any medication or had a history of immunotherapy. All of the LC cases were Child's grade B or C.

Assays for IL-2 and sIL-2R

Serum levels of IL-2 and sIL-2R were measured using commercially available ELISA kits. Both assays were performed on the same day and in a single batch to avoid variability. The assay methods are described in detail in a previous publication[1]. Briefly, quadruplicate samples of peripheral blood mononuclear cells (PBMCs) which had been separated by density gradient centrifugation were cultured with phytohemagglutinin (PHA) for 72 h. Following culture, the cells were washed, placed in microtubes, and then incubated with fluorescein-labelled anti-IL-2R monoclonal antibodies. After incubation, the cells were washed 3 times with Hank's balanced salt solution, and then gently resuspended with a Pasteur pipette. One drop of liquid with cells was examined using a fluorescence microscope equipped with a barrier filter. The number of lymphocytes that displayed immunofluorescence staining for IL-2R was recorded, and expressed as a percentage of total cultured PBMCs.

Statistical analysis

The data were analyzed using the *t*-test, and results are expressed as the mean ± SD

RESULTS

The serum levels of IL-2 and sIL-2R and the levels of mIL-2R

Table 1 Serum levels of IL-2 and sIL-2R, and expression of mIL-2R in patients with liver cirrhosis or hepatocellular carcinoma

	IL-2 (μ/mL)	sIL-2R (μ/mL)	mIL-2R (%)
LC	80.1 ± 15.2	264.2 ± 51.3	39.9 ± 7.2
HCC	42.5 ± 11.7	340.7 ± 63.9	33.1 ± 6.4

expression are shown in Table 1. The results showed that IL-2 levels and mIL-2R expression were both significantly lower in patients with HCC, when compared to those parameters in patients with LC ($P < 0.01$) as well as control subjects ($P < 0.01$). Furthermore, IL-2 levels and mIL-2R expression in LC patients were also lower when compared with those parameters in normal subjects (both P-values < 0.05). In contrast, the serum levels of sIL-2R in HCC patients were significantly higher when compared with those in both LC patients, ($P < 0.05$) and control subjects ($P < 0.01$), and the serum levels of sIL-2R in LC patients were also higher than those in control subjects ($P < 0.05$).

DISCUSSION

IL-2 secreted by activated T-lymphocytes interacts with specific membrane receptors (mIL-2R) to upregulate immune reactions in an autocrine manner[2]. In addition to mIL-2R, both *in vivo* and *in vitro* studies have identified a soluble form of IL-2R (sIL-2R) in the supernatant fractions of activated mononuclear cells[3]. This molecule apparently represents the Tac chain of the heterodimeric high affinity IL-2R complex found on the surface of activated T-lymphocytes. sIL-2R displays a lower binding affinity for supernatant IL-2 than it does for mIL-2R. The release of sIL-2R from activated T-lymphocytes may occur due to either proteolysis of mIL-2R or as the result of an alternative mRNA-related process[4].

The decreased IL-2 levels and higher sIL-2R levels in the PBMCs of some individuals may result from several types of liver diseases, including acute and chronic viral hepatitis[5]. High levels of sIL-2R in cases of acute or chronic HBV infection appear to be directly related to the activity of liver diseases, and therefore may reflect the activation of effector cells which help kill the infected hepatocytes. One possible explanation for the reduced IL-2 activity might be that if reflects the compartmentalization of active cells in the liver, and the down-regulated or inhibited production of soluble factors by PBMCs. Similar to hepatitis patients, patients with LC or HCC also show reduced IL-2 activity and mIL-2R expression, accompanied by higher serum levels of sIL-2R. When considering the etiologic and morphologic characteristics of cirrhosis, LC appears to be the most important risk factor for HCC, and HBsAg positivity is one of several factors that increase the risk for HCC in patients with cirrhosis. Our results suggest that patients with LC or HCC have a similar type of immune dysfunction; however, HCC patients have a higher degree of the dysfunction. This similarity in immune disturbances may be an important factor affecting the progression of liver cirrhosis to liver cancer. Our results also indicate that an evaluation of the IL-2/IL-2R system can provide reliable information when selecting an immunotherapy or evaluating the effects of immunotherapy.

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Assessment of natural and interleukin-2-induced production of interferon-gamma in patients with liver diseases

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Abstract

AIM: To determine whether the production of lower interferon gamma (IFNλ) by lymphocytes in patients with liver diseases is due to defects of the lymphocytes themselves or to other cofactors, such as interleukin-2 (IL-2).

METHODS: Peripheral blood mononuclear cells (PBMCs) from patients with various liver diseases were cultured with or without phytohemagglutinin (PHA) and IL-2. The cells were harvested and counted, and the supernatants were tested for IFNλ by a sensitive and quantitative ABC-enzyme-linked immunosorbent assay (ELISA).

RESULTS: IFNλ was not found in serum samples from patients or normal individuals. However, IFNλ was detectable in supernatants of non-induced and induced PBMCs by ABC-ELISA. In non-induced PBMCs (group 1), the content of IFNλ in supernatants from control, chronic active hepatitis (CAH), chronic persistent hepatitis (CPH), and hepatocellular carcinoma (HCC) was 8.72/L, 5.03/L, 6.02/L, and 4.91/L, respectively. The production of IFNλ in liver disease was significantly decreased compared to control. In PBMCs stimulated with PHA (group 2), the content of IFNλ was 22.71/L, 17.12/L, 14.54/L, and 17.63/L, respectively. In PBMCs induced by IL-2 (group 3), the amount of IFNλ in supernatant from control (60.67/L) was much larger than those from CAH (21.70/L), CPH (24.00/L), and HCC (19.15/L) ($p < 0.01$). When comparing the amount of IFNλ in group 3 with that in group 1, we found that IFNλ production was enhanced by nearly 4-folds in liver diseases and by over 7-fold in control. In contrast, the number of PBMCs, whether from liver diseases or from control, was increased by only approximately 3-fold.

CONCLUSION: The decreased production of IFNλ in liver diseases, including HCC, is mainly due to endogenous defects of lymphocytes but may also involve a decrease in stimulating cofactors, such as IL-2.

Key words: Liver disease; Interleukin-2; Interferon type Ⅱ

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INTRODUCTION

Abnormal immune function is an important mechanism of hepatitis B virus (HBV) infection-related liver diseases and hepatocellular carcinoma (HCC)[1]. It was previously shown that disruption of cellular-mediated immune function, including defects in the production of lymphokines, is involved in HBV infection and HCC[2,3]. Interferon-gamma (IFNγ), one of the most important cytokines with various activities, plays a key role in immunoregulation. It has been suggested that IFNγ production by lymphocytes is correlated with the cause, development, and prognosis of chronic liver diseases[4]. However, it is not clear whether the decrease of IFNγ production is caused by defective secretion of IFNγ producing lymphocytes, by decreased levels of inducing cofactors, such as interleukin-2 (IL-2), or both. In the present study, we measured IL-2-induced IFNγ production in peripheral blood mononuclear cells (PBMCs) in order to evaluate the relationship between IFNγ production and liver diseases.

MATERIALS AND METHODS

Subjects

Twenty-one patients with chronic HBV infection (19 males and two females, 15-63 years old), including 12 cases of chronic persistent hepatitis (CPH) (three cases with cirrhosis) and nine cases of chronic active hepatitis (CAH), and nine patients with HCC (eight males and one female, 15-57 years old) were studied. The diagnoses were confirmed by clinical characteristics, laboratory analyses, and imaging examinations. Ten healthy blood donors served as controls.

Preparation of PBMCs

Five milliliters of venous blood were collected and heparinized. PBMCs were isolated from whole heparinized blood by centrifugation over Ficoll, washed three times with Roswell Park Memorial Institute

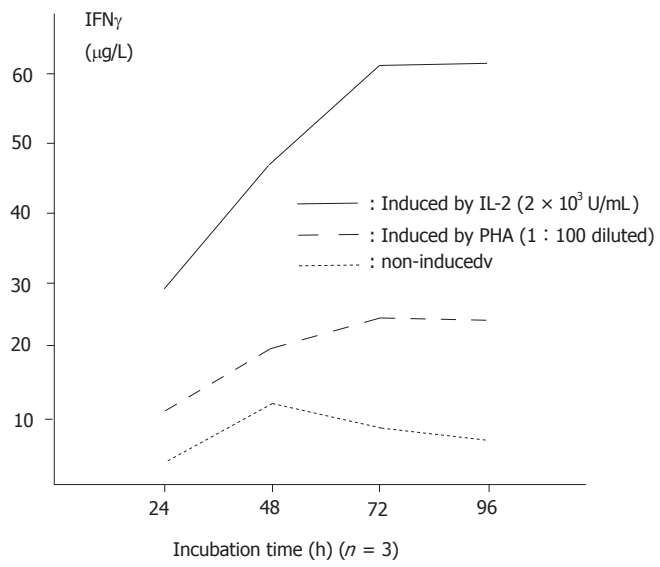

Figure 1 Interferon gamma (IFNγ) production by normal peripheral blood mononuclear cells (PBMCs) over time.

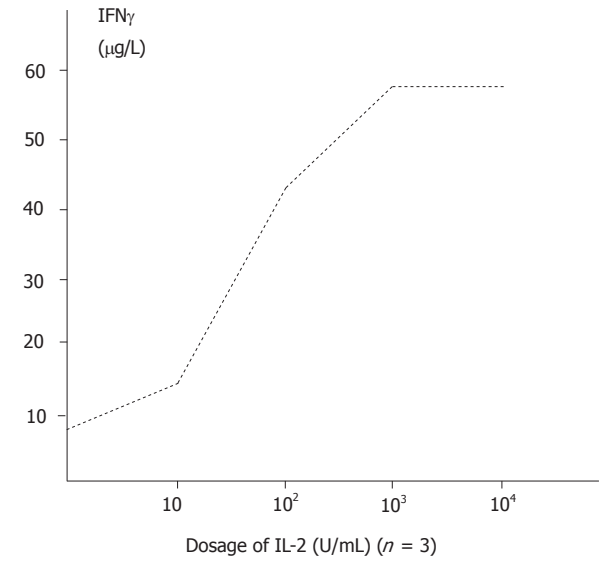

Figure 2 IFNγ production by normal PBMCs induced by various dosages of interleukin (IL)-2.

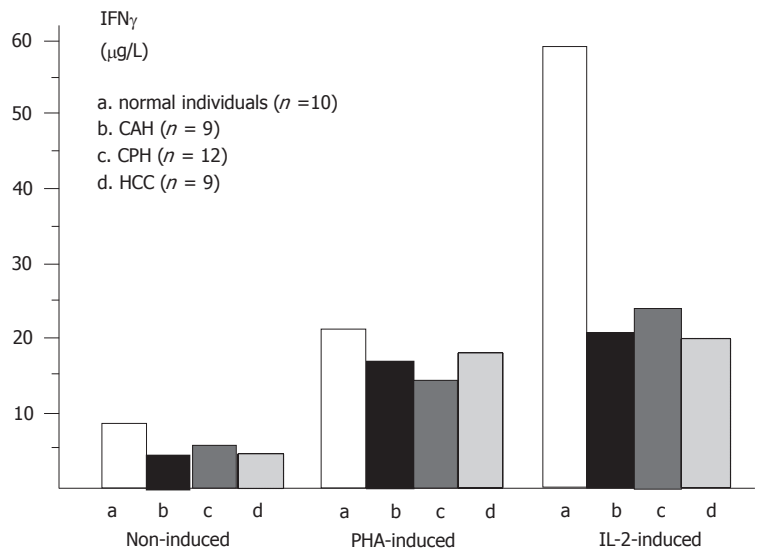

Figure 3 IFNγ production by PBMCs from patients with liver diseases and from normal individuals.

(RPMI)-1640 medium (Gibco, Carlsbad, CA, United States), and then suspended at 2 × 10^6 cells/mL in RPMI-1640 medium containing 10% fetal calf serum. The viability of cells was higher than 95% by the trypan blue exclusive test.

Induction of IFNγ from PBMCs

Each PBMC sample was placed in a 96-well plate (2 × 10^5 cells/100 μL per well) in three groups. For each group, double wells were designed. In group 1, 100 μL of medium was added and cultured without any inducers. In group 2, 100 μL of 1:100 diluted phytohemagglutinin (PHA) (type M. Sigma, St Louis, MO, United States) was added. In group 3, 100 μL of IL-2 (gift from the Department of Microbiology, SMMC) with the activity of 2 × 10^3 U/mL was added. The cells were incubated at 37 ℃ in 5% CO_2 for 72 h, and the culture supernatants were removed and tested for IFNγ. The cells were counted under a light microscope.

Detection of IFNγ

IFNγ in supernatants was detected by a quantitative ABC-enzyme linked immune sorbent assay (ELISA), as we previously described[5].

Statistical analysis

Data were analyzed by Student's *t* test and *F* test.

RESULTS

IFNγ in serum samples

IFNγ was not detected in the sera of 21 patients with chronic HBV infection, the nine patients with HCC, or any of the controls.

IFNγ production by PBMCs in normal individuals

The amount of IFNγ produced in non-induced supernatants was 8.72 ± 0.65 μg/L (n = 10). Stimulation with PHA and IL-2 with increasing time durations and dosages significantly enhanced the production of IFNγ ($p < 0.01$). The relationships between course and effectiveness and between dosage and effectiveness are indicated in Figures 1 and 2.

Comparison of IFNγ production in various liver diseases

IFNγ production in either PHA-induced or non-induced PBMCs in liver diseases was significantly lower than that in normal individuals ($P < 0.01$). Although the production of IFNγ was the lowest in HCC patients, there was no significant difference in IFNγ production among various liver diseases ($P > 0.05$). The level of IFNγ was increased in the supernatants of IL-2 induced PBMCs from patients with CPH, CAH, and HCC, respectively, compared with non-induced PBMCs (Figure 3).

The number of IL-2 induced PBMCs

The number of IL-2 induced PBMCs was increased approximately 3 times compared to non-induced PBMCs in liver diseases and in control. When comparing the increase of IFNγ production (by 6.9 times in control, 4.3 times in CAH, 4.0 times in CPH and 3.9 times in HCC) with the increase in the number of PBMCs, we found that there was a difference between them.

DISCUSSION

Recently, the importance of cytokines in immunoregulation has been recognized, and the correlation between cytokines and body disorders is continuing to be elucidated[6]. The dysfunction of cellular immunity is an important mechanism of HBV infection-related chronic liver diseases and is associated with persistent HBV infection[7].

Some new therapies for HBV infection, such as the application of IFNs and lymphokine activated killer (LAK) cells, are partially effective for the clearance of HBV[8]. More recently, cytokine gene transfer therapy for HBV infection and HCC has also been reported[9]. All these therapies are based on findings that the production of cytokines is decreased in hepatitis B and that the replacement of cytokines is an effective way to maintain balance of the immune system. In this study, we found that IFNγ production by non-induced and lymphoblastized PBMCs from patients with various chronic liver diseases and HCC was significantly lower than that in normal individuals, with the lowest in patients with cirrhosis. These findings suggest that IFNγ producing cells, *i.e.*, T helper and natural killer (NK) cells, may be defective in the production of IFNγ. Among the factors that stimulate the production of IFNγ, IL-2 is the most important[10]. In this study, when PBMCs were cultured with IL-2, the secretion of IFNγ was enhanced 3.9 (in HCC) to 6.8

(in control) fold, whereas the number of PBMCs was increased by only 3 times, suggesting that the enhancement of IFNγ production was related not only to the increase in PBMC number but also to the enhancement in the IFNγ producing ability of PBMCs. It was reported that LAK cell therapy was shortly effective for suppression of HBV duplication. These authors suggested that the increase in cytokine production of LAK cells was involved in the anti-HBV mechanism[7]. Our results are consistent with these findings.

However, the inducing effects of IL-2 on IFNγ production are not the same in patients with liver diseases and normal controls. In patients with liver diseases, the amount of IFNγ produced by IL-2 induction in PBMCs was nearly 7 times greater than that in non-induced PBMCs; whereas in normal controls, the former is about 4 times greater than the latter. We conclude that the decreased production of IFNγ in patients with liver diseases is mainly due to endogenous defects of lymphocytes but may also be due to a decrease in some stimulating cofactors.

Notably, we failed to detect IFNγ directly from the sera of patients or normal individuals by a sensitive and quantitative ABC-ELISA established in our laboratory. Some other authors reported similar results by CPE. It may be concluded that the level of IFNγ secreted in serum is too small to be detected by the present methods. Moreover, the amount of IFNγ in serum may be influenced by many factors, such as the half-life of IFNγ, liver and renal function, and the time of collecting blood. Therefore, we recommend for future studies on cytokines, including IFNγ, the preparation of lymphocytes or PBMCs rather than serum.

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Effects of acute hepatic damage on natriuresis and water excretion after acute normal saline loading in rats

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Abstract

AIM: To investigate the relationship between liver functional impairment and sodium and water retention.

METHODS: An animal model of acute liver damage model was established by administering carbon tetrachloride (CCl_4) to male Sprague-Dawley rats. Twenty-four and 48 h after CCl_4 administration, the excretion of acute sodium and water load was measured. In controls, the excretion of acute sodium and water load was measured 24 h after administration of normal saline. In addition, the concentration of plasma caffeine was analyzed using high pressure liquid chromatography (HPLC). The half-life of plasma caffeine (Caf $t_{1/2}$) served as a quantitative index of hepatic function. Plasma alanine aminotransferase (ALT) was measured using the Reitman method. Hepatic tissue sections from the same site were used for water content measurement and pathological observation. The serum and urinary sodium levels were measured with flame photometry.

RESULTS: Twenty-four hours after CCl_4 administration, plasma ALT level (n = 6, 37.5 ± 12.6 → 189.4 ± 34.4 U, P < 0.01) and water content of hepatic tissue (n = 6, 70.0% ± 0.11% → 73.0% ± 1.0%, P < 0.01) were significantly increased, and Caf $t_{1/2}$ was prolonged significantly (94.9 ± 18.9 → 326.4 ± 85.8 min, P < 0.01) compared to saline treated control. Renal function, as assessed by excretion of acute salt and water load, was significantly decreased (n = 6, Na^+: 92.4% ± 14.1% → 50.1% ± 13.1%, P < 0.01; H_2O: 86.3% ± 14.3% → 42.1% ± 8.8%, P < 0.01). The above indices had recovered somewhat 48 h later but were still markedly different from those of control. In addition, the relationships between Caf $t_{1/2}$ and ALT (r = 0.752, P < 0.01) and between Caf $t_{1/2}$ and excretory rate of sodium (r = 0.634, P < 0.05) and water remained significant (r = 0.612, P < 0.01) at 48 h.

CONCLUSION: Caf $t_{1/2}$ is a good index to assess the degree of hepatic damage. Hepatic dysfunction may contribute to impairments in renal excretion following acute sodium and water load.

Key words: Liver disease; Water-electrolyte imbalance; Kidney/Metabolism

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INTRODUCTION

Most data to date have shown that there is a significant relationship between hepatic functional damage and sodium retention, but it remains unclear whether acute liver dysfunction results in sodium and water retention. To understand further the relationship between liver function per se and sodium retention, we investigated renal excretion following acute salt and water load in a rat model of acute hepatic damage.

MATERIALS AND METHODS

Acute hepatic damage model

Eighteen male Sprague Dawley rats weighing 270-320 g were divided into three groups: control (n = 6), experimental group 1 (n = 6), and experimental group 2 (n = 6). Reduced salt chow (12.74 mmol/kg) and re-distilled water were available *ad libitum*. Three days later, carbon tetrachloride (CCl_4) (0.02 mL/100 g body wt) was administered intraperitoneally to experimental groups, and normal saline (0.02 mL/100 g body wt) was administered to the control group. Acute normal saline loading was conducted in experimental groups 2 and 3 24 and 48 h after CCl_4 administration, respectively, and 24 h after saline administration in the control.

Acute sodium and water loading experiment

Twelve hours after removing water and food, the animals received under urethane anesthesia normal saline (4 mL/100 g body wt) through the femoral vein. Urine was collected for 4 h and stored in an Eppendorf tube at -20 ℃ until analysis. One percent caffeine, 0.1 mL (1 mg/rat), was then administered to the femoral vein for

Table 1 Lesion indices of hepatic tissue and liver dysfunction after CCl_4 administration (n = 6, $\bar{x} \pm s$)

Group	Water content (%)	ALT (Reitman U)	Caf $t_{1/2}$ (min)
Control	70.1 ± 1.1	37.5 ± 12.6	94.8 ± 18.9
CCl_4 24 h	73.0 ± 1.0[b]	189.4 ± 34.4[b]	326.4 ± 85.8[b]
CCl_4 48 h	72.0 ± 0.8[b]	126.1 ± 59.1[a]	169.5 ± 37.9[b]

[a]$P < 0.05$; [b]$P < 0.01$ *vs* control. Liver water content (%)

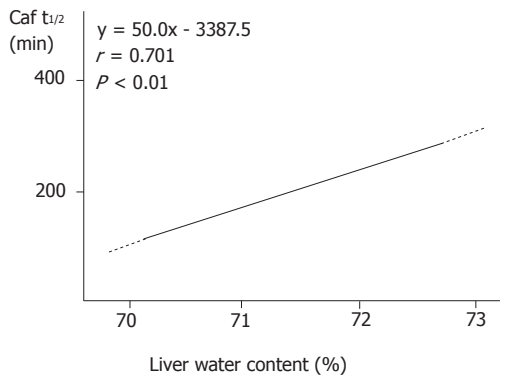

Figure 1 Relationship between liver water content and liver function

Table 2 Serum Na^+ and water salt excretory rate (n = 6, $\bar{x} \pm s$)

Group	serum Na^+ (mmol/L)	Na^+ excretory rate (%)	H_2O excretory rate (%)
Control	145.7 ± 5.7	92.4 ± 14.1	86.3 ± 14.3
CCl_4 24 h	143.0 ± 5.6	50.1 ± 13.1[b]	42.1 ± 8.8[b]
CCl_4 48 h	139.8 ± 2.1	64.3 ± 14.1[a]	56.6 ± 12.4[a]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control

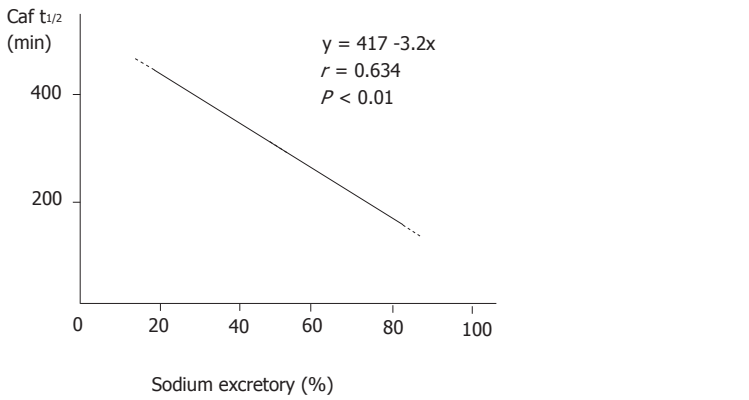

Figure 2 Relationship between excretory rate of salt load and liver function

3 h. Four milliliters of blood were taken from the inferior vena cava and anti-agglutinated with heparin. The plasma was separated to measure caffeine, ALT, and sodium. At this time, liver tissues were taken for pathologic examination and water content calculation.

Examination method

The plasma caffeine concentration was analyzed with high pressure liquid chromatography (HPLC). Plasma ALT was examined with Reitman method, and serum and urinary sodium levels were measured with flame photometry.

Calculation and statistics

The Caf $t_{1/2}$ was calculated using the following formula:

$$\text{Caf } t_{1/2} = \ln 2 \times (t / \ln (D / (avd \times \text{body wt}) \ln C_{caft})$$

where D = the dosage of caffeine (1 mg in this experiment); t = time from caffeine injection to blood collection (180 min); C_{caft} = caffeine concentration when blood was taken (mg/L); adv = apparent distribution volume (0.64 mL/kg). Data for each parameter were compared between two groups with a t test and among the three groups with an F test. Regression analysis was used to correlate Caf $t_{1/2}$ and water content in hepatic tissue, sodium excretory rate, water excretory rate, and plasma ALT. p values less than 0.05 were considered to be statistically significant.

RESULTS

The changes of hepatic tissue

In the control group, the hepatic cells were arranged regularly, and the plates radiated from the central vein to the portal canals. Twenty four hours after CCl_4 administration, the hepatic tissue exhibited extensive necrosis, hepatocyte swelling, and vacuolization under light microscopy. In the 48 h group, hepatocyte necrosis, swelling, and vacuolization were still clearly seen but to a lesser extent than the 24 h group. Consistent with the histological changes in the hepatic tissue, water content was significantly higher in both experimental groups than the control group.

Liver function decline

Following structural damage to the liver tissue, the capability of the liver to metabolize caffeine was impaired. The Caf $t_{1/2}$ was prolonged significantly in the 24 h and 48 h groups compared to that in the control group (Table 1). Caf $t_{1/2}$ and water content in hepatic tissue were significantly correlated (r = 0.701, $P < 0.01$, Figure 1).

Consistent with our findings on water content in hepatic tissue and Caf $t_{1/2}$, plasma levels of ALT rose significantly in both experimental groups. There was a significant positive correlation between ALT levels and Caf $t_{1/2}$ (r = 0.753, $P < 0.01$).

Relationship between liver dysfunction and sodium and water excretion

Following the impairment in liver function, the capability of the kidney to excrete acute water and sodium load declined significantly. Although this decline recovered somewhat in the 48 h group, it remained significantly different from that of the control (Table 2). The relationship between Caf $t_{1/2}$ and renal excretory rate of sodium (Figure 2) and water was significantly correlated (r = -0.612, $P < 0.01$). There was no significant difference among the three groups in serum sodium level (F = 2.34, $P > 0.05$, Table 2).

DISCUSSION

Recently, different models of hepatic damage[1-3] have demonstrated that the decline in liver function is an important cause of salt and water retention. Caffeine is metabolized by hepatic cytochrome P-450, and the ability of the body to clear caffeine reflects metabolic function of the liver. Jost *et al*[4] showed that the clearance rate of plasma caffeine is in significant agreement with the aminopyrine breath test (r = 0.80, $P < 0.01$), prothrombin time (r = 0.59, $P < 0.01$), indo cyanogreen test (r = 0.51, $P < 0.01$), and galactose clearance rate (r = 0.46, $P < 0.01$). Therefore, use of Caf $t_{1/2}$ to quantitate liver function is reliable; HPLC is an efficient and stable means to measure caffeine. Our results found that 24 and 48 h after CCl_4 administration, Caf $t_{1/2}$ was prolonged significantly. Changes in ALT levels and hepatic tissue water content paralleled Caf $t_{1/2}$. There was a significant relationship between Caf $t_{1/2}$ and ALT and between Caf $t_{1/2}$ and water content of hepatic tissue. These results illustrate that the decrease in liver function is linked to the degree of hepatic tissue damage. Thus, Caf $t_{1/2}$ may be considered a sensitive index that reflects the degree of acute hepatic tissue damage.

Importantly, following pathological damage of the liver, the plasma Caf $t_{1/2}$ was prolonged significantly and the ability of the kidney to excrete acute water and sodium load was declined significantly. Caf $t_{1/2}$ and renal excretory rate of sodium and water were negatively correlated. These results are consistent with the report by Wong *et al*[5] and identity hepatic tissue damage and liver function decline as important contributors to salt and water retention.

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Effect of interferon in combination with ribavirin on the plus and minus strands of HCV RNA in patients with chronic hepatitis C

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Abstract

AIM: To probe the effect of interferon in combination with ribavirin on the plus and minus strands of hepatitis C virus RNA (HCV RNA).

METHODS: Twenty-three cases diagnosed as chronic hepatitis C (CHC), according to positive HCV RNA/anti-HCV, fluctuating levels of aminotransferase activities (< 1 year), and absence of other hepatitis virus marker, were studied. Among them, 13 patients received combined antiviral therapy (subcutaneous injection of 3 MU of interferon-α three times per week for 3 months and intravenous drip of 1 g of ribavirin per day during the first month of treatment with interferon), and 10 patients received single interferon therapy, as described above, as control. The plus and minus strands of HCV RNA in sera and peripheral blood mononuclear cells (PBMCs) of these patients were tested by nested reverse transcription-polymerase chain reaction (nested RT-PCR).

RESULTS: At the end of therapy, the abnormal alanine amino-transferase (ALT) levels decreased to normal range in nine (69.23%) cases in the combined antiviral group. Of them, five (55.56%) experienced post-therapy relapse, and four (44.44%) were complete responders. In the interferon group, ALT decreased to normal in six (60%) cases, of which, four (66.67%) had post-therapy relapse and two (33.33%) were complete responders. The differences between the two groups were not significant ($P < 0.05$). At the end of therapy, the positive rate of the plus strand in sera decreased from 92.30% to 38.46% ($P < 0.05$) and that of the minus strand in PBMCs from 76.92% to 38.46% ($P < 0.05$) in the combined antiviral group. In the interferon only group, the former decreased from 100% to 50% ($P < 0.05$) and the latter, from 90% to 40% ($P < 0.05$). Again, no significant differences were found between groups ($P < 0.05$). Relapse occurred in patients whose plus strand HCV RNA in PBMCs remained after treatment.

CONCLUSION: Ribavirin did not enhance the antiviral effect of interferon when the plus and minus strands of HCV RNA were measured. The absence of HCV RNA in serum does not mean complete clearance of HCV, and its value for evaluating antiviral effects and prognosis is limited. Therefore, it is essential to measure the plus and minus strands of HCV RNA in sera and PBMCs simultaneously.

Key words: hepatitis C; RNA, viral; interferon-alpha; antiviral agents

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INTRODUCTION

Recently, interferon has been used to treat chronic hepatitis C (CHC). Abnormal alanine aminotransferase (ALT) levels become normal in 50%-70% of treated patients, and replication of hepatitis C virus (HCV) RNA in these patients is suppressed to various degrees[1]. However, approximately 50% of CHC patients experienced post-therapy relapse after interferon withdrawal[2]. Whether the therapeutic effect of combination of antiviral drugs is superior to monotherapy is rarely reported. In this study, we used interferon in combination with ribavirin to treat patients with CHC and investigated the effect of the combined antiviral therapy on the plus and minus strands of HCV RNA in sera and peripheral blood mononuclear cells (PBMCs) using nested reverse transcription polymerase chain reaction (RT-PCR) and serum ALT.

MATERIALS AND METHODS

Subjects

Twenty-three patients with CHC (29 to 55 years old, nine male and 14 female) were studied. All patients had positive HCV RNA/anti-HCV and fluctuating levels of aminotransferase activities (> 1 year) and did not have any hepatitis B virus (HBV) related markers, positive anti-hepatitis E virus (HEV), and anti-hepatitis A virus (HAV) immunoglobulin (Ig)M. Chronic active hepatitis (CAH) was confirmed in eight of the HCV patients by biopsy. Thirteen of

Table 1 The results of alanine aminotransferase (ALT) and hepatitis C virus (HCV) RNA before and after treatment

Cases	Type	Biopsy	Therapeutic effect	Before therapy			End of therapy			24 wk after therapy		
				HCV Serum	RNA PBMC	ALT U/L	HCV Serum	RNA PBMC	ALT U/L	HCV Serum	RNA PBMC	ALT U/L
1 IFN	CHC	NT	NR	+ (-)	+ (+)	230	+ (-)	+ (+)	77	+ (-)	+ (+)	112
2 IFN	CHC	NT	NR	+ (-)	+ (+)	146	+ (-)	+ (+)	68	+ (-)	+ (+)	57
3 IFN	CHC	CAH	NR	+ (-)	+ (+)	60	+ (-)	+ (+)	50	+ (-)	+ (+)	88
4 IFN	CHC	NT	NR	+ (-)	+ (+)	126	+ (-)	+ (-)	46	+ (-)	+ (+)	97
5 IFN	CHC	CAH	SR	+ (-)	+ (+)	175	- (-)	+ (-)	25	+ (-)	+ (+)	92
6 IFN	CHC	NT	SR	+ (-)	- (-)	108	- (-)	+ (-)	38	+ (-)	+ (-)	66
7 IFN	CHC	NT	SR	+ (-)	+ (+)	88	+ (-)	+ (-)	30	+ (-)	+ (+)	120
8 IFN	CHC	CAH	SR	+ (-)	+ (+)	105	- (-)	- (+)	40	+ (-)	+ (+)	68
9 IFN	CHC	NT	CR	+ (-)	+ (+)	200	- (-)	- (-)	28	- (-)	- (-)	27
10 IFN	CHC	CAH	CR	+ (-)	+ (+)	220	- (-)	- (-)	30	- (-)	- (-)	40
11 I+R	CHC	NT	NR	+ (-)	+ (+)	166	+ (-)	+ (+)	188	+ (-)	+ (+)	100
12 I+R	CHC	NT	NR	+ (-)	+ (+)	250	+ (-)	+ (+)	142	+ (-)	+ (+)	320
13 I+R	CHC	CAH	NR	+ (-)	+ (+)	98	+ (-)	+ (+)	66	+ (-)	+ (+)	146
14 I+R	CHC	CAH	NR	+ (-)	+ (+)	125	- (-)	- (+)	55	- (-)	- (+)	70
15 I+R	CHC	NT	SR	+ (-)	+ (+)	241	- (-)	+ (-)	26	- (-)	- (-)	67
16 I+R	CHC	NT	SR	+ (-)	+ (+)	225	+ (-)	- (+)	31	+ (-)	+ (-)	560
17 I+R	CHC	CAH	SR	+ (-)	- (-)	290	- (-)	+ (-)	20	+ (-)	+ (-)	50
18 I+R	CHC	NT	SR	+ (-)	+ (+)	90	+ (-)	+ (-)	34	+ (-)	+ (+)	60
19 I+R	CHC	NT	SR	+ (-)	+ (+)	110	- (-)	+ (-)	32	+ (-)	+ (+)	72
20 I+R	CHC	NT	CR	+ (-)	- (-)	450	- (-)	- (-)	16	- (-)	- (-)	34
21 I+R	CHC	NT	CR	+ (-)	+ (+)	140	- (-)	- (-)	36	- (-)	- (-)	40
22 I+R	CHC	NT	CR	+ (-)	+ (+)	204	- (-)	- (-)	30	- (-)	- (-)	28
23 I+R	CHC	CAH	CR	+ (-)	- (-)	200	- (-)	- (-)	28	- (-)	- (-)	28

IFN: Interferon; I + R: Interferon and ribavirin; NT: Not tested. Marks in parentheses are the results of minus strand.

them received the combined antiviral therapy, *i.e.*, interferon plus ribavirin (I + R group), which consisted of subcutaneous injection of 3 MU of interferon-α (α-2b interferon, Schering Corporation, Kenilworth, NJ, United States) three times per week for 3 mo and intravenous drip of 1 g of ribavirin (Wuhan Second Pharmaceutical Factory, Wuhan, China) per day during the first month of interferon therapy. The remaining 10 patients received single interferon therapy, as described, as control (IFN group). Serum ALT and plus and minus strands of HCV RNA in sera and PBMCs in all patients were detected before therapy, at the end of therapy, and 24 wk after therapy.

Primers

Primer sequences were selected from the highly conserved 5′ noncoding region of the HCV RNA and were synthesized by the Institute of Immunology, Essen University, Germany. Two outer primers were sense: r-kf-10, 5′GGCGACACTCCACCATAGAT and antisense: r-kf-11c, 5′GGTGCACGGTCTACGAGACC. The inner sense primer was r-kf-15: 5′GGAGGATCCACTCCCCTGT, and the antisense primer was r-kf-16a: 5′ GGAAAGCTTGAATTCACCCTATCAGGCAGT. The size of the expected amplification product was 295 bp.

Nested RT-PCR

PBMCs were separated by density gradient centrifugation on ficoll hypaque from 4 mL EDTA blood samples and then washed three times in 10 mL of 0.9% NaCl. The extraction of HCV RNA in sera and PBMCs and the synthesis of complementary DNA (cDNA) of the plus and minus strands of HCV RNA were performed, as described previously[3]. Three microliters of cDNA were added to 25 μL of PCR reaction mixture and amplified for 40 cycles. Next, 3 μL of the first PCR products were added to 25 μL of the second PCR reaction mixture, with inner primer instead of outer primers, and nested RT-PCR was performed in the same way as described above.

Judgement of antiviral effect

The patients were divided into three groups based on changes of HCV RNA and ALT levels before and after antiviral therapy. Patients whose sera ALT level and status of HCV RNA had not changed after antiviral therapy were named nonresponders (NR). Patients whose ALT level decreased to within the normal range, whose plus and/or minus strands of HCV RNA became negative at the end of the treatment, and who experienced post therapy relapse 24 wk after therapy were named short-term responders (SR). The third group consisted of complete responders (CR), who had sustained normal ALT levels and had undetectable plus and/or minus strands not only at the end of therapy but also 24 wk after cessation of the therapy.

Statistics

Chi-square test was used to statistically analyze the percentages. $P < 0.05$ was considered significant.

RESULTS

Dynamics of ALT levels before and after antiviral therapy

A 295 bp fragment was found in the positive blood samples from the cases and the positive control serum but not in the negative control serum, which was an aliquot from the last wash of PBMCs and PCR reaction mixture without cDNA or primer.

The results (Table 1) showed that by the end of treatment, the increase in ALT levels in nine (69.23%) cases in the I+R group were restored to within the normal range, which took an average of 23.8 days. Of the nine cases, five (55.56%) were SR and four were CR. In the IFN group, the ALT level of six (60.00%) cases decreased to normal, taking an average of 29.6 days. Four (66.67%) of the six cases were SR and two (33.33%) were CR. There were no significant differences between the two groups ($P > 0.05$).

The effect of antiviral therapy on the plus and minus strands of HCV RNA

The minus strand of HCV RNA in sera was negative in all patients. At the end of therapy in the I+R group, the positive rate of the plus strand in sera (92.31%) decreased to 38.46% ($P < 0.05$) and that of the minus strand in PBMCs (76.92%) decreased to 38.46% ($P < 0.05$). In the IFN group, the former decreased from 100% to 50% ($P < 0.05$) and the latter from 90% to 40% ($P < 0.05$). Although the results indicated that antiviral therapy suppressed the reproduction of HCV, the differences between the two groups were not significant ($P > 0.05$). Regarding the percentage of HCV RNA turned negative at the end of the therapy in the I + R group (Table), we found that if the plus strand of HCV RNA in sera alone was tested, HCV RNA of seven (58.33%) cases turned negative; and if the plus and minus strands of HCV RNA in sera and PBMCs were simultaneously tested, HCV RNA of only four (30.77%) cases became negative. Regarding the relationship between HCV RNA and relapse, we found that if the plus strand of HCV RNA in sera alone was tested, HCV RNA of three out of five patients who had relapsed turned negative. When the plus and minus strands of HCV RNA in PBMCs were simultaneously detected, the plus strand of HCV RNA of four out of five and the minus strand of HCV RNA of one out of the five patients remained

positive. These data indicate that the plus strand of HCV RNA in the sera of the three cases turned negative and that HCV was actually not cleared. In contrast, the four cases whose plus and minus strands of HCV RNA in sera and PBMCs remained negative 24 wk after the treatment, no post-therapy relapse occurred.

DISCUSSION

It is now clear that when HCV is replicated, a minus strand of HCV RNA, *i.e.*, the replicative form of HCV RNA, can be detected[4,5]. Here, we showed that interferon in combination with ribavirin made the replicative form of HCV RNA negative in more than 50% of patients with CHC, confirming that combined antiviral therapy suppressed the replication of HCV. About half of the responders, as defined as those in whom the plus strand of HCV RNA in sera became negative, had relapse after interruption of the treatment. In these patients, however, the plus strand in PBMCs remained positive during and after treatment. Importantly, in the cases that did not have relapse, the plus and minus strands of HCV RNA in sera and PBMCs were both negative, indicating that (1) the absence of HCV RNA in serum did not mean complete clearance of HCV, and its lack of detection was due primarily to the low serum concentration of HCV following antiviral therapy that suppressed the replication of HCV; and (2) HCV in extrahepatic sites, such as PBMC,s is difficult to eliminate, which might serve as a source of reinfection of the liver[6] and might be one of the causes of relapse. It has been suggested that the value of the plus strand of HCV RNA in sera alone for evaluating the antiviral effect and prognosis is limited. Therefore, it is essential to measure the plus and minus strands of HCV RNA in sera and PBMCs simultaneously to identify complete clearance of HCV.

Falling serum ALT levels means a reduction of hepatocellular damage, which might be related to the ability of antiviral therapy to suppress the replication of HCV or to eliminate HCV. Thus, diminishing or eradicating the viral load of the liver may improve liver disease[2].

Although the percentage of and average time for restoring ALT levels to the normal range and the percentage of HCV RNA turned negative in the I + R group were slightly better than those in the IFN group, the differences were not statistically significant. The reasons for this lack of significance might be that (1) the course of treatment with ribavirin was too short or the dosage of ribavirin was too small; and (2) ribavirin could not enhance the antiviral effect of interferon.

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REVIEW

Recent advances in the application of cultured hepatocytes into bioartificial liver

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INTRODUCTION

Over the past 3 decades, various experimental liver support systems have been studied. Early artificial liver support systems included hemodialysis, extracorporeal liver perfusion, human cross circulation, charcoal hemoperfusion, hepatodialysis, fresh blood, and plasma exchange transfusion. These systems were developed to remove toxic substances from the blood. Clinical trials showed that these detoxification systems could promote the recovery of consciousness in patients with deep coma, although the survival of patients was not improved. Recently, advances in biotechnology and tissue engineering have brought into focus the importance of biological components for a hybrid artificial liver support system (HALSs). Biological components may include isolated enzymes, cellular components, slices of liver, or cultured hepatocytes. Hepatocyte systems have shown the greatest promise for HALSs. The advantages of hepatocyte systems may be summarized as follows: (1) supplying crucial liver specific metabolic functions; (2) being easily scaled up; (3) having immunoisolation from the host defenses by semipermeable membrane; and (4) being cryopreserved for later use. The disadvantage of hepatocyte systems is the problem of maintaining normal hepatocyte viability and function at high cell density necessary for clinical application. With the development of cell culturing technology and cell engineering technology, great progress has been made in the research of using cultured hepatocytes as a bioreactor to provide hepatic support.

Current situation of cultured hepatocytes system research

Cultured hepatocytes must maintain good viability and function to be used in HALSs. However, when they are cultured on a plastic surface with standard cell-culture medium, they flatten and become agranular; tissue specific functions are lost in 3 to 5 d, followed by hepatocyte death within 1 to 2 wk[1]. Therefore, the techniques of cell culture should be improved. The approaches include: (1) addition of growth factors and hormones to culture medium; (2) co-culturing of hepatocytes with nonparenchymal liver cells; (3) using biologic gel or matrix; and (4) cultivation of cells in hollow fiber.

Addition of growth factors and hormones to culture medium

It has been reported that hormones and growth factors, such as insulin, glucagon, dexamethasone, epithelial growth factor (EGF), and hepatocytes growth factor (HGF), may play an important role in modulating the differentiation and proliferation of cultured hepatocytes as well as maintaining tissue-specific functions.

According to Hamad's results, EGF can prolong the survival of cultured hepatocytes and stimulate hepatocyte proliferation[2]. The DNA contents of hepatocytes were reported to be well preserved in media supplemented with insulin and EGF by Hamad[2] and Dich *et al*[3] respectively.

It has also been reported that glucagon is necessary for maintaining the synthesis of urea by cultured hepatocytes for about 2 wk. Moreover, Takahashi *et al*[4] reported in 1993 that the growth and differentiation functions of hepatocytes cultured at different densities were modulated by EGF and insulin when hepatocytes were cultured at a low cell density of 2.5×10^4 cells/cm^2. Conversely, cellular functions were induced by hormones at a high cell density of 12.5×10^4 cells/cm^2, whereas the hepatocyte proliferation was suppressed.

Co-culture with nonparenchymal liver cells (NPC)

Co-culturing is a technique in which two more cell types are cultured together, resulting in cell behavior and physiological responses that would not occur if the cell types were cultured alone. It was first reported by Puck and Marcus in 1955 in studies with Hela cells. One of the most successful methods of co-culturing is using mito-mycin-C-treated "feeder layers" of another cell type (often fibroblasts), which are thought to supply the primary cells with nutrients or factors. Begue *et al*[5] found that the cytochrome P-450 content in adult rat hepatocytes was maintained over a 10-d period when these cells were co-cultured with another rat liver epithelial cell type.

Use of biologic gel and cell engineering techniques

Hepatocytes are anchorage dependent. Currently, biologic gel, microcarriers, micro-encapsules, and Poly-N-P-Vinybenzyl-D-

Lactomide (PVLA) are used to culture hepatocytes with high density and slight differentiation.

Immobilized hepatocytes in hydrogel or microencapsule

Miura *et al*[6] demonstrated the long term maintenance of the capacity to synthesize urea, albumin, and glucose as well as the ability to detoxify hepatic toxins like phenols and fatty acids when isolated hepatocytes were encapsuled within calcium alginate gel. The encapsulating technique can avoid immunological hazards and maintain the cellular function of isolated hepatocytes. Micro-encapsulated hepatocytes are mainly used in hepatocytes transplantation, and HALSs produced using such cells would be large and, therefore, inconvenient for clinical use.

Microcarrier hepatocytes

Van Wezel reported the microcarrier technique in 1967. It has the advantage of increasing the adhesive area of culturing cells (the surface area of 1 g dextran is 0.6 m^2), thus facilitating transplantation of large quantities of hepatocytes in very small volumes of microcarrier suspension. Being cultured on the collagen-covered microcarriers, hepatocytes can maintain long-term cellular function and growth. Kasai *et al*[7] found that the metabolic activity of rat hepatocytes attached to collagen-coated multiporous cellulose microcarriers can be preserved for at least 1 week. Nezuil *et al*[8] developed a HALSs using porcine hepatocytes attached to microcarriers and placed on the outer surface of hollow fibers. The HALSs was used to treat a 33-year-old male patient with alcohol induced cirrhosis. The patient's mental status greatly improved 2 h after initiation of HALSs treatment. Three weeks later, the patient' s liver function improved gradually and steadily. He underwent orthotopic liver transplantation 6 months later.

PVLA hepatocytes

Recently, rapid progress has been made in research on using PVLA to culture hepatocytes. In hepatocytes cultured on the PVLA coated dishes, growth and functional activity was regulated by the simulated three-dimensional environment *in vivo*[9]. Hepatocytes cultured on PVLA coated dishes are able to maintain a high level of differentiation functions and longevity because PVLA has the ligand, β-galactose for asialogly protein receptors on hepatocytes[10,11]. Kobayashi *et al*[12] found that the regulation of differentiation function and proliferation of hepatocytes is low for cells cultured on dishes coated with high level PVLA (200 μg/mL) and is high for those with low level PVLA (1 μg/mL). Moreover, bile acid release was maintained at higher levels in hepatocytes attached on dishes coated with a high level of PVLA.

Toke *et al*[13] found that the hepatocytes initially attached onto PVLA substratum and then migrated together to form multilayer aggregations by the stimulation of growth factors, such as EGF and insulin. They observed many orifices of tube-like structures on the surface of the multilayer aggregation by scanning electron microscopic analysis. Transmission electron microscopic analysis of the aggregation revealed the maintenance of endoplasmic reticulum and the appearance of bile canalicular-like structure. This tissue-reconstruction of primary cultured rat hepatocytes exhibited long-term maintenance of specific cellular functions, such as the secretion of albumin and bile acid, and retained mitochondrial enzyme activity. These data showed that PVLA could potentiate the differentiation and proliferation of hepatocytes. However, hepatocytes cultured with high proliferation and high maintenance of differentiation function could not be achieved with the same coating concentration. Further refinement of the PVLA hepatocytes systems is expected to assist in the development of HALSs.

Hollow fiber hepatocytes

Hollow fiber hepatocyte bioreactors consists of two compartments: (1) an intraluminal compartment within the fibers and (2) an extraluminal compartment outside the fibers and within the rigid housing. Hepatocytes are cultured within the extraluminal compartment, and the patient's blood is circulated within the capillaries. Through pores in the walls of the hollow fiber, communication between the compartments occurs. Toxic substance in the patient's blood permeates through the pores and is acted on by the hepatocytes attached on the hollow fibers.

Hollow fiber systems have several advantages, such as immunological separation between the patient and the support hepatocytes and providing a large membranous area for hepatocytes to attach. Recently, several versions of hollow fiber membrane based systems have been reported in the literature[14-16]. Sussman *et al*[15] cultured a CAS cell line on the fibers, and Nyberg *et al*[16] proposed a novel hollow fiber based HALSs. The primary hepatocytes were entrapped in cylindrical gels inside the lumen of the hollow fibers.

Recent experimental studies with these devices have demonstrated their efficacy in animal models of acute liver failure. Rozga *et al*[17] treated seven patients with acute liver failure with a HALSs based on hollow fiber bioreactor. These patients recovered gradually and underwent liver transplantation. However, randomized clinical trials are still needed to evaluate these forms of HALSs.

Problems and future aspects of the cultured hepatocytes systems

In the last 5 years, research on cultured hepatocytes in bioartificial liver has made rapid progress with the aid of recent advances in cryobiology and tissue culture. Isolated hepatocytes or differentiated cell lines can be cultured at a density of 10^7/mL. However, for clinically applicable HALSs, the cell density needs to be increased to 10^8/mL. Therefore, much effort should be made to obtain the effective HALSs.

As mentioned above, only hepatocytes have been used in HALSs to date. Nonparenchymal liver cells were confined to co-culturing. Some experts proposed that the ideal HALSs may be developed with co-cultured hepatocytes systems. Thus, further studies are needed to ascertain its feasibility.

The development of HALSs is still in its infancy. Some of the HALSs designs using cultured hepatocytes have satisfied the rigors of animal testing and now are being studied in phase I clinical trials. We expect that the randomized clinical trials will establish the value of bioartificial liver therapy for patients with hepatic failure.

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BRIEF REPORT

Combination assay for serum tumor markers in patients with hepatic carcinoma

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INTRODUCTION

In general, hepatic solid space occupying lesions (HSSOL) can be found by both ultrasonoscopy (US) and computed tomography (CT). If the customary alpha-fetoprotein (AFP) standard for diagnosis of primary hepatic carcinoma is used, those cancers with lower AFP concentration may go undiagnosed[1]. On the other hand, using the standard AFP-positive (≥ 20 μg/L) + HSSOL to diagnose primary hepatocellular carcinoma (PHCC) may yield false positive results[2, 3]. In order to elevate both the preoperative and differential diagnosis levels for hepatic carcinoma, we assayed the preoperative levels of serum AFP, carbohydrate antibody (CA) 19-9, and carcinoembryonic antigen (CEA) in patients with HSSOL.

SUBJECTS AND METHODS

Subjects

Four groups of patients (30 patients in each group) with benign HSSOL (BHSSOL group; including 17 cases of hepatic cyst, 10 of benign hepatic tumor, and three of hepatic tuberculosis), secondary hepatic carcinoma (SHC group; the cancer stemmed from the stomach in 12 cases; the pancreas in eight cases; the colon in seven cases; the bile duct in two cases; and the uterus in one case), primary non-hepatocellular carcinoma (PNHCC group; including 29 cases of intrahepatic duct cell carcinoma and one case of mixed type cancer), and primary hepatocellular carcinoma (PHCC group) were verified by percutaneoustranshepatic biopsy and/or hepatic postoperative pathology. All patients met the following criteria: (1) HSSOL was confirmed by US or CT. (2) The peripheral venous blood samples were collected before the operation. The concentrations of serum AFP, CA19-9, and CEA were assayed. Patients with serum AFP levels between 20 μg/L and 200 μg/L were reexamined by the same method 1 mo later. And s(3) The main organs, including stomach, pancreas, biliary tree, colon, lung, kidney, uterus, etc. were examined by endoscopy, X-ray, or CT combined with biopsy to check for the existence of tumor lesions. In this series, 74 patients were male and 46 were female, and they were between 17 and 76 years old, with a mean age of 53.5.

Methods

CA19-9 radioimmunoassay (RIA) kit was purchased from Symtron (USA), and other kits were provided by the Institute of Chinese Atomy (China). Serum AFP, CA19-9, and CEA levels were measured according to the manufacturer's protocols.

Judging

Serum AFP ≥ 200 μg/L or between 20 μg/L and 200 μg/L, which was enhanced or unchanged after 1 month, was defined as AFP positive. Antigen levels higher than the cutoff value (serum CA19-9 ≥ 37 KU/L and CEA ≥ 15 μg/L) or a single item two times higher than the cutoff value were considered positive.

Statistical analysis

Analysis of variance was used for the multivariant measurement data, and a *t* test was used to make comparisons between two groups.

RESULTS

Serum AFP

Using a cutoff value of 20 μg/L, the sensitivity of AFP to PHCC was 100% (30/30 cases, Table 1), and the specificity was 85.7% (78/90 cases). With a cutoff of 200 μg/L, the sensitivity was 76.7% (23/30 cases), and the specificity was 100% (90/90 cases).

Serum CA19-9

With a cutoff value of 37 KU/L for CA19-9, the sensitivity in detecting PNHCC was 93.3% (28/30 cases, Table 1), and the specificity was 65.6% (59/90 cases). In SHC, the sensitivity and specificity of CA19-9 were 90.0% (27/30 cases) and 63.3% (57/90 cases), respectively. At a cutoff value of 74 KU/L, the sensitivity and specificity of CA 19-9 were 43.3% (13/30 cases) and 77.8% (70/90 cases), respectively, in detecting PNHCC, and 13.3% (18/30 cases)

Table 1 Comparison serum alpha-fetoprotein, carbohydrate antibody19-9 and carcinoembryonic antigen in patients with hepatic solid space occupying lesions ($\bar{x} \pm s$)

Grouping	*n*	AFP (μg/L)	CA19-9 (KU/L)	CEA (μg/L)
1 BHSSOL	30	11.07 ± 4.30	28.43 ± 15.76	10.49 ± 6.93
2 SHC	30	15.23 ± 13.13	231.20 ± 196.64	27.08 ± 13.35
3 PNHCC	30	14.03 ± 8.13	169.67 ± 140.08	19.27 ± 8.67
4 PHCC	30	320.65 ± 180.12	23.73 ± 12.91	10.73 ± 3.94

Notes: The comparison of AFP levels between group 4 and groups 1, 2, and 3: $P < 0.01$; among groups 1, 2, and 3: $P > 0.05$. CA 19-9 levels in groups 2 and 3 were higher than those in groups 1 and 4: $P < 0.01$; between group 2 and 3 and between group 1 and 4: $P > 0.05$. CEA levels in groups 2 and 3 were higher than those in groups 1 and 4 ($P < 0.01$); between group 2 and 3 and between group 1 and 4: $P > 0.05$. AFP, alpha-fetoprotein; BHSSOL, benign HSSOL; CA19-9, carbohydrate antibody 19-9; CEA, carcinoembryonic antigen; HSSOL, hepatic solid space occupying lesion; PHCC, primary hepatocellular carcinoma; PNHCC, primary non-hepatocellular carcinoma; SHC, secondary hepatic carcinoma

Table 2 Serum alpha-fetoprotein and carbohydrate antibody19-9 + carcinoembryonic antigen in patients with hepatic solid space occupying lesions

Grouping	No. of cases	AFP		CA19-9 + CEA	
		Positive	Negative	Positive	Negative
BHSSOL	30	0	30	0	30
SHC	30	3	27	30	0
PNHCC	30				
Biliary cell cancer	29	0	29	29	0
Mixed cancer	1	1	0	1	0
PNCC	30	30	0	0	30

and 81.1% (73/90 cases), respectively, in detecting SHC.

Serum CEA

At a cutoff of 15 μg/L, the sensitivity of CEA in detecting PNHCC was 73.3% (22/30 cases, Table 1), and the specificity was 63.3% (58/90 cases). In SHC, the sensitivity and specificity were 80.0% (24/30 cases) and 66.7% (53/90 cases), respectively. At a cutoff of 30 μg/L, the sensitivity and specificity of CEA were 16.7% (5/30 cases) and 84.4% (76/90 cases), respectively, in detecting PNHCC; and 13.3% (18/30 cases) and 83.3% (75/90 cases), respectively, in detecting SHC.

Combined assay

The results from the combined assay are shown in Table 2. With US, X-ray, CT, and endoscopy in combination with biopsy, 29 cases (96.7%) of extrahepatic primary cancer nests were clinically discovered in the SHC group; and four cases (13.3%) of extrahepatic transition cancer nests were discovered in the PNHCC group. The difference between the two groups was significant ($P < 0.05$, Table 2).

DISCUSSION

There are four types of HSSOL, namely, PHCC, PNHCC, SHC, and BHSSOL. We found that serum AFP level was much higher in PHCC than in the other three types ($P < 0.01$, Table 1), while there was no statistically significant difference among the other three groups ($P > 0.05$). By raising the cutoff value of AFP, the specificity for detecting cancer was improved, but the sensitivity was reduced. Using the standard in our study (serum AFP ⩾ 200 μg/L or between 20 μg/L and 200 μg/L, which was elevated 1 mo later), specificity and sensitivity for diagnosing PHCC were the highest. Therefore, we recommend that this standard be adopted as the diagnosis standard of PHCC. Assaying serum AFP level helps to differentiate PNHCC and SHC from BHSSOL and PHCC. Serum CA19-9 levels were much higher in the SHC and PNHCC groups than in PHCC and BHSSOL groups ($P < 0.01$). CA19-9 is a gastrointestinal tumor marker, and its content is the highest in tumor epithelial cells of the digestive tract[4,5]. Since PNHCC is an abdominal adenocarcinoma, the serum CA19-9 level in PNHCC is elevated, which is normal in PHCC and BHSSOL. Therefore, these diseases can be differentiated by assaying serum CA19-9 levels. Serum CEA levels were higher in the PNHCC and SHC groups than in the PHCC and BHSOL groups ($P < 0.01$). It has been reported that serum levels of CEA might be elevated significantly in patients with digestive tract tumors 2-18

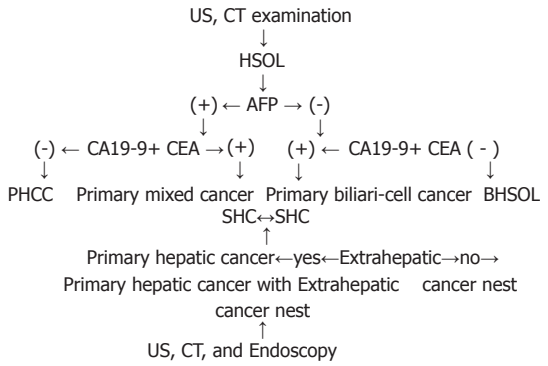

Figure 1 A flow chart for preoperative and differential diagnosis of hepatic carcinoma.

mo before the lesions were found clinically by X-ray examination[6,7]. CEA is an important marker for the diagnosis of liver metastasis. Sometimes the level of CEA is elevated by 100% in patients with hepatic metastasis. CEA may also be a marker of intestinal tumors. Therefore, we can assay serum CEA levels to differentiate between SHC and PNHCC, and BHSSOL and PHCC.

As shown in Table 2, negative-AFP and positive CA 19-9 + CEA occurred in 90% of SHC and primary biliary cell cancer (96.7%) among PNHCC. Both positive AFP and CA19-9 + CEA occurred in 10% of SHC and mixed cancer (3.3%) among PNHCC. SHC usually originated from the tumors of lower organs, *i.e.*, stomach, colon, pancreas, biliary tree, *etc*. As some of the tumors of the digestive system belong to hepatoid adenocarcinomas in SHC, AFP levels were positive[8]. Nagai *et al*[9] reported that serum AFP was elevated in 5.4% of gastric carcinoma, and liver metastasis occurred in 72% of these patients. The appearances of SHC and PNHCC intersect, making differentiation between the two difficult. Our study indicates that extrahepatic cancer nests (OTCN) are more common in SHC than in PNHCC ($P < 0.01$). In general, patients with OTCN discovered by US, CT, X-ray, and endoscopy may be considered to have SHC, while those without OTCN may be considered to have PNHCC. Strictly speaking, however, OTCH transferred from primary hepatic cancer should be differentiated from primary OTCN by pathologic examination alone. Based on the above results, the procedure of early diagnosis and differential diagnosis of hepatic carcinoma is shown by the Figure 1. By detecting serum levels of AFP, CA19-9, and CEA in patients with BHSOL, preoperative and differential diagnosis of PHCC, PNHCC, SHC, and BHSSOL might be improved.

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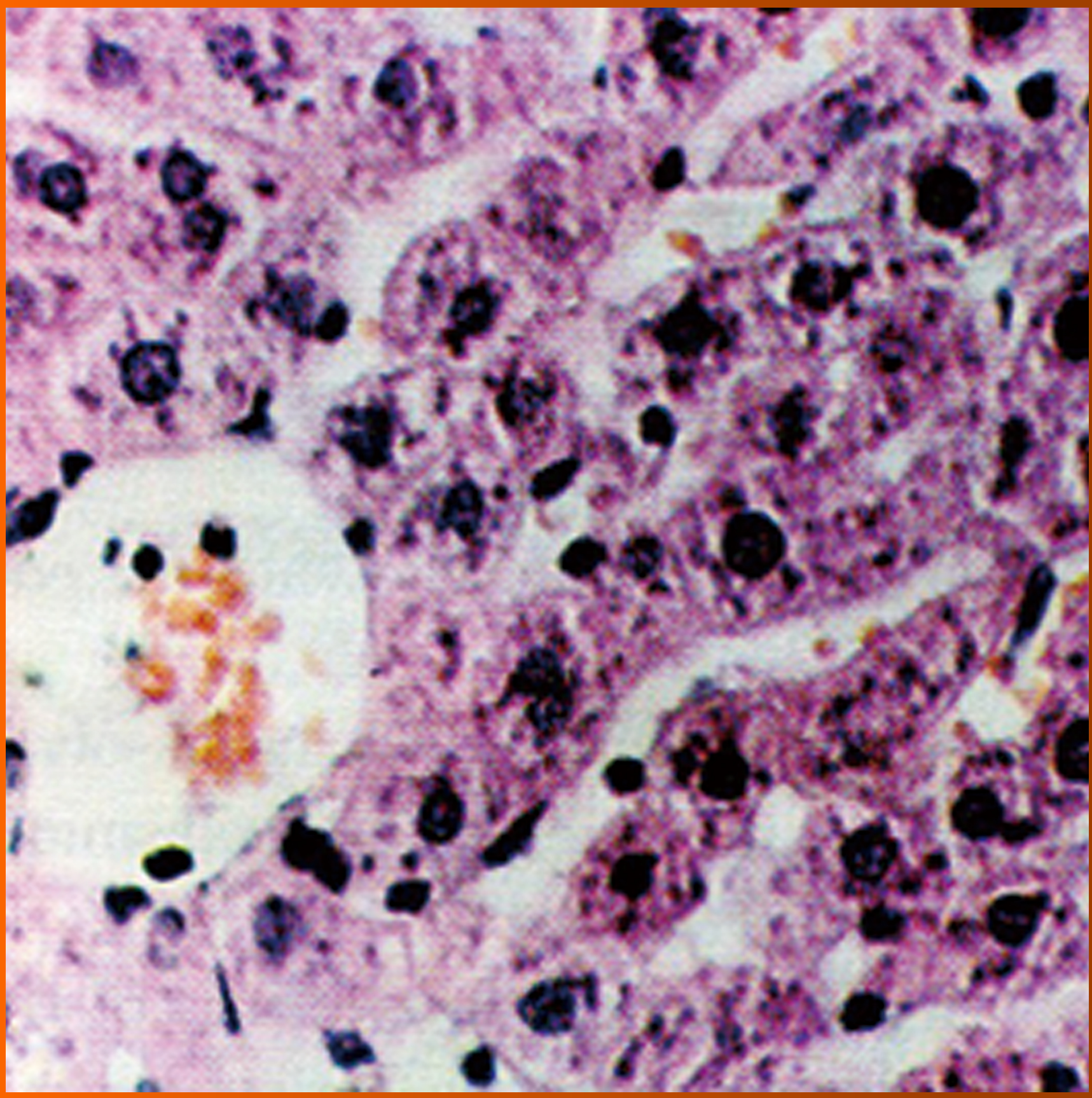

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Detecting the localization of hepatitis B and C viruses in hepatocellular carcinoma by double *in situ* hybridization

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Abstract

AIM: To detect whether hepatitis B virus (HBV) and hepatitis C virus (HCV) can infect the same host cell.

METHODS: We established a method of non-isotope double *in situ* hybridization by combining a degoxigenin-labeled probe with a biotin-labeled probe, and detected HBV and HCV infections in 10 cases of hepatocellular carcinoma.

RESULTS: It was found that HBV and HCV can infect the same host cell, which is a unique biological phenomenon.

CONCLUSION: Infections with HBV and HCV might not always follow the rule of "viral interference", which provides a new clue for investigating the synergistic action of HBV and HCV in the pathogenesis of hepatocellular carcinoma.

Key words: *In situ* hybridization; Liver neoplasms; Hepatitis B virus; Hepatitis C virus

Liu YJ, Cong WM, Xie TP, Wang H, Shen F, Guo YJ, Chen H, Wu MC. Detecting the localization of hepatitis B and C viruses in hepatocellular carcinoma by double *in situ* hybridization. *World J Gastroenterol* 1996; 2(4): 187-189 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/187.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.187

INTRODUCTION

Hepatocellular carcinoma (HCC) in Mainland China is closely associated with hepatitis B virus (HBV)[1]. The relationship between HCC and hepatitis C virus (HCV) has attracted more and more attention and about 20% of HCC patients have HBV and HCV co-infection in their sera[2]. An investigation has indicated that HBV and HCV co-infection accounted for about 14% of chronic hepatitis patients in Shanghai[3]. However, little is known about whether or not synergistic actions of HBV and HCV exist in the pathogenesis of HCC.

People generally believe that two sorts of viruses could not infect the same host cell, and this is the phenomenon of "viral interference"[4]. Direct methods of detecting HBV and HCV co-infection to identify whether HBV and HCV infect different cells respectively or the same cell simultaneously are still lacking. So in this study, we first established a method of non-isotope double *in situ* hybridization for detecting the two sorts of target nuclear acids in one cell, and then studied whether HBV and HCV co-infection would obey the rule of "viral interference". Finally, we discuss the biologic significance of HBV and HCV co-infection.

MATERIALS AND METHODS

Materials

The PUC18 plasmid carrying full length HCV cDNA was given by professor Qi-Zhong Tian (Department of Microbiology, Second Military Medical University). The PBR322 plasmid carrying full length HBV cDNA was provided by professor Gu-Jian Ren (Institute of Tumor, Shanghai). Specimens of HCC and their surrounding tissues were obtained from Eastern Hospital of Hepatobiliary Surgery, Second Military Medical University.

Preparation of probes

HBV and HCV plasmids were transformed into *E. coli* DH5α. Plasmids were prepared from *E. coli* by alkaline lysis method after bacteria had been amplified. The target gene fragments were cut by specific restriction endonuclear enzymes (HBV cDNA length is 3.4 kb, and the restriction enzyme used is *EcoR* I; HCV cDNA length is 0.54 kb, and the restriction enzymes used are *EcoR* I and *Pst* I). HBV and HCV cDNA probes were labeled by digoxigenin (DIG) using a DIG-Labelling and Detection Kit (Boehringer Mannheim Inc.); HCV cDNA probe was also labeled by biotin with the help of a Nick Translation Kit (Enzo Inc.).

Double hybridization in situ

Sections of HCC and their surrounding tissues, 6 μm thick, were cut by a cryostat microtome, fixed in 4% paraformaldehyde (pH 7.4) for 10 min, and washed with Poly Butylenes Succinate (PBS) for 2 × 5 min. They were then incubated in 0.2 mol/L HCl for 20 min, washed with 2 × SSC for 2 × 5 min, treated with 2 μg/mL

Table 1 The background information of 10 cases of hepatitis B virus patients

Specimen number	Age	Sex	HBV serum marker	AFP ($\rho B/\mu g \cdot L^{-1}$)	Pathological diagnosis
1	48	Male	HBsAg (+) HBeAb (+) HBcAb (+)	42	HCC, chronic hepatitis
2	27	Male	HBsAg (+)	900	HCC, hepatitis in porta tract
3	61	Male	HBsAg (+) HBcAb (+)	260	HCC, cirrhosis
4	38	Male	(-)	12000	HCC, cirrhosis
5	44	Male	HBeAb (+) HBcAb (+)	40	HCC, cirrhosis
6	46	Male	(-)	150	HCC, non-cirrhosis
7	45	Male	(-)	< 25	HCC, cirrhosis
8	62	Male	(-)	52	HCC, cirrhosis
9	40	Male	HBsAg (+)	-	HCC, cirrhosis
10	58	Male	-	< 25	HCC, cirrhosis

HBV: Hepatitis B virus; AFP: Alpha fetal protein; HCC: Hepatocellular carcinoma.

Table 2 Results of single *in situ* hybridization

Specimen number	Dig-HBV		Dig-HCV	
	C	S	C	S
1	+	+	-	-
2	+	+	+	+
3	+	+	-	-
4	+	+	+	+
5	+	+	-	-
6	-	-	-	-
7	+	+	-	-
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-

C: Cancer; S: Tumor surrounding tissues; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Table 3 Results of double *in situ* hybridization of Dig-HBV and Bio-HCV

Specimen number	Dig-HBV		Bio-HCV	
	C	S	C	S
1	+	+	-	-
2	+	+	+	+
3	+	+	-	-
4	+	+	+	+
5	+	+	-	-
6	-	-	-	-
7	+	+	-	-
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-

C: Cancer; S: Tumor surrounding tissues; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

proteinase K (containing 10 mmol/L Tris-HCl and 2 mmol/L $CaCl_2$, pH 7.4) for 15 min at 37 ℃, washed with 2 mg/mL glycine in PBS, fixed in 4% paraformaldehyde (pH 7.4) for 10 min, and washed with PBS for 2 × 5 min. Finally, sections were prehybridized at 42 ℃ in prehybridization buffer for 2 h (the buffer was composed of 4 × SSC, 50% formamide, 5 × Denhardt's solution, 5% dextran sulfate and predenatured 0.5 mg/mL ssDNA). After prehybridization buffer was removed, hybridization buffer (5 ng/μL DIG-HBV and BIO-HCV cDNA probes, 4 × SSC, 50% formamide, 5 × Denhardt's solution, 5% dextran sulfate and 0.5 mg/mL ssDNA) was added to slides which had already been incubated for 15 min at 100 ℃. Then these slides were placed into a humid chamber for 10 min at 100 ℃, followed by incubation in the humid chamber at 42 ℃ overnight. Colour detection of biotin system (ABC method with substrates of DAB and H_2O_2) was done before that of digoxigenin system (based on the procedure of DIG-labelling and detection kit, Boehringer Mannheim Inc., Cat. No. 1093657, with the substrates of NBT and BCIP).

Controls

DIG-PBR328 and BIO-PBR328 were adopted as negative controls instead of target probes. The results of single *in situ* hybridization were taken as parallel controls for the results of double *in situ* hybridization.

RESULTS

The background information of 10 cases of HCC patients is shown in Table 1.

Results of single in situ hybridization

HBV or HCV positive cells showed diffuse distribution in HCC; most HBV or HCV positive cells in tumor surrounding tissues showed local distribution, concentrating to the porta tract (Table 2).

Results of double in situ hybridization

HBV and HCV co-infection was detected only in No. 2 and No. 4 cases. HBV or HCV positive cells were distributed diffusely in HCC and locally in tumor surrounding tissues, some of them were shown at single cell level. The signal of BIO-HCV was weaker than that of DIG-HBV.

Negative controls

Results of negative controls with single or double *in situ* hybridization were negative (Table 3).

DISCUSSION

HBV infection is the most important factor associated with HCC in Chinese people. With the improvement of sensitivity of detecting methods, the importance of HCV infection and HBV/HCV co-infection in the pathogenesis of HCC has gradually been recognized.

Scientists from Taiwan discovered that both HBsAg and anti-HCV were important risk factors for HCC (relative risk ratio, 13.96 and 27.12, respectively), and the risk ratio was elevated significantly to 40.05 when HBsAg and anti-HCV were considered simultaneously, which suggested that the two viruses might have independent but synergistic effects on the pathogenesis of HCC[5].

The results of double *in situ* hybridization conform with those of single *in situ* hybridization. We found that there were two positive cases of HCV infection accompanied by HBV infection with the help of single and double *in situ* hybridization, respectively. These results directly provide experimental evidence that HBV and HCV may play a synergistic role in the pathogenesis of HCC (Figures 1-3).

People generally believe that two kinds of viruses could not infect the same host cell simultaneously (the phenomenon of viral interference). There are two sorts of viral interference, homologous and heterologous, and the latter is more common[4]. HBV is a double stranded DNA virus, and HCV is a single stranded RNA virus. There is no homology between them. In 1983, Bradley noted that non-A non-B hepatitis virus (NANBV) infection could resist hepatitis A virus (HAV) infection in two cases of chimpanzees, and symptoms of one HBV infected chimpanzee were relieved and the level of serum HBsAg reduced after infection with NANBV. So he suggested that NANBV could interfere with HBV and HAV infections[6]. Brotman *et al*[7] indicated that HCV could inhibit the replication of HBV. Dr. Du *et al*[8] discovered that acute HAV infection could obviously affect the level of HBV markers, and particularly reduce the level of serum HBsAg. But these results which came from serum detection, for example, the reduction or disappearance of serum HBsAg, could not accurately show the actual change of liver cells.

The result that HBV and HCV could co-infect the same host cell indicates that HBV and HCV might not always follow the rule of "viral interference"; the above conclusion conforms with the results that liver functions of HBV patients were severely damaged by HCV

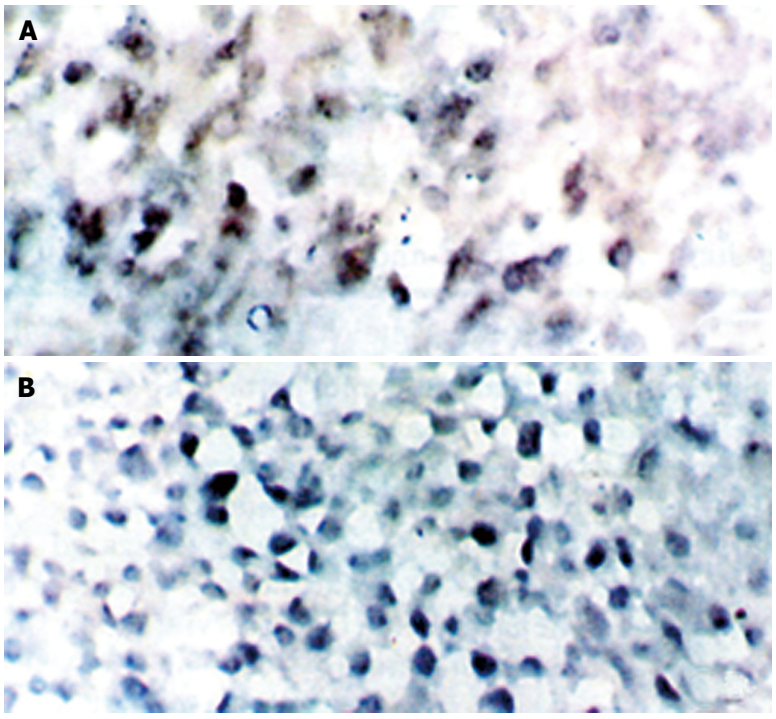

Figure 1 Single *in situ* hybridization (× 40). A: HBV positive cells are distributed diffusely in HCC; B: HCV positive cells are distributed diffusely in HCC. HBV: Hepatitis B virus; HCV: Hepatitis C virus. HCC: Hepatocellular carcinoma.

Figure 3 Negative controls (Dig-PBR328 and Bio-PBR328) (× 40).

infection caused by blood transfusion. So it laid down a theoretical basis that HBV and HCV might play a synergistic role in the pathogenesis of HCC, which indicates that vaccines of HBV and HCV will not interfere with each other in their preventive effects.

We noted that there were 5 cases positive for HBV serum markers, 4 cases negative and one case undetected; but the results of *in situ* hybridization showed that 9 cases were positive with hybridized signals of HBV both in HCC and their surrounding tissues, and one case was negative. Considering their pathologic diagnoses, we found that 3 cases negative for HBV serum markers but positive for *in situ* hybridization showed liver cirrhosis, and one case negative for these two methods showed no liver cirrhosis. These results suggest that

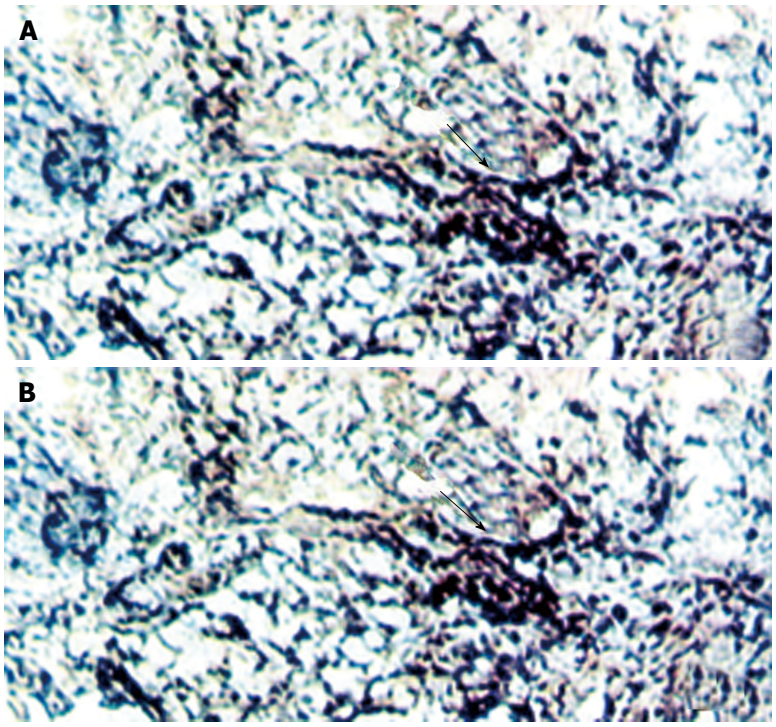

Figure 2 Double *in situ* hybridization (× 40). A: HBV or HCV positive cells are distributed diffusely in HCC; some of them present co-localization of both viruses at single cell level; B: HBV or HCV positive cells are distributed locally in tumor surrounding tissues, especially the porta tract; some of them present co-localization of both viruses at single cell level. HBV: Hepatitis B virus; HCV: Hepatitis C virus. HCC: Hepatocellular carcinoma.

in situ hybridization is more sensitive than serum immunodetection for HBV infection. It was a pity that we could not compare *in situ* hybridization with serum detection for HCC because we lack these patients' background information of HCV serum markers.

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Protective action of various doses of compound zinc preparation on cadmium-induced liver damage

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Abstract

AIM: To investigate the protective action of various doses of compound zinc (Zn) preparation on liver damages induced by cadmium (Cd) in pregnant rats.

METHODS: The changes in the microstructure and ultrastructure of hepatic cells were observed by microscopy and electron microscopy.

RESULTS: In Cd-toxic group, severe damage of hepatic cells was observed. Electron microscopic analysis showed extensive irreversible pathological changes, such as nearly dissolved hepatic cells with a lot of vacuoles, swelling endoplasmic reticulum and partial degeneration of mitochondrial cristae. However, in 300 μg/mL Zn group, the microstructure and ultrastructure were normal.

CONCLUSION: Zn has a remarkable protective effect on the Cd-induced hepatic cell damage in pregnant rats, and the effect is dose-dependent. Zn at a dose of 300 μg/mL has a significant protective action.

Key words: Zinc; Cadmium; Liver pathology; Rats; Inbred strain

Han GA, Jiang HM, Zhen XM, Zhou HJ, Xu CZ, Tian JX. Protective action of various doses of compound zinc preparation on cadmium-induced liver damage. *World J Gastroenterol* 1996; 2(4): 190-191 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/190.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.190

INTRODUCTION

Cadmium (Cd) is a heavy metal that can cause carcinomas, malformation and mutation. It usually accumulates in the liver and kidney and causes serious damage to the liver and kidney. Zinc (Zn) has an obvious protective effect on Cd-induced liver damage in mice[1]. The present study investigated the protective action of Zn on Cd-induced hepatic cell damage in pregnant rats by observing the microstructure and ultrastructure of hepatic cells.

MATERIALS AND METHODS

Materials

Sixty Wistar rats weighing 200-240 g were provided by the Experimental Animal Center of Shandong Medical University with a sex ratio of 2 females to 1 male. Self-made Zn preparation containing 450 μg/mL, 400 μg/mL and 300 μg/mL Zn, respectively, $CdCl_2 \cdot 2.5H_2O$ (AR grade), optical microscope, JEM-120EX transmission electron microscope and AOE ultramicrotome were used.

Methods

Twenty-eight pregnant rats were divided randomly into five groups: A, B, C, D and E. There were 5 rats in each group except that group A had 8 rats. In group A, rats with Cd toxication were given tap water; In groups B, C and D, rats with Cd toxication were given water containing 450 μg/mL, 400 μg/mL and 300 μg/mL Zn, respectively, on the day of gestation, and each was given 29.30 mL per day; In group E (control group), normal rats were given tap water. The pregnant rats in groups A-D were injected with 2 mg/kg Cd into the abdominal cavity on the 7^{th}, 9^{th}, and 11^{th} d of gestation, while the pregnant rats in group E were injected with equal volume of saline solution. All rats were killed on the 20^{th} d of gestation

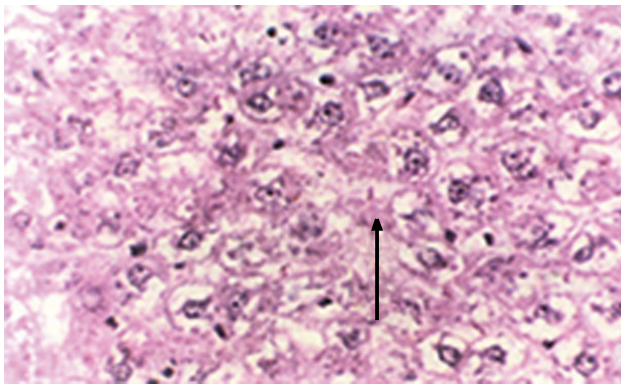

Figure 1 Cd toxic group, showing severe hydropic degeneration and sporadic lytic necrosis of the hapatic cells (× 600).

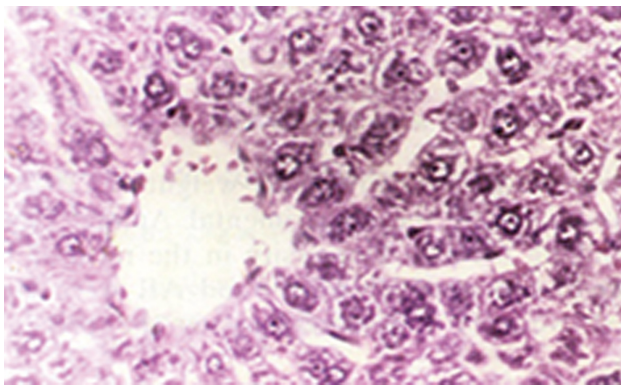

Figure 2 300 μg/mL Zn group, only showing edema of the hepatic cells (× 600).

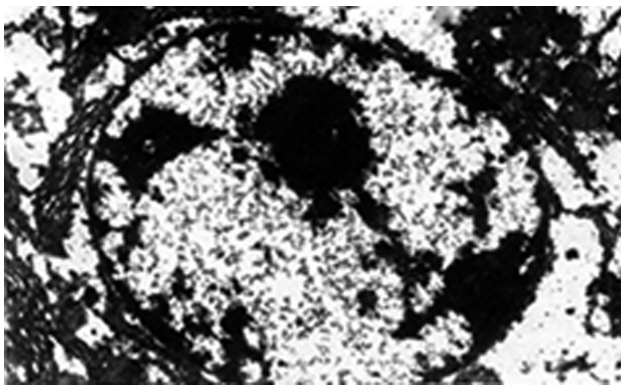

Figure 3 Cd toxic group; the hepatic cells are nearly dissolved with a lot of vacuoles, flocculent chromatin, swelling endoplasmic reticulum and degenerated mitochondria (× 8000).

and their livers and kidneys were taken out. Routine sections were observed and photographed by microscopy and electron microscopy.

RESULTS

Changes in the microstructure of rat hepatic cells

In group E, light microscopic examination revealed that the structure of hepatic cells was normal. Group A showed severe hydropic degeneration and sporadic lytic necrosis of hepatic cells (Figure 1). Group B exhibited diffuse hydropic degeneration of hepatic cells and focal inflammatory cellular infiltration. Group C revealed slight expansion in the central vein of the hepatic lobule, edema and necrosis of hepatic cells, and infiltration of lymphocytes. Group D demonstrated swelling of the hepatic cells and slight hydropic degeneration, which indicated that Zn at a dose of 300 μg/mL had obvious antagonism against Cd-induced damage (Figure 2).

Changes in the ultrastructure of hepatic cells

In group E, the ultrastructure of the hepatic cells was normal. Group A revealed the nearly dissolved hepatic cells with a lot of vacuoles, flocculent chromatin in the cytoplasm, unclear karyotheca, swelling endoplasmic reticulum and degenerated mitochondria (Figure 3). In groups B and C, the ultrastructure showed no significant differences compared with that of group A. In group D, the karyotheca of the hepatic cells was clear and regular, the endoplasmic reticulum was almost normal, the bilaminar membrane and cristae of mitochondria were clearly seen but vacuoles were still visible in the cytoplasm (Figure 4). Electron microscopic observations showed that group D had the obvious protective action on Cd induced damage to the ultrastructure of hepatic cells of pregnant rats, while groups B and C had no obvious antagonism, which is consistent with light microscopic examination.

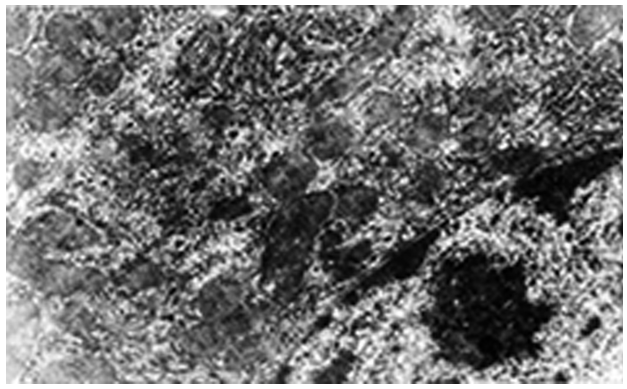

Figure 4 300 μg/mL Zn group; the karyotheca of the hepatic cell is clear and regular, the endoplasmic reticulum is nearly normal, but vacuoles are still seen in the cytoplasm (× 16000).

DISCUSSION

In the present study, various doses of zinc preparation were given orally on the day of gestation before Cd toxication to antagonize Cd-induced damage to the liver of pregnant rats. Groups with 450 μg/mL and 400 μg/mL zinc preparation showed an unremarkable protective effect, but in group with 300 μg/mL zinc preparation, the microstructure and ultrastructure of the hepatic cells remained nearly normal, which suggests that Zn antagonism against Cd-induced damage was closely related to the dose of zinc preparation. The dose of 300 μg/mL Zn is consistent with the dose used in the animal experiment to antagonize Cd-induced malformation[2]. Therefore, it is very necessary to select the proper dose of zinc preparation to get best effect but low toxicity.

The antagonistic mechanism of Zn against Cd-induced liver damage is that one of the important biological functions of Zn in the organism is to induce the formation of metallothionein (MT). Taking zinc before Cd toxication greatly increases the synthesis of MT in the organism. Since Cd has greater power to synthesize Cd-MT, it will replace Zn in Zn-MT, form stable Cd-MT and reduce the concentration of free Cd[1]. Furthermore, taking zinc ahead of time can decrease the quantity of the toxic Cd-high molecular weight protein[3], so the ultrastructure of hepatic cells can be nearly normal. Another biological function of zinc is to protect cells so that they may divide and reproduce in accordance with physiological process[4].

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Protective effect of various doses of selenium on cadmium-induced hepatic cell damage in pregnant rats

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Abstract

AIM: To investigate the protective effect of various doses of selenium (Se) on cadmium (Cd)-induced hepatic cell damage in pregnant rats.

METHODS: The changes in the microstructure and ultrastructure of hepatic cells were observed by microscopy and electron microscopy.

RESULTS: Groups of 16 μg/mL and 8 μg/mL Se had no remarkable protective effects on Cd-induced hepatic cell damage in pregnant rats, while the microstructure and ultrastructure of hepatic cells in the 4 μg/mL Se group were nearly normal.

CONCLUSION: The results show that the antagonism of Se on Cd-induced hepatic cell damage in pregnant rats is related to the dose of Se given. The addictive action of Se and Cd toxicity was found in group B, but group D showed significant protection against Cd-induced damage of hepatic cells.

Key words: Selenium; Cadmium; Liver pathology; Rats; Inbred strain

Han GA, Jiang HM, Zhen XM, Zhou HJ, Xu CZ, Tian JX. Protective effect of various doses of selenium on cadmium-induced hepatic cell damage in pregnant rats. *World J Gastroenterol* 1996; 2(4): 192-193 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/192.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.192

INTRODUCTION

Selenium (Se) is able to inhibit the toxicity of cadmium (Cd). For instance, it is able to antagonize Cd-induced malformation and absorption[1], but this is related to the way, the dose and the time that Se is given. The safety range of Se is very narrow. To investigate the protective action of various doses of Se taken orally on the day of gestation on Cd-induced hepatic cell damage in pregnant rats, and to select the effective and safe dose of Se, we performed this study.

MATERIALS AND METHODS

Materials

Sixty Wistar rats weighing 220-250 g were provided by the Experimental Animal Center of Shandong Medical University with a sex ratio of two females to one male. Na_2SeO_3 (AR grade) was used, and the rest materials were in the same way as described in the preceding paper.

Methods

Groups B, C and D were given various doses of Se. The rats in these groups were given water containing 16 μg/mL, 8 μg/mL and 4 μg/mL Se, respectively. The rest was dealt with in the same way as that in the preceding paper.

RESULTS

Changes in the microstructure of hepatic cells

The results of groups A and E were the same as those in the preceding paper. Group B revealed edema of hepatic cells, sporadic acidophilic degeneration, fragmented hepatic cells and bridging necrosis. The infiltration of lymphocytes was found in the necrotic

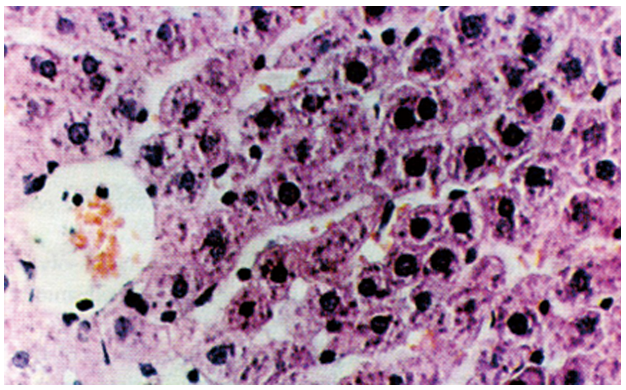

Figure 1 4 μg/mL Se group. Hepatic cells in the mid-lobular area show slight edema, with necrosis of a few hepatic cells visible. (× 600)

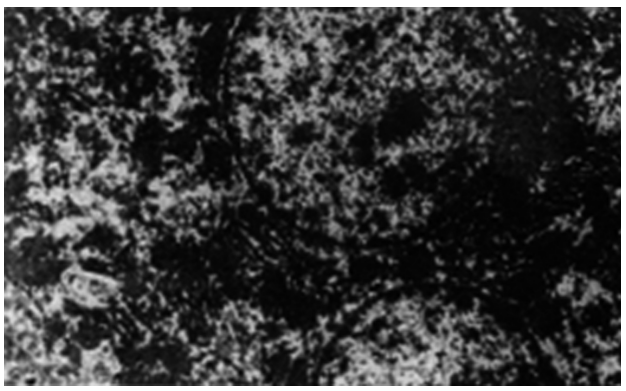

Figure 2 4 μg/mL Se group. The karyotheca of the hepatic cell is clear and regular, double nuclei are visible, most of membrane and mitochondria are clear, and the endoplasmic reticulum is normal. (× 16000)

area, which caused the disturbance in the hepatic lobular structure. In group C, hepatic cells showed edema and most of the lobular necrosis foci were visible in the mid-lobular area with infiltration of lymphocytes. In group D, hepatic cells in the mid-lobular area showed slight edema and necrosis of hepatic cells was rarely visible (Figure 1).

Changes in the ultrastructure of hepatic cells

In group B, the hepatic cells were dissolved with a lot of vacuoles, the karyotheca of the hepatic cell was not clear, mitochondria swelled greatly, cristae vanished and endoplasmic reticulum expanded seriously; in group C, the karyotheca of hepatic cell was irregular, few vacuoles were visible in the cytoplasm, parts of mitochondria degenerated and endoplasmic reticulum expanded slightly; in group D, the karyotheca of the hepatic cells was clear and regular, double nuclei were visible, the composition of subcellular structures in the cytoplasm was basically normal, most of the membrane and cristae of mitochondria were clear and the endoplasmic reticulum was normal (Figure 2).

DISCUSSION

In the organism, Se has antagonism against the toxicity of Cd, mercury (Hg) and arsenic (As). Gunn *et al* found that among the few known substances having antagonistic action on Cd-induced testicle damage, such as Zn, cobalt (Co), BAL and cysteine, Se has the most effective protective action. Its effect is 100 times more than that of Zn[2,3]. The mitochondrium is a sensitive and most easily damaged cell organ and it can exhibit the severity of cell damage. The order of mitochondrial damage severity for all groups is: B > A > C > D. Group B exhibited an addictive effect of Se and Cd toxicity, while group D showed basically normal microstructure and ultrastructure of liver cells, which suggests that a low dose of Se had remarkable protective action on Cd-induced damage of hepatic cells. However, since the safety range of Se is rather narrow, it is extremely necessary to make further selection of a highly effective, low toxic and safe dose of Se. The antagonistic mechanism of Se on Cd-induced liver damage is as follows. First, Se can cause Cd to be redistributed in tissues and cells[1-3]. Taking Se 4 μg/mL orally could greatly lower Cd contents in the liver, kidney and blood of the pregnant rats by 50.3%, 53% and 78.03%, respectively, in comparison with the Cd-toxication group[1]. Second, taking Se orally at a low dose on the day of pregnancy has made Se in the rat body remain in normal equilibrium. At first, SeO_3 is metabolized into selenide; when Cd comes into the body, Se in selenide form can combine Cd to form a compound, which decreases the concentration of free Cd and reduces Cd toxictiy[1,2]. Finally, taking Se 4 μg/mL orally can make the activity of GSH-Px enzyme in the liver, kidney and blood significantly higher than that in the Cd-toxic group and control group[1,4,5], and it can also inhibit Cd-induced lipid peroxidation and effectively clean up free radicals.

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Purification and activity of human fetal hepatic stimulating substance

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Abstract

AIM: To purify human fetal hepatic stimulating substance (hHSS) and to determine its activity.

METHODS: hHSS was purified by ion exchange chromatography, nondenaturing polyacrylamide gel electrophoresis (PAGE), and ISCO (*In situ* chemical oxidation) concentrator electrophoresis. The molecular weight was determined by sodium dodecyl sulfate (SDS)-PAGE and the activity of hHSS was examined by both ^{3}H-thymidine incorporation assay and enzyme linked immunosorbent assay (ELISA).

RESULTS: The purity of hHSS was increased along with the purification process. After many steps, the major band of nondenaturing PAGE showed a high activity and purity, and two bands were revealed by SDS-PAGE.

CONCLUSION: The molecular weight of hHSS is about 70 kDa, and it consists of two subunits with molecular weights of 18 kDa and 53 kDa, respectively. The activity of hHSS was assayed by both ^{3}H-TdR incorporation assay and ELISA, and good consistence was seen.

Key words: Liver stimulating; Substance; Molecular weight

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INTRODUCTION

Hepatic stimulating substance (HSS) stimulates specifically DNA synthesis in liver cells both *in vivo* and *in vitro*[1]. It has organ specificity but no species specificity. Although HSS purification has been studied using rat livers, only crude human fetal hepatic stimulating substance (hHSS) was used for clinical treatment and research. The purification of human HSS remains to be further developed. In this study, hHSS was purified to near homogeneity by ion exchange chromatography, nondenaturing polyacrylamide gel electrophoresis (PAGE) and ISCO (*In situ* chemical oxidation) concentrator electrophoresis.

MATERIALS AND METHODS

Animals

Kunming mice were purchased from the Department of Experimental Animal of China Medical University.

Instruments and reagents

RC5C centrifuge was purchased from Dupont Company, United States; 2023 MinicoldLab from LKB Co., Sweden; and UV-260 spectrophotometer from Shimadzu Co., Japan. Liquid Scintillation system model 1801 was obtained from Beckman Co., United States; EL-309 Microplate Reader from BIO-TEK Co., United States; and ^{3}H-thymidine (^{3}H-TdR) (20 Ci/mmol) from the Institute of Atomic Energy of China. Antibody of hHSS was from the Department of Immunology, General Hospital of Chinese PLA (Nanjing, China). Cell culture reagents and Williams' Medium E were purchased from Sigma Co., United States

Purification of hHSS

A fresh fetal liver (from a mouse aged 5 mo) was excised and immediately homogenized in ice-cold 0.9% NaCl containing EDTA (Ethylenediaminetetraacetic acid) 10 mmol/L and PMSF 0.09 mmol/L with a polytron. Then it was centrifuged at 400 r/min for 20 min. The supernatant was heated at 95 ℃ for 15 min and centrifuged at 45000 *g* for 20 min, then 40% cold ethanol was added to the supernatant and stirred at 4 ℃ for 2 h[1]. After centrifugation, the precipitate was dissolved in 0.02 mol/L Tris-HCl buffer, and centrifuged at 12000 *g* for 20 min.

The supernatant was purified by chromatography on a DEAE-Sephadex A50 ion exchange column in Tris-HCl (0.02 mol/L, pH 7.8) buffer and eluted with a continuous gradient of 0-0.6 mol/L NaCl. The active peak of DEAE-Sephadex A50 was further purified by nondenaturing gradient PAGE. Following the electrophoresis, the major protein band was sliced and homogenized. Elution and concentration were done by the ISCO concentrator. The recovered protein was tested for hHSS activity using both enzyme linked immunosorbent assay (ELISA) and ^{3}H-TdR incorporation assay. The molecular weight was determined by sodium dodecyl sulfate-PAGE (SDS-PAGE).

Table 1 Assay of human fetal hepatic stimulating substance activity by ^{3}H-TdR incorporation method

Sample	Sample volume (μL)	Concentration of protein (mg/mL)	cpm (mean ± SD)	IS	Specific activity (IS/mg protein)	Fold of purification
Control (0.9% NaCl)	25	-	1275 ± 203	-	-	-
Homogenization	25	52.600	3162 ± 329	2.48	1.89	1.00
ETOH-ppt hHSS	25	8.280	3683 ± 401	3.03	14.64	7.74
DEAE A50 peak V	25	3.470	2920 ± 251	2.29	26.40	13.97
Nondenaturing PAGE major band	25	0.169	2039 ± 114	1.60	326.89	172.76

Any value > 1.3 is significant; P = 0.05; Nondenaturing PAGE utilized DEAE-Sephadex A50 peak V as a starting material. Its purity had been improved obviously. hHSS: Human fetal hepatic stimulating substance; PAGE: Polyacrylamide gel electrophoresis; DEAE: Diethylaminoethyl; ETOH: Ethyl alcohol; IS: Index of stimulation.

Table 2 Assay of human fetal hepatic stimulating substance by enzyme linked immunosorbent assay method

Sample	Abs at 630 nm	Abs at 280 nm	Abs at 630 nm/Abs at 280 nm	Fold of purification
Control (0.9% NaCl)	0.077	0	-	-
Homogenization	0.141	2.790	0.051	1.00
ETOH-ppt hHSS	0.159	0.964	0.165	3.24
DEAE A50 peak V	0.284	0.236	1.200	23.52
Nondenaturing PAGE major band	0.197	0.019	10.368	203.29

Abs at 280 nm is absorbent value of samples at 280 nm, which represents the concentration of the samples. Abs at 630 nm represents the hHSS content in samples. hHSS: Human fetal hepatic stimulating substance. ETOH: Ethyl alcohol; DEAE: Diethylaminoethyl; PAGE: Polyacrylamide gel electrophoresis.

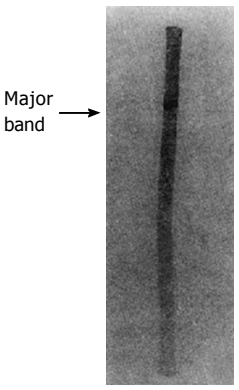

Figure 1 Nondenaturing gradient PAGE. Sample: DEAE-Sephadex A50 peak V.

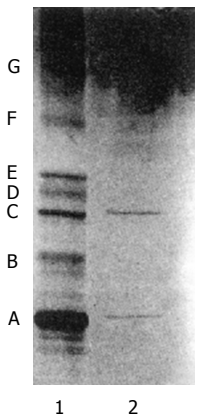

Figure 2 The sample was prepared in 0.1% SDS and SDS-PAGE was run. Silver staining was performed. Lane 1 is molecular weight standards: A = 18 kDa; B = 34 kDa; C = 53 kDa; D = 76 kDa; E = 116 kDa; F = 170 kDa and G = 212 kDa. Lane 2 is major bands of nondenaturing PAGE.

Assay of hHSS activity

^{3}H-TdR incorporation assay: This assay was performed according to the methods described by LaBrecque[5] and Schwarz[6]. An index of stimulation (IS) was then determined as: IS = Count per minute (cpm) for experimental hepatocytes/cpm for control hepatocytes.

Assay of hHSS by ELISA

hHSS- antibody diluted to 1:1000 with coating buffer was added to a 96-well microplate (0.2 mL/well) and kept at 4 ℃ overnight. After absorption, blocking solution was added to the wells at 0.4 mL/well and set at room temperature for 4 h. After the blocking solution was removed, 0.1 mL of sample was added to the coated wells and allowed to react at 37 ℃ for 1 h. After washing 5 times, 0.1 mL of peroxidase-conjugated anti-rabbit IgG secondary antibody was added to the testing wells and incubated at 37 ℃ for 1 h. After washing, the wells were subjected to chromogenic reaction.

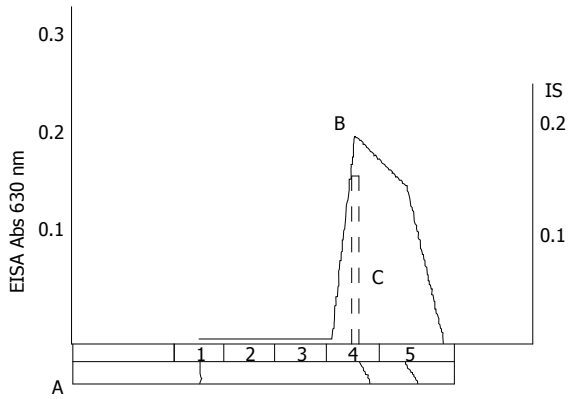

Figure 3 After ISCO concentrator electrophoresis, the activity showed: A: the protein bands on the tube gel; B: the content of hHSS by ELISA method; C: the value of activity (IS) by ^{3}H-TdR incorporation method.

RESULTS

Purification

A combination of the above steps produced an almost 200-fold purification of hHSS. The results of purification are shown in Table 1 (by ^{3}H-TdR incorporation method) and Table 2 (by ELISA method).

Nondenaturing gradient PAGE

Peak V of DEAE-Sephadex A50 was loaded on a nondenaturing gradient polyacrylamide tube gel (Figure 1). After electrophoresis, three bands were shown on the tube gel. Assay of hHSS activity showed that the major band had a very high specific activity.

Determining the molecular weight of hHSS by SDS-PAGE

The major band of nondenaturing PAGE was subjected to SDS-PAGE, and the molecular weight of hHSS was shown as two bands with molecular weights of 18 kDa and 53 kDa, respectively (Figure 2).

DISCUSSION

hHSS is able to initiate and/or stimulate hepatic cell growth and regeneration. Many techniques of purification and assay were studied in many laboratories[1-7], but few were concerned with the purification of hHSS. From the process of isolation, it was shown that large amounts of contaminated protein was removed after heating treatment and ISCO concentrator electrophoresis (Figure 3). SDS-PAGE showed that only two bands with molecule weights of 18 kDa and 53 kDa were found. So the molecular weight of hHSS may be 70 kDa, and it

consists of two subunits with molecular weights of 18 kDa and 53 kDa, respectively.

^{3}H-TdR incorporation assay is a standard method for the assay of HSS activity, but it is difficult to control when a large number of samples are assayed simultaneouly. In 1991, Tsubouchi used ELISA for measuring HSS in blood[7]. In the present study, we utilized ELISA for measuring the hHSS content in every step of the purification, and compared the measured values with those by ^{3}H-TdR incorporation method. The values measured by both methods were basically parallel, and the ELISA method is easier to perform and is suitable for measuring a large number of samples simultaneously.

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Effects of Jiawei Sijunzi Decoction on migrating myoelectric complex in 8.0 Gy irradiated rats

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Abstract

AIM: To investigate the changes of small intestinal migrating myoelectric complex (MMC) and regulatory effect of Jiawei Sijunzi Decoction in 8.0 Gy irradiated rats.

METHODS: The MMC of the intestine of rats was recorded using chronically implanted electrodes at different parts of the small intestine to analyse duration of four phases of MMC and its cycle before and after irradiation and to investigate the regulatory effect of Jiawei Sijunzi Decoction.

RESULTS: From 1 h to 7 d after 8.0 Gy irradiation the MMC cycle in most of rats disappeared and only phase II existed with minute' s rhythm. From 1 h to 3 d after irradiation, the MMC cycle appeared in a few rats with the duration of phase II shortened significantly ($P < 0.05$). Results of observation on effects of Jiawei Sijunzi Decoction on MMC after irradiation showed that the changes in phase and cycle of MMC were normalized basically by medication.

CONCLUSION: Jiawei Sijunzi Decoction can improve the intestinal disturbances caused by radiation, and this might be one of the reasons for its alleviating effect on radiation induced diarrhea.

Key words: Myoelectric complex; Sijunzi Decoction; Radiation, ionizing; Small intestine

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INTRODUCTION

Diarrhea induced by ionizing radiation is one of the gastrointestinal reactions occurring in radiotherapy of tumors (especially tumors of the lower abdomen) and in acute radiation sickness[1]. Investigation of pathophysiology of the motor function of the small intestine during diarrhea bears significance for adopting therapeutic measures. For this reason, we took migrating myoelectric complex (MMC) of the small intestine as an index to study its change during diarrhea induced by irradiation with ^{60}Co γ-rays and the regulatory effect of Jiawei Sijunzi Decoction on it.

MATERIALS AND METHODS

Materials

Sixteen Wistar male and female rats, aged 10-12 wk and weighing 200-240 g, were provided by the Animal Center of the Academy of Military Medical Sciences.

Jiawei Sijunzi Decoction is composed of Radix Astragali seu Hedysari, Radix Codonopsis Pilosulae, Poria, Rhizoma Atractylodis Macrocephalae, Radix Glycyrrhizae, Pericarpium Citri Reticulatae, and Semen Cuscutae. The decoction was prepared by adding the above-mentioned herbs in proportion to water for boiling, then dry extract powder was made from the decoction (1 g of the powder equivalent to 5 g crude drugs). The powder was dissolved in distilled water for use in experiments.

Methods

One pair of needle-shaped electrodes made of platium wire were implanted each in the muscular layer of the duodenum, jejunum, and ileum of a rat narcotized by conventional method used in this laboratory[2]. Seven days later the experiment was begun to be carried out.

MMC of the small intestine of normal rats was first recorded, then the animal was irradiated with 8.0 Gy of ^{60}Co γ-rays (dose rate, 104.8 r/min). The irradiated rats were randomly divided into a treatment group (n = 7) and a control group (n = 9). Jiawei Sijunzi Decoction was administered intragastrically (1.6 g/kg) to rats of the treatment group immediately and on days 1, 2, and 3 after irradiation, once daily. Equal amount of distilled water was given intragastrically to rats of the control group. MMC of the small intestine was recorded on the day of irradiation and on days 1, 3, 5, and 7 after irradiation. Recording was done after fasting for 16-24 h and lasted 60 min each time. The instrument used was a Model RM-6000 Multichannel Recorder. The time constant was 0.01 s, and the frequency of filtered wave was 10 Hz. Data of the same group

Table 1 Effect of Jiawei Sijunzi decoction on various phases and duration of cycle of duodenal migrating myoelectric complex of rats irradiated with 8.0 Gy (min, mean ± SD)

Group	Time		No. of rats	MMC				Duration of cycle
				I	II	III	IV	
Control	Before irradiation		9	3.8 ± 0.2 (4)	15.5 ± 4.7 (33)	2.6 ± 0.7 (27)	0.6 (1)	11.9 ± 4.5 (21)
	After irradiation	1 h	9 [3]	2.4 ± 0.9 (6)	6.3 ± 2.7 (20)*	3.1 ± 0.6 (19)	0.6 ± 0.3 (3)	9.4 ± 1.8 (5)
		1 d	9 [1]	-	14.7 ± 2.7 (4)	1.7 ± 0.7 (3)	-	18.4 ± 1.8 (2)
		3 d	7	60 (1)	60 ± 0 (6)	-	-	-
		5 d	3	60 ± 0 (2)	60 (1)	-	-	-
		7 d	1	-	60 (1)	-	-	-
Treated	Before irradiation		6	1.6 ± 0.24 (4)	9.8 ± 2.8 (29)	2.4 ± 0.5 (26)	0.6 ± 0.3 (3)	10.4 ± 3.6 (20)
	After irradiation	1 h	6 [3]	-	21.2 ± 17.4 (7)	2.8 ± 0.6 (6)	-	9.8 ± 23 (3)
		1 d	6	60 (1)	60 ± 0 (5)	-	-	-
		3 d	6 [2]	-	37.5 ± 27.6 (5)[a]	1.7 ± 0.1 (4)	-	15.6 ± 1.8 (2)
		5 d	6 [2]	-	26.7 ± 11.2 (2)[a]	2.1 ± 0.6 (2)	-	37.0 ± 4.5 (2)[b]
		7 d	1		60 (1)	-	-	-

Note: Compared with pre-irradiation: [a]$P < 0.05$, [b]$P < 0.01$; [ ]: No. of animals showing MMC cycle; ( ): Appearance time; MMC: Migrating myoelectric complex.

Table 2 Effect of Jiawei Sijunzi decoction on various phases and duration of cycle of jejunal migrating myoelectric complex of rats irradiated with 8.0 Gy (min, mean ± SD)

Group	Time		No. of rats	MMC				Duration of cycle
				I	II	III	IV	
Control	Before irradiation		9	3.2 ± 1.3 (11)	10.0 ± 4.2 (41)	3.1 ± 0.5 (35)		15.8 ± 5.8 (26)
	After irradiation	1 h	9 [3]	2.5 ± 0.4 (17)	3.5 ± 0.9 (20)[a]	3.9 ± 1.0 (18)	0.6 (1)	8.8 ± 1.3 (15)
		1 d	9 [7]	3.1 ± 0.9 (16)	11.9 ± 7.4 (31)	2.7 ± 0.7 (26)		12.7 ± 1.9 (20)
		3 d	6	60 (1)	60 ± 0 (5)	-	-	-
		5 d	3	-	60 ± 0 (3)	-	-	-
		7 d	1	-	60 (1)	-	-	-
Treated	Before irradiation		7	2.5 ± 1.0 (15)	9.5 ± 4.2 (29)	2.9 ± 0.6 (29)	0.6 ± 0.2 (5)	14.5 ± 5.1 (26)
	After irradiation	1 h	7 [3]	2.4 ± 0.6 (6)	14.7 ± 12.4 (11)	2.8 ± 0.5 (10)	0.5 ± 0.2 (5)	13.0 ± 5.5 (8)
		1 d	7 [4]	1.8 ± 0.1 (6)	14.5 ± 9.1 (16)	2.3 ± 0.4 (15)	0.9 ± 0.3 (2)	14.4 ± 6.8 (11)
		3 d	6 [2]	-	18.8 ± 12.3 (7)	2.2 ± 0.4 (6)	-	12.0 ± 5.2 (4)
		5 d	6 [1]	2.9 ± 1.3 (2)	9.9 ± 3.7 (4)	3.3 ± 0.3 (4)	-	13.4 ± 1.5 (3)
		7 d	1		60 (1)	-	-	-

Note: Compared with pre-irradiation: [a]$P < 0.05$; [ ]: No. of animals showing MMC cycle; ( ): Appearance time; MMC: Migrating myoelectric complex.

Table 3 Effect of Jiawei Sijunzi decoction on various phases and duration of cycle of ileal migrating myoelectric complex of rats irradiated with 8.0 Gy (min, mean ± SD)

Group	Time		No. of rats	MMC				Duration of cycle
				I	II	III	IV	
Control	Before irradiation		5	3.5 ± 1.2 (2)	25.2 ± 5.3 (12)	5.8 ± 1.7 (7)	0.7 (1)	23.0 ± 11.9 (5)
	After irradiation	1 h	5 [2]	-	7.8 ± 1.2 (11)	3.0 ± 1.1 (9)	-	10.2 ± 5.2 (6)
		1 d	5 [2]	5.6 ± 1.7 (2)	24.3 ± 2.2 (4)	4.1 ± 0.2 (4)	-	32.0 ± 6.3 (2)
		3 d	4	-	60 ± 0 (4)	-	-	-
		5 d	3	60 (1)	60 ± 0 (2)	-	-	-
		7 d	1	60 (1)	-	-	-	
Treated	Before irradiation		3	0.8 (1)	20.5 ± 6.2 (8)	3.2 ± 0.3 (7)	0.8 ± 0.2 (2)	29.4 ± 11.9 (5)
	After irradiation	1 h	3 [1]	-	18.4 ± 16.1 (3)	2.8 ± 0.5 (2)	-	13.1 (1)
		1 d	3 [2]	13.2 ± 8.8 (2)	18.3 ± 7.8 (4)	3.3 ± 0.7 (2)	-	35.2 ± 2.9 (2)
		3 d	3 [1]		26.0 ± 0.1 (2)	3.1 ± 0.1 (2)	-	28.9 (1)
		5 d	1 [1]	-	18.1 ± 12.5 (3)	2.5 ± 0.1 (2)	-	15.3 (1)
		7 d	1	60 (1)	-	-	-	-

[ ]: No. of animals showing MMC cycle; ( ): Appearance time; MMC: Migrating myoelectric complex.

were subjected to *t*-test.

RESULTS

The effects of Jiawei Sijunzi Decoction on small intestinal MMC of rats irradiated with 8 Gy are shown in Tables 1-3. Compared with pre-irradiation, the rat small intestine of the control group exhibited an excitatory state during the first three days after irradiation, which was manifested as follows: (1) The cyclic pattern of small intestinal MMC disappeared and was replaced by phase II in the majority of rats; (2) In isolated rats showing MMC cycle, time duration of phase II was significantly reduced ($P < 0.05$) and the number of cycles increased; and (3) Appearance of minute's rhythm in the phase II occurred in some rats. All MMC cycles disappeared beginning from the third day after irradiation in the survived rats and only phase I or II remained. At that time diarrhea occurred in most rats with an incidence of 93%. In rats of the treatment group, the small intestine also showed an excitatory state similar to that of the control group during the first 2 d after irradiation. However, starting from the third day, MMC cycle still appeared in a part of survived rats. Besides the phase II of the duodenum was still different from that of preirradiation ($P < 0.05$), all other parameters were normalized. The number of rats suffering from diarrhea was remarkably reduced (incidence, 60%).

DISCUSSION

MMC of the small intestine is composed of four phases, among which phase II can effectively transport the content of the small intestine into the large intestine. When the duration of phase II prolongs too much, water and nutrients in the small intestine cannot be absorbed sufficiently and are pushed into the large intestine. Too much water entering lager intestine will readily induce diarrhea. Phaes III has the action similar to that of an "house-keeper". It

contracts the small intestine violently with certain intervals of time to clean up thoroughly the food, secreted substances, and exfoliated cells remaining in the small intestine, and pushes them into colon. Lack of phase III wave will facilitate hyper-proliferation of bacteria in the small intestine, and thus diarrhea occurs. Duration of phase II of rat small intestine MMC prolonged evidently and phase III disappeared starting from the 3rd day after irradiation with 8.0 Gy. These could be the causes of occurrence of diarrhea. The mechanism for obvious disturbance in small intestinal MMC, especially disappearance of phase III is not yet clarified. It could be related with damage to smooth muscle cells caused by radiation to change their electrophysiological activities, or disorder in vegetative nerve function induced by radiation[3].

After treatment of 8.0 Gy-irradiated rats with Jiawei Sijunzi Decoction, MMC cycle of the small intestine reappeared starting from the 3rd day. Except that there was still difference in phase II of the duodenum from that before irradiation, all other parameters had been normalized and the number of rats suffering from diarrhea evidently was reduced. This finding indicates that: (1) Jiawei Sijunzi Decoction can promote normalization of the small intestine lacking in MMC and regulate motor function of the small intestine; and (2) Reduction of the number of diarrheal rats supports indirectly the concept of phase III of MMC has house-keeper function and lacking of it can induce diarrhea[4]. The regulatory action on small intestinal MMC by prescriptions having the function of strengthening the spleen and stomach such as Jawei Sijunzi Decoction could be related to acetylcholine receptors in the intestinal tract or they could act as the antagonists against M1 receptor or as the agonists for the M2 receptor as reported in the literature[5]. Further studies are needed to clarify these.

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Changes of neurotensin and endotoxin in rats with intestinal ischemia

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Abstract

AIM: To investigate the changes of neurotensin (NT) and endotoxin in rats with segmental intestinal ischemia.

METHODS: The distal ileal mesenteric arteries in rats were ligated to make segmental intestinal ischemia models. At the 2nd, 6th and 12th hours after intestinal ischemia, endotoxin levels in portal blood were tested by limulus lysate test and NT levels in plasma from the heart and in intestine tissues (ischemia and peri-ischemia areas) were assayed by radioimmunoassay. Histological changes of the mucosa were examined under light and electron microscopes.

RESULTS: NT levels decreased significantly in intestinal ischemia and peri-ischemia areas (34.07 ± 5.93 *vs* 40.14 ± 5.38, $P < 0.05$; 7.47 ± 1.38 *vs* 40.14 ± 5.38, $P < 0.01$), especially lower in ischemia area (34.07 ± 5.93 *vs* 7.47 ± 1.38, $P < 0.05$. However, NT level increased obviously in plasma (0.76 ± 0.16 *vs* 0.47 ± 0.10, $P < 0.05$). Levels of endotoxin elevated obviously in portal blood (389.0 ± 105.0 *vs* 55.1 ± 6.7, $P < 0.01$), and the mucosa was injured both in ischemia and peri-ischemia areas.

CONCLUSION: Intestinal ischemia injures intestinal mucosa and leads to decrease of intestinal NT level, which is accelerated by endotoxemia and increase of blood NT level.

Key words: Neurotensin; Endotoxin; Intestinal ischemia

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INTRODUCTION

Intestinal ischemia may cause impairment of gut barrier and leads to systemic dissemination of bacteria and endotoxin from the gastrointestinal (GI) tract, which is called bacterial translocation[1]. The GI mucosa is a complicated multifunctional tissue that not only serves as the major site of digestion and absorption of nutrients, but also has metabolic, endocrine and immune functions, all of which play important roles in gut barrier. In order to study the endocrine changes in bacterial translocation, we adopted the model of segmental intestinal ischemia to investigate changes of endotoxin in portal blood and neurotensin (NT) in plasma and intestinal tissues.

MATERIALS AND METHODS

Animals

Ninety SD rats were acclimated for 1 wk and fed standard rat chow ad libitum. After acclimation, the rats were weighed (300-350 g) and randomly assigned into six groups ($n = 15$, each): intestinal ischemia for 2 h, 6 h, and 12 h (experiment groups) and corresponding controls (2, 6, and 12 h). The distal ileal mesenteric arteries of rats in control groups were not ligated.

Animal models and sample processing

After being fasted overnight with free access to water, the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (30 mg/kg body weight). The abdomen was opened and 2-3 distal ileal mesenteric arteries were ligated to make a 20 cm intestinal ischemia area, then the abdomen was closed. The rats were allowed to stay for 2, 6 and 12 h according to each group before undergoing the second stage of surgical procedure.

After anesthesia, the abdomen was reopened and the samples were taken in the following procedure: (1) Blood samples of 5-6 mL from the heart were collected in tubes containing trasylol (200 U each), and the plasma was immediately separated by centrifugation, then was frozen and stored at -20 ℃ until analysis; (2) Portal blood (2 mL) was collected in heparinized tubes; (3) The small intestine tissues (both in ischemia and peri-ischemia aresa) were removed, rinsed, weighed, and then were put into a tube with boiling water (W/V = 1/2). The tube was plunged into vigorously boiling water for 10 min, then it was cooled down and homogenized for 10 min. After centrifugation at 3000 r/min for 5 min, the supernatant was saved and stored at -20 ℃ until assay; and (4) Samples of the small intestine for histological study were taken immediately in the same parts stated above. The tissue from the ischemia area for light microscopy was fixed in 10% buffered formalin and embedded in

Table 1 Changes of NT in plasma (10^{-3} mg/L, mean ± SD)

Group	2 h	6 h	12 h
Control	0.45 ± 0.08	0.49 ± 0.09	0.47 ± 0.10
Experiment	0.74 ± 0.08[a]	0.81 ± 0.18[a]	0.76 ± 0.16[a]

[a]$P < 0.05$, *vs* control.

Table 2 Changes of NT in intestinal tissues (10^{-3} mg/L, mean ± SD)

Group	2 h	6 h	12 h
Control	39.38 ± 5.80	38.94 ± 4.76	40.14 ± 5.38
Ischemia	11.71 ± 1.33[bd]	6.23 ± 0.79[bd]	7.47 ± 1.38[bd]
Peri-ischemia	33.25 ± 3.70[a]	32.42 ± 4.10[a]	34.0 ± 5.93[a]

[b]$P < 0.01$, *vs* control; [a]$P < 0.05$, *vs* control; [d]$P < 0.01$, *vs* peri-ischemia.

Table 3 Changes of endotoxin in portal blood (10^{-3} mg/L, mean ± SD)

Group	2 h	6 h	12 h
Control	50.4 ± 8.2	54.3 ± 9.8	55.1 ± 6.7
Experiment	297.0 ± 47.0[a]	336.0 ± 97.0[a]	389.0 ± 105.0[a]

[a]$P < 0.01$, *vs* control.

paraffin, then sections (4 μm) were stained with hematoxylin and eosin. Samples from the peri-ischemia area for electron microscopy was fixed in phosphate-buffered glutaradehyde (2.5%) and osmium tetroxide (1%), dehydration of the mucosa was accomplished with acetone solutions of increasing concentrations. The tissue was embedded in epoxy resin. Semithin (1 μm) sections through the mucosa were then cut and stained with toluidine blue. Then 600 angstrom-thin sections were made from a selected area of tissue defined by the semithin section, and these sections were stained with lead citrate and uranyl acetate.

Morphologic observation and tissue assay

Histological changes were observed with a light microscope in ischemia area and with an electron microscope in peri-ischemia area. NT levels in plasma and intestine tissue were determined by radioimmuno assay[2]. Endotoxin level in portal blood was assayed by limulus lysate test.

Statistical analysis

Data are expressed as mean ± SD and analyzed by Student's *t*-test. *P*-values < 0.05 were considered significant.

RESULTS

Histological features

Histological features were observed with a light microscope in ischemia area. Two hours after intestinal ischemia, the mucosal villi denuded, part of which degenerated and necrosed. Six hours later, the whole layer of the mucosa necrosed. Twelve hours later, the mucosa was digested and its construction disappeared. Microvilli observed had edema and part of them lifted down, and the degree of microvillus damage was positively correlated with the time of intestinal ischemia. No similar changes of the mucosa were found in the control groups.

Changes of NT in plasma

The NT levels in plasma are shown in Table 1. In the intestinal ischemia groups, statistical differences were found when compared with control groups ($P < 0.05$). The plasma NT level elevated obviously during intestinal ischemia.

Changes of NT in ischemia and peri-ischemia areas

The NT values in ischemia and peri-ischemia areas are summarized in Table 2. In peri-ischemia area, the NT values were significantly lower than those in the control groups ($P < 0.05$). In ischemia area, significant differences were found when compared with values of the control groups ($P < 0.01$) and peri-ischemia area ($P < 0.01$). NT values in ischemia area decreased and were even lower than those in peri-ischemia area.

Changes of endotoxin in portal blood

As shown in Table 3, endotoxin in portal blood increased obviously ($P < 0.01$) during intestinal ischemia.

DISCUSSION

This study demonstrates that NT levels increased obviously in plasma and decreased in intestinal tissues (ischemia and peri-ischemia areas) with particularly low level in ischemia area, levels of endotoxin elevated significantly in portal blood, and mucosa was injured in ischemia and peri-ischemia areas.

NT is a tridecapeptide originally isolated from bovine hypothalamus and is subsequently found to be distributed widely in the GI. NT-like immunoreactivity has been localized to a specific mucosal endocrine cell type (N cells), which is found in the highest density in the ileum, and in lower densities in the duodenum, jejunum and colon. From the duodenum to ileum, the level of NT elevates gradually. Because N cells are impaired by intestinal ischemia, NT level in ischemia area will decrease; but decrease of NT level in peri-ischemia area is related to endotoxemia, because a large amount of endotoxin from the GI entered blood due to impairment of gut barrier. Endotoxin can cause constriction of visceral blood vessel, and impair intestinal epithelial cells, including N cells.

Elevation of NT in plasma may be related to: stress caused hypersecretion of adrenal medulla; and entering of NT into bloodstream from the ruptured N cells.

In conclusion, intestinal ischemia injures the intestinal mucosa and leads to a decrease of intestinal NT accelerated by endotoxemia. NT has several protective functions on the gut barrier. It can stimulate small bowel and colonic mucosal growth[3,4], and affect different aspects of immune function by regulating neuroendocrine immune axis. For example, NT stimulates the chemotactic properties of leukocytes[5], augments phagocytosis by macrophages and neutrophils[6], and maintains the secretion of IgA in the GI[7]. Increase of blood NT during intestinal ischemia may benefit the gut barrier. Further studies are required to evaluate the significance of NT level changes in the intestine and plasma.

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Effects of electro-needling at Zusanli on stress gastric ulcer in rats: Changes in nitric oxide and catecholamine

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Abstract

AIM: To investigate the effects of electro-needling at Zusanli (ST36) on changes of nitric oxide (NO), dopamine (DA) and norepinephrine (NE) in rats with stress gastric ulcer.

METHODS: By biochemical methods the changes in contents of NO, DA and NE in gastric antral mucosa, corporal ventriculi mucosa and in blood after electro-needling (EN) were analysed. For observation all animals were randomly divided into four groups: stress group, EP after stress group, stress after EP group and control group.

RESULTS: NO content in serum of stress gastric ulcer rats (5.78 ± 1.49 μmol/L) was significantly decreased, as compared with the control group (13.30 ± 2.75 μmol/L; $P < 0.01$). DA content in gastric antral mucosa was markedly decreased (3.31 ± 0.67 ng/mg *vs* 6.78 ± 4.65 ng/mg, $P < 0.05$). DA in corporal ventriculi mucosa showed a tendency to increase. EN at Zusanli made NO level recover in stress gastric ulcer rats (7.91 ± 1.11 μmol/L), as compared with the stress group ($P < 0.01$). EN regulated DA and NE contents in antrum and corpora ventriculi mucosa bi-directionally, *i.e.*, the contents were decreased when they formerly rose, and increased when formerly fell, respectively, as compared with the stress group ($P < 0.01$).

CONCLUSION: EN has a protective effect on the gastric mucosa. EN bi-directionally regulates DA and NE, and the vascular relaxation partially brought about by NO regulates the gastric mucosal blood flow, and strengthens the defensive function of the gastric mucosa.

Key words: Nitric oxide; Dopamine; Norepinephrin; Zusanli; Electro-needling; Gastric ulcer

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INTRODUCTION

Clinical practice has proved that acupuncture is effective in the treatment of gastrointestinal diseases, for which Zusanli is always first selected, but its mechanism is not yet clear completely. It is known that nitric oxide (NO) plays an important role in gastrointestinal physiology and pathology, while dopamine (DA) and norepinephrine (NE) serve as important neuro-transmitters in the gastrointestinal tract[1]. How these bioactive molecules exert their action in the course of electro-needling (EN) has not been reported. In this study, we investigated the changes of contents of NO and catecholamines (DA and NE) in stress gastric ulcer rats after EN at Zusanli.

MATERIALS AND METHODS

Model of stress gastric ulcer

Healthy male Wistar rats, weighing 160-230 g, were used to develop a model of stress gastric ulcer using restraint-cold method. The damaged gastric mucosa showed spotty or patchy hemorrhagic necrosis. The gastric antral mucosa and corpora ventriculi mucosa samples were taken, absorbed out of water, weighed, and stored in a freezer (-20 ℃) until analysis. The serum samples were taken from eyeball blood.

Experimental groups

All rats were randomly divided into four groups: stress group, EN after stress group, stress after EN group and control group.

EN method

In the experiments, EN at Zusanli was applied to both sides of the rat using Model PCG-1 acupuncture therapy instrument, with a frequency of 10 Hz, stimulus strength of 10-20 V up to trembling of double lower limbs, totally given for 10 min.

Assay of biochemical indexes

Using the method described by Green *et al*[2], the contents of NO in serum were assayed with a 752 model ultraviolet grating spectrophotometer. The contents of DA and NE were assayed with

Table 1 Effects of electroneedling on contents of nitric oxide, dopamine and norepinephrine in stress gastric ulcer rats (serum: μmol/L; tissue: ng/mg; wet weight)

	Control		Stress		EN after stress		Stress after EN	
	n	Content	*n*	Content	*n*	Content	*n*	Content
NO	7	13.30 ± 2.75	6	5.78 ± 1.49[b]	6	42 ± 1.31	6	7.91 ± 1.11[c,b]
DA								
Gastric antrum	9	6.78 ± 4.65	9	3.31 ± 0.67[a]	6	4.78 ± 0.89[d]	6	3.14 ± 0.80
Corporal ventriculi	9	3.70 ± 1.62	9	4.66 ± 1.71	6	3.24 ± 0.77	6	2.33 ± 0.92[d]
Serum	6	10.32 ± 3.25	6	6.94 ± 4.15	6	4.74 ± 2.59[b]	3	5.06 ± 1.95[a]
NE								
Gastric antrum	9	6.33 ± 3.77	9	6.63 ± 1.72	6	7.11 ± 4.46[c]	6	3.14 ± 0.80
Corporal ventriculi	9	2.65 ± 1.51	9	3.73 ± 1.81	6	2.03 ± 1.52	6	2.05 ± 1.19
Serum	6	2.84 ± 0.88	6	2.72 ± 1.00	6	2.07 ± 1.18	3	2.60 ± 1.42

[a]$P < 0.05$, [b]$P < 0.01$, *vs* control group; [c]$P < 0.05$, [d]$P < 0.01$, *vs* stress group. NO: Nitric oxide; DA: Dopamine; NE: Norepinephrine; EN: Electroneedling.

a HITACHI MPE-4 model fluorescence spectrophotometer using the method described by Shellenberger *et al*[3]. All values are given as mean ± SD, and the data were statistically analyzed by *t*-test.

RESULTS

The effects of EN on contents of NO, DA and NE in stress gastric ulcer rats are shown in the Table 1.

The NO content in serum of stress gastric ulcer rats showed a significant decrease, as compared with the control group ($t = 5.97$, $P < 0.01$). DA and NE contents in the gastric antrum mucosa showed a tendency to decrease, and the decrease of DA had statistical significance ($t = 2.22$, $P < 0.05$). DA and NE in the corpora ventriculi mucosa showed a tendency to increase.

EN at Zusanli made NO level recover in the stress gastric ulcer rats. The NO level in the stress after EN group was significantly higher compared with the stress group ($t = 2.84$, $P < 0.05$). Although it was still markedly lower than that of the control group, it approached the level of the control group. EN regulated DA and NE contents in the gastric mucosa bi-directionally, *i.e.*, DA in the antral mucosa was decreased when it had a rise formerly, and DA content of the EN after stress group was especially increased, as compared with that in the stress group ($t = 0.68$, $P < 0.01$). DA in the corpora ventriculi mucosa was increased when it decreased formerly, in the stress after EN group it was especially decreased compared with the stress group ($t = 3.04$, $P < 0.01$), all of these were tending to a level of the control group. DA level in serum was continuously decreased in the EN after stress group compared with the control group ($t = 3.20$, $P < 0.01$). The changes of NE in the mucosa were similar to the changes of DA. The level of NE in serum had not changed basically.

DISCUSSION

In long time practice, clinical acupuncturists have treated gastrointestinal diseases by using Zusanli as the main acupoint. The data have shown[4-6] that needling at Zusanli has yielded obvious bi-directional function in regulating gastric motivity. It is no doubt related to the nervous and humoral factors. In recent years the study on NO has become a hot point in medical investigation. Because NO functions as a vasodilator, it acts on regulating the gastric mucosal blood flow and maintaining the mucosal integrity and defense. It is closely related to the protection of the gastric mucosa.

In this study we observed the changes of NO, DA and NE in stress gastric ulcer rats resulting from the damage to the gastric mucosa. DA has drawn more attention as a gastrointestinal neurotransmitter in recent years[7]. A decrease of DA contents in antral mucosa caused a decrease of blood flow and brought about poor perfusion to tissues, so that gastric ulcer occurred. DA produced NE through the action of dopamine-β-carboxylase, while the decrease of DA content led to a corresponding change of NE. NE is one of the agents that induce endotheliocytes to release NO[8]. When the gastric mucosa is damaged, the release of endogenous NO was decreased and normal biochemical metabolism was obstructed. It was disadvantageous for maintaining vessels in a relaxation state. The mechanism of DA to regulate the blood flow of the mucosa suggests that acting on DA_1 receptor in peripheral vascular wall should directly cause the expansion of blood vessels, and that, stimulating DA_2 receptor at sympathetic nerve ending should decrease the release of NE and increase the blood flow. The increase of DA content in the corpora ventriculi mucosa in the ulcer rats may possibly be a factor strengthening the gastric capacity for relaxation, on the other hand, may possibly be a factor yielding actions on different receptors. Therefore, contents of catecholamines (DA and NE) in the gastric antrum and corpora ventriculi showed different change tendencies.

The DA and NE contents in the ulcer rats after EN at Zusanli nearly recovered to normal, which showed a bi-directional regulatory effect. This indicates that acupuncture can protect the gastric mucosa from damage. The effect of acupuncture at Zusanli is closely associated with the nervous system, especially the autonomic nervous system. It may lower down the rising excitation of sympathetic nerve or parasympathetic nerve. Our study showed that the changes of the catecholamine content were consistent with the excitation of the nervous system. It also suggests that the effects of EN at Zusanli are due to the above-mentioned bio-active molecules, and other transmitters such as VIP (intrestinal peptide), ATP (adenosine triphosphate) and GABA (γ-aminobutyric acid) may take part in, but NO may be the final transmitter. From the change of NO, it was revealed that the EN applied before stress was more favourable than that applied after stress in ulcer rats. It further confirmed the protection of EN for the gastric mucosa.

In this study, DA content in serum of ulcer rats was found still continuously decreased after EN, and the NE in serum did not. It suggests that the damage to the gastric mucosa possibly speeds up the metabolic inactivation of peripheral catecholamines. DA decreased but NE remained in kinetic equilibrium, implying that the formation of NE from DA and the further analytic metabolism of NE were speeded up so as to fit the changes of the body in stress state. EN at Zusanli may give rise to the response of the body to the stress state.

To sum up, EN at Zusanli is able to cause the changes of NO and catecholamines in stress gastric ulcer rats, suggesting that EN is effective in protecting the gastric mucosa. It is regarded that the effect is brought about by the bi-directional regulatory effect of acupuncture on DA and NE, the regulation from DA, the changes of NE levels and the vascular relaxation due to NO, which all in turn regulate the gastric mucosal blood flow, and strengthen the defensiveness of the gastric mucosa.

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Therapeutic effects of rhubarb on gastrointestinal failure

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Abstract

AIM: To investigate the effects of rhubarb on gastrointestinal failure and the underlying pharmaceutical mechanism.

METHODS: Ninety-seven patients in intensive care unit were divided into a treatment group (76 critically ill patients complicated with gastrointestinal failure) and a control group (21 recovered patients). The effects of rhubarb on stress ulcer and toxic paralytic ileus in the patients were observed. The rectal and gastric intramural pH values, cardiac index, oxygen delivery, and oxygen consumption were measured.

RESULTS: Treatment with rhubarb achieved a significant curative effect in 30 of the 36 cases of stress ulcer complicated with gastrointestinal hemorrhage. Ha-2-receptor blocking agent had a poor effect on them ($P < 0.05$). Among the 49 cases of toxic paralytic ileus treated with rhubarb, peristalsis was recovered in 41, and gastrointestinal nutrition could be tolerated in 24, while other medicine had no effect on them. According to gastric and rectal intramural pH, rhubarb could improve gut mucosa perfusion. Among the 23 cases of multiple organ dysfunction syndrome who received treatment with rhubarb, 9 survived.

CONCLUSION: This study suggests that rhubarb has a good curative effect on gastrointestinal failure.

Key words: Rhubarb; Multiple organ failure; Gastrointestinal disease

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INTRODUCTION

Gastrointestinal failure is common in critical illness and potentially influences its pathophysiological process. Bacterial translocation and endotoxemia related to gut barrier damage result in activation of the white blood cell system which can release large amounts of cytokines, and cause systemic inflammatory response syndrome (SIRS) and ultimately, multiple organ dysfunction syndrome (MODS). Prevention of gastrointestinal failure is the key to preventing the occurrence of fatal complications in critical illness. Since 1991, we have been using a combination of western medicine with traditional Chinese medicine to treat gastrointestinal failure and have achieved good curative effects. The aim of the present study was to investigate the therapeutic effects of rhubarb on gastrointestinal failure and the underlying mechanism.

MATERIALS AND METHODS

Subjects

Ninety-seven patients in intensive care unit (ICU) were enrolled. Twenty-one recovered patients were included as controls while the others complicated with gastrointestinal failure were contained in a rhubarb treated group. The diagnoses of gastrointestinal failure and MODS were in accordance with the guidelines set in 1992[1,2].

Devices and materials

Devices and materials included multifunction monitor (Siemens 961, Germany); Swan-Ganze catheter (ARROW CO., United States); ABL-300 blood-gas analyzer (Radiometer CO., Denmark); gas exchange membrane (Fudan University, China); raw rhubarb powder (granted by Rhubarb Lab, Shanghai Xiang Shan Traditional Medical Hospital).

Treatment

Rhubarb was given to patients at a dose of 12 g, 1/6 h, after 12, 24, 48 h. The effects on gastrointestinal bleeding and toxic paralytic ileus were monitored and recorded.

Measurement of intramural pH

Gastric and rectal intramural pH values (pHi) were studied in 43 patients, including the control group (21 cases) and the rhubarb treated group (22 cases, who suffered from stress ulcer). Before treatment, the pHi measurement was performed in all the patients, then rhubarb at a dose of 12 g was given to the patients in the treatment group and the same dose was repeated 6 h later, while the patients in the control group only took the same volume of 0.9%

Table 1 Effects of rhubarb on stress ulcer accompanied with gastric hemorrhage

Group	*n*	Hemostasis	Rebleeding
H_2-RBA	8	2	2
H_2-RBA plus Cold water and NE	12	5	5
Rhubarb	36	30[ae]	6

[e]$P < 0.005$ *vs* H_2-receptor blocking agent group (RBA); [a]$P < 0.05$ *vs* H_2-RBA plus cold water and nonadrenaline (NE) group.

Table 2 Effects of rhubarb on toxic paralytic ileus

Group	*n*	Peristalsis	Diet tolerance
H_2-RBA	27	0	0
Rhubarb	49	41[e]	24[e]

[e]$P < 0.005$ *vs* H_2-RBA. RBA: Receptor blocking agent group.

Table 3 Effects of rhubarb on gastric intramural pH (pHi) (mean ± SD)

Group	*n*	pHi
Pre-treatment	27	7.018 ± 0.117
Control	21	7.335 ± 0.180
Post-treatment	24	7.305 ± 0.095[f]

[f]$P < 0.001$ *vs* pre-treatment.

Table 4 Effects of rhubarb on rectal pHi (mean ± SD)

Group	*n*	pHi
Pre-treatment	27	7.071 ± 0.1860
Post-treatment	19	7.268 ± 0.0785[f]

[f]$P < 0.001$ *vs* pre-treatment.

Table 5 Hemodynamic parameters and gastrointestinal pHi (n = 8, mean ± SD)

Parameter	Before rhubarb treatment
CI (L. min^{-1}/m^2)	5.06 ± 0.73
DO_2 (ML. min^{-1})	977.75 ± 157.35
VO_2 (ML. min^{-1})	375.86 ± 161.34
Gastric pHi	7.02 ± 0.11
Rectal pHi	7.04 ± 0.14

Table 6 Effects of the rhubarb on multiple organ dysfunction syndrome

Involved organs	*n*	Rhubarb treatment		Non-rhubarb treatment	
		Sum	Surviving	Sum	Surviving
2	8	5	4	3	2
3	11	7	3	4	2
4	16	11	2	5	0

sodium chloride instead of rhubarb. The pHi was again measured 12 h after intake of medicine. The method of pHi measurement was in accordance with that established by Le Bo.

Evaluation of effects of rhubarb

The effects on stress ulcer were determined on the basis of occult blood examination (OBE) of gastric contents 48 h after treatment, OBE result changing from ++++ to "++" or from +++ to + represented effectiveness. Negative OBE was regarded as relatively good effects. Effects on toxic paralytic ileus referred to the recovery of gut peristalsis and diet tolerance. Bowel sound was weak or less than 3 times per minute was defined as treatment failure, more than 4 times/min of bowel sound represented effectiveness, and active peristalsis and tolerance of more than 2090 KJ/d of essential diet stood for good response to rhubarb.

Statistical analysis

All values are expressed as mean ± SD. The unpaired Student's *t*-tests were used to compare the quantitative data, and chi-square tests were used to compare the qualitative data. *P*-values less than 0.05 were considered significant.

RESULTS

Thirty-six patients with stress ulcer and gastric bleeding were treated with rhubarb, which was effective in 30 patients and had better effects in 18 patients. Two out of 8 patients received H_2-receptor blocking agent which proved to be effective. However, they all rebled later. Among 12 patients treated with H_2-receptor blocking agent plus cold water containing nonadrenaline, gastric bleeding ameliorated in 5 cases, but rebleeding occurred in all later (Table 1).

Forty-eight patients suffering from toxic paralytic ileus received rhubarb treatment and gut peristalsis recovered in 41 cases. Twenty-four cases could tolerate 2090 KJ/d of essential diet, while 12 patients inflicted with same disorder were treated with H_2-receptor blocking agent, and none of them recovered from toxic paralytic ileus (Table 2).

Twenty-seven samples of the stomach and rectum for measurement of pHi were obtained in 22 patients before rhubarb treatment, including 5 samples collected from 5 patients who were harassed by gastric rebleeding and 24 and 19 samples were collected fter treatment. The results showed that rhubarb had a good curative effect on stress ulcer (Tables 3 and 4).

Hemodynamic monitoring was performed in 8 patients. The hemodynamic parameters revealed that all the patients were in hyperdynamic condition and the cardiac index, oxygen delivery and oxygen consumption were far beyond the normal ranges, meanwhile gastric and rectal intramural pH values were obviously lower (Table 5). These findings mean that hypoperfusion existed in the gut mucosa.

In this study, out of 35 patients suffering from MODS, 23 received rhubarb treatment. Among whom 5, 7 and 11 cases had 2, 3 and more than 4 organs involved, respectively. Four, three and two patients survived for each, respectively (Table 6).

DISCUSSION

Gut failure is common in critical illness, which is characterized by toxic paralytic ileus and stress ulcer usually accompanied with gastric bleeding. According to traditional opinion, pathophysiological basis of the latter is damage of the barrier which is resistant to retroflow of H^+. However, recent studies have proved that stress ulcer not only occurs in the stomach, but in the whole digestive tract from the mouth to rectum. Mucosal blood hypoperfusion is its main pathophysiological mechanism. Stress can result in low basic and maximal secretion of gastric acid and hike of intragastric pH[3]. Change of gastric microecological environment promotes bacterial reproduction, bacterial translocation and endotoxin absorption. Through 5 years of study, we found that the traditional Chinese medicine rhubarb has a good curative effect on gut failure and can protect the gastrointestinal mucosa, and meanwhile has a unique capacity to ameliorate stress ulcer and to relieve toxic paralytic ileus. In this study, 30 out of 36 patients with gastric stress ulcer accompanied with hemorrhage had hemostasis. Among 49 cases of toxic paralytic ileus, peristalsis was recovered in 41 cases, and enteric nutrition could be tolerated in 24 cases, while other medicines had a poor effect on them.

Gut is a sensitive organ to MODS. It is the first organ to be easily damaged under some pathophysiological circumstances. The present study revealed that although the patients were in hyperdynamics, the gastric and rectal intramural pH values were much lower (gastric pHi, 7.018 ± 0.186 *vs* 7.335 ± 0.180 in control, $P < 0.001$), indicating that the blood flow supplied to the gut was compromised and the gut was in hypoperfusion and oxygen defect. Blood redistribution from the gut to the vital organs initiated by the neuro-endocrine system was implicated in the pathogenesis of gut failure[4]. The current research also proved that rhubarb could

markedly improve gastrointestinal ischemia and increase oxygen delivery (gastric and rectal pHi were 7.305 ± 0.095 and 7.268 ± 0.079 respectively). The pharmaceutical effects of rhubarb are conducive to gut recovery from toxic paralytic ileus and protection of gut barrier. This provides an important method to prohibit critically ill patients from fatal complications.

In general, gut communicates with the exterior, and a large amount of bacteria and endotoxin exists in the intestinum crassum, whereas the stomach and intestinum tenue are relatively sterile. However, some pathophysiological factors, such as surgical stress and use of H_2-receptor blocking agent, can damage the gastrointestinal microecological environment, thus causing bacterial colonization and reproduction in the stomach and intestinum tenue, meanwhile bacterial translocation and endotoxin absorption occur, inducing monocyte phagocytic system activation, especially hepatic Kuppfer's cells, which release a large amount of inflammatory factors, resulting in SIRS. Border[5] regarded bacterial translocation and endotoxin absorption as the key factors which result in constant activation of white blood cell system. So the gut is not passively sacrificed, on the contrary, it actively participates in the pathophysiological process of SIRS and MODS. Clinical information showed that the patients inflicted with MODS involving two organs were treated simply, while patients had more than three organs involved were critically ill and difficult to treat. Rhubarb was used to relieve toxic paralytic ileus. Several advantages of rhubarb are as follows: (1) The patients recovering from toxic paralytic ileus can obtain benefits from decreased intraabdominal pressure, which can potentially affect cardiac-pulmonary function; (2) Recovery of gut peristalsis can promote excretion of endotoxin contained in the gut and is conducive to stability of gut microecologic environment; (3) Recovery of enteric nutrition can improve nutritional conditions, upgrade host defense and protect the gut barriers[6]; and (4) Rhubarb can prohibit some bacteria from reproduction[7] and maintain balance of gut flora. We observed that once the gut recovered from toxic paralytic ileus in critically ill patients, their pulmonary and cardiac function could be improved immediately. We once treated 16 patients who suffered from MODS involving more than four organs, among whom 11 cases were treated with rhubarb, two of them survived, which had broken through the deadline that the mortality rate of the patients suffering from MODS involving more than four organs was 100%. The mechanism may be that rhubarb can prevent bacterial translocation and endotoxin absorption, and resolve the pathophysiological basis which results in continuous activation of white blood cell system and SIRS. Animal experiments displayed that rhubarb could prevent bacterial translocation and endotoxin absorption and scavenge oxygen free radicals in hemorrhagic shock models[8,9].

Rhubarb is a kind of traditional Chinese herb, and gut is its main target organ. Its pharmaceutical mechanism is to promote gut peristalsis, protect the gastrointestinal mucosa, improve gut blood perfusion, promote recovery of cellular digestion, secretion and absorption of the gastrointestinal mucosa, and increase tolerance to enteric nutrition.

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EXPERIMENTAL PAPERS

Precancerous lesions in the glandular stomach of Wistar rats induced with dimethylamine hydrochloride and sodium nitrite

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Abstract

AIM: To observe the effects of both dimethylamine hydrochloride and sodium nitrite on the glandular stomach of Wistar rats.

METHODS: Both dimethylamine hydrochloride and sodium nitrite were administered to male Wistar rats at a concentration of 0.06% in the drinking water ad libitum for 56 wks, and the rats were killed subsequently. The sequential histological changes induced in the glandular stomach of the rats by both dimethylamine hydrochloride and sodium nitrite were also observed.

RESULTS: It was found that the rate of intestinal metaplasia in the glandular stomach of rats was 88.9% (40/45), 55.0% (22/40) of which had the sulphomucin-positive intestinal metaplasia. The rates of mild, moderate and severe dysplasia were 44.5% (20/45), 33.3% (15/45), and 22.2% (10/45), respectively. Cystic dilatation of gastric glands was found in 28.9% (13/45). No gastric cancer occurred. The sequential histological changes were also reported.

CONCLUSION: Both dimethylamine hydrochloride and sodium nitrite can induce precancerous lesions of the glandular stomach in Wistar rats and may be one of the pathogenic factors of gastric cancer.

Key words: Dimethylamine hydrochloride; Precancerous lesions; Stomach neoplasms; Sodium nitrite

Li CQ, Liu WW. Precancerous lesions in the glandular stomach of Wistar rats induced with dimethylamine hydrochloride and sodium nitrite. *World J Gastroenterol* 1996; 2(4): 209-211 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/209.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.209

INTRODUCTION

Since the demonstration by Magee *et al*[1] in 1956 of the carcinogenicity of N-nitrosodimethylamine (NDMA), the carcinogenic potential of over 200 N-NCs has been established in all 40 animal species so far tested with varying degrees of susceptibility in different organs including the liver, stomach, oesophagus, intestines, bladder, brain, lung and kidney[2,3]. There is now convincing experimental epidemiological evidence that N-NCs associated with human cancer affect the oral cavity, urinary bladder and oesophagus, and their possible role in the pathogenesis of gastric cancer has also been strongly postulated[4]. Recently NDMA and some other volatile N-nitrosamines and the precursors of N-NCs were detected in fish-sauce by Chinese researchers in the high-risk areas of gastric cancer[5,6]. Lu *et al*[7] further found that the concentration of N-NCs was relatively low in foods but that in the fasting gastric juice was very high in the high-risk population of oesophageal cancer, postulating that the endogenous synthesis *in vivo* was the main source of N-NCs. Both dimethylamine and sodium nitrite are the precursors of NDMA synthesis, and whether they can induce gastric cancer is unknown. In the present study, we observed the effects of both dimethylamine hydrochloride and sodium nitrite on the glandular stomach of rats.

MATERIALS AND METHODS

One hundred and forty male Wistar rats, weighing 120-140 g, were randomized into three groups. Groups 1 and 2 consisted of 50 rats, respectively. They were given a standard diet and received a mixed drinking water containing 0.06% dimethylamine hydrochloride or 0.06% sodium nitrite ad libitum. The control group consisted of 40 rats, and was fed a regular diet and water. All the rats were housed in plastic cages (ten animals in each cage) in a room with controlled temperature and humidity. Animals were maintained under the guidance set forth in the "Guide for the Care and Use of Laboratory Animals" by the National Laboratory Animal Administration Council, China. From the beginning of experiments every 2-4 wk, 3-4 rats in group 1 were killed during 56 wk. The rats in group 2 and control group were killed at the 56th week.

The stomaches of the rats were quickly excised and opened along the great curvature. Each stomach was washed several times with normal saline for gross examination, and fixed in 10% neutral formalin and cut into 8 longitudinal strips about 2 mm in width. The strips were embedded in paraffin and serially sectioned at 4 μm in thickness. Sections were routinely stained with hematoxylin

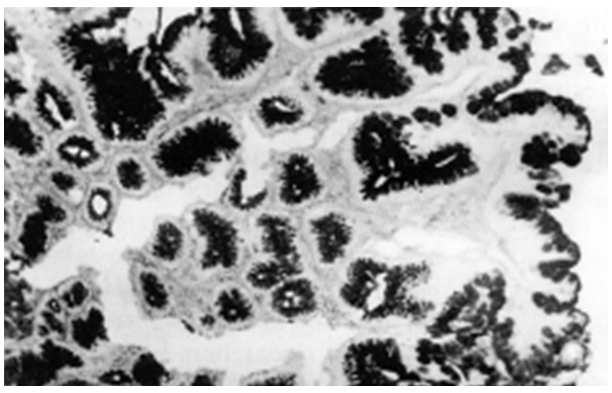

Figure 1 IM of the gastric mucosa was observed. Goblet cells and columnar cells secreting abundant acid mucin mixed with neutral mucin are shown. [AB (pH 2.5)/PAS, × 192]

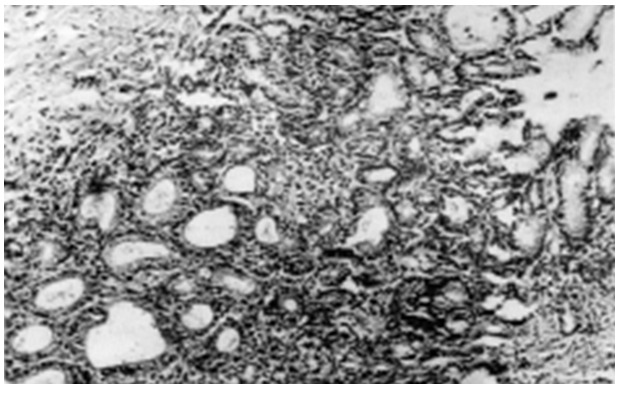

Figure 2 Gastric dysplasia with inflammatory cell infiltration and glandular atrophy. (HE, × 100)

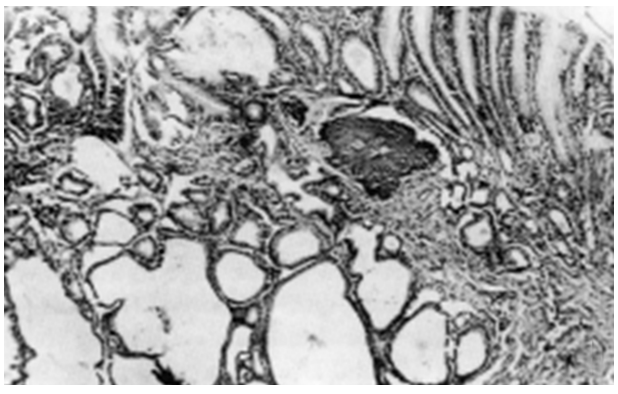

Figure 3 Cystic dilatation of gastric glands. [AB (pH 2.5)/PAS, × 63]

and eosin, and with alcian blue/periodic acid-Schiff (AB/PAS) and high iron diamine/alcian blue (HID/AB). The livers of the rats were also treated routinely for the histopathological observations. The pathological diagnosis and the grading of the lesions were performed according to the criteria of the National Anti-cancer Association of China in 1990.

RESULTS

Group 1

During the 2nd to 12th week, the gastric mucosal congestion, edema and erosion were grossly observed under a light microscope. There was a lot of inflammatory cell infiltration. Intestinal metaplasia (IM) of the glandular stomach was first found in the glandular neck with characteristic goblet cells at the 18th week and afterwards extended to the surface or bottom of the mucosa (Figure 1). Mild dysplasia of the gastric mucosa occurred at the 24th week, moderate dysplasia at the 32th week and severe dysplasia at the 46th week (Figure 2). Single and multiple cystic dilatation of the gastric glands was noted at the 22nd week (Figure 3). In the forestomaches of rats, hyperplasia or mild dysplasia of squamous epithelia appeared after the 36th week. At the 48th week, under a light microscope the obvious degeneration and necrosis of liver cells and inflammatory cell infiltration in the portal area were observed. At the 54th week, greyish white nodules in the liver could be seen at gross examination and under a light microscope, and the most typical feature was the hyperplasia of liver cells and fibro-tissues and adenoma-like dilatation of some intrahepatic biliary ducts.

Group 2

Total intake of dimethylamine hydrochloride or sodium nitrite was 5.88 g and the mean daily intake was 15 mg for a rat. Five rats died in the course of experimentation. It was found that the rate of IM in the glandular stomaches of the rats was 88.9% (40/45), 55.0% (22/40) of which was the sulphomucin-positive IM. The rates of mild, moderate and severe dysplasia were 44.5 (20/45), 33.3% (15/45) and 22.2% (10/45), respectively. Cystic dilatation of gastric glands was found in 28.9% (13/45). No gastric cancer occurred. Hyperplasia of the squamous epithelia was 26.7% (12/45) and mild dysplasia 17.8% (8/45) in the forestomaches of the rats. Ten (22.2%) cases of liver cirrhosis without liver cancer were diagnosed.

Control group

IM was observed in 3 (7.5%) rats in the pyloric gland areas of stomaches.

DISCUSSION

N-NCs have been identified in various environmental situations, such as food products, water, drugs, cosmetics, tobacco products, agricultural and industrial products and so on. Exposure in human beings may be through ingestion, inhalation, skin contact and *in vivo* formation, with the latter being mainly in the gastrointestinal tract which probably represents the main source of human exposure. The site most commonly regarded as risk from endogenous N-NC synthesis *in vivo* is the stomach. N-NC synthesis *in vivo* can be carried out by chemical or biological synthesis[8]. Dimethylamine hydrochloride or sodium nitrite itself is not carcinogenic but they can be changed into NDMA by chemical synthesis in the acidic environment of the stomach.

It is widely accepted that epithelial dysplasia of the gastric mucosa is a precancerous lesion and IM, especially sulphomucin-positive IM, is closely related to gastric cancer[9-11]. We previously found that the sequential lesions of gastric glandular mucosa induced with both N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and ranitidine were from IM, dysplasia to gastric cancer or from dysplasia to gastric cancer[12]. In this study both dimethylamine hydrochloride and sodium nitrite were administered to Wistar rats in the drinking water ad libitum for 56 wk, and the incidences of IM and moderate and severe dysplasia were 88.9% and 55.5%, respectively, suggesting that the two precursors of NDMA synthesis can induce the precancerous lesions of the stomach in rats. This finding enriches the contents of etiology of gastric cancer and supplies new experimental evidence for the primary prevention of gastric cancer.

We previously reported that after 0.01% NDMA liquid was administered to rats for 16 wk ad libitum, the incidence of liver cancer was 100% at the 18th week[13]. In the present study, both dimethylamine hydrochloride and sodium nitrite, the precursors of NDMA synthesis, were fed to the rats for 56 wk, and 22.2% of the rats developed liver cirrhosis. Epidemical investigations suggested that NDMA could be one of the pathogenic factors of oesophageal cancer. Oesophageal lesions were not observed in the study but only hyperplasia and mild dysplasia of the squamous epithelium were found in forestomaches of the rats. We supposed that this may be related to the low synthesizing quantity of NDMA in the stomach of the rats. The lesions of the liver and forestomach may be more severe if higher concentrations of both dimethylamine hydrochioride and sodium nitrite are administrated to the rats.

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Mechanism of cryopreserved porcine hepatocyte xenotransplantation in treating acute hepatic failure

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Abstract

AIM: To study the effect of hypothermia on structure of membrane proteins of pig hepatocytes and the mechanism of transplantation of cryopreserved porcine hepatocytes in treating acute hepatic failure in Wistar rats.

METHODS: Isolated porcine hepatocytes were cryopreserved in liquid nitrogen for 150 d and then transplanted to the rat abdominal cavity. Three days later, 80% of rat liver by weight was resected. Conformation of membrane proteins of the porcine hepatocytes, liver function and weight of the remaining liver in the rats were measured.

RESULTS: Viability of the cryopreserved hepatocytes was 78%. Light and electron microscopy showed that the hepatocytes were morphologically intact. The cells were rich in glycogen and glucose-6-phosphatase. The α-helix content in the membrane proteins of fresh cells was 48%. After freezing the cells, the content was increased to 59.5%. Survival rate of rats in the transplanted group was 16/17, significantly higher than 3/9 in the control group (P = 0.0022). However, there were no significant differences in the weight of the remaining liver or indices of liver function between the two groups.

CONCLUSION: Transplant of frozen porcine hepatocytes is effective in treating acute hepatic failure. The mechanism is suggested to be a result of multiple effects. Hypothermia could increase α-helix content in the cellular membrane proteins, which might be related to the report that hypothermia was able to reduce the immunogenicity of the transplanted tissues and cells.

Key words: Liver failure, acute; Cryopreservation, Liver/cytology; Transplantation, Heterologous; Membrane proteins

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INTRODUCTION

Hepatocyte transplantation has been applied to the treatment of acute hepatic failure (AHF) and congenital disturbed hepatic diseases[1]. It was shown that after long-term cryopreservation of hepatocytes, the shape and functions of hepatocytes could be maintained[2] and AHF could be treated effectively[3]. The immunogenicity of tissues and cells decreased obviously after being frozen[4], but the mechanism is not known completely. In this study, we explored the effect of hypothermia on antigen structure of pig hepatocellular membranes and the mechanism of transplantation of cryopreserved porcine hepatocytes in treating AHF in Wistar rats.

MATERIALS AND METHODS

Preparation of pig hepatocytes and histologic examination

A Chinese experimental minipig[5] weighing 7 kg was used as the donor. After gradient cooling, the isolated porcine hepatocytes[6] were preserved in liquid nitrogen (-196 ℃) for 150 d[3]. Before transplantation, the cell viability was measured and the hepatocytes were observed under light and electron microscope. HE staining and histochemical staining for glycogen were used in light microscopic observations. The percentage of cells containing glucose-6-phosphatase (G6Pase; ++ - ++++) was counted. Fresh and cryopreserved hepatocytes of the pig were cultured routinely for 48 h.

Preparation of hepatocellular membrane proteins and conformational measurement

Porcine hepatocytes were washed with a solution containing 0.05 M boric acid, 0.15 mol/L NaCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$ and 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF) (pH 7.2). The cells were centrifuged at 450 g for 5 min and then lysed with 100 volumes of 0.02 mol/L boric acid, 0.2 mmol/L EDTA and 0.1 mmol/L PMSF (pH 10.2). Whole cells were removed by centrifugation at 12000 *g* for 30 min. Cell membranes were then suspended in PBS (pH 7.4), layered on 35% (w/v) sucrose in PBS and centrifuged at 7000 g for 30 min. The material at the sucrose-PBS interface was

Table 1 Assay of biochemical values of sera after liver failure ($\bar{x} \pm s$)

	Group IIt	Group IIc	Normal rats
2 d after liver failure			
ALB (g/L)	23.62 ± 3.69 (13)	24.13 ± 3.56 (8)	33.87 ± 2.36[b] (15)
TBr (μmol/L)	20.30 ± 13.23 (13)	24.00 ± 17.22 (8)	2.29 ± 1.61[b] (15)
DBr (μmol/L)	12.89 ± 9.12 (13)	15.61 ± 13.63 (8)	0.40 ± 0.39[b] (15)
7 d after liver failure			
ALB (g/L)	30.00 ± 4.22 (12)	26.00 ± 3.00 (3)	33.87 ± 2.36[b] (15)
TBr (μmol/L)	2.97 ± 1.02 (11)	2.90 ± 0.36 (3)	2.29 ± 1.61 (15)
DBr (μmol/L)	1.14 ± 0.49 (11)	0.80 ± 0.56 (3)	0.40 ± 0.39 (15)

Note: group IIt : Transplant group in the part two; group IIc: Control group in the part two. Numbers in parentheses are numbers of rats. [b]$P < 0.01$, group IIt *vs* group IIc, and group IIc *vs* normal rats. ALB: Albumin. TBr: Total bilirubin. DBr: Direct bilirubin.

membrane proteins[7]. Their conformations were calculated from their circular dichroism spectra.

Animal groups and pig hepatocyte transplantation

Wistar rats weighing 200-250 g were used, and 80% of the liver by weight was resected to induce AHF. The AHF rats usually died within 7 d after the operation[3]. The experimental animals were divided into two parts. The first part was used to observe the survival rate and living situation during the survival for one month after 80% hepatectomy. This part was further divided into two groups: transplant group (I_t, 17 rats) and control group (I_c, 9 rats). The second part was sacrificed on the 2nd or 7th d following AHF to measure the liver function and the weight of the remaining liver in the AHF rats, and to do histological examination. This part was divided into two groups too: transplant group (II_t, 37 rats) and control group (II_c, 27 rats). Fifteen normal rats provided comparative standards of the liver weight and function. 4×10^7 cryopreserved hepatocytes in 2 mL were injected into the abdominal cavity of the rats in the two transplant groups before and after 80% hepatectomy[6]. Normal saline was injected into the rats of the two control groups.

Biochemical values of liver function

Biochemical values of liver function included serum concentrations of albumin (ALB), total bilirubin (TBr) and direct bilirubin (DBr).

Statistical analysis

Data were processed by variance analysis and *q*-test.

RESULTS

The viability of fresh pig hepatocytes was 84%, and that of cryopreserved cells was 78%. Light microscopy showed that the frozen hepatocytes had a spherical shape and were rich in glycogen and G6Pase. The hepatocytes containing G6Pase (++ - ++++) accounted for 97% of total liver cells. There was no apparent difference between the cryopreserved cells and fresh ones under an electron microscope. The cells grew very well in culture and there was no difference either.

As regards to the conformation of membrane proteins, the α-helical structure accounted for 48% in fresh cells, and 59.5% in frozen cells.

The survival rate of group I_t was 16/17, which was significantly higher than that of group I_c (3/9) ($P = 0.0022$, exact probability). The AHF rats showed low consciousness and appetite, but the situation in the transplant group was a little better than that in the control group. Twelve rats of group II_t and 16 of group II_c died before sacrifice. Varying degrees of light yellow ascites were found by autopsy in the dead rats. The remaining livers of 10 out of 13 sacrificed rats in group II_t and of 6 out of 8 sacrificed rats in group II_c were weighed 2 d after the 80% hepatectomy. The same procedures were performed in another set of rats and in 10 normal rats 7 d after the 80% hepatectomy. The weights of remaining livers in groups II_t and II_c were 6.34 ± 0.88 g ($n = 10$) and 6.22 ± 0.95 g ($n = 6$), respectively, 2 d after the 80% hepatectomy, and 8.80 ± 1.02 g ($n = 10$) and 8.22 ± 0.50g ($n = 3$), respectively, 7 d after the 80% hepatectomy. There was no significant difference between groups II_t and II_c ($P > 0.05$), but the weights of livers at 7 d in both groups were significantly lower than that of the normal rats (13.27 ± 1.99 g, $n = 10$) ($P < 0.01$). Histological examination revealed that the structure of the remaining livers was normal. The results of the hepatic function are showed in the Table 1.

DISCUSSION

The survival rate (16/17) of the AHF rats after the transplantation was significantly higher than that of group I_c (3/9), and the living situation of the former during the survival was apparently superior to that of the latter. We found that after the 30th d of cryopreservation in liquid nitrogen, the viability of hepatocytes no longer decreased in subsequent freezing up to 180 d, and the appearance and function of the hepatocytes remained well[3]. In view of the above, it is possible to cure AHF using pig hepatocytes cryopreserved for 150 d.

The transplanted cells will be destroyed by immunological rejection. To enable the grafted porcine hepatocytes to play the therapeutic role in the body of a xenogeneic animal, we adopted the transplanting method before and after the 80% hepatectomy, respectively. Moreover, as hypothermia is able to reduce the immunogenicity of cells[4], and thus delay or abate the rejection, the hepatocytes can gain more time to recover their functions. The conformational investigation of membrane proteins revealed that the content of α-helical structure increased from 48% to 59.5% after freezing. This result was possibly responsible for the report that hypothermia was able to reduce the immunogenicity of cells. The conformational change of membrane proteins might subsequently affect the characteristics of their functioning domains.

The possible mechanism of treatment of AHF with hepatocyte transplantation was thought to be that the transplanted hepatocytes produce hepatocyte growth factor which promotes liver regeneration and recovery of liver function, and the cells act as a substitution for the AHF liver. From our experiment, ALB concentrations of groups II_t and II_c 2 d after the 80% hepatectomy were significantly lower than that of the normal rats. TBr and DBr increased rapidly, but there was no significant difference between groups II_t and II_c , neither was there any significant difference in the weight of remaining livers between the two groups 2 and 7 d after the 80% hepatectomy. However, the survival rate of the transplant group was significantly higher than that of the control group. The results suggest that the mechanism of hepatocyte transplantation in treating AHF may not only be the effects of the substitution for the liver and the hepatocyte growth factor, but also be the effect of other unknown factors. It may be a result of multiple effects.

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Effect of aging on Kupffer cell membrane phospholipid function: Modulation by vitamin E

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Abstract

AIM: To investigate the mechanisms leading to the disordered response to aging liver Kupffer cells (KCs).

METHODS: The effects of aging on KC membrane signal transduction and eicosanoid production and their management with vitamin E (VE) were assessed by measuring inositol phospholipid (PI) metabolism, intracellular calcium responses, and prostaglandin Ea-2 (PGEa-2) production in response to the inflammatory signals endotoxin (LPS) and platelet activating factor (PAF).

RESULTS: Aging resulted in a significant alteration in signal transduction of PAF as both PI turnover and calcium response were significantly reduced in the 18- and 24-mo old groups, compared with the 6 mo-old group. Aging significantly reduced PGEa-2 production in response to LPS. VE pretreatment resulted in an increased PI turnover, calcium response and PGEa-2 production.

CONCLUSION: The aging KCs have a disordered membrane phospohlipid function and VE is an effective modulator on it.

Key words: Liver/cytology; Phospholipids; Calcium; Prostaglandin; Vitamin E; Aging

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INTRODUCTION

Liver Kupffer cells (KCs) play an important role in the inflammatory process through the production of eicosanoids and monokines. Aging has an important effect on KC, resulting in a disordered function, which is related with the susceptibility of the defense system of the aged host[1]. Membrane phospholipids provide structural integrity to the cell, and have many additional functions. These include: (1) serving as substrate for inflammatory mediators, *i.e.*, eicosanoid production, and (2) transducing extracellular signals into appropriate intracellular responses. We examined the effect of aging on membrane phospholipid function of KCs and its management with vitamin E (VE) to elucidate the nature of their relationship.

MATERIALS AND METHODS

Animals and groups

Forty Wistar rats (Chinese Herb Research Institute of Sichuan Province) were divided into four groups (6, 12, 18 and 24-mo age groups). Each group was further randomly subdivided into two groups: VE pretreated group (VEG) and non-VE pretreated group (NVEG). Each group has 5 animals. Animals in the VEG were given an introperitoneal injection of 5% VE solution for 3 months (500 mg/kg/wk, 2 injections/wk) before experiments. The NVEG was given normal saline.

KC isolation

Liver non-parenchymal cells were isolated by a collagenase-perfusion method[2]. After anaesthesia (30 mg of barbital/kg body weight, intraperitoneally), the liver was perfused in situ with Ca^{2+}-free Hanks balanced salt solution at 37 ℃ for 3 min. Then 0.05% collagenase (Sigma, type IV) was added and the liver was perfused for 4 min with Hanks balanced salt solution. After gentle shaking, the suspension was filtered and hepatocytes were sedimented at 50 g for 3 min. Non-parenchymal cells were collected and sedimented at 300 g for 10 min. The pellets of non-parenchymal cells were resuspended and cultured with RPMI 1640 medium containing 15 mmol/L HEPES, 0.05 U/mL insulin, 15 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin supplemented with 10% newborn calf serum. After 30 min, the non-adherent cells were deleted. The viability of KCs was greater than 90% as determined by trypan blue exclusion.

Measurement of prostaglandin E_2 (PGE_2) production

KCs (1×10^6/mL) were plated in a 2-mL volume of medium in six-well plastic tissue culture trays (New York). The plated cells were

Table 1 Prostaglandin E_2 production capacities of Kupffer cells from various age groups after lipopolysaccharide stimulation

Group	0 ng/mL	10 ng/mL	100 ng/mL	10 μg/mL
6 mo				
NVEG	921.78 ± 102.46	836.47 ± 90.18	3224.42 ± 396.53[d]	2926.02 ± 323.47[d]
VEG	985.23 ± 89.34	1011.47 ± 104.15	3553.64 ± 304.81	3018.74 ± 297.57
24 mo				
NVEG	315.94 ± 26.54[b]	1013.19 ± 97.47[d]	634.73 ± 56.07[bd]	194.91 ± 21.78[bd]
VEG	713.48 ± 65.50[bf]	1278.78 ± 113.21[bdf]	1455.67 ± 121.34[bdf]	1337.46 ± 122.59[bdf]

[b]$P < 0.01$ compared with 6 mo; [d]$P < 0.01$ compared with 0 ng/mL; [f]$P < 0.01$ compared with NVEG. NVEG: non-VE pretreated group; VEG: VE pretreated group.

Table 2 Intracellular calcium concentrations in various age groups before and after platelet activating factor stimulation (mean ± SD, pg/mL, $n = 5$)

Group (mo)	PAF	[Ca++]i		Δ[Ca++]		%	
		NVEG	VEG	NVEG	VEG	NVEG	VEG
6	-	105.7 ± 16.1	111.5 ± 12.1				
	+	266.1 ± 31.3[d]	285.3 ± 27.5	160.4 ± 22.4	173.8 ± 19.7	151.8	155.8
12	-	111.4 ± 19.3	109.8 ± 9.7				
	+	262.8 ± 27.6[d]	276.3 ± 30.3	151.4 ± 21.9	166.5 ± 16.0	135.9	151.6
18	-	151.4 ± 21.4	118.4 ± 10.8[f]				
	+	254.5 ± 36.7[db]	255.9 ± 27.0	103.1 ± 27.9[a]	137.5 ± 14.8[a]	68.1[b]	116.1[bf]
24	-	164.3 ± 20.8	130.7 ± 12.1[f]				
	+	249.3 ± 28.4[db]	255.7 ± 24.1	85.0 ± 24.3[b]	125.0 ± 14.0[bf]	51.7[b]	95.6[bf]

[a]$P < 0.05$ compared with 6 mo; [b]$P < 0.01$ compared with 6 mo; [d]$P < 0.01$ compared with PAF (-); [f]$P < 0.01$ compared with NVEG. NVEG: non-VE pretreated group; VEG: VE pretreated group; PAF: Platelet activating factor

allowed 6 h to adhere and recover from the isolation procedure, and then non-adherent cells were washed away. The adherent monolayers were then stimulated with lipopolysaccharide (LPS) from Escherichia coli $O_{111}B_4$ (Sigma). In 90 min, the conditioned supernatants were removed, filtered, and stored at -70 ℃. The PGE_2 level of each conditioned supernatant was determined by radioimmunoassay according to the manufacturer's protocol (Scientific Institute of Military Medicine).

Measurement of intracellular calcium

Intracellular calcium was measured by a method previously described[3]. KC suspensions were loaded with the fluorescent calcium probe Fura-2 for 40 min in culture medium. Uptake of the dye was confirmed by fluorescent microscopy. The cells were washed twice and resuspended in HEPES-Krebs-Rins buffer before measurement. Intracellular calcium was measured on a MPF-4 spectrofluorimeter at an excitation wavelength of 380 nm and an emission wavelength of 510 nm. Macrophages were added to the cuvette (2 million/mL) and kept in suspension by constant stirring. Fluorescence was recorded. Changes in intracellular calcium in response to the added signals were calculated from the changes in fluorescence after calibration. Triton X-100 (final concentration 0.09%) was used in calibration for Fmax and Fmin is determined by addition of 9 mmol/L EGTA.

Determination of inositol phospholipid turnover

KCs (2×10^6/mL) were cultured overnight in PRMI 1640 with 5% calf serum containing 3.0 μCi/mL of [^{3}H]inositol. After washing, the cells were incubated for 15 min in culture medium containing 10 mmol/LiCl. The signal to be tested for stimulation of phospholipid (PI) turnover was added and the cells were incubated for 1-5 min. The cell monolayers were placed on ice, the medium was removed, and the cells were extracted with chloroform:methanol:water (1:1:0.9) into aqueous and lipid phases. The counts in the aqueous phase corresponded to total inositol phosphates[4]. The increases in total counts compared to controls indicated a signal-induced turnover of inositol phospholipid.

RESULTS

PGE_2 productions in various groups

The production of PGE_2 in response to LPS was significantly reduced in KCs from the 18- and 24-mo age groups in comparison with the 6-mo age group. In addition, with the increase of LPS concentration, the production of PGE_2 of the 6-mo group increased with the peak at 100 ng/mL and that of the 24-mo group peaked at 10 ng/mL. VE pretreatment had no significant effect on the production of PGE_2 by KCs from the 6-mo age group, but had an apparent effect on that by KCs from the 24-mo age group (Table 1).

Intracellular calcium responses of each age group

Table 2 shows that the basal level of [Ca^{++}]i increased with age. After the stimulation of platelet activating factor (PAF), the [Ca^{++}]i levels of four age groups were comparable with significant decreases of the net increase in the 18- and 24-mo age groups. KCs of 18 or 24 months old from vitamin E-fed animals showed a significant attenuation of basal level of [Ca^{++}]i and a better calcium response in comparison with NVEG.

Inositol phospholipid metabolism of each age group

It was found that the total inositol phospholipid decreased with age and that of the 18- or -24-mo old group was significantly decreased compared with that of the 6 mo-old group. When stimulated with PAF, KCs of the four groups had significantly increased levels with a fact that the total inositol phospholipid of the 18- or -24-mo old group was still significantly lower. VE pretreatment resulted in significant elevations in the 18- and/or 24-mo old groups in the control and PAF groups compared with NVEG. In addition, LPS stimulation resulted in no change of inositol phospholipid in each group (Table 3).

DISCUSSION

Recent interest has focused on the effect of aging on the defense system of the host, in which liver macrophages (KCs) play an important role. It has been found that the aged KCs have disordered responsiveness and phagocytosis function whose mechanisms are thought to arise from altered composition and function of cell membrane phospholipids[1,5]. In addition to providing structural integrity to the cell, membrane phospholipids and the fatty acids provide the precursors to a number of inflammatory mediators and are involved in signal transduction pathways that initiate inflammatory responses. The aged KCs have altered phospholipid functions as demonstrated by the reduced production of PGE_2 and disordered signal transduction response to PAF.

The responsiveness of an inflammatory cell to an extracellular signal depends on its ability to transduce the signal into appropriate intracellular messengers that propagate the signal into the desired response. Membrane inositol phospholipids are intimately involved in the signal transduction process. When the appropriate signal

Table 3 Total inositol phospholipid in various age groups (mean ± SD, cpm × 1000, *n* = 5)

	6 mo	12 mo	18 mo	24 mo
NVEG				
Control	48.5 ± 4.3	46.3 ± 4.5	39.5 ± 4.3[a]	30.8 ± 4.4[b]
LPS	52.4 ± 3.8	49.7 ± 5.6	42.8 ± 4.6[a]	34.8 ± 3.9[b]
PAF	78.7 ± 6.1[f]	78.1 ± 8.4[f]	62.9 ± 6.5[bf]	49.5 ± 5.4[bf]
VEG				
Control	53.1 ± 4.0	56.2 ± 6.3	49.9 ± 5.0[c]	39.3 ± 2.9[bc]
LPS	51.8 ± 3.4	53.2 ± 4.9	48.8 ± 4.6	38.1 ± 4.0[b]
PAF	80.5 ± 7.3[f]	77.2 ± 8.1[f]	72.4 ± 6.8[f]	63.8 ± 6.4[bdf]

[a]$P < 0.05$ compared with 6 mo; [b]$P < 0.01$ compared with 6 mo; [c]$P < 0.05$ compared with NVEG; [d]$P < 0.01$ compared with NVEG; [f]$P < 0.001$ compared with control. NVEG: non-VE pretreated group; VEG: VE pretreated group; LPS: Lipopolysaccharide; Platelet activating factor.

is encountered with membrane receptor, inositol phospholipids are first phosphorylated and hydrolyzed to produce intracellular second messengers in a receptor-mediated and G-protein-coupled event. The second messengers include inositol phosphates, of which the triphosphate has been demonstrated to result in an increased intracellular calcium concentration; and diacylglycerol, which activates protein phosphorylation *via* protein kinase C (PKC). Eventually, through intermediary steps, the changes in calcium concentration and activation of PKC produce the desired cellular response. Platelet activating factor activates macrophages through this signal transduction pathway. Studies have demonstrated that PAF signals an increase in intracellular calcium and inositol phospholipid metabolism in macrophages[4]. This study showed that ageing altered the intracellular calcium and inositol phospholipid metabolism of KCs exposed to PAF.

The ability to modulate intracellular calcium responses to extracellular signals has sweeping implications on the functional responsiveness of macrophages since intracellular calcium plays an important role in several functions. These include superoxide production for killing, chemotaxis, phagocytosis, activation of phospholipases for arachidonate metabolism, and interleukin-1 and tumor necrosis factor production.

Our investigation suggests that the disordered functions of aged KCs may contribute to the suppression of eicosanoid production by KCs as well as the attenuation of KC responsiveness due to a reduced ability to transduce the inflammatory response.

It has been widely accepted that the age-associated damage to membrane phospholipids is mediated by free radicals which, once generated, are capable of initiating random chain reactions and destroying the integrity of the substance[6]. VE is thought to act as a biological antioxidant by protecting polyunsaturated lipids against peroxidative attack[7]. Our results showed that the pretreatment of VE protects the KC membrane phospholipids from age-related damage, implying that vitamin E is an effective modulator for KC membrane phospholipids.

KCs contribute to hepatic inflammation and cytotoxicity through the production of several proinflammatory cytokines, including eicosanoids. It has been postulated that KCs are responsible for hepatic dysfunction and liver failure in progressive sepsis and inflammation. Our results showed that VE offers the potential ability to regulate the inflammatory response by increasing the PGE_2 production, which would be of great significance in clinical practice.

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Expression of metastasis suppressor gene *nm23* in human hepatocellular carcinoma

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Abstract

AIM: To investigate the relationship between the expression of *nm23-Hi* mRNA and the metastatic potential of hepatocellular carcinoma (HCC).

METHODS: The expression of *nm23-H1* mRNA was detected in 24 cases of HCC by *in situ* hybridization using digoxigenin-labeled nm23-H1 antisense cRNA probe. Twenty-four HCC specimens were divided into two groups according to the following criteria: (1) metastasis in portal lymph nodes; (2) the number of tumors in the liver; (3) cancerous emboli in the portal vein; and (4) the existence of satellite lesions. We named those meeting criteria (1) or (2) and (3), or (3) and (4) high metastatic potential (n = 6); and the others formed the low metastatic potential group (n = 18).

RESULTS: Positive results of *in situ* hybridization showed granules or masses in the cytoplasm. In the low metastatic potential group strong staining was obtained in ten specimens, while in the high metastatic potential group there was none. Three negative results were found in the high metastatic potential group, and one in the low metastatic potential group (P < 0.05). The expression of *nm23-H1* mRNA was not correlated with some clinical factors, such as tumor size or the background liver disease.

CONCLUSION: The expression of *nm23-H1* mRNA is inversely correlated with HCC metastatic potential, and can be considered as an index which indicates the metastatic potential of HCC.

Key words: Liver neoplasms; *In situ* hybridization; Neoplasms metastasis; RNA; Messenger

Chen XD, Dai YM, Yang JM, Bao JZ, Wang JJ, Chong WM. Expression of metastasis suppressor gene *nm23* in human hepatocellular carcinoma. *World J Gastroenterol* 1996; 2(4): 218-219 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/218.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.218

INTRODUCTION

nm23, a novel gene associated with low tumor metastatic potential, was isolated from a cDNA library of murine melanoma cells by Steeg *et al*[1] in 1988. Since then, a large number of investigations have been made by various methods. Although many studies have demonstrated that the *nm23* gene is differentially expressed in tumors with different metastatic potential[2-5]. Some researchers still have different viewpoints[6,7]. This preliminary study was designed to investigate the relationship between the expression of *nm23-H1* mRNA and the metastatic potential of human hepatocellular carcinoma (HCC).

MATERIALS AND METHODS

Materials

Twenty-four HCC specimens were obtained from the patients by operation. All the specimens contained cancer tissues and non-cancerous tissues. We divided the specimens into two groups according to the following criteria: (1) metastasis in portal lymph nodes; (2) ≥ 2 tumors in the liver; (3) cancerous emboli in the portal vein; and (4) the existence of satellite lesions. We named those meeting the criteria (1) or (2) and (3), or (3) and (4) high metastatic potential (n = 6); and the others formed the low metastatic potential group (n = 18). *Nm23-H1* cDNA was obtained as a gift from Rosengard. Dig-RNA labeling kits were purchased from Boehringer Mannheim Co., and *nm23-H1* cRNA probes were prepared according to the manufacturer's instructions.

Methods

In situ hybridization method was derived from several published papers with some modifications[8,9]. Frozen sections were fixed in 4% paraformaldehyde/PBS for 30 min at 4 ℃ and then rinsed sequentially with PBS, glycine/PBS, and Triton X-100/PBS. Hybridization buffer contained 2 ng/μL *nm23-H1* cRNA probes and the sections were hybridized in a 25 μL system. Optimal hybridization temperature

Table 1 Staining intensity of *nm23-H1* mRNA in the two groups

	Staining intensity		
	-	+	+ +
Low metastatic potential group	1	7	10
High metastatic potential group	3	3	0

$P < 0.05$, *vs* high metastatic potential group.

Table 2 Staining intensity of *nm23-H1* mRNA according to some clinical features

	Staining intensity		
	-	+	+ +
Liver tumor (s)			
Solitary	1	9	10
Multiple	3	1	0
Tumor size (cm)			
≤ 5	3	3	4
> 5	2	5	7
Associated liver disease			
Chronic hepatitis	1	2	5
Liver cirrhosis	6	5	11
Serum alpha-fetoprotein (ng/mL)			
≤ 100	2	6	7
> 100, ≤ 1000	2	3	3
> 1000, ≤ 10000	0	1	0

$P < 0.05$, compared with multiple group. $P > 0.05$

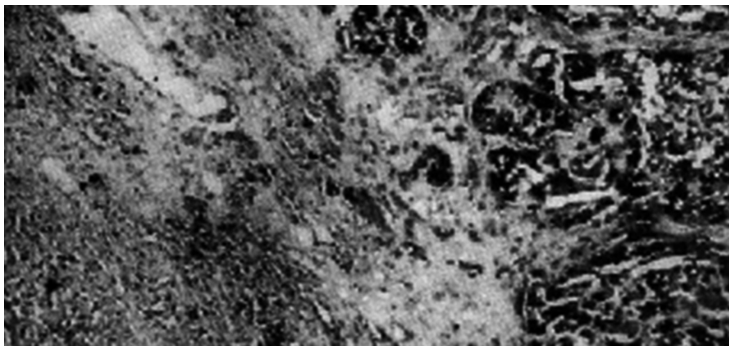

Figure 1 Staining intensity in hepatocellular carcinoma tissue (right panel) is higher than that in the adjacent nontumorous liver tissue (left panel). (Magnification, × 100)

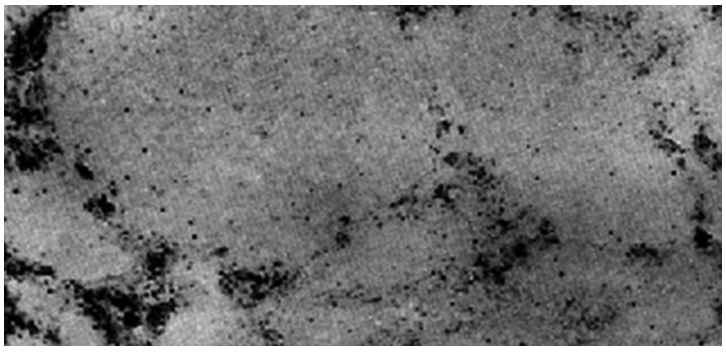

Figure 2 Cancerous nodules showing negative staining. (Magnification, × 100)

was about 50 ℃. As a negative control, the sections were hybridized with the sense probe or incubated with hybridization buffer only. To confirm the reactivity of the probes to tissue RNAs, the sections were digested with RNase (100 μL/mL) at 37 ℃ before hybridization. Statistical analyses were conducted by Ridit test. Results were considered significantly different when *P*-values were less than 0.05.

RESULTS

The positive results of *in situ* hybridization showed blue granules or masses in the cytoplasm. Staining intensity was graded as follows: (-), staining less intense than in adjacent non-tumorous tissue; (+), staining intensity similar to or slightly more intense than that in adjacent non-tumorous tissue; (++), staining more intense than in adjacent non-tumorous tissue (Figures 1-3). The results are shown in Tables 1 and 2. The *nm23-H1* mRNA expression in the high metastatic potential group differed significantly from that of the low metastatic potential group ($P < 0.05$).

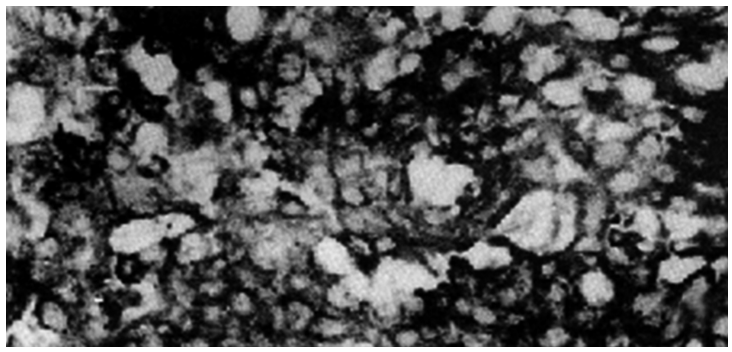

Figure 3 The positive signal of nm23-H1 mRNA is mainly distributed in the cytoplasm. (Magnification, × 200)

DISCUSSION

Many investigations have demonstrated that the *nm23* gene is closely related with tumor metastasis[4]. It has been widely accepted that *nm23* is the best candidate for a metastasis suppressor gene. The results of this study also showed that the expression of *nm23-H1* mRNA was inversely correlated with HCC metastatic potential. Rosengard found that nm23 protein existed in the cytoplasm and nucleus, but did not explain the specific functions of nm23 protein in different sites. Our study indicated that *nm23-H1* mRNA expression mainly existed in the cytoplasm. *Nm23-H1* cDNA probe was used in our study. Labeling kits used were DIG-DNA Labeling and Detecting kits (Boehringer Mannheim). The results of using *nm23-H1* cDNA are far from satisfaction. Possible reasons may be: (1) the sensitivity of detecting mRNA using cDNA probe is low; and (2) the quantity of *nm23-H1* mRNA is very small. In conclusion, our study shows an inverse relationship between the expression of *nm23-H1* mRNA and the metastatic potential of human HCC. The results of this paper warrant further studies with more cases and more detailed clinical features of each case. Our current efforts are focused on the detection of NDPA-A subunit, a product of *nm23-H1* gene, whose relationship with HCC metastasis deserves further studies.

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Proliferating cell nuclear antigen and P53 protein expression and prognosis in primary liver cancer

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Abstract

AIM: To study the individual difference and prognosis of primary liver cancer (PLC) after hepatectomy.

METHODS: Two hundred specimens obtained from PLC patients were examined immunohistochemically using anti-P53 and anti-PCNA monoclonal antibodies. The mutations of p53 in exons 5-8 of the gene were examined using the polymerase chain reaction/single-stranded conformational polymorphism (PCR/SSCP) method in 60 of the 200 specimens.

RESULTS: The results showed that the labeling index of proliferating cell nuclear antigen (PCNA) and expression of p53 had a parallel correlation ($P < 0.001$). The high labeling index of PCNA or overexpression of p53 was associated with a high risk of tumor recurrence, more aggressive growth and poor survival.

CONCLUSION: Immunohistochemical assessment of PCNA and P53 can provide very useful information for the prognosis of PLC.

Key words: Prognosis; P53 protein; Liver neoplasms; Monoclonal antibodies; Immunohistochemstry; Polymerase chain reaction

Li JQ, Zhang CQ, Feng KT. Proliferating cell nuclear antigen and P53 protein expression and prognosis in primary liver cancer. *World J Gastroenterol* 1996; 2(4): 220-222 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/220.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.220

INTRODUCTION

With the development of diagnostic techniques, many cases of primary liver cancer (PLC) can be resected at an early stage[1,2]. Because of the biologic behavior of PLC, the recurrence rate in the patients who underwent hepatectomy is very high[3-5]. Some authors have described the factors influencing HCC recurrence, such as tumor size, capsule formation, differentiation of tumor cells, clinicopathologic stage, proliferating cell nuclear antigen (PCNA), and mutation of p53[5-9]. To study the individual difference and prognosis of PLC after hepatectomy, 200 specimens obtained from PLC patients were examined immunohistochemically using anti-P53 and anti-PCNA monoclonal antibodies. The mutations of p53 in exons 5-8 of the gene were examined using the polymerase chain reaction/single-stranded conformational polymorphism (PCR/SSCP) method in some of the 200 specimens.

MATERIALS AND METHODS

Patients

From August 1992 to December 1995, 200 PLC patients who underwent hepatectomy were recruited to this study. One hundred and eighty-eight cases were male and 12 cases were female with ages ranging from 20 to 72 years. One hundred and thirty-four cases underwent radical hepatectomy and 66 cases underwent palliative resection. Forty-seven cases had small tumor(s) (diameter < 5 cm), and hepatocellular carcinoma was confirmed in all of the patients.

Immunohistochemical staining

Formalin-fixed and paraffin-embedded specimens were examined immunohistochemically using anti-PCNA monoclonal antibody Pc-10 and anti-P53 monoclonal antibody DO-7 (LSAB kit, Dako). The deparaffinized tissue sections had to be treated in a microwave oven before the immunohistochemical staining procedure for the P53[9,10].

The criteria of nuclear labeling index for PCNA and expression of p53 in this study were as follows: the positive nuclei number was semiquantitatively evaluated by counting the number of positive nucleus in 8-10 randomly-chosen medium power (× 160 magnification) fields; four degrees of positive nuclei for PCNA were identified:"+" < 24%, "++" = 25%-50%, "+++" = 51%-74%, "++++" > 75%; four degrees for P53 were identified: "-" no positive cell, "+" < 30%,"++" = 31%-70%, "+++" > 71%.

PCR/SSCP

PCR/SSCP method[11,12] was used to examine the mutations of p53 in exons 5-8 of the gene in 60 of the 200 specimens.

Table 1 Proliferating cell nuclear antigen labeling index and tumor size

	Cases	PCNA labeling index	
		Low index (+, ++)	High index (+++, ++++)
Small tumor	47	32	15[a]
Large tumor	152	88	65[a]

[a]$P > 0.05$, small tumor *vs* large tumor. PCNA: Proliferating cell nuclear antigen.

Table 2 P53 expression and tumor size

	Cases	P53 expression	
		Low expression (-, ++)	Overexpression (++,+++)
Small tumor	47	31	16[a]
Large tumor	153	90	63[a]

[a]$P > 0.05$, small tumor *vs* large tumor.

Table 3 Proliferating cell nuclear antigen labeling index and tumor recurrence

	Cases	PCNA labeling index	
		Low index (+, ++)	High index (+++, ++++)
Recurrence	38	11	27[e]
No recurrence	96	42	26[e]

[e]$P > 0.001$, recurrence *vs* no recurrence. PCNA: Proliferating cell nuclear antigen.

Table 4 p53 expression and tumor recurrence

	Cases	p53 expression	
		Low expression (-, ++)	Overexpression (++, +++)
Recurrence	38	17	21[e]
No recurrence	96	64	32[e]

[e]$P > 0.001$, recurrence *vs* no recurrence.

Table 5 Comparison of the survival rates of patients with low or high labeling index of proliferating cell nuclear antigen after palliative hepatectomy

PCNA index	Cases	Survival rate (%)			
		6 mo	12 mo	18 mo	Median time
Low index (+, ++)	39	89.7	76	69	14
High index (+++, ++++)	27	50.9	11.8	3.9	5

PCNA: Proliferating cell nuclear antigen.

Table 6 Comparison of the survival rates of patients with weak expression or overexpression P53 after palliative hepatectomy

PCNA index	Cases	Survival rate (%)			
		6 mo	12 mo	18 mo	Median time
Low index (-, ++)	39	81.4	63.5	55.3	11
High index (++, +++)	27	52.9	11.8	5.9	6

$P = 0.0079$, Mantel-cox; $P = 0.0051$ Breslow. PCNA: Proliferating cell nuclear antigen

Statistical analysis

The data were analyzed statistically by the chi-square test and the spearman rank correlation coefficient test. The Bio Medical Data Processing (BMDP) Statistical Software was used to compare the survival rates of the patients in different groups (Breslow and Mantel-Cox). *P*-values < 0.05 were considered statistically significant.

RESULTS

Labeling index of PCNA and mutations of p53 gene in PLC

Using the PCR/SSCP method, we found mutations in 20 (33.3%) of the 60 cases. Eighteen mutations were found in exon 7 and two in exon 8. No mutations were found in exon 5 or 6. The P53 protein expression was also positive in the 20 cases. There were no significant differences in labeling index of PCNA or expression of P53 between the small and large tumors ($P > 0.05$) (Tables 1 and 2).

The labeling index of PCNA correlated with expression of P53 ($r = 0.3814$, $t = 3.9004$, $P = 0.0003$).

Recurrence and labeling index of PCNA and expression of P53

By June 1996, tumor recurrence occurred in 38 of the 134 patients who underwent radical resection. PCNA high labeling index (+++, ++++) accounted for 71.1% (27/38). Overexpression of P53 accounted for 55.3% (21/38). In 96 cases who had not tumor recurrence, PCNA high labeling index accounted for 27.1% (26/96). Overexpression of P53 accounted for 33.3% (32/96). There were significant differences in high PCNA labeling index and P53 overexpression between the recurrence and non-recurrence groups ($P < 0.01$) (Tables 3 and 4).

Survival rates and labeling index of PCNA and expression of p53

The different survival rates of the patients with low or high labeling index of PCNA, low expression or overexpression p53 who underwent palliative hepatectomy are listed in Tables 5 and 6.

DISCUSSION

Some authors reported that the labeling index of PCNA and overexpression of P53 might provide information about clinical outcomes. PCNA labeling index correlated with the degree of histologic differentiation. Labeling index of PCNA was significantly low in small tumors and encapsulated tumors and high in large tumors and tumors without capsule[7-9]. But Ng *et al*[10] reported that the presence of p53 mutations did not show a significant association with tumor size, sex, age, tumor invasiveness, microsatellite lesion, venous permeation, cirrhosis or encapsulation[7,8]. This study showed that the high labeling index of PCNA and overexpression of p53 were frequent in large tumors, but there was no statistical difference ($P > 0.05$).

Recurrence was closely related to the biologic behavior. Labeling index of PCNA was correlated with the recurrence. The tumor with a high index of PCNA had more aggressive growth and recurrence, resulting in low survival rates[9-12]. This study indicated that tumors with a high labeling index of PCNA and overexpression of p53 tended to have a high risk of recurrence.

Even some small tumors may show rapid recurrence and poor survival rate. In this study, 3 cases of small tumor and 11 cases of large tumor had both a high labeling index of PCNA and overexpression of p53. They underwent radical hepatectomy, but all of them experienced tumor recurrence 3-5 mo later after the operation. A low recurrence rate was found in the tumor with low labeling index, and even after recurrence, the tumor grew slowly and had a good survival. For the patients with a high risk of recurrence, hepatic arterial chemoembolization 3-4 wk after hepatectomy could decrease the recurrence rates and improve the survival[6]. This study also showed that the immunohistochemical assessment of PCNA and P53 could provide very useful information for the prognosis of PLC.

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Restriction fragment length polymorphism of pepsinogen C gene in patients with gastric carcinoma and in high risk population of gastric carcinoma

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Abstract

AIM: To analyze the difference of distribution of the pepsinogen C (*PGC*) gene polymorphism among different groups and to study its application value in screening of the high risk population of gastric carcinoma and its value as an indicator for gene diagnosis.

METHODS: The study consisted of two parts: study on the restriction fragment length polymorphism (RFLP) of *PGC* gene in normal individuals and patients with gastric cancer, and study on the *PGC* gene polymorphism in members from a family at a high risk for stomach carcinoma and the follow-up survey. A total of 40 cases were analyzed including 11 healthy blood donors, 10 gastric carcinoma patients, and 19 members from a high risk family of stomach carcinoma. The Southern blot method was adopted in the study. The probe and restriction endonuclease used were *PGC* 301 and *EcoR* I, respectively. Gastroscopy and gastric mucosa biopsy were performed in the follow-up study.

RESULTS: There were three kinds of common *PGC EcoR* I allelic fragments (20 kb, 5.7 kb and 3.6 kb) and only one rare fragment (3.5 kb) in the normal subjects, and there was no difference in allelic fragments between the normal subjects and the patients. The incidence of the rare fragment and rare hybrid band type in the patients was higher than that in the normal subjects. The incidence of rare fragment and rare hybrid band type in the members from a high risk family was a little higher than that in normal subjects. After the 3-year follow-up by gastroscopy, one of the four members from the high risk family with *PGC EcoR* I rare fragment was found to suffer from early stomach cancer. The hybrid signal of *EcoR* I common allelic fragments in the gastric tumor tissue was weakened, even disappeared.

CONCLUSION: *PGC EcoR* I rare fragment and rare hybrid band type in the early diagnosis and screening of high-risk population of stomach carcinoma are probably of important application value. There is gastric normal differentiation gene (*PGC* gene) deletion in the stomach tumor tissue.

Key words: Stomach neoplasms; PGC; Restriction fragment length polymorphism

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INTRODUCTION

In recent ten years, with the application of molecular biological techniques in clinical medicine, as a method of gene diagnosis, the applying value of restriction fragment length polymorphism (RFLP) analysis in the diagnosis of cancer as well as the screening of high risk population of cancer has called wide attention of scholars. In 1985, Krontiris *et al*[1] first reported that the polymorphism of ras gene could be used in predicting the risk of cancer occurrence. From then on, there have been many studies focusing on the polymorphism analysis of tumor genes in hopes of finding out the specific genetic susceptible marker of cancer[2-5]. However, in few studies the polymorphism was analysed using some normal tissue differentiation genes. In fact, compared with the common tumor gene detection method, the polymorphism analysis of some normal tissue differentiation genes is even more specific for the diagnosis of some kinds of cancer.

In 1988, Azuma *et al*[6] first reported the polymorphism of pepsinogen C (*PGC*) gene. In 1993, Azuma *et al*[7] studied the ulcer

Table 1 The distribution of pepsinogen C 301 alleles

Allele	Size (kb)	Normal subjects *n* (%)	Patients *n* (%)
a1	20.0	22 (100)	16 (80)
a2	5.7	22 (100)	20 (100)
a3	3.6	20 (90)	14 (70)
a4	3.5	2 (10)	6 (30)

Table 2 The distribution of DNA band types

Hybrid band type	Normal subjects *n* (%)	Patients *n* (%)
Common type		
a1/a2/a3	9 (82)	4 (40)
Rare type		
a1/a2/a3/a4	2 (8)	3 (30)
a1/a2/a4	0	1 (10)
a2/a3/a4	0	1 (10)
a2/a3	0	1 (10)

Table 3 The distribution of pepsinogen C 301 alleles

Allele	Size (kb)	Family members *n* (%)
a1	20.0	38 (100)
a2	5.7	38 (100)
a3	3.6	36 (86.8)
a4	3.5	5 (13.2)

Table 4 The distribution of DNA band types

Hybrid band type	Family members *n* (%)
Common type	
a1/a2/a3	15 (78.94)
Rare type	
a1/a2/a3/a4	3 (15.79)
a1/a2/a4	1 (5.26)

located in the body of the stomach using the *PGC* polymorphism, and found that the frequency of *PGC EcoR* I rare fragment of the patients was significantly higher than that of the normal population. They thought that the *PGC* polymorphism could be regarded as a genetic susceptible marker of ulcer in the body of the stomach. So far there has been no report on the *PGC* polymorphism in patients with gastric carcinoma and in the high risk population. It is unknown if there is any difference between the polymorphism distribution of *PGC*, a gastric normal differentiation gene, in normal subjects and in patients with gastric carcinoma, and if the detection of the difference could be regarded as a marker in the early diagnosis of gastric carcinoma and in the screening of high risk population.

MATERIALS AND METHODS

Materials

The study consisted of two parts. The first part was study on the *PGC* gene polymorphism in normal individuals and in patients with gastric cancer. The second part was study on the *PGC* gene polymorphism in members from a high risk family of gastric carcinoma and the follow-up survey. A total of 40 cases were analysed. Ten gastric tumor tissues were obtained surgically at the First Affiliated Hospital of China Medical University, and 11 normal blood samples were obtained from healthy donors without family histories of gastric cancer. Nineteen peripheral blood samples for the study were obtained in February 1990 from a high risk family of gastric carcinoma in Zhuanghe City of Liaoning Province which is a high risk area of gastric carcinoma. By that time, there had been four deaths due to gastric carcinoma in three generations in the family. Except the aged and the children, 11 cases were examined by means of gastroscopic biopsy. The results showed that one case suffered from atrophic gastritis and the other 10 suffered from superficial gastritis of different stages. No case of gastric cancer was found.

Methods

The Southern blot method was adopted in the study. The gene probe used in the experiment was *PGC* 301 which was supplied by Dr. Azuma of Kyoto Prefectural University of Medicine, Japan. Gastric cancer genomic DNA was extracted from the surgical specimens using sodium dodecyl sulphate (SDS), EDTA, proteinase K, phenol and chloroform. After removing RNA with RNA enzyme, DNA was precipitated with alcohol of two times in volume, mixed in proper TE buffer solution, and kept for use at 4 ℃. The DNA of normal people and members from a high-risk family was obtained from peripheral blood white cells. The extracting method was the same as that used in previous work[8]. Genomic DNA was digested with the stated restriction endonuclease *EcoR* I and electrophoresed on a 0.8% agarose gel. After electrophoresis, DNA was denatured, neutralized and transferred to nitrocellulose membranes according to the method of Southern blot. The α-32p dCTP PGC probe (3000 Ci/mmol, Amersham's product) was labelled using a Random Primer Labelling Amersham's Kit to a specific activity of 2×10^9 dpm/μg. Then, the nitrocellulose membranes were hybridized in a water bath at 42 ℃, agitated overnight, and washed, followed by autoradiography at -80 ℃ for 3 to 5 d.

RESULTS

EcoR I RFLP analysis of *PGC* gene was performed in a total of 11 normal subjects, 10 patients with gastric carcinoma and 19 members from a high-risk family. The results showed that there were three kinds of common allelic fragments (20 kb, 5.7 kb and 3.6 kb) and only one rare fragment (3.5 kb) in the normal subjects. There was no difference in allelic fragments between the normal subjects and the patients, but the hybrid signal of common fragments of the patients was weakened and with deletion. Two patients had no 20 kb fragment, one had no 3.6 kb fragment, and five had 3.5 kb fragment (Table 1). The hybrid DNA bands were classified into five types. The common type was 20 kb/5.7 kb/3.6 kb which appeared in nine normal subjects and four patients. There were four rare types. One was 20 kb/5.7 kb/3.6 kb/3.5 kb which appeared in two normal subjects and four patients, and the other three were 20 kb/5.7 kb/3.6 kb/3.5 kb; 5.7 kb/3.6 kb/3.5 kb; and 5.7 kb/3.6 kb which appeared only in the patients (Table 2).

There were three generations and 19 members in the high risk family of gastric carcinoma. The first generation had 2 members, the second had 12 and the third had 5. After examination of the *PGC* gene *EcoR* I RFLP, we found that the incidences of the three common allelic fragments, 20 kb, 5.7 kb and 3.6 kb, in the family members were 100%, 100% and 86.6%, respectively. The 3.5 kb rare fragment appeared in four family members (Table 3). There were three hybrid band types in the family, one common type (20 kb/5.7 kb/3.6 kb) and two rare types (20 kb/5.7 kb/3.6 kb/3.5 kb and 20 kb/5.7 kb/3.5 kb) (Table 4). We examined by means of gastroscopic biopsy and pathology and followed the four family members (all in the second generation) who had *PGC* gene *EcoR* I rare fragment. Two and a half years later, one of them, a 54-year-old male, was found to have early gastric carcinoma. This man underwent gastroscopic examination and was diagnosed as having atrophic gastritis in March 1990. He also underwent the examination of *PGC* gene *EcoR* I RFLP in the same period, and the results showed that the band type was 20 kb/5.7 kb/3.6 kb/3.5 kb. In October 1992, gastroscopic reexamination revealed that there was an irregular superficial erosion with unclear border on the back wall of the upper part of the lesser curvature of the stomach body. It was 3 cm × 2 cm in size with gray-yellow mucus on its surface and hyperemic edge. The lesion was diagnosed as early stomach carcinoma of type II_c and confirmed by gastric mucosa pathology as lowly differentiated carcinoma and signet ring cell carcinoma.

DISCUSSION

It was reported[9,10] that *PGC* was located on 6p21.1-Pter with 9 exons and *PGC* 301 was a 1224-bp cDNA clone containing exons

2-9 of the human *PGC* gene coding sequence. There was *EcoR* I RFLP between exons 7 and 8 in normal subjects because of DNA polymorphism. The frequencies of its polymorphic fragment were 82.5% (3.6 kb) and 17.5% (3.5 kb). In the present study, the analysis of *EcoR* I RFLP of normal subjects showed that there were 20 kb and 5.7 kb fragments in all normal subjects, but 3.6 kb and 3.5 kb fragments were distributed in polymorphism. The allelic fragment of 3.6 kb was 90% while that of 3.5 kb was 10%, which was a little different from 17.5% reported in previous studies. The reason may be the small sample size and different subjects. By increasing the sample size, its allelic fragment might be more accurately illustrated. No 20 kb fragment was found in 2 (20%) patients with gastric cancer, which might be due to the partial deletion of the *PGC* gene in gastric cancer. This is probably of important significance in the occurrence of gastric cancer. In addition, the frequency of 3.5 kb polymorphism fragment in gastric cancer patients was significantly higher than that in normal subjects, and the frequency of rare band type in patients was also higher than that in normal subjects. The atrophic gastritis in a member of high-risk family with 3.5 kb fragment finally developed into early gastric cancer. The results suggest that it is necessary to increase the sample number to investigate the significance of *PGC* rare fragment and rare hybrid band type for the early diagnosis of gastric cancer, and to further compare the changes and polymorphism of *EcoR* I fragment in gastric tumor tissue and other tissues of the patient. These are essential for illustrating the significance of changes in *PGC* gene structure in the occurrence of gastric cancer.

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Changes of plasma cAMP and cGMP levels in chronic atrophic gastritis patients with spleen-qi deficiency

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Abstract

AIM: To investigate the relationship between plasma cAMP and cGMP levels and spleen-qi deficiency in chronic atrophic gastritis.

METHODS: Plasma cAMP and cGMP levels were detected in chronic atrophic gastritis patients with spleen-qi deficiency (n = 87). Thirty patients underwent self-controlled study prior to and after 1-2 courses of Weiweian therapy, with each course lasting 3 mo. The samples were detected using ^{125}I-labelled cAMP and cGMP kits.

RESULTS: Plasma cAMP and cGMP levels in all cases of chronic atrophic gastritis patients with spleen-qi deficiency syndrome were lower than or at the lower margin level. In the 30 patients receiving Weiweian therapy, both plasma cAMP and cGMP levels increased significantly after the therapy as compared with those prior to the therapy (P < 0.01, and < 0.05).

CONCLUSION: Measurements of plasma cAMP and cGMP levels are of great importance in CAG patients with spleen-pi deficiency, and Weiweian therapy can correct the spleen-qi deficiency. Furthermore, plasma cAMP may serve as a prognostic factor in CAG, and it might prevent CAG from progressing into gastric cancer since plasma cAMP is low in gastric cancer.

Key words: Gastritis, atrophic; Spleen-qi deficiency; cAMP; cGMP

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INTRODUCTION

It had been proved that plasma cAMP level was lowered in experimental animals with spleen-deficiency syndrome[1,2]. Both plasma cAMP and cGMP levels were decreased in experimental qi deficiency (*i.e.*, energy deficiency)[2], thus providing valid clues to the insight of this syndrome. This study mainly explored the relationship between plasma cyclic nucleotides and spleen-qi deficiency in chronic atrophic gastritis (CAG).

MATERIALS AND METHODS

Patients

Eighty-seven patients were diagnosed with CAG by fibergastroscopy and pathologic examination. Of these patients, 57 were male and 30 were female with an average age of 47.9 years (range, 21 to 72 years). Thirty of the 87 patients were subjected to self-controlled study prior to and after one or two courses of Weiweian therapy, with each course lasting 3 mo. Among these 30 patients, 17 were male and 13 female with an average age of 50.2 years (range, 36 to 72 years).

Method

The samples were detected using ^{125}I-labelled cAMP and cGMP kits provided by the Nuclear Laboratory of Shanghai Traditional Chinese Medicine College. The samples were pretreated with anticoagulant and detected according to the instructions of the kits and counted by Beckman 5800 liquid scintigraphy.

RESULTS

Plasma cAMP and cGMP levels in 87 CAG patients are shown in Table 1.

The plasma cAMP and cGMP levels in 30 patients with CAG prior to and after therapy are shown in Table 2.

DISCUSSION

The cAMP is universally present in the body cells and body fluids, through which the neurohumoral functions were mediated. The cAMP and cGMP were released by the cells and tissues into blood. The evaluation of cAMP and cGMP is of importance in the study of the physiology and pathophysiology of human diseases. CAG patients usually manifest spleen-qi deficiency syndrome which is caused not only by gastric mucosal glandular atrophy, but also by the abnormal neurohumoral function. Decrease in plasma cAMP reflects hypofunction of sympathetic nervous activity and relative hyperfunction of parasympathetic nervous activity. Spleen-qi deficiency is characterized by epigastric fullness or aching, loose

Table 1 Plasma cAMP and cGMP levels in 87 chronic atrophic gastritis patients

	Normal value	CAG patients	*t*
cAMP	23.1 ± 7.7	14.629 ± 4.307[b]	6.742
cGMP	4.6 ± 0.7	4.668 ± 1.317	0.449

[b]$P < 0.01$. CAG: Chronic atrophic gastritis

Table 2 Plasma cAMP and cGMP levels in 30 patients with chronic atrophic gastritis prior to and after Weiweian therapy

	Prior to therapy	After therapy	*t*
cAMP	13.234 ± 3.968	27.285 ± 7.330[b]	8.955
cGMP	4.159 ± 1.494	7.366 ± 7.290[a]	2.217

[a]$P < 0.05$, [b]$P < 0.01$.

bowel movement, weakness, poor appetite, swollen tongue with teeth printing and thready pulse, which is called spleen-stomach-qi deficiency symptom complex. In all cases, plasma cAMP was lower than normal (Table 1) ($P < 0.01$), and cGMP was at its lower normal limit. The spleen deficiency syndrome of CAG is closely related to the plasma levels of cAMP and cGMP which can be modulated by the body's neurohumoral function. The 30 patients were treated with Weiweian powder, and the results were satisfactory. The effective rate of symptom relief was 90% with regression of pathologic lesions in 73.3%. Through this self-controlled study, we observed a remarkable increase of plasma cAMP which was of marked statistical significance ($P < 0.01$), so was that of cGMP ($P < 0.05$). Weiweian powder has the effects of strengthening spleen and benefiting qi, so that the spleen-qi deficiency syndrome can be improved remarkably. It was also found that spleen-qi deficiency with low cAMP usually occur in gastric cancer[3].

In conclusion, modulating the plasma cAMP and cGMP levels is of importance in correcting spleen-stomach-qi deficiency. Therefore, the increase of cyclic nucleotides in CAG is of prognostic significance. Meanwhile the increase of the concentration of plasma cAMP is conducive to preventing CAG from progressing into gastric cancer, since plasma cAMP level is usually low in patients with gastric cancer.

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Study on content of serum epidermal growth factor, gastric acid secretion and serum gastrin in duodenitis

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Abstract

AIM: To study whether there is EGF secreting abnormality in duodenitis and its relationship with gastric acid output and serum gastrin, so as to further explore the pathogenesis of duodenitis.

METHODS: Twenty-five duodenitis patients were confirmed by electrogastroscopy and biopsy, with an average age of 35.9 ± 7.0 years (range, 24-52 years). The control group consisted of 20 healthy volunteers (10 females, 10 males), with an average age of 34.4 ± 7.6 years (range, 23-48 years). Twenty duodenal ulcer patients (10 females, 10 males), with an average age of 35.0 ± 7.6 years (range, 24-52 years), were also included. Serum EGF and gastrin were measured using radioimmunoassay. Intragastric acidity was determined by pentagastrin method. Statistical analysis was performed using Student's *t*-test.

RESULTS: In comparison with those in the control group, the contents of serum EGF and serum gastrin in duodenitis patients were all significantly increased. In comparison with those in the duodenal ulcer group, serum EGF was significantly increased, basal acid output and peak acid output were decreased, and serum gastrin was increased significantly in duodenitis patients. Serum EGF was negatively correlated with gastric acid output and positively correlated with serum gastrin.

CONCLUSION: In duodenitis, serum EGF concentration was increased, which was positively correlated with serum gastrin content, but was negatively correlated with gastric acid output. This indicates that EGF plays a protective role in the pathogenesis of duodenitis, which provides a new clue to pathogenesis study of duodenitis.

Key words: Duodenitis; Epidermal growth factor; Gastric acid; Gastrin

Gong LB, Yang X, Zhang WW, Li SL, Sun SY. Study on content of serum epidermal growth factor, gastric acid secretion and serum gastrin in duodenitis. *World J Gastroenterol* 1996; 2(4): 228-229 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/228.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.228

INTRODUCTION

Epidermal growth factor (EGF) is a polypeptide consisting of 53 amino acids, which was first isolated from the mouse submandibular gland by Cohen[1] and was also found in human submandibular gland, Brunne's gland of the duodenum, pancreas, breast gland, kidney, *etc*. EGF has the function of inhibiting gastric acid secretion and promoting the synthesis of mucosa DNA, RNA and protein. It has certain protective function on gastroduodenal mucosa against multiple stimulators[2,3]. Now, the pathogenesis of duodenitis is still unclear. The increase of gastric acid is a key factor[4]. We assumed that in duodenitis, the increased secretion of EGF may enhance the defensive function of duodenal mucosa and inhibit hypersecretion of gastric acid.

MATERIALS AND METHODS

Subjects

A duodenitis group (DI group) consisted of 25 patients (13 males and 12 females) with an average age of 35.9 ± 7.0 years. Chronic superficial duodenitis was proven by endoscopic mucosa biopsy in accordance with the pathologic diagnostic criteria of the National Gastric Carcinoma Protection and Treatment Cooperation Group[4]. A control group consisted of 10 males and 10 females; the average age was 34.4 ± 7.6 years (range, 23-48 years). A fuodenal ulcer group (DU group) consisted of 20 patients (10 males and 10 females), with an average age of 35.0 ± 7.6 years (range, 24-52 years), and all were at the active stage. Diseases of the liver, gallbladder, pancreas, kidney and thyroid gland were excluded. The patients had not any history of smoking and did not take any drug during thelast one week.

Methods

Five milliliters of venous blood sample were taken from each subject in the morning (fasting,with in 3 d after electrogastroscopic examination). After standing and centrifugation, serum was separated and 1 mL serum was stored at -70 ℃ for EGF detection while another 1 mL was stored at -20 ℃ for gastrin determination.

Table 1 Content of serum epidermal growth factor, gastric acid output and serum gastrin in duodenitis patients (mean ± SD)

Group	*n*	Serum EGF (ng/mL)	Gastric acid output		Gastrin (ng/mL)
			BAO	PAO (mmol/h)	
DI	25	1.62 ± 0.64[bd]	5.91 ± 2.52[ba]	24.07 ± 5.84[ba]	102.17 ± 16.24[bd]
Control	20	0.02 ± 0.34	3.64 ± 1.38	19.76 ± 2.34	84.94 ± 15.63
DU	20	0.41 ± 0.24[b]	7.68 ± 3.25[b]	30.23 ± 5.50[b]	74.64 ± 9.10

[b]$P < 0.01$ compared with control group; [d]$P < 0.01$ compared with DU group; [a]$P < 0.05$ compared with DU group. BAO: Basic acid output; PAO: Peak acid output.

Gastric acid output was determined by pentapeptide gastrin method. RIA kits were provided by Beijing Haikerui Biological Technique Center (for EGF) and Beijing Atomic Energy Institution (for gastrin). Pentapeptide gastrin was provided by Shanghai Biochemical Institution (950514).

Statistical analysis

All data are expressed as mean ± SD, and *t*-test was used to compare the differences between groups. Linear regressive analysis and relative coefficient test were used to study the relationship between variables.

RESULTS

In comparison with normal controls, serum EGF ($P < 0.01$), gastric acid output ($P < 0.01$), and serum gastrin ($P < 0.01$) in the duodenitis group were highly increased. In comparison with the duodenal ulcer group, serum EGF ($P < 0.01$) and serum gastrin ($P < 0.01$) were highly increased, too, while gastric acid output was decreased. In the duodenitis group, serum EGF was negatively correlated with gastric acid output (peak acid output: $r = -0.716$, $P < 0.01$; linear regressive equation, $Y = 3.2269 - 0.07X$), but was positively correlated with serum gastrin ($r = 0.7434$, $P < 0.01$; linear regressive equation, $Y = 0.0297X - 1.3637$). All data are shown in Table 1.

DISCUSSION

Etiology of duodenitis is not very clear. The inflammation may be due to the co-action of many factors, just like esophagitis and gastritis. Hypersecretion of gastric acid has long been thought of one of the most important factors. EGF has the function of inhibiting gastric acid secretion and promoting the synthesis of mucosa DNA, RNA and protein. And it has a certain protective function on gastroduodenal mucosa injured by multiple factors. EGF has a promoting effect on epithelial cell proliferation[5], and it can also promote regeneration of damaged duodenal epithelia. In this study, serum EGF in the DI group was significantly higher than that in the healthy control group and DU group, which suggested that the compensatory increase of EGF secretion was present in duodenitis. For gastric acid secretion, including basic acid output and peak acid output , it was in the order of healthy control group < DI group < DU group, which was the same as the viewpoint in Baron's report[4]. Serum EGF was negatively correlated with gastric acid secretion, which showed that EGF could inhibit gastric acid secretion, which was the suggestion by Korturek's research[6]. Animal experiments show that acidic substance in the duodenum may cause the release of VIP, which is an important neurotransmitter capable of regulating duodenal gland secretion. We deduce that the increase of gastric acid in duodenitis can cause the release of VIP, promoting the secretion of EGF by the duodenal gland[7] (mainly referring to Brunner's gland)[8]. This may be the reason for the increase of serum EGF, but the mechanism is unclear. The increase of EGF can protect the duodenal mucosa by stimulating alkaline secretion and mucosa formation in the duodenum, and can decrease gastric acid level[9,10]. Therefore, it plays a protective role in the pathogenesis of duodenitis.

In our study, serum gastrin level in the DI group was much higher than that in the control group, which is in agreement with Sun Su-Yun's report. The mechanism may be the same as that of EGF, for VIP promotes the secretion of G cells in the Brunner's gland. It was reported that gastrin can promote the EGF secretion[11], which is consistent with our result that serum gastrin was positively correlated with the EGF increase.

In general, the bottom of peptic ulcer can penetrate the submucosa layer, and may go deep into the muscular layer, even to the serosa layer. The submucosa layer, even the muscular layer, is completely eroded and replaced by granular tissues and scar tissues, so the Brunner's gland in the duodenal submucosa layer can be injured. An animal study shows that semicystamine can induce duodenal ulcer by inhibiting the Brunne's gland[11]. Many animal experiments have shown that infusion of exogenic EGF to the stomach or duodenum could prevent experimental ulcer or promote the healing of ulcer[3], which indicates that endogenous EGF deficiency can decrease the defensive function of the mucosa against harmful factors. Furthermore it shows that EGF plays an important protective role for the duodenal mucosa.

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Correlation between Hepatitis delta virus infection and heptitis B virus serum markers

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Abstract

AIM: To analyse the correlation between HDV infection and HBV serum markers.

METHODS: Patients who were positive for HBV serum markers were selected and HDV infection was examined in them. Blood donors were used as a control group. Both HDV infection and HBV serum markers were tested by enzyme-linked immunosorbent assay.

RESULTS: HDV infection was detected in 40 of 289 patients who were positive for HBV serum markers. The overall positive rate of HDV infection was 13.8%. The positive rates of HDV infection in HBsAg(+) group, HBcAb(+) group and HBeAb(+) group were 17.6%, 18.8% and 25.2%, respectively, which were higher than that in HBeAg(+) group (10.9%), and none was detected in HBsAb(+) group. HDV infection appeared in HBsAg(+)HBcAb(+)HBeAb(+) patients with a positive rate of 26.2%, which was much higher than that in HBsAg(+)HBcAb(+)HBeAg(+) patients (10.9%).

CONCLUSION: HDV coinfection is more frequent in HBsAg(+) HBcAb(+)HBeAb(+) patients than in BsAg(+)HBcAb(+)HBeAg(+) patients. HDV infection is not completely related with the speed and amount of HBV replication.

Key words: Hepatitis delta virus infection; Hepatitis B; Enzyme linked immunosorbent assay

Song CH, Wu MY, Wang XL, Dong Q, Tang RH, Fan XL. Correlation between HDV infection and HBV serum markers. *World J Gastroenterol* 1996; 2(4): 230-231 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/230.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.230

INTRODUCTION

Hepatitis delta virus (HDV) is a defective RNA virus which is related to viroids and requires HBsAg synthesis for its assembly and infectivity. HDV occurs either in a coinfection form with heptitis B virus (HBV) resulting in acute or fulminant hepatitis, or in a superinfection form in a chronic HBV carrier resulting in severe chronic hepatitis B and liver cirrhosis[1]. There is a high rate of HBV infection in China and many reports have reported HDV infection. However, these reports concentrated much on the observation of symptoms of hepatitis D, and on epidemiological study of HDV infection in the patients with acute or chronic hepatitis B[2]. In China, the serological diagnosis of HBV infection plays an important role in the diagnosis of hepatitis B, and HBV serum markers can to some extent reflect the HBV replication state, HBV infectivity and progress of the patients. In order to draw some hints about the relation between HDV infection and active state of HBV replication, we observed the positive rate of HDV infection in 289 patients with HBV who were positive for serum markers and analysed the correlation between HDV infection and HBV serum markers.

MATERIALS AND METHODS

Patients

From June 1995 to December 1995, a total of 289 serum samples from HBV carriers visiting our hospital were examined. There were 195 males and 94 females, ranging in age from 5-62 years (mean, 32.6 years). The selected patients were positive for at least 1 of 5 HBV serum markers (HBsAg, HBsAb, HBcAb, HBeAg, and HBeAb), including 60 patients with only HBsAb(+) who refused to be vaccinated (group A), 21 patients with only HBsAg(+) (group B), 107 patients with HBsAg(+)HBcAb(+)HBeAb(+) (group C) and 101 patients with HBsAg(+)HBcAb(+)HBeAg(+) (group D).

Methods

Five HBV serum markers were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Kohua, Shanghai). HDAg, total anti-HD and anti-HD IgM were also measured in the 289 serum samples by ELISA (Yelikan Biological Company Ltd., Yangzhou, China). The patients whose sera were positive for one or more than one of the 3 HDV serum markers were considered as having HDV infection. Positive results were verified by a repeat test. A control group consisted of normal sera from blood

Table 1 Positive rate of HDV infection in each HBV serum marker (+) group *n*(%)

	HBsAg (*n* = 227)	HBsAb (*n* = 64)	HBcAb (*n* = 208)	HBeAg (*n* = 101)	HBeAb (*n* = 111)
HDAg	17	0	17	8	9
Total anti-HD	5	0	5	0	5
anti-HD IgM	19	0	18	3	15
Total	40 (17.6)	0	39 (18.8)	11 (10.9)	28 (25.2)

Table 2 Positive rate of HDV in common HBV serology pattern groups *n*(%)

	Group A (*n* = 60)	Group B (*n* = 21)	Group C (*n* = 107)	Group D (*n* = 101)
HDAg	0	0	9 (8.4)	8 (8.0)
Total anti-HD	0	0	5 (4.7)	0
anti-HD IgM	0	1 (4.8)	15 (14.0)	3 (3.0)
Total	0	1 (4.8)	28 (26.2)	11 (10.9)

donors.

Statistical analysis

U-test of rates between two groups was used for statistical analysis. $P < 0.05$ was considered significant.

RESULTS

Positive rate of HDV infection in patients positive for HBV serum markers

HDV infection was detected in 40 of 289 patients with HBV serum markers(+). All had only one positivity of HDV serum indicator except that one had both total anti-HD and anti-HD IgM. The overall positive rate of HDV infection was 13.8%, HDAg positive rate was 5.9%, total-anti-HD was 1.7% and anti-HD IgM 6.6%.

Positive rate of HDV infection in each serum marker group

HDV infection was detected in 28 (25.2%) of 111 sera from patients with HBeAb(+), 39 (8.8%) of 208 sera with HBcAb(+), 40 (17.6%) with HBsAg(+) and 11 (10.9%) with HBeAg(+). However, none of the 64 patients with HBsAb(+) was found positive. The statistical analysis was carried out between every two groups, and a significant difference ($P < 0.01$) between all of them except between HBsAg(+) group and HBcAb(+) group was seen (Table 1).

Positive rate of HDV infection in common HBV serology pattern

HDV infection was found in 28 (26.2%) of 107 patients in group C, 11 (10.9%) of 101 patients in group D, and 1 (4.8%) in group B. The differences of positive rates between every two groups were significant ($P < 0.01$) (Table 2).

DISCUSSION

Clinical recognition of HDV infection is complex because of its diverse manifestations and variable outcomes. The detection of HDV serum indicators is a helpful measure, though the problems of the test sensitivity and sampling time could lead to false negative results. HDAg appears transiently during the earliest phase of HDV infection prior to the onset of symptoms. IgM or anti-HD IgG may also appear either transiently or late in the course of the disease[3]. Smedile found that anti-HD IgM appeared in both acute and chronic hepatitis D, and the patients with total anti-HD used to be grouped into chronic hepatitis D[4]. Now it has been proven that the presence of either IgM or anti-HD IgG is in accord with active HDV replication, so the patients positive for one of HDV serum markers can be considered as having HDV infection[3]. Our results showed that the positive rates of HDAg and anti-HD IgM were higher than that of total anti-HD (mainly IgG type), possibly because most of the patients tested were with acute hepatitis D or in the early phase of HDV infection, or because the sensitivity of total anti-HD detection was lower than that of the others.

HDV requires the help of HBV for its replication. HBsAg in HBV infected cells is used by HDV, and it is important for the carrying of chronic HDV[1]. These facts can explain why all the 40 cases of HDV infection in our study appeared in the HBsAg(+) patients, but none in the infected patients positive for only HBsAb. Interestingly, the positive rate of HDV infection varied significantly in different HBV serum marker groups (Table 1) and in common HBV serology pattern groups (Table 2). It could be easily understood that no HDV infection was detected in patients positive for only HBsAb because there was no HBV replication, and that much lower positive rate of HDV infection in patients positive for only HBsAg (group B) was because of less HBV replication. These results suggest that HDV infection has relations with HBV replication. However, the positive rate in HBsAg(+)HBcAb(+)HBeAb(+) patients (group C) was higher than that in HBsAg(+)HBcAb(+)HBeAg(+) patients (group D), in whom HBV replicates more rapidly and has a greater quantity. This reveals that HDV infection is not completely related with the amount and speed of HBV replication. Until now, the mechanism of this phenomenon is unclear, and it is possibly because there exists a co-interference between HDV and HBV replication. It has been found that HDV replication not only depends on HBV replication, but also can suppress the expression of HBsAg and HBcAg in hepatocytes[5].

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Preliminary study on mechanism of drug resistance in human colon cancer cell line HCT$_{V2000}$

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Abstract

AIM: To investigate the mechanisms of multidrug resistance in human colon cancer cell line HCT$_{V2000}$.

METHODS: P-glycoprotein (P-GP) was detected by immunocytochemical staining. Expression and amplification of *mdr1* gene were detected by nucleic acid hybridization. Cytotoxicity was measured by MTT method.

RESULTS: HCT$_{V2000}$ cells showed a positive response to monoclonal antibodies against P-GP encoded by the *mdr1* gene. Overexpression of *mdr1* mRNA was found, but no evidence of *mdr1* gene amplification or rearrangement was suggested. Verapamil increased the accumulation of ^{3}H-VCR inside the cells and partially reversed drug resistance to VCR.

CONCLUSION: Overexpression of *mdr1* mRNA is one of the important mechanisms of drug resistance in HCT$_{V2000}$ cell line; however, there may be some other mechanisms which are also involved in this.

Key words: Colon neoplasms; Drug resistance; Cell line

Zhang XH, Li XT, Ji XJ, Zhu YJ. Preliminary study on mechanism of drug resistance in human colon cancer cell line HCT$_{V2000}$. *World J Gastroenterol* 1996; 2(4): 232-235 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/232.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.232

INTRODUCTION

Investigating the mechanisms of multidrug resistance (MDR) is one of the methods of raising chemotherapy effects in cancer. Drug-resistant cell lines are an important model system for the analysis of *mdr1* gene in human cancer cells. The protein product of *mdr1* gene, P-glycoprotein (P-GP), is an energy-dependent drug efflux pump, which decreases intracellular drug accumulation. MDR cell lines have wide cross-resistance to hydrophobic compounds[1]. Verapamil (VRM) may reverse MDR in these cells by competing with antitumor drugs for binding to P-GP[2]. HCT$_{V2000}$, an MDR cell line of human colon cancer HCT-8 cells, was developed by stepwise selection on exposure to increasing doses of vincristine (VCR). In this report we aimed to study expression and amplification of *mdr1* gene, and the effect of reversal of VCR resistance with verapamil, as well as to analyse the mechanisms of drug resistance in HCT$_{V2000}$ and its parent line.

MATERIALS AND METHODS

Cell lines and cell culture

The HCT-8 line of human colon cancer cells was obtained from the American Type Culture Collection. HCT$_{V2000}$ cells were 147-fold more resistant to VCR than HCT-8 cells. Cells were grew in monolayer in RPMI-1640 with 10% newborn calf serum, penicillin (100 U/mL) and streptomycin (100 μg/mL). The cultures were incubated at 37℃ in a humidified atmosphere of 5% CO_2.

Survival assay by MTT assay[3]

Cells were seeded onto 96-well plates at a density of 2000 cells per well. Drugs were added 24 h later. After incubation at 37℃ for 96 h, 100 μL of 0.5 mg/mL MTT reagent (FLUKA) was added to each well. The plates were then incubated for 4 h, and DMSO was added before absorption at 540 nm was measured with a Dynatech MR700 ELISA plate reader.

Immunocytochemical staining

JSB-1 (1:120) and MRK-16 (5 μg/mL), monoclonal antibodies against P-GP were provided by Dr. Scheper and Dr. Tsuruo, respectively. Expression of P-GP was detected with avidin-biotin peroxidase complex (ABC) method as described by Grogan *et al*[4]. ABC kits were produced by Vector Laboratories, Inc.

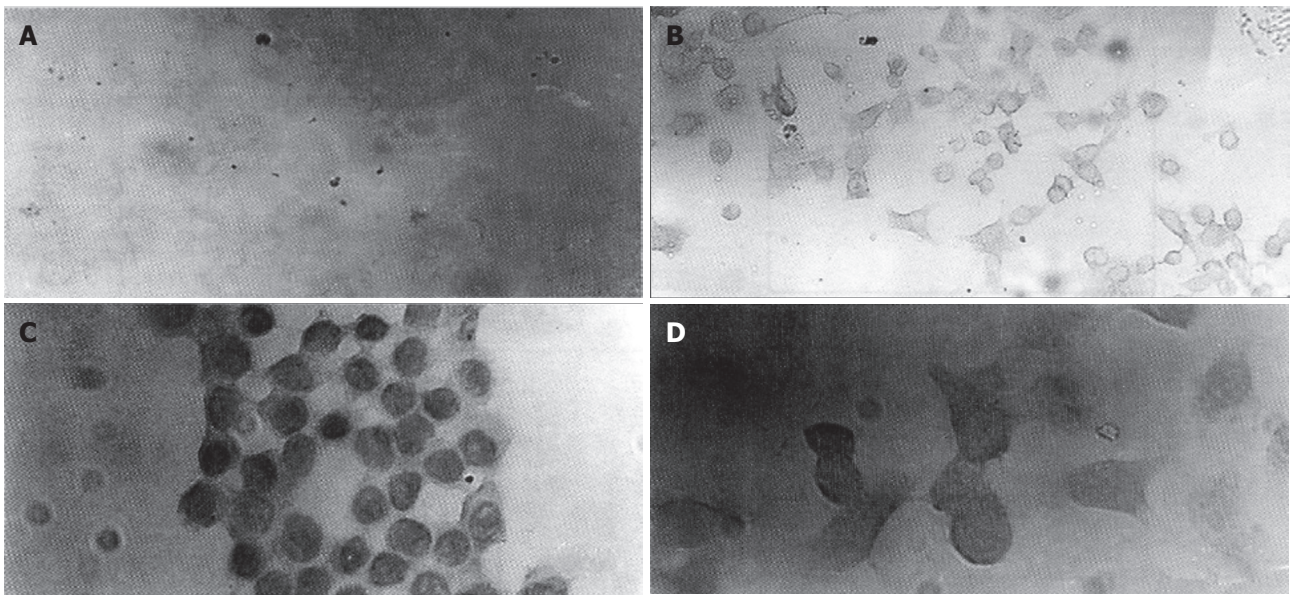

Figure 1 Demonstration of P-glycoprotein in HCT-8 (A and C) and HCT$_{V2000}$ cells (B and D) by immunocytochemical staining with JSB-1 (A and B) and MRK-16 (C and D).

DNA and RNA isolation

Total cellular RNA was prepared by acid-phenol extraction method[5]. Genomic DNA was isolated from cells by the method of Maniatis *et al*[6].

DNA and RNA analysis[6]

DNA (10 μg) was digested with the restriction endonuclease *Eco*R Ⅰ, electrophoresed on a 0.7% agarose gel, and transferred to nylon filters for Southern blot analysis. Slot blotting filters were prepared with DNA using a BRL blot apparatus. RNA (10 μg) was separated on 1.2% agarose gels containing formaldehyde, and then transferred to a nylon filter. DNA and RNA were cross-linked to the filter by baking for 1 h at 80℃ in an oven. A ^{32}P-labeled DNA probe, a 1.383-kb *Eco*R Ⅰ fragment containing the *mdr1* coding regions from PHDR5A, was labeled by the random priming method. PHDR5A was provided by Dr. Gottesman (NCI, Bethesda, MD, United States).

Drug accumulation

Cells were plated at a density of 5 × 10^5 cells/mL and incubated overnight at 37 ℃. Then the medium was replaced with serum-free RPMI1640, and the cells were incubated for 1 h with ^{3}H-VCR (4.6 × 10^{10} Bq) in the absence or presence of VRM. The cells were washed three times with cold PBS. NaOH solution (1 mol/L) was added to dissolve the cells, and the solution was neutralized with acetic acid. The radioactivities were determined by liquid scintillation counting.

RESULTS

Expression of P-GP

Immunocytochemical staining showed that P-GP could be detected in plasma membrane and cytoplasm of HCT$_{V2000}$ cells with both JSB-1 and MRK-16. However, in HCT-8 cell line, a low level of P-GP expression was detected with MRK-16, but not with JSB-1 (Figure 1).

Expression of mdr1 mRNAt

Northern blot analysis was conducted with RNA isolated from the two cell lines. The *mdr1* specific probe was hybridized to a 4.5 kb RNA species, which showed high expression in HCT$_{V2000}$, but no *mdr1* mRNA was detected in HCT-8 (Figure 2).

Amplification of mdr1 gene

To determine the possibility that overexpression of *mdr1* mRNA in HCT$_{V2000}$ cell line might result from *mdr1* gene amplification or rearrangement, genomic DNA from HCT$_{V2000}$ and HCT-8 was analyzed. DNA slot blot analysis showed that the signal intensity from HCT$_{V2000}$ was the same as that from HCT-8 cells, and there was no apparent amplification of *mdr1* gene in HCT$_{V2000}$ cells (Figure 3). Hybridization of the Southern blot revealed that *mdr1* probe, pHDR5A, was hybridized to five *Eco*R Ⅰ fragments in both cell lines and the size and the signal intensity of the five bands from the two cell lines were similar (Figure 4). It is therefore suggested that neither amplification nor rearrangement exists in the HCT$_{V2000}$ cell line and that overexpression of the *mdr1* mRNA in HCT$_{V2000}$ cells may be correlated with activated transcription of *mdr1* gene.

Effect of VRM

Reversal of multidrug resistance. Nontoxic or slightly toxic (growth inhibition did not exceed 10%) concentrations of VRM were used in these experiments. The IC$_{50}$ values of HCT$_{V2000}$ and HCT-8 cells to VCR were 3651.8 and 24.9 nmol/L respectively, and the index of resistance was approximately 167 when compared with each other. VRM enhanced the cytotoxicity of VCR in both cell lines. A 84- and 4-fold increase in cytotoxicity was produced by 10 μg/mL VRM in HCT$_{V2000}$ and HCT-8 cells, respectively. Reversal of MDR by VRM in the HCT$_{V2000}$ cell line was more effective than that in the HCT-8 cell line (Figures 5 and 6).

Increase of ^{3}H-VCR intracellular accumulation. The intracellular accumulation of ^{3}H-VCR in HCT$_{V2000}$ cells was lower than that in HCT-8 cells. VRM increased the accumulation of ^{3}H-VCR in both cell lines. According to the drug sensitivity test (Table 1), the drug resistance of HCT$_{V2000}$ cells was related to the decrease in intracellular drug accumulation. The ability of VRM to enhance VCR cytotoxicity was directly related to the ability to increase intracellular drug accumulation. VRM at 10 μg/mL increased ^{3}H-VCR accumulation in HCT$_{V2000}$ to a level comparable to HCT-8. Although the intracellular concentration of ^{3}H-VCR was similar in both cell lines, HCT$_{V2000}$ cells maintained 8-fold higher resistance to VCR than HCT-8 cells did.

DISCUSSION

Human colon cancer is intrinsically resistant to chemotherapeutic agents and the exact mechanism of drug resistance is not clear yet. There existed different results in studying the mechanisms of multidrug resistance in the human colon cancer cell line HCT-8. Klohs's study on HCT-8 cells showed that although verapamil increased adriamycin accumulation and cytotoxicity, surface labelling failed to detect a glycoprotein with appropriate molecular size due to the insensitivity of the technique used[7]. Hunter *et al*[8], however, detected the expression of P-GP in HCT-8 cells by immunoprecipitation with MAb JSB-1. In this report overexpression of P-GP in both cell lines was observed, and the intensity of the protein staining in HCT$_{V2000}$ cells was much stronger than that in HCT-8 cells. Drug resistance in both cell lines can be overcome by VRM, which inhibits the function of P-GP. These results can be used to explain the P-GP mechanism of intrinsic drug resistance and acquired drug resistance in this kind of colon cancer cells. JSB-1 and MRK-16 recognize different epitopes in plasma membrane or within cytoplasm and the results would be different under different experimental conditions[4].

Table 1 Effect of Verapamil on intracellular accumulation and cytotoxicity of vincristine (mean ± SD)

VRM (μg/m)	HCT-8 cell line		HCT$_{V2000}$	
	Drug accumulation[1]	Relative ID50	Drug accumulation[1]	Relative ID50
0	0.60 ± 0.07	1	0.42 ± 0.03	146.66
5	1.49 ± 0.05	0.32	1.08 ± 0.05	2.05
10	2.06 ± 0.21	0.2	1.89 ± 0.13	1.73

[1]Pmol/5 × 10^5 cells, n = 3.

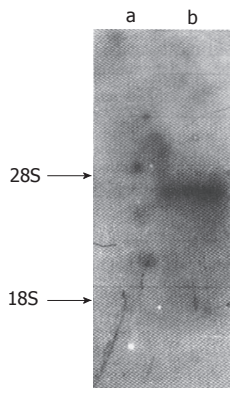

Figure 2 Northern hybridization. Ten micrograms of total cellular RNA were loaded for each line. a. HCT-8 cell line; b. HCT$_{V2000}$ cell line.

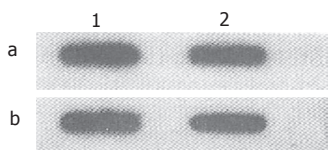

Figure 3 Slot blot hybridization. Twenty micrograms (1) or 10 μg (2) DNA from HCT-8 cell line (a) and HCT$_{V2000}$ cell line (b) were analyzed.

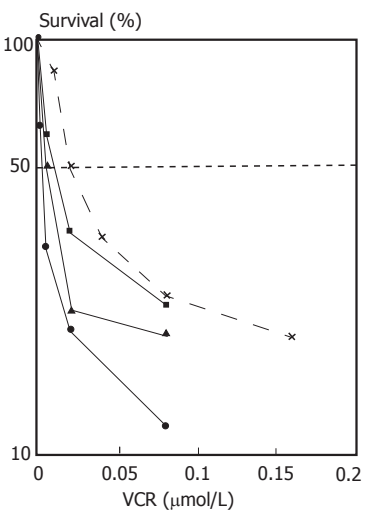

Figure 5 Effect of VRM on sensitivity of HCT-8 cells to VCR. The doses of VRM were 0 (x), 2.5 (■), 5 (▲) and 10 (●) μg/mL.

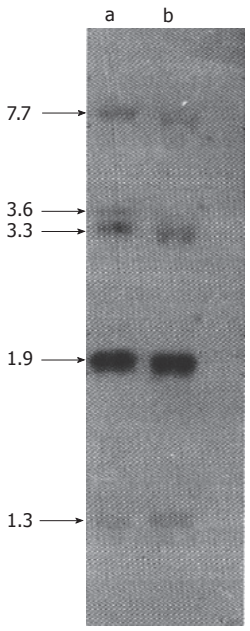

Figure 4 Southern hybridization of mdr1 gene. Ten micrograms of DNA extracted from HCT-8 cell line (a) and HCT$_{V2000}$ cell line (b) were digested with *Eco*R Ⅰ and separated on a 0.7% agarose gel. The arrows indicate the position of hybridization.

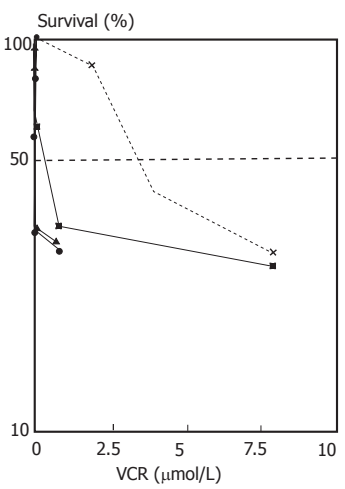

Figure 6 Effect of VRM o-n sensitivity of HCT$_{V2000}$ cells to VCR. The doses of VRM were o (x), 2.5 (■), 5 (▲) and 10 (●) μg/mL.

Our results suggest that combination of different monoclonal antibodies could reduce false negative rate.

The drug resistance mediated by P-GP is considered as typical MDR. P-GP is encoded by *mdr1* gene in human MDR cells. Overexpression of *mdr1* gene is frequently found in some types of human tumors, including colon cancer, but the amplification of *mdr1* gene has not been reported yet. In this study, we failed to detect the expression of *mdr1* mRNA in HCT-8 cells by Northern blot hybridization probably due to the low levels of mRNA in this cell line and insensitivity of the method. Expression of *mdr1* mRNA was at a high level but no gene amplification or rearrangement was observed in HCT$_{V2000}$ cells, being consistent with observations of clinical specimens. The overexpression of P-GP in this cell line may be due to activated transcription of *mdr1* gene. Shen *et al* reported that KB cell lines with low level of drug resistance did overexpress *mdr1* mRNA without *mdr1* gene amplification. However, expression of *mdr1* mRNA was increased simultaneously with amplification of *mdr1* gene in high-level drug resistant KB cell lines (40-fold)[9]. No *mdr1* gene amplification was found in HCT$_{V2000}$ cell line, which is about 146-fold more resistant to VCR than HCT-8 cell line. There may be two possibilities: expression of *mdr1* mRNA may not always be accompanied by amplification of *mdr1* gene in cells with high levels of drug resistance, and the activation of *mdr1* gene may not be the only mechanism for the drug resistance in this cell line.

Drug accumulation and cytotoxicity assays showed that VRM increased VCR intracellular accumulation and reversed drug resistance in HCT$_{V2000}$ cell line. This provides evidence for the P-GP mediated mechanisms of drug resistance of colon cancer cells. But there is not much correspondence between the amount of ^{3}H-VCR intracellular accumulation and the level of drug resistance in HCT$_{V2000}$ cell line. VRM can only reverse drug resistance partially while VCR intracellular accumulation is increased perfectly in this cell line. All of these assays demonstrate that other mechanisms of drug resistance besides P-GP may exist in this cell line. The cell line HCT$_{V2000}$ may be a useful model

for studying the mechanisms of drug resistance in human colon cancer.

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Expression of P53 oncoprotein in benign and malignant lesions of large bowel

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Abstract

AIM: To investigate the expression of P53 oncoprotein in benign and malignant lesions of the large bowel as well as the relationship between p53 expression and clinicopathological factors.

METHODS: Immunohistochemistry was used to detect P53 protein in large bowel tissues of 146 cases with benign and malignant lesions.

RESULTS: All normal large bowel mucosae and non-neoplastic polyps were negative for P53 protein. However, the positive rates of P53 protein in adenomas, paracancerous mucosae and carcinomas were 18.18% (2/11), 13.21% (7/53) and 42.11% (32/76), respectively. The P53 expression in both adenomas and paracancerous mucosae presented only weak staining, whereas 75% of p53 positive cancers displayed very intense staining (++ or +++). The rates of P53 protein detection in poorly differentiated carcinoma and mucous carcinoma were 63.64% (7/11) and 62.5% (10/16), respectively, which were much higher than that of well/moderately differentiated carcinomas (30.16%, 15/40) ($p < 0.05$), and the carcinomas with marked positive p53 expression were more likely to penetrate the bowel wall and metastasize to lymph nodes ($p < 0.05$). However, no relationship between p53 expression and massive type, tumor size, location, Dukes stage or 3-year survival was found in this study.

CONCLUSION: *P53* gene mutation and overexpression are common in colorectal cancers, and seem to be associated with histological type, progression and lymph node metastasis of colorectal cancer.

Key words: Colorectal neoplasms; P53 protein; *P53* gene; Imuno-histochemistry

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INTRODUCTION

The p53 suppressor gene is the most frequently altered gene in solid human malignancies[1,2]. In colorectal neoplasia, p53 dysfunction is important in the progression from adenomas into invasive carcinomas[3,4], and mutation of p53 seems associated with tumor biology of colorectal and other neoplasias[5,6]. In this study, an immunohistochemical method was used to detect p53 protein in 76 cases of colorectal cancers and 70 cases of other large bowel tissues.

MATERIALS AND METHODS

Materials

Specimens of 76 colorectal carcinomas, 11 adenomas, 6 non-neoplastic polyps, and 53 paracancerous mucosae were obtained from Yijishan Hospital. The tissues were fixed in 10% neutral buffered formalin. Paraffin sections (5 μm) were cut and used for HE staining and immunohistochemistry. Mouse antibody against P53 protein DO7 and S-P Kits were purchased from Maxim Co.

Methods

S-P immunohistochemistry was used in this study, and both negative and positive controls were set. The staining patterns observed 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), < 30% cells stained (1), 30%-70% cells stained (2), and > 70% cells stained (3). Then the cases were divided into four stages according to the scores obtained by adding the two above-mentioned scores: those not stained at all being negative (-), those scoring 2 points grade (+), those scoring 3-4 points grade (++) and those scoring 5-6 points grade (+++).

RESULTS

P53 protein positive rates and intensities in colorectal cancers and other large bowel tissues are shown in Table 1.

P53 protein in colorectal cancer cells was mainly located in nuclei (Figure 1) and the distribution of stained cells was very variable. In some tumors the majority of cells and sometimes all cells showed overexpression at a very high level, indicating very intense P53 staining. In some tumors there were only occasionally stained cells. But in the majority of p53 positive cases, the percentage of stained cells ranged from 30% to 70%. However, p53 expression in both adenomas and paracancerous mucosas presented occasional cell staining in some cases.

The correlations between p53 expression and clinicopathological

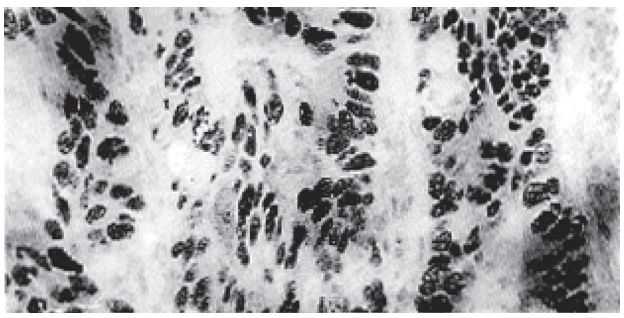

Figure 1 Moderately differentiated adenocarcinoma. The P53 protein is located in nuclei and nearly all cells are stained(S-P, ×200).

Table 1 P53 protein expression in colorectal cancer and other tissues of large bowel

Group	*n*	-	+	+ +	+ + +	Positive rate (%)
NMOLB	13	13	0	0	0	0
Non-neoplastic polyps	6	6	0	0	0	0
Paracancerous mucose	53	46	3	0	0	13.21
Adenomas	11	9	2	0	0	18.18
Carcinomas	76	44	8	16	8	42.11[b]

[b]$P < 0.01$. NMOLB: Normal mucosae of large bowel.

Table 2 Correlation between P53 expression and tumor differentiation

Group	*n*	-	+	+ +	+ + +	Positive rate (%)
Well/moderately-differentiated	49	33	3	8	4	30.61
Poorly-differentiated	11	4	2	3	2	63.64[a]
Mucous	16	6	2	6	2	62.50[a]

[a]$P < 0.05$, compared with well/moderately differentiated.

Table 3 Correlation between P53 expression and serosal invasion and lymph node metastasis

P53 expression	Serosal invasion		Lymph node metastasis	
	-	+	-	+
-	21	23	37	5
+	3	5	5	3
++, +++	5	19[a]	16	8[c]

[a]$P < 0.05$, P53(++, +++) *vs* P53(-) or P53(+); [c]$P < 0.05$, P53(+-+++) *vs* P53(-).

parameters of colorectal carcinoma are shown in Tables 2 and 3. No significant relation was found between P53 expression and tumor size, location, massive type, Dukes stage or 3-year survival ($P > 0.05$). However, P53 expression in colorectal cancer was associated with tumor differentiation, histological type, serosal invasion and lymph node metastasis. Poorly differentiated and mucous carcinomas possessed much higher rates of P53 detection than well and moderately differentiated cancer, and the cancers with marked positive p53 expression were more likely to penetrate the bowel wall and metastasize to lymph nodes ($P < 0.05$).

DISCUSSION

Growing evidence suggests that the accumulation of multiple genetic events is responsible for the pathogenesis and/or progression of tumors. The abnormalities of the *p53* gene were found to represent the most common molecular change in human cancer[1-4]. Such abnormalities can be detected in many ways. For example, mutations can be demonstrated by sequencing of the commonly mutated exons or inferred by SSCP analysis. The mRNA expression can be detected by hybridization and the expression of oncoprotein can be investigated by immunochemical methods, including immunohistochemistry. Of all the above-mentioned methods, immunohistochemistry is valuable in clinical practice. Recent studies have shown that there are several mechanisms to stabilize P53 protein[7]. In addition to *p53* gene mutation, SV40 and other viral genomes encoded proteins can bind to, stabilize and inactivate P53 protein. It has been reported thatthe product of the *mdm2* gene may directly interact with P53 and stabilize it[8]. However, Bass *et al*[9] used six kinds of antibodies for P53 (DO7, Pab1801, Pab240, Bp_{53-12}, CM_1, and Signet) on 19 archival colorectal neoplasms and compared the results with mutation status of the *p53* gene and 17p allelic deletion status. They found that there was a very close correlation between overexpression of P53 and *p53* genetic alterations, which produced the same results. They suggested that immunohistochemistry is valuable for assessing *p53* gene mutation in colorectal neoplasms.

In our study, we used DO7 antibody for P53 protein and found that overexpression of P53 protein can be detected in colorectal cancers in 32 of 76 cases, and that most of them presented far intensive staining (++ or +++), but only 18.18% of adenomas and 13.21% of paracancerous mucosas presented occasionally weak staining. This agreed with the results of other studies[10].

We found that the positive rates of P53 in poorly differentiated and mucous carcinomas were much higher than those in well/moderately differentiated cancers, and that carcinomas with positive P53 protein were more likely to invade the serosa and metastasize to lymph nodes. Our results suggest that overexpression of P53 protein in colorectal cancers is associated with tumor histological differentiation, and may play an important role in tumor progression and biological behavior.

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Scanning electron microscopic study of lymphatic corrosion casts in the rabbit appendix

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Abstract

AIM: To study the three-dimensional structure and distribution of the lymphatics in the rabbit appendix, and to reveal the correlation between the perifollicular lymphatic sinus (PLS) and lymphatics.

METHODS: Freeze-fractured tissues and lymphatic corrosion cast with the Mercox were used for scanning electron microscopy (SEM), and histologic and semithin sections were used for light microscopy. The Mercox was diluted and injected intraparenchymally into the appendix wall. The injected appendixes were cut and put in a concentrated NaOH solution until the tissues were corroded away.

RESULTS: The lymphatic capillary networks were found in the superficial layer of the mucosa and the lymphatic capillary plexuses were observed in the deep layer of the mucosa. From the plexuses, the short grove-like lymphatic capillaries were connected with the PLS. The luminal side of the sinus looked like a flower basket. Short lymphatic capillaries arising from the bottom of the PLS were continuous with the lymphatics of the submucosa. The lymphatics of the submucosa were connected with the lymphatics running in the muscular layer, then they were led into the serosal lymphatics and drained into the lymphatics in the mesoappendix.

CONCLUSION: The PLS and rich lymphatics in the rabbit appendix may play an important role in the drainage of lymph and the immune function.

Key words: Appendix; Lymphatic corrosion; Microscopic; Eectron; Scanning

Tang FC, Zhang YF, Xu YD, Zhong SQ, Wang XP, Wang YX. Scanning electron microscopic study of lymphatic corrosion casts in the rabbit appendix. *World J Gastroenterol* 1996; 2(4): 238-240 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/238.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.238

INTRODUCTION

The appendix is one of the gut-associated lymphoid organs. The lymphatics and perifollicular lymphatic sinus (PLS) in the appendix have been studied by many authors[1-4]. However, using SEM technique and the lymphatic corrosion cast with Mercox to study the three-dimensional structure of lymphatics in the appendix and to reveal the relationship between the PLS and the lymphatics has not been reported in China. In our study, we used the lymphatic corrosion cast with Mercox, fracture specimens and semithin section methods to investigate the intramural lymphatics in the rabbit appendix and their three-dimensional structure.

MATERIALS AND METHODS

Fifteen healthy rabbits of either sex, weighing 2.5-4.0 kg, were used: one for light microscopy with histologic sections, two for scanning electron microscopy (SEM) with fractured tissues, two for semithin sections, and ten for lymphatic corrosion cast.

Corrosion cast specimens

About 2-3 mL Mercox (CL-2B-5, Velene Hospital, Tokyo, Japan) was diluted to 30%-40% (V/V) solution, then it was injected intraparenchymally into the lower part of the mucosa of the appendix wall. After the injected medium had filled the lymphatics of the rabbit appendix, the injected appendix was cut and put into 5%-20% NaOH solution until tissue elements were corroded away. Thus the corrosion casts were obtained by washing in water. The casts were cut into blocks of appropriate size, then they were dried, coated with gold and observed by SEM[5].

Freeze-fractured and semithin section specimens

The rabbits were anesthetized with pentobarbital sodium and perfused with 0.85% NaCl and 2.5% PBS through the superior mesenteric artery. The appendixes were cut into small pieces, and freeze-fractured specimens were obtained by conducting electronic staining, dehydrating in a graded series of ethanol, immersing in isoamyl acetate, fracturing with the solution of liquid nitrogen and drying at critical point with liquid CO_2. The dried specimens were mounted on a specimen holder, coated with gold and observed by

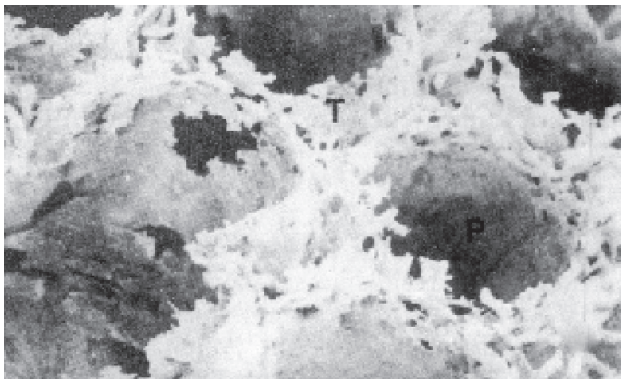

Figure 1 A scanning electron micrograph of lymphatic corrosion cast. The lymphatic capillary plexus (T) in the TDA is connected with a perifollicular lymphatic sinus (P). (SEM, × 40)

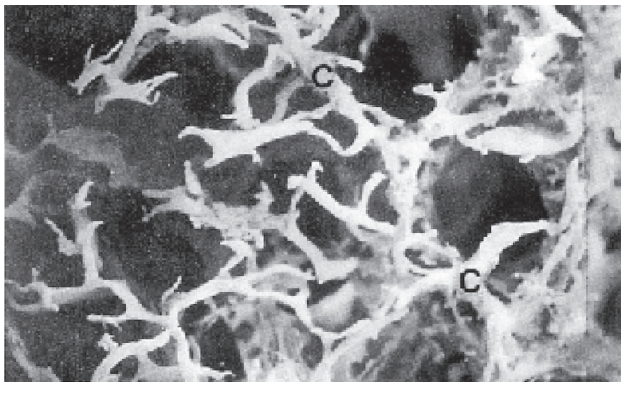

Figure 2 A scanning electron micrograph of lymphatic corrosion casts. A well-developed network of lymphatic capillaries (C) exists in the superficial layer of the appendix mucosa. (SEM, × 30)

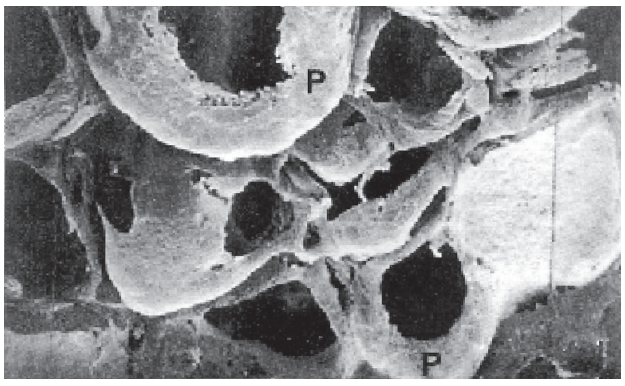

Figure 3 A scanning electron micrograph of lymphatic corrosion cast, displaying the bottoms of the perifollicular lymphatic sinuses (P). (SEM, × 40)

SEM with increasing voltage of 10-20 kV. Semithin section samples were completed by using usual TEM technique, and were observed with a light microscope.

RESULTS

SEM of lymphatic casts showed that the PLSs were not continuous along the entire wall of each follicle but surrounded the lateral surface of the follicle (Figure 1). The upper end of each sinus possessed dense short grove-like lymphatic capillaries that projected toward the thymus-dependent areas (TDA). In the TDA, they formed dense lymphatic capillary plexuses (Figure 1). The straight lymphatics arose from the upper ends of the sinuses, and they were thicker than the lymphatic capillaries in the TDA. The straight lymphatics were continuous with the lymphatic capillaries in the mucosa. The latter interconnected and formed a well-developed network of lymphatic capillaries in the superficial layer of the mucosa (Figure 2). The bottoms of the PLS gave the appearance of incomplete sleeve. In many cases, the sleeves were round in shape (Figure 3). The fine lymphatics arose from the bottom of the sinuses and were continuous with the submucosa lymphatics. The submucosa lymphatics were connected with the lymphatics of the muscular layer, then they were led into the subserosa lymphatics and drained into the lymphatics in the mesoappendix. The fusiform impression of the endothelial nuclei was found on the casts. The casts of the thicker lymphatics showed notches corresponding to the bicuspid valves of the lymphatics.

The PLS and lymphatics in rabbit appendix demonstrated by SEM of fractured appendix and the semithin section observations by light microscopy were also similar to the scanning electron microscopic images of the lymphatic corrosion cast.

DISCUSSION

In general, the lymphatics do not exist in the superficial layer of the mucosa, but between the bottom of the large intestine glands and the mucosa muscle, there were the lymphatic capillaries[6,7]. In our study, a large number of lymphatic capillaries in the superficial layer of the mucosa in the rabbit appendix were observed. They connected with each other in circle, surrounded the mucosa glands and projected to the luminal side. The results are similar to those reported by Hirashima *et al*[8]. These lymphatic capillaries drain the lymph and may transfer some immune substances from the TDA to the lumen, which plays an important role in immune function. Ohtani[4] observed mucosa lymphatics (140 μm in diameter), but we only found the lymphatic capillaries (20-40 μm in diameter) circling the top of the PLS in the deep layer of the mucosa, and many lymphocytes in the lumen were identified. Our results may support the Waksman's[3] opinion that the TDA was the place of the lymphocyte recirculation. We suggest that the lymphatic capillaries in TDA could transfer the lymphocytes not only to the PLS, but also to the lymphatic capillary networks in the superficial layer of the mucosa, then to the lumen of the intestine.

The PLS

As we have described, each follicle was surrounded by a well-developed PLS as reported by Ohtani in 1984. Ohtani considered that two adjacent follicles frequently shared one PLS. But we found that each follicle had one PLS; rarely did two adjacent PLS fuse to form a large sinus.

According to our observation, we consider that PLS may have extensive drainage. They communicate with (1) the adjacent one by anastomotic channels; (2) the network of lymphatic capillaries in the mucosa by straight channels; (3) the plexuses of the lymphatic capillaries in TDA by short anastomotic channels; and (4) submucosal lymph channels by short lymphatics. It is suggested that the PLS may transport immune substances and lymphocytes to the network of the superficial layer in the mucosa by straight lymphatics. The immune substances may be supplied by TDA, transferred to the local lymph nodes, then to the lymph circulation. In general, the sinuses may act as a reservoir.

Baba[9] has reported that few lymphatics go to the lymphatic follicle. We have not observed intrafollicle lymphatics in the rabbit appendix.

Submucosal, muscular and subserosal lymphatics

Our observation is the same as that described by великоречин. In the submucosal layer, the lymphatic capillaries and lymphatics were situated at the same level. In the muscular layer, the lymphatics and lymphatic capillaries were also found. Baba[9] described that there were only lymphatics in the muscular layer. Sui Guang-Zhi reported that the lymphatic capillaries located in the superficial layer of the subserosa, and lymphatics in the subserosal deep layer. We have not found the lymphatics and lymphatic capillaries in the superficial layer of the subserosa.

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Tissue isoantigens A, B and H in primary carcinoma of the pancreas

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Rong-Hua Li, Leture, having 10 papers published, 2 of 10 were collected by BA and CA.

Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To investigate the quantity of demonstrable tissue isoantigens A, B and H in primary carcinoma of the pancreas and their relationship with the degree of anaplasia.

METHODS: The pathological classification of 26 primary carcinomas of the pancreas and their anaplasia were studied by light microscopy with HE staining. The quantity of isoantigens A, B and H in carcinomas of the pancreas and their adjacent normal pancreas tissues were studied by immunohistochemical method.

RESULTS: Twenty-six primary carcinomas of the pancreas were classified as follows: Two acinar cell carcinomas, 22 adenocarcinoma, 1 adenocanthoma and 1 undifferentiated carcinoma. One of 2 acinar cell carcinomas, 6 of 22 adenocarinomas and 1 adenocanthoma were well differentiated; 1 of 2 acinar carcinomas and 12 of 22 adenocarcinoma were moderately differentiated; 4 of 22 adenocarcinoma and 1 undifferentiated carcinoma were poorly differentiated. The results of the immunohistochemical method showed that the ABH positive cells were more frequent in acinar cells and ductal epithelial cells in the adjacent normal pancreas than those in carcinoma of the pancreas. In 22 adenocarcinoma, the ABH positive cells were more frequent in well differentiated and moderately differentiated cancer cells than in poorly differentiated cells. In 11 invasive adenocarcinoma positive cells (++ or +++) were more frequent in primary foci than in metastatic foci. Twenty of 26 carcinomas of the pancreas belonged to A, B and AB blood groups. Most H isoantigens were converted to A or B or AB substances in 8 well differentiated, 7 moderately differentiated and 2 poorly differentiated carcinomas, while less transformation of H to A or B antigen was observed in 3 poorly differentiated carcinomas of the pancreas. Furthermore, it was observed that the secretion of isoantigens A, B, and H was less in carcinomas of the pancreas than in normal pancreas.

CONCLUSION: The loss of demonstrable isoantigens ABH parallels morphological anaplasia. Furthermore, we found that the functions of transformation of H substance into A and B substances as well as the secretion of ABH substances into the pancreatic duct were hampered.

Key words: Pancreatic neoplasms; ABH antigens; Immunohistochemistry

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INTRODUCTION

The isoantigens A, B and H are not only present on red blood cells of groups A, B, O and AB individuals, but also present in normal human tissues and body fluids[1-3]. The pancreas is one of the organs in which large quantities of these antigens are present in the epithelial cells of the exocrine glands and the pancreatic ducts. Szulman[1] was the first to study the histological distribution of ABH isoantigens in human tissues by immunofluresence technique. Some authors have investigated the effect of cancerous transformation on tissue isoantigens A, B and H[4-8]. This article is an extension of our previous studies on the distribution and cellular and subcellular localization of ABH isoantigens in normal human tissue cells by immunohistochemical and immunoelectro-microscopy[3,9]. The aim of this study was to investigate the relationship between the quantities of ABH antigens present in normal pancreas and anaplasia in cancerous transformation.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded tissue sections (5 μm) were made from 26 cases of carcinoma of the pancreas. Five sections of each case were used for study. One section was stained with HE for determining the type of cancer and the degree of anaplasia. Three sections were used for immunohistochemical study using avidin-biotin complex/HRP (ABC) technique. After deparaffinization, rehydration, and treatment with H_2O_2 to block the intrinsic peroxidase activity and with bovine serum albumin, the sections were incubated with McAb > A, McAb > B and McAb > H (1:50, Dako), respectively, for 60 min at room temperature. After washing with PBS-T, sections were incubated with rabbit anti-mouse Igs/biotin (1:300, Dako) for 50 min, conjugated with ABC/HRP for 30 min, treated with H_2O_2-DAB (3,3'-dianimo-benzidine) for 3 min, counter-stained with hematoxylin, and mounted. The interal positive controls were: (1) erythrocytes and endothelial cells in vessels; and (2) the epithelial cells of the exocrine gland and the

Table 1 Type and differentiation degree of 26 cases of primary carcinoma of the pancreas

Type	Acinar cell carcinoma	Adeno-carcinoma	Undifferentiated carcinoma	Adenoa-canthoma	Total
Well differentiated	1	6		1	8
Moderately differentiated	1	12			13
Poorly differentiated		4	1		
Total	2	22	1	1	26

Table 2 The frequency of ABH positive cells in 26 normal pancreatic ducts and in ducts of 22 adenocarcinoma

Frequency of ABH positive cells	4/4	3/4	2/4	1/4	< 1/4	0	Total
Normal pancreatic ducts	10	5	4	3	2	2	26
Adenocarcinoma (cases)							
Well differentiated	4	1	1				6
Moderately differentiated	5	3	2	2			12
Poorly differentiated	1	1	2				4

Note: 4/4 indicates that the epithelial cells of the whole circumference of duct were ABH positive, and others were analyzed in this way.

pancreatic duct adjacent to the carcinoma. The internal negative controls were (1) the connective tissue; and (2) the islands of Langerhans.

RESULTS

ABO blood groups of 26 cases of carcinoma of the pancreas

The ABO blood groups of 26 cases of carcinoma of the pancreas were as follows: A, 8; B, 9; AB, 3; and O, 6.

Type and degree of anaplasia of carcinoma of the pancreas

The type of carcinoma and the degree of anaplasia are shown in Table 1.

Relation of ABH positive cells to the anaplasia of carcinoma of pancreas

Eight of 26 cases of carcinoma of the pancreas had adjacent normal pancreas in which 26 normal interlobular pancreatic ducts were found. ABH positive epithelial cells (+-+++) were demonstrated in 24 ducts. The positive epithelial cells were more frequent in them than in adenocarcinoma, and were more frequent in well and moderately differentiated adenocarcinoma than in poorly differentiated adenocarcinoma (Table 2).

Relation of ABH antigens present in primary foci to those in metastatic tumors

Twelve of 26 cases of carcinoma of the pancreas had the adjacent normal pancreas in which carcinoma invasion was found in 11 cases. It was found that the ABH positive staining was stronger in cells of primary foci (+-+++) than in the invasive tumor (Table 3).

Comparison of secretion of ABH isoantigens between normal pancreatic ducts and in ducts of adenocarcinoma

In general, the ABH antigens were located in infra-nuclear regions, supranuclear regions, brush borders, and cytoplasm as well as in cellular secretions. Secretions of ABH antigens in 26 normal pancreatic ducts and in 26 ducts randomly selected from 5 cases of well differentiated and moderately differentiated adenocarcinoma each were compared. The adenocarcinoma cells secreted less ABH antigens in their secretions than those in the normal pancreatic ducts (Table 4).

Table 3 Comparison of the staining intensity of ABH positive cells in primary foci and in the invasive carcinoma

Intensity of ABH positive staining of cells in		Cases (n = 11)
Primary foci	Invasive carcinoma	
+-+++	0	7
+-+++	0-++	3
++-+++	0-+	1

Table 4 The localization of ABH antigens in 26 normal pancreatic cells and in 26 ducts of adenocarcinoma

Localization of ABH antigens on cell	Whole cytoplasm	Infranuclear region	Supranuclear region	brush border	Secretion
Normal ducts	6	11	11	24	24
Well differentiated	10	12	15	18	8
Moderately differentiated	5	8	14	14	8

DISCUSSION

Our results revealed that the quantity of ABH antigens in the pancreas was related with the anaplasia in cancerous transformation. It was less in carcinomas of the pancreas and their ductal secretions than in normal pancreatic tissue and their secretions, less in undifferentiated adenocarcinoma than in moderately and well differentiated adenocarcinoma, less in invaded tumors than in primary foci. It is suggested that ABH antigens in the pancreas are related to the cancerous transformation of the pancreas. That is to say, in carcinoma of the pancreas partial loss of ABH antigens occurred. These results agree with Davidsohn's research[6,7]. Furthermore, we found a phenomenon of hindering A or B antigens from H antigens in carcinoma of the pancreas.

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Histopathological study of pancreatic duct in acute fulminant pancreatitis

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Abstract

AIM: To study the pathological changes in the pancreas of acute fulminant pancreatitis patients suffering from sudden death.

METHODS: Pancreatic duct specimens taken at autopsy from 30 patients with acute fulminant pancreatitis were examined under a light microscope for histopathological changes, with those of 30 other patients who died of other diseases as controls.

RESULTS: Among the 30 cases of acute fulminant pancreatitis, 29 (96.7%) were non-inflammatory including hemorrhagic necrotic type (2 cases) and one were inflammatory. The pathological findings showed that all the above types had pancreatic ductal changes, including the accumulation of shed ductal epithelial cells, eosinophilic materials in the ductal lumen and the presence of ductules in the wall of the pancreatic duct. In the control group, except for 1 case presenting ductules, there were no other changes observed.

CONCLUSION: Abnormal structural changes can cause damage to the ductal system of the pancreas, leading to an escape of activated proteolytic and lipolytic enzymes into the interstitial tissues and resulting in edema, ischemia, hemorrhage, necrosis and development of acute fulminant pancreatitis, which is an important cause of sudden death.

Key words: Sudden death; Pancreatitis/Pathology

Hu SX,Tang RJ,Zuo DY,Chen PH. Histopathological study of pancreatic duct in acute fulminant pancreatitis. *World J Gastroenterol* 1996; 2(4): 243-245 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/243.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.243

INTRODUCTION

There have been no uniform explanations for the causes of acute fulminant pancreatitis[1]. We have suggested that there must be certain relationship between the development of acute fulminant pancreatitis and structural abnormalities of the pancreatic duct[2]. For further observations of the abnormal structural characteristics of the pancreatic duct, a more detailed histopathological study was made on 30 cases of acute fulminant pancreatitis, so as to find out the pathological basis of sudden death in acute fulminant pancreatitis.

MATERIALS AND METHODS

Specimens of pancreatic tissues from 30 patients were collected during the period of 1956-1987 by autopsy within 3-7 h after death. All the diagnoses were proved to be acute fulminant pancreatitis through autopsy, and their records were relatively complete. Specimens were taken from the head, body and tail of the pancreas, fixed in formalin solution and embedded in paraffin. Serial sections and HE staining were made for all; Foot Argyrol Fiber and van Gieson collagen staining for some. All the specimens were examined under a light microscope. Specimens of 30 other patients who died of diseases not involving the pancreas were taken as controls. The patients studied were 22 men and 8 women, ranging in age from 3 to 65 years (mean age: 30.2; 25 patients under 20). All the patients died within a few minutes to 24 h after the onset of symptoms, which occurred after playing cards, or watching performance or manual labour each in 3 cases; intake of a large amount of cold boiled water and a full meal each in 2 cases; ascariasis in the pancreatic duct, pregnancy, surgical operation, blood transfusion, drug injection, drinking wine, sexual intercourse, making speech in public and playing basket ball each in 1 case. No predisposing causes were found in 8 cases. Clinical symptoms included roaring in 5 cases, moaning, dyspnea and convulsion each in 4 cases, restlessness in 3 cases, sighing in 2 cases, coma in 1 case and records of symptoms not available in 8 cases. Twenty-five patients died during sleep at night, 2 patients died during a nap after lunch in the afternoon and 3 patients died suddenly at rest after manual labour.

RESULTS

Among the 30 cases of acute fulminant pancreatitis, 29 (96.7%) were non-inflammatory. Of which, 23 (79.3%) belonged to hemorrhagic necrotic type (Figure 1), characterized by the accumulation of a large number of shed epithelial cells in the

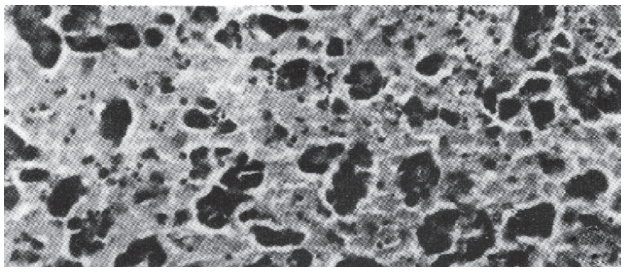

Figure 1 Hemorrhagic necrosis of the pancreas.

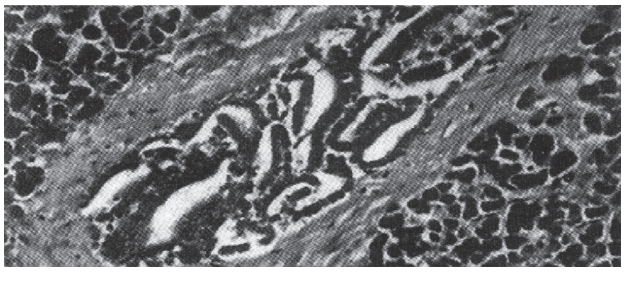

Figure 2 Shed epithelial cells and pseudoglandular formation.

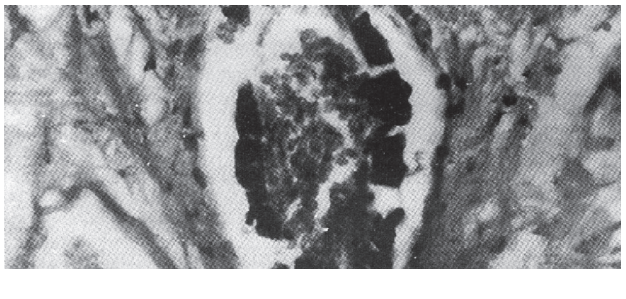

Figure 3 Massive blood in the pancreatic duct.

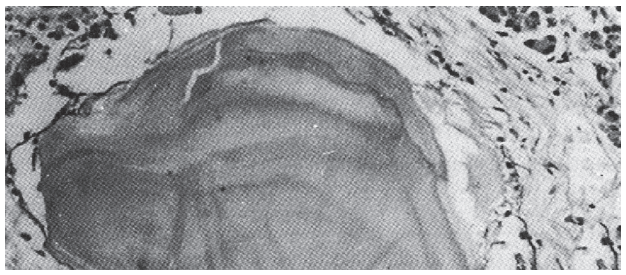

Figure 4 Eosinophilic material in the lumen of the pancreatic duct.

pancreatic duct lumina in all cases (Figure 2), a plenty of red blood cells in 9 cases (Figure 3), eosinophilic material in 6 cases (Figure 4), and eosinocytes in 4 cases (Figure 5), and the presence of necrosis of the pancreatic duct epithelium in the hemorrhagic areas, papillary proliferation or squamous epithelial metaplasia of the pancreatic duct epithelium in 17 cases (Figures 6 and 7). Pancreatic ductules in the wall of pancreatic ducts were present in 20 cases (Figures 8 and 9), with as many as 6 intramural ductules seen in 1 case (Figure 10). And hemorrhage occurred in the wall of pancreatic ducts in 12 cases (Figure 11). Four cases (13.8%) were of lipo-necrotic type with necrosis and shedding of the epithelial cells of the pancreatic ducts occurring in all the cases; eosinophilic material in the pancreatic duct lumina in 3 cases and pancreatic ductules in the wall of pancreatic ducts in 1 case. Two cases (6.9%) were of edematous type with eosinophilic material presenting in the pancreatic duct lumina in all and the shedding of epithelial cells in 1 case. Only 1 (3.3%) of the 30 cases belonged to the inflammatory type, which was of hemorrhagic necrotic type with the accumulation of eosinophilic material and inflammatory cells in the pancreatic duct lumina and the shedding of non-necrotic epithelial cells; infiltration of inflammatory cells was found in and around the wall of pancreatic ducts with ductules presenting in the wall of pancreatic ducts. In the control group, except for intramural ductules found in 3 cases, no other pathological changes were seen.

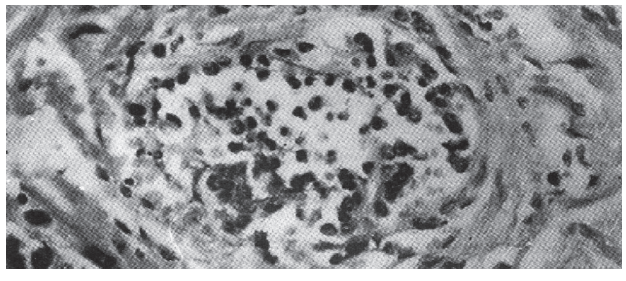

Figure 5 Eosinocytes in the lumen of the pancreatic duct.

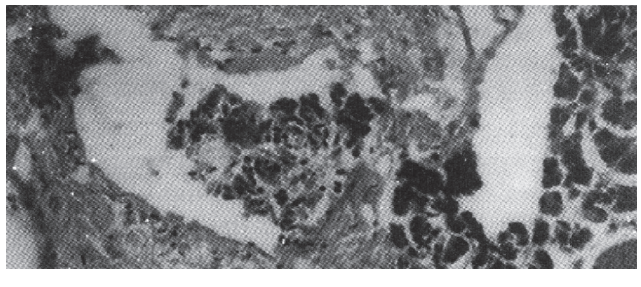

Figure 6 Papillary hyperplasia and neoplastic plug appearance.

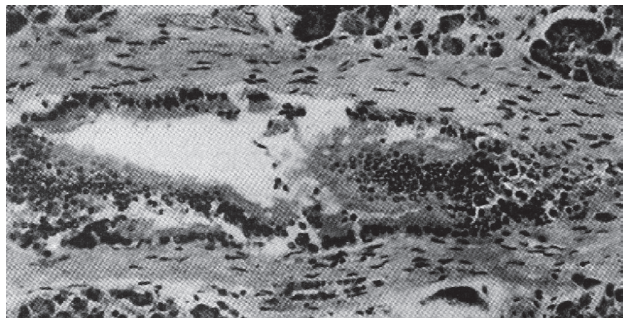

Figure 7 Squamous metaplasia of the epithelial cells of the pancreatic duct.

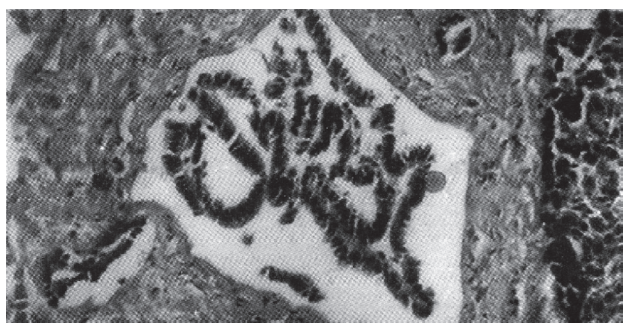

Figure 8 Shedding of the epithelium and intramural ductule formation in the wall of the pancreas.

DISCUSSION

Our study showed that the histopathological changes in the pancreatic ducts in non-inflammatory and inflammatory pancreatitis of the same hemorrhagic necrotic type were essentially the same. As to non-inflammatory pancreatitis, changes in different types were markedly different, *e.g.*, hemorrhagic necrotic type, lipo-necrotic type and edematous type. These findings are of considerable prophylactic and therapeutic significance for clinical practices.

There have been various theories about the etiology of acute fulminant pancreatitis in the literature[3-5], but most were based on experimental materials, only a few on autopsy data. The results of our study showed that there were characteristic lesions of the pancreatic duct in acute fulminant pancreatitis, and would provide a histopathological basis for interpretation of these theories. Specially worthy of notice is the presence of pancreatic ductules in the wall of pancreatic ducts as revealed in this study. Are they structural abnormalities or just normal branches of the pancreatic duct? We consider them as structural abnormalities, not normal branched pancreatic ducts, since (1) they were observed in cross sections, not in longitudinal sections; (2) in one cross section, 6

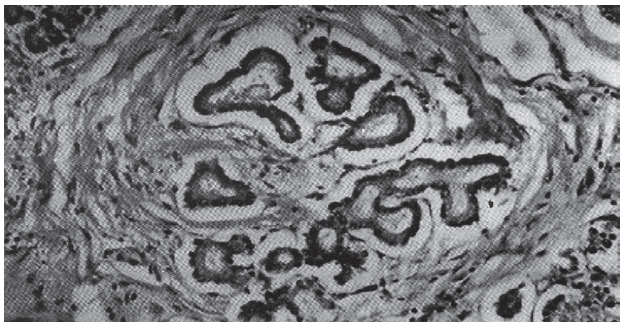

Figure 9 Multiple intramural ductules lined with a layer of the epithelial cells with basement membrane.

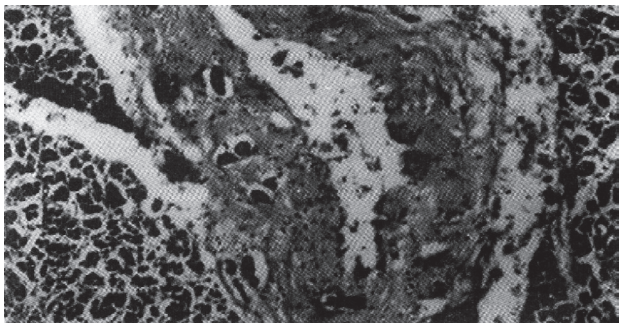

Figure 10 As many as 6 intramural ductules.

tubules may be seen at most; and (3) 22 (73.3%) of the 30 cases of acute fulminant pancreatitis developed intramural pancreatic ductules, whereas in the control group intramural ductules were only seen in 3 cases. The differences are statistically significant ($P < 0.01$). We conclude that the abnormal structural changes of the pancreatic duct as described above are important etiologic factors

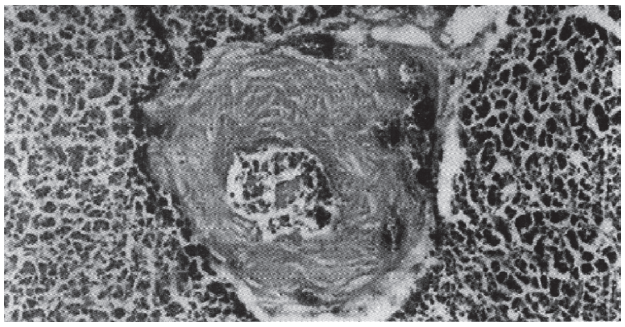

Figure 11 Intramural hemorrhage of the pancreatic duct.

responsible for the damage to the ductal system of the pancreas, which makes the activated proteolytic enzymes escape into the interstitial tissues of the gland and results in the development of edema, ischemia, hemorrhage and necrosis of the pancreatic tissues. This may also bean important cause of sudden death in acute fulminant pancreatitis.

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BRIEF REPORT

Efficacy of six therapies for chronic hepatitis B

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Hong-Tian Zhang, Associate Chief Physician, has published 23 papers.

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INTRODUCTION

There are many therapies for chronic hepatitis B at present. However, the efficacy of most of them has not been confirmed. In order to select several effective therapies from them, we treated 302 chronic hepatitis B patients with six different therapies, observed their efficacy and side effects, and then followed the patients for 9 mo.

MATERIALS AND METHODS

Patients

A total of 302 patients (282 males and 20 females) with chronic hepatitis B were treated from 1992 to 1994. Their age ranged from 20 to 60 years. The diagnoses were established according to the standard developed at the "6th China Symposium on Virus Hepatitis". There were 92 cases of chronic persistent hepatitis and 210 cases of chronic active hepatitis. The course of diseases was within five years. Patients in this group had no family history of hepatitis. Before treatment, HBsAg, HBeAg, anti-HBC and HBV-DNA were all positive, and ALT was abnormal in all patients. During the 6 mo before treatment, no patient had been treated with antiviral drugs or immune drugs. The 302 patients were divided into six groups with comparable age, sex, hepatitis type, ALT and HBV-marker.

Methods

In group Ⅰ (n = 45), Zhuling Duotang was given at a dose of 40 mg (i.m.) per day for 60 days (the drug was used for 20 days and then discontinued for 10 days, and the course was repeated three times). In group Ⅱ (n = 63), Zhuling Duotang was given at the same dosage as in group Ⅰ, and hepatitis B vaccine was given at a dose of 30 μg (i.m.) every two weeks for a total of six times. All patients of groups Ⅲ, Ⅳ and Ⅴ had been treated with arabinofuranosy adenine monophosphate (ARA-AMP) for 28 days. From the first to the fifth day, the dosage of ARA-AMP was 10 mg/kg (i.m.) per day; and from the sixth to the 28th day, it was 5 mg/kg (i.m.) per day. Patients in group Ⅲ (n = 42) were treated with ARA-AMP alone. Group Ⅳ (n = 42) was given thymosin 10 mg (i.m.) daily for 3 mo besides ARA-AMP. Group Ⅴ (n = 58) received hepatitis B vaccine 30 μg (i.m.) every 2 weeks for a total of six times besides ARA-AMP. Group Ⅵ (control group, n = 42) was given diisopropylamine ascorbatis 180 mg (i.m.) daily for 3 mo.

Table 1 HBsAg negative cases and anti-HBs positive cases after treatment

Group	(n)	End of treatment		3 mo after treatment		9 mo after treatment	
		HBsAg(-)	Anti-HBs(+)	HBsAg(-)	Anti-HBs(+)	HBsAg(-)	Anti-HBs(+)
Ⅰ	45	1	0	2	1	2	1
Ⅱ	63	2	0	2	1	2	2
Ⅲ	52	1	0	2	1	1	1
Ⅳ	42	3	2	3	3	2	3
Ⅴ	58	3	0	3	2	2	2
Ⅵ	42	0	0	0	0	0	0

Table 2 HBeAg negative rate (%), anti-HBe positive rate (%) and HBV-DNA negative rate (%) after treatment

Group	(n)	End of treatment			3 mo after treatment			9 mo after treatment		
		HBsAg (-)	Anti-HBs(+)	DNA (-)	HBsAg (-)	Anti-HBs(+)	DNA (-)	HBsAg (-)	Anti-HBs(+)	DNA (-)
Ⅰ	45	44.4	33.3	46.7	46.7	40.0	48.8	42.2	35.6	40.0
Ⅱ	63	53.9	36.7	55.6	53.9	37.9	57.1	44.4	38.1	40.6
Ⅲ	52	51.9	25.0	55.6	50.0	28.8	52.8	30.8	28.8	32.7
Ⅳ	42	61.9	54.8[b]	71.4	64.9	56.8[b]	75.7	62.1[d]	62.1[b]	72.4
Ⅴ	58	55.2	37.9	70.7	56.9	41.1	74.1	57.7[a]	41.4	48.3
Ⅵ	42	14.3	11.9	14.3	19.0	16.3	19.0	16.7	16.7	16.7

[b]P < 0.01, group Ⅳ *vs* other groups; [d]P < 0.01, group Ⅳ *vs* group Ⅲ; and [a]P < 0.05, group Ⅴ *vs* group Ⅲ.

Observation methods

Before and after treatment, HBV markers, liver function, HBV-DNA and BUN were detected, and then the patients were followed for 9 mo. HBV markers were detected by ELISA, and HBV-DNA by dot hybridization. Before treatment, 154 cases were diagnosed both by liver biopsy and clinically.

RESULTS

After treatment, HBsAg and anti-HBs changed in only a few cases in each treatment group, but did not change in the control group (Table 1). At the end of treatment and during the follow-up, the negative seroconversion rate of HBeAg, positive seroconversion rate of anti-HBe and negative seroconversion rate HBV-DNA in each treatment group were higher than those in the control group (P < 0.01). The results of group Ⅳ were the best among all groups (Table 2). In the treatment groups, ALT recovery rates were from 97.6% to 84.5% at the end of treatment, while in the control group, it was 64.2% (P > 0.05). At 3 mo of follow-up, ALT recovery rates were from 77.6% to 91.9% in the treatment groups, but was 69.0% in the control group (P < 0.05). There was no severe side effects in all groups.

DISCUSSION

The present study showed that the five therapies were effective against HBV and in reducing ALT, and produced no severe side effects. Zhuling Duotang alone or in combination with hepatitis B vaccine was both effective in treating hepatitis B, and there was no significant difference between them (P > 0.05). Good efficacy and no severe side effects were observed in the treatment of chronic hepatitis B with ARA-AMP alone or in combination with other drugs. ARA-AMP combined with thymosin had the best therapeutic effect. ARA-AMP was used only intramuscularly in this study. The results showed that the clinical effects were even better and there were no severe side effects, thus the patients are able to be treated at the outpatient department. Nine months of follow-up revealed that the long-term efficacy of ARA-AMP combined with thymosin was the best, therefore this therapy is effective and safe in treating chronic hepatitis B.

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CASE REPORTS

Laparoscopy guided combined treatment of advanced hepatic carcinoma

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INTRODUCTION

Laparoscopic surgery, owing to its advantages of minor trauma and rapid recovery, has been accepted by surgeons all over the world in a quite short time. The technique has been used in the field of hepatic surgery. Laparoscopic liver resection[1,2], liver cyst fenestration[3] and liver abscess drainage[4] have been performed in recent years. Here we report a case of unresectable liver cancer treated by laparoscopic technique which includes laparoscopic hepatic artery ligation, alcohol and interleukin (IL)-2 injection into the tumor and a drug delivery system (DDS) implant through an omental vein.

CASE REPORT

A 47-year-old woman was admitted with a 1-mo history of pain in the right upper quadrant of the abdomen in March 1996. She had neither fever nor jaundice. Physical examination showed good general condition, and no enlarged lymph nodes were palpable. The sclerae were anicteric and the heart and lungs were normal. The liver was palpable 3 cm below the hypochondrium and 8 cm below the xiphoid process. Computed tomography (CT) showed a 12 cm × 10 cm × 8 cm mass in the right hepatic lobe. The porta hepatis and inferior vena cava were pushed to the left. There was no carcinomatous embolus in the portal vein as determined by ultrasonography. Blood tests showed normal liver function and serum bilirubin level. The concentration of alpha-feto protein (AFP) was 27180 μg/mL. The diagnosis of primary hepatic carcinoma was made according to the above-mentioned data. Preoperative CT scan showed that the tumor was unresectable. It was determined that hepatic artery ligation, alcohol and IL-2 intratumor injection and a DDS implant through an omental vein would be performed laparoscopically.

SURGICAL TECHNIQUE

The operation was performed with the patient in the supine position, the surgeon standing on the left side of the patient and an assistant on either side. The videomonitor was set on the right. After induction of general anesthesia, pneumoperitoneum was created by umbilical puncture using a Veress needle, and intra-abdominal pressure was controlled at 14 mmHg. A straight 0° telescope attached to the videocamera (Karl Storz, Tuttlingen, Germany) was inserted through a 10-mm umbilical trocar (port A). Under the guidance of a laparoscope, four other cannulas were inserted. A 10-mm trocar (port B) inserted 5 cm below the xyphoid was used to provide an auxiliary operating channel for grasping instruments first and then be enlarged with an 18-mm trocar used to pull out the omentum for insertion of the DDS tube. A 5-mm cannula in the right hypochondrium (below the enlarged liver) was for the assistant' s grasping forceps for operating field exposure (Port C). Port D (10 mm) was used as the primary operating channel and port C for insertion of a 10-mm five-blade laparoscopic retractor (Figure 1).

After insertion of the trocars, the table was tilted 20° head-up. Laparoscopic inspection showed that the whole right lobe was occupied by the tumor; the liver was translocated to the left; and the porta hepatis was displaced to the left of the round ligament. An assistant grasped the funder of the gallbladder and turned the liver upward. The stomach and duodenum were pushed down with the five-blade retractor and the hepatoduodenal ligament was exposed. The hepatic artery pulsation could be seen clearly on the videomonitor. The serous coat of the hepatoduodenal ligament was opened up, the fat tissue was pushed away, and the proper hepatic artery was identified and ligated with two titanium clips. The color of the liver darkened after the blood stream was blocked. Percutaneous puncture of the liver tumor was performed with a long fine needle from several different points. Forty milliliters of absolute alcohol and one million unit of IL-2 were injected into the tumor. A rubber drainage tube was placed beside the porta hepatis which was delivered out of the abdominal cavity through port C. Port B was dilated with an 18-mm trocar and the greater omentum was pulled out. A larger omental vein was separated and the DDS tube was inserted into the vein about 20 cm long along the way of right gastroepiploic vein to the portal vein. The tube was fixed properly and the body of DDS was fixed near the port. Finally, the trocars were removed. The operation lasted three hours. The postoperative recovery was uneventful.

DISCUSSION

The most effective treatment for liver carcinoma is resection. The

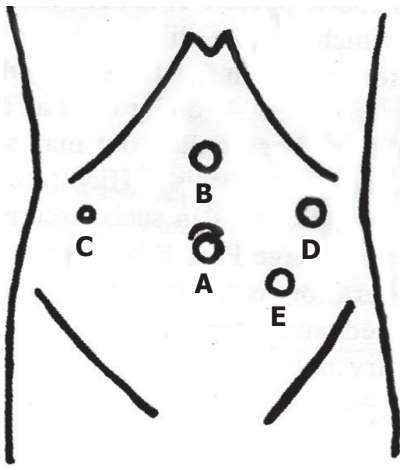

Figure 1 The positions of trocars.

hepatic artery blocking is a choice of treatment for unresectable hepatic tumor at present. About 90%-95% of tumor blood supply comes from the hepatic artery. The blood stream in the normal part of the liver decreases by only 35%, while that in the tumor part declines by over 90% after the hepatic artery ligation. Thus, the tumor tissue necroses owing to ischemia. Intratumor alcohol injection is an effective approach to promoting the necrosis of tumor, therefore it can be an aspect of the combined treatment for liver cancer. The omental vein tube placement provides an approach to regional chemotherapy which makes high concentrations of antineoplastic agents accumulate in the tumor tissue, therefore the general toxic reactions can be diminished. Completing the above-mentioned operations under the guidance of a laparoscope is in accordance with the principle of treatment for advanced liver cancer. The indications of surgery are the same as those of open surgery, that is, no jaundice, ascites or cancerous embolus in the main portal vein so as to prevent irreversible postoperative hepatic failure.

Determining the positions of ports is the prerequisite for a successful operation. The basic principle is to place the ports below the edge of the enlarged liver except those for insertion of laparoscope and retractor. The main operating port can only be placed in the left hypochondrium because of obvious left translocation of porta hepatis. It is convenient to place port B on the midline, which can be dilated with an 18-mm trocar to pull out the greater omentum for the DDS tube placement into the omental vein, apart from being used as an auxiliary port. The omentum that has been pulled out may sometimes become contracture and pachynsis. It is difficult to find appropriate vessels to insert the DDS tube in such circumstances. Hence it may be essential to enlarge port B by a small incision.The key point for this operation is to free the hepatic artery which should be identified and operated carefully. The portal vein and bile duct injury must be avoided. It is quite important to prepare enough blood and get everything ready for open surgery. The tumor, due to its huge and fragile properties, is easy to bleed when injured. Under such circumstances, iatrogenic diffusion may occur. Therefore, great attention should be paid to avoiding injury to the tumor by trocars and instruments.

Laparoscopic surgery has been accepted widely owing to its advantages of less trauma and rapid recovery. It has special merits for patients with advanced liver cancer whose general resistance and tolerance to operative strike have been greatly decreased. We conclude that it is feasible to perform the combined operation guided by laparoscope in patients with unresectable liver cancer.

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