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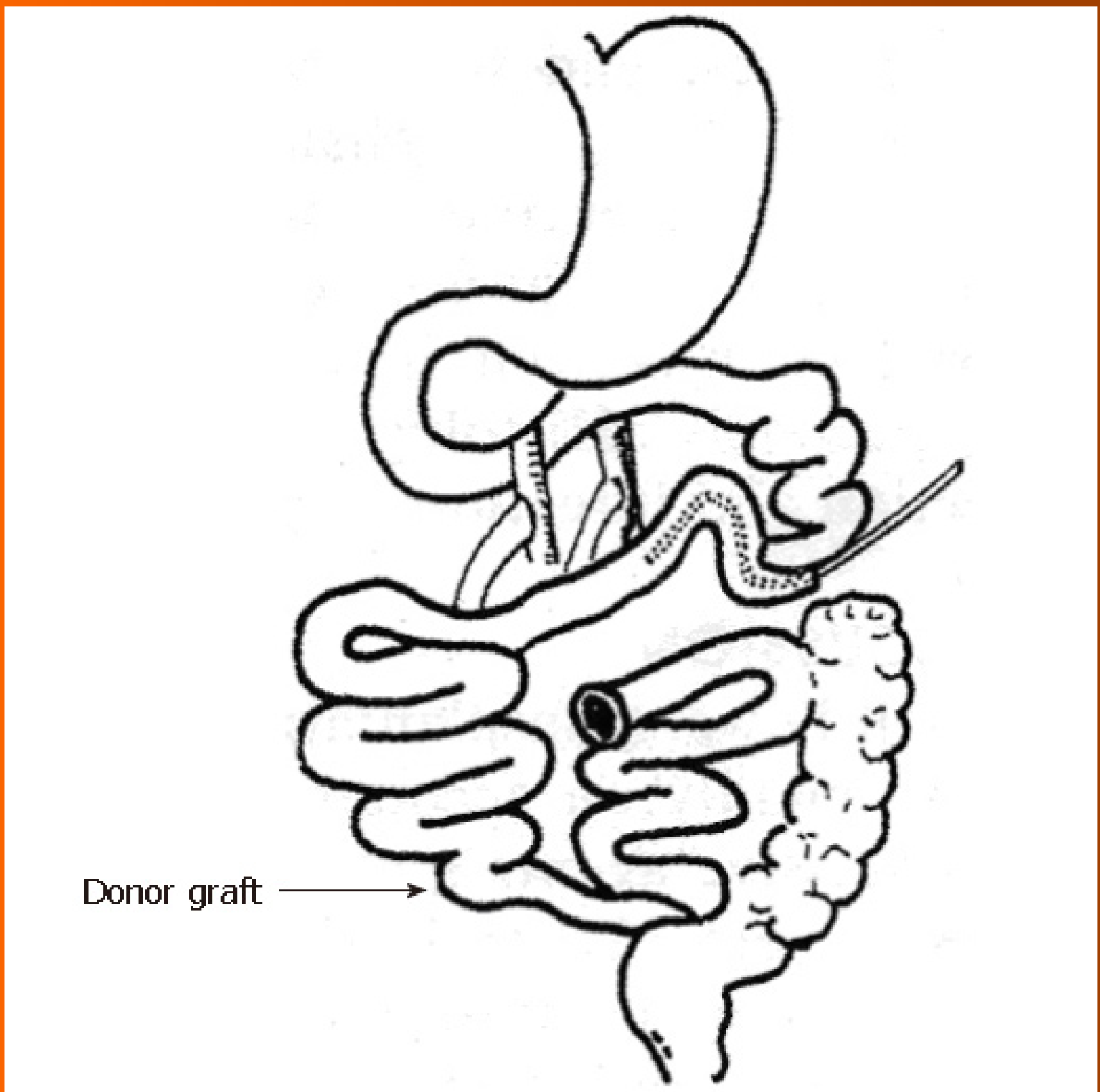

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AIMS AND SCOPE

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COMMENTARY

Evolution of surgical treatment of intrahepatic lithiasis in China

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Cholelithiasis in China has undergone marked changes in its character since the 50 s, when the main features associated with biliary disease were recognized as biliary stones, infection, and parasitic infestation. However, at that time, cases of cholelithiasis accounted for only 40%-60% of the cases of biliary surgery, and 50% of them were associated with primary bile duct stones. This condition remained unchanged until the early 80 s. The Biliary Surgical Society of the Chinese Association of Surgery conducted a nationwide survey of 11342 surgical cases of cholelithiasis enrolled from 146 hospitals between 1983 and 1985. This survey revealed that the relative incidence was 52.8% for cholecystolithiasis, 11.0% for secondary common bile duct stones, 16.1% for intrahepatic duct stones, and 20.1% for extrahepatic bile duct stones. Thus, the relative incidence of primary bile duct stones (36.2%) had decreased. Ten years later, the survey was repeated in 1992, and the results showed a tendency of drastic decrease in the occurrence of bile duct stones, evidenced by the decrease to 5% in the relative incidence of intrahepatic bile duct stones.

However, intrahepatic bile duct stones are not completely eliminated in our country and in the Far East. A comparative survey by Nakayama (1986) revealed that the relative incidence of intrahepatic bile duct stone was 4.1% in Japan, while the relative incidence of hepatic bile duct stone in Taiwan is reported to be the highest in the world (53.3%). However, in a retrospective review of cases of cholelithiasis from 28 hospitals in Taiwan during a 20-year period (1971-1990), Su *et al* found that intrahepatic bile duct stones accounted for 20.3% of 17182 surgical patients of cholelithiasis. This indicates that the incidence of intrahepatic lithiasis is slightly higher than that in the mainland of China.

Regional differences in the prevalence of intrahepatic bile duct stones in our country are remarkable. The incidence of intrahepatic bile duct stone among autopsy and clinical cases of cholelithiasis was 38% and 24.6%, respectively, in Chongqing; 18.7% and 31% in Chengdu and Luzhou, respectively; 43.1% in Shantou; and only 4.5% in Shanghai. It was low in the northwest and north of China (4.8% and 4.1%, respectively). A general tendency of decrease in the relative incidence of intrahepatic bile duct stones and reduction in the number of new patients diagnosed was noted. However, this change was not paralleled in different areas of the country; for example, in a 10-year survey (1981-1991) conducted in Guangxi Province, the relative incidence of gallbladder stones rose from 12.7% to 19.8% in the latter 5 years, while the incidence of bile duct stone decreased only from 55.2% to 41.8% during the same period. Therefore, intrahepatic bile duct stone was still a common disease that remains difficult to treat in many inland provinces, where the disease is not only disappearing, but also attracted more attention than before.

The basic principles of surgical treatment for intrahepatic bile duct stones are relief of obstruction, elimination of lesion, and adequate drainage. The key aspect of surgical treatment is "elimination of lesion". Intrahepatic bile duct stone is a condition with strictly intrahepatic segmental distribution; corresponding pathological changes have been reported to occur in different areas of the liver, *e.g.*, fibrosis, atrophy, and dysfunction. Huang ZQ (1957) advocated the use of planned hepatic lobectomy for treating intrahepatic bile duct stones and reported one case of left lateral hepatic lobectomy and another case of right hepatic lobectomy. Hepatic lobectomy has been gradually and widely been accepted as the treatment for intrahepatic bile duct stones, and it is now a common procedure for treating gallstones in the left lateral lobe of the liver. In a national survey on surgical therapy for intrahepatic bile duct stones occurring in 4197 patients, hepatic lobectomy was used for the treatment of 728 cases (17.3%). According to a recent report, hepatectomy rates ranged from 11% to 32% in 4 groups of surgical patients with hepatic bile duct stones, with the mean rate being 18%. The percentage of hepatectomy among the surgical procedures performed in cases of intrahepatic stones were 49.8% and 56.6% in two core hepatobiliary centers in the mainland and about 50% in Taiwan. However, as for the location of hepatectomy, 85% of the cases were of lateral left lobectomy and 95% were of left hepatic lobectomy, 10% and 17% were of right hepatic lobectomy in some hepatobiliary centers. A discrepancy was noted in the number of cases of right-sided hepatectomy between the location of hepatectomy and distribution of gallstones. Patients with intrahepatic bile duct stones often had serious

Table 1 The surgical therapeutic outcomes for intrahepatic bile duct stones (1963～1975)

Surgery	*n*	A%	B%	C%	D%
Hepatic lobectomy	43	58.1	32.6	9.3	0.0
Cholangiojejunostomy	33	51.5	24.2	21.2	3.0
Cholangioduodenostomy	22	31.8	31.8	22.7	13.6
Choledocholithotomy	32	34.4	21.9	12.5	31.3
Total	130	46.2	27.7	15.4	10.8

Table 2 The different surgical outcomes of 80 cases intrahepatic duct stones (1975～1981)

Surgery	*n*	A%	B%	C%	D%
Hepatic lobectomy	23	26.1	56.5	13.0	4.3
Cholangiojejunostomy	43	27.9	48.8	18.6	4.6
Cholangioduodenostomy	7	14.3	57.1	28.6	0.0
Choledocholithotomy	7	57.1	42.8	0.0	0.0

complications before hospital admission, and the operation only provided symptom relief. Therefore, the rates of infection in the biliary tract, residual gallstones, and reoperation were high. Thanks to the development and popularization of modern imaging technology, *i.e.* ultrasound and computed tomography, early diagnosis has become possible for the patients with intrahepatic bile duct stones since the 1980s. Hepatolithiasis in early phase usually presents with mild symptoms or is asymptomatic; the symptoms of infection are also mild because of the use of antibiotics. Gallstones were often limited to 1-2 hepatic segments of the liver without involvement of extrahepatic bile duct as noted in these patients. Therefore, the concept of surgical therapy for intrahepatic lithiasis should now be modified. The surgical treatment for intrahepatic bile duct stones in its early phase should be "radical" rather than targeted at achieving symptom relief. It would be reasonable if hepatic segmental or subsegmental resection were selectively performed during the early phase of the condition. Intrahepatic bile duct stones in their early phase are often restricted to the posterior segment of the right lobe and laterosuperior segment of the left lateral lobe, without gallstones in the extrahepatic bile duct.

Stricture of hepatic bile duct is an important factor influencing the operative effect of intrahepatic bile duct stones, and its incidence is sometimes high. For example, among 3938 surgical patients with intrahepatic bile duct stones in this country, 956 (24.28%) had hepatic hilar bile duct stricture; but the corresponding rates were 41.94%, 41.76% and 40.17%, respectively, in Guangdong, Hunan and Sichuan provinces. Among patients who underwent reoperation, the incidence rate of hepatic bile duct stricture was proportional with the frequency of reoperation. The main causes of failure of surgical treatment for intrahepatic bile duct stones were incomplete correction of the stricture and presence of residual stones in the liver. Thirty-four patients in a group of 130 patients who received surgical treatment for intrahepatic stones and had been followed for an average of 8 years, did not give satisfactory results; in 82% of the cases, the failure was due to the presence of uncorrected hepatic bile duct strictures.

Strictures associated with intrahepatic bile duct stones often occur in the hepatic hilum and the left hepatic duct, which appear narrow and ring-like with dilatation of the bile duct on both ends or nearly normal appearance of the bile ducts. Therefore, the narrowing of the bile duct may be corrected by proper surgery. Necessary steps for correcting the stricture for hepatic bile duct include wide incision of the stricture site to make a new posterior wall by suturing the bile duct wall and repairing the anterior bile duct wall with a Roux en Y-jejunal loop, in addition to performing extensive cholangiotomy and cholangioenterostomy. To ensure complete removal of the hepatic lesion, hepatic lobectomy was performed simultaneously during the operation; this combined operation was called "combined surgery". If the hepatic bile duct stricture could be corrected, the surgical outcomes for intrahepatic bile duct stones could be significantly improved. For example, in a series of 107 patients with intrahepatic duct stones and hepatic duct stricture followed up for an average of 4.5 years, 87.8% of the patients obtained satisfactory results; however, two patients died at the end stage of the disease, yielding a mortality rate of 2%. Among the various surgical procedures, hepatic lobectomy achieved the best results, with the success rate being 93.0%. This indicates that the outcome of the main hilar duct stricture would not interfere with that of the intrahepatic bile duct stone if the case was managed effectively. However, if hepatic duct stricture remained untreated, the prognosis would be poor. For example, in a group of 14 cases with the stricture *in situ*, 3 died, thereby indicating a mortality rate of 21.5%.

If intrahepatic bile duct stones can be completely eliminated in patients with mild hepatic hilar duct strictures, pediculated tissues were selectively used to repair the bile duct defect after incision of the stricture. The tissues used are gallbladder wall, serosal patch of the stomach, jejunum, or round ligament flap with blood supply. The repair yielded good outcomes in the early phase, owing to the preservation of the function of Oddi's sphincter and decrease in retrograde biliary infection; however, it is still difficult to arrive at a final conclusion because the number of the patients treated in this manner was small and the follow-up period was short.

Occasionally, intrahepatic bile duct stones may be extensively distributed in the liver, and in such cases, to gain access to all the major hepatic ducts, the hilar bile duct may need to be widely incised to remove stones under the field of vision. Investigations in autopsied liver specimens and clinical experiences indicated that it is possible to make a wide incision linking the right anterior inferior hepatic bile duct, right hepatic duct, left hepatic duct, and left medial hepatic bile duct. Through this 5-8 cm-long incision, the openings of 2-3 grade intrahepatic ducts can be directly visualized. Sometimes the resection of the quadratus lobe may also be performed to further widen the operative field of the hilar biliary tract, thus making the operation easier and more perfect.

Biliary tract carcinoma complicated with gallstones is an important concern to be considered during the surgical treatment of intrahepatic bile duct stones. According to a national survey of 826 surgical cases of extrahepatic biliary carcinoma studied between 1977 and 1989, 140 (16.9%) cases were associated with gallstone. In another report on 4197 cases of intrahepatic bile duct stones, 14 (0.68%) cases were associated with biliary carcinoma. The incidence of coexisting biliary carcinoma with intrahepatic duct stones varied between 0.36% and over 10%; this variation may be attributed to the differences in the subjects involved, methods of diagnosis, and the length of follow-up. Sanes and McCallum (1942) first reported two cases of intrahepatic bile duct stones associated with biliary carcinoma. Subsequently, Koga and Chijiiwa reported an incidence of 2% and 7.3% of biliary carcinoma in intrahepatic bile duct stones in Japan, respectively. A study from Taiwan indicated an incidence of 5.0%, where peripheral cholangiocarcinoma was detected during autopsy in 10% of the patients with intrahepatic bile duct stones. Cholangiocarcinoma may be discovered during surgery performed for intrahepatic bile duct stones or may develop several years after the operation; therefore, it is called delayed hepatic duct carcinoma. We investigated 6 cases of hilar cholangiocarcinoma coexisting with intrahepatic bile duct stones, accounting for 1.46% of the surgical cases of intrahepatic duct stones. Guo Hong Guang *et al* reviewed 12 cases of delayed hepatic duct carcinoma after the surgery for primary intrahepatic duct stones between 1981 and 1994, accounting for 1.5% of the operative cases of intrahepatic duct stones during the same period. Hepatic duct carcinoma was noted 3-40 years (average, 10 years) after the first biliary operation for hepatic duct stones. All the patients had history of biliary disease spanning 10-40 years, and 8 of them showed nothing remarkable in endoscopic retrograde cholangiopancreatography (ERCP) before the diagnosis of the carcinoma was established.

If the resected liver specimens for intrahepatic stones were examined, the incidence of associated hepatic duct carcinoma could be even higher. In six recently published reports in this country encompassing 661 cases of hepatic lobectomy for removal of intrahepatic duct stones, 16 (2.4%) of the cases were of cholangiocarcinoma. Chang Hai Hospital in Shanghai and the 47th Hospital of PLA reported hepatic cancer in 3.33% and 3.36%, respectively, of their resected liver specimens, while 3 (4.7%) of

Table 3 The surgical outcomes of intrahepatic duct stones (Cai-Jing Xiu, 1983～1994)

Surgery	*n*	A%	B%	C%	D%
Hepatic lobectomy	181	75.7	17.1	4.4	2.7
Hepatic lobectomy and cholangioenterostomy	192	58.3	33.0	7.3	2.0
Cholangioenterostomy	220	49.1	40.9	5.5	4.5
Choledocholithotomy	156	42.9	28.8	21.1	7.0
Total	749	56.6	30.4	8.6	4.0

63 cases of liver specimens exhibiting malignant changes at Queen Mary Hospital in Hong Kong. Chronic intrahepatic duct stones, biliary infection, and cholestasis can result in atypical hyperplasia of the epithelial mucosa of the hepatic bile duct, which may occur as part of precarcinomatous changes.

Surgical treatment of intrahepatic bile duct stones have improved over the last few decades. However, current treatment options are far from achieving complete cure, and problems such as residual stones, recurrence and progressive liver damage still await solution. At present, multiple surgical procedures for intrahepatic bile duct stones are in use, but they may be broadly classified under three main categories, namely, hepatic lobectomy, cholangioenterostomy, cholangiotomy with T-tube drainage. In fact, the above methods are often used in combination. Our experiences with various surgical techniques over 40 years of clinical practice can be chronologically arranged into three stages: 1963-1975, 1975-1981, 1983-1994. Since all the practice was centralized and carried out under a stable leadership, the stages were comparable (Tables 1-3). The success rates (A + B) of the three stages were 73.9%, 80.1% and 87.05%, respectively. Unfortunately, only half the patients became completely asymptomatic after the operation, and the frequency of symptom recurrence might increase over a prolonged follow-up period.

The relationship between the therapeutic effect and different operative methods were affected by several factors. If the liver lesion was not removed by liver resection, the outcome of choledochoduodenostomy was usually poor, with patients frequently developing serious postoperative retrograde biliary infection.

Hepatic lobectomy has the best outcomes, with a long-term success rate of 91.16% among 439 cases. The better result of hepatectomy was confirmed by many other reports from our country. Hepatic lobectomy is usually combined with cholangioen terostomy to solve cholestasis. For example, 368 (81.3%) of 482 cases of hepatic lobectomy were associated with various cholangioenterostomies at the Chongqing Southwest Hospital. However, the patients with limited intrahepatic bile duct stones who were treated only by hepatic lobectomy gave better long term outcomes than the patients by combined cholangioenterostomy.

Although hepatic lobectomy may remove lesions in the liver, it does not eliminate the possibility of recurrent stones in the remnant portion of the liver; this was shown in the study conducted at Queen Mary Hospital in Hong Kong, which showed that 16% of the 63 cases of hepatic lobectomies had recurrent gallstones in a new area of the liver after a median follow-up period of 47 mo. Thus, the continuation of treatment even after hepatic lobectomy is necessary, and further investigations on the prevention of the recurrence of gallstones are imperative.

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ORIGINAL RESEARCH

Ultrastructural observation of liver tissue ablation induced by high-intensity focused ultrasound

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Abstract

AIM: To observe the ultrastructural changes of liver tissues on normal rabbit ablated by high-intensity focused ultrasound (HIFU).

METHODS: A single shot of 1.1 MHz focused ultrasound at an intensity of 500 W/cm^2 with 20-s duration of continuous exposure was applied intraoperatively in normal rabbit livers. Ultrastructural changes of the sonoablated lesion, as viewed by light and electron microscopy, were observed.

RESULTS: Liver cells at the center of the sonoablated lesion showed irreversible degeneration immediately after HIFU treatment; electron microscopy showed that although the liver cells appeared normal histologically, irregularly shaped cavities of about 0.3-0.5 μm in diameter were present in the cytoplasm.

CONCLUSION: Thermal damages may be the main mechanism of HIFU-induced ablation of liver tissues besides cavitation effect.

Key words: Liver/ultrastructure; Ultrasonic therapy; Eletron microscopy

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INTRODUCTION

High-intensity focused ultrasound (HIFU) is a new method of ablating non-invasively a selected volume of tissue at a certain depth within the body whilst sparing overlying tissues. This technique was originally developed as a method of selective brain tissue destruction in neurosurgical research[1]. Recently, it has been successfully applied in ophthalmology for the treatment of glaucoma[2] and is undergoing clinical trials for application in the prostate[3]. We have previously used this principle for the ablation of liver tumors in a rabbit model with encouraging results[4]. In this paper, the ultrastructural changes of the ultrasonic lesion in normal rabbit livers, as viewed by light and electron microscopy, are described.

MATERIALS AND METHODS

HIFU instrument

The HIFU instrument developed by the Biomedical Ultrasound Laboratory, Shanghai Jiaotong University, is composed of the following: a signal generator, power amplifier, phase-control system, transducer motion control, microcomputer, and a transducer. The transducer had an array of 16 pistons distributed on the concave spherical surface and was 14 cm in diameter; it produced an acoustic beam with half-intensity axial and lateral dimensions of 10 mm and 8 mm, respectively, at a focal distance of 11 cm (Figure 1).

The energy was generated by the amplifier delivering 500 W/cm^2 at a frequency of 1.1 MHz. For experiments on the rabbit, the transducer was fixed to a motorized, three-dimensional coordinate system; this enabled a movement of 0.1 mm in accuracy. A microcomputer controlled the parameters of phase, power, emitting time, and mode.

HIFU treatment

Five New Zealand white rabbits, weighing 2.5 ± 0.24 kg, were anesthetized by administration of 3% pentobarbital (1 mL/kg of body weight, administered intravenously) and a subxiphoid midline abdominal incision was made. The hepatic lobe of the right center was exteriorized and exposed to the transducer hung by the motorized three-dimensional coordinate system at the focal point of the beam. A thin latex bag filled with thermostatical degassed water, which served as the acoustic coupling medium, was placed between the transducer and the liver. Then, a single shot of 20 s in the continuous wave mode was administered, followed by closure of the abdomen. The animals were allowed to recover. The surviving rabbits were killed at various intervals after HIFU treatment.

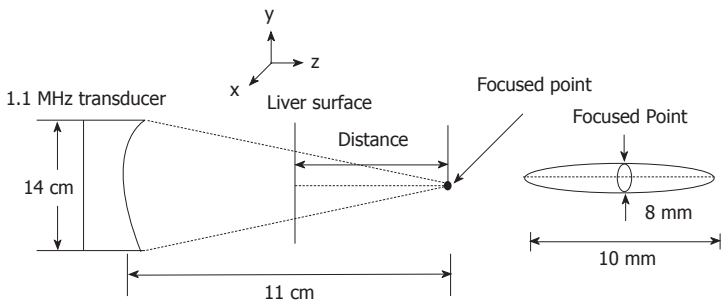

Figure 1 Beam characteristics of the 1.1 MHz high intensity focused ultrasound transducer.

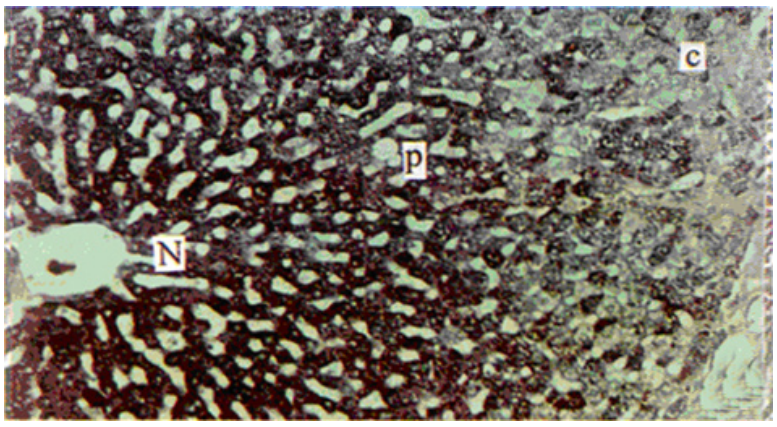

Figure 2 Light micrography showing the cytoplasmic glycogen decreased in ablated lesion (c, p) immediately after high intensity focused ultrasound (HIFU) exposure by stained PAS. N = Normal liver; C = Lesion centre; P = Lesion edge. × 66.

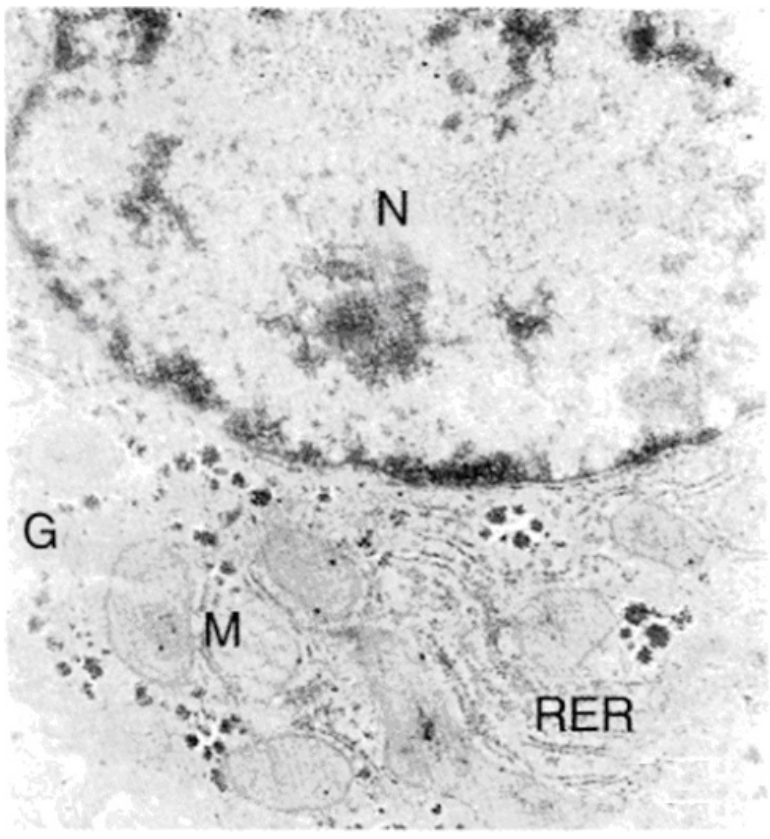

Figure 3 Electron micrography of a normal rabbit hepatocyte. N = Nucleus; M = Mitochondria; RER = Rough endoplasmic reticulum; G = Glycogen.

Histological study

After the post-mortem excision of the liver, it was fixed immediately in 10% methacarn, embedded in wax, and thin sections were prepared. They were stained with hematoxylin and eosin (HE) and reticulin and periodic acid Schiff's base reaction (PAS).

Electron microscopy

Immediately after HIFU exposure, tissue samples in the centre and edge of the lesion (Figure 2) were placed in 2.5% glutaraldehyde, fixed overnight, and postfixed in 1% osmium tetroxide. The fixed samples were dehydrated using a graded series of ethanol and acetone and were embedded in Epon 618 resin. Ultrathin sections were then cut, double-stained with uranyl acetate and lead citrate, and viewed under a JEM-1200EX transmission electron microscope.

Figure 4 Electron micrography from the edge region of the lesion immediately after HIFU treatment showing the destruction of organelles and massive vacuolization of the cytoplasm as a sign of irreversible cell death.

RESULTS

Histological findings

Immediately after HIFU exposure, a sharply defined, grey-white block with diameter 8 mm was noted in the liver. The liver cells showed enlargements and sinus showed stenosis on HE staining, but the liver plate still appeared regular and the cytoplasm and the nucleus appeared normal. PAS staining showed that the cytoplasmic glycogen had decreased but had not disappeared (Figure 2). After 24 h, the cells in this lesion died with cell debris, pyknotic nuclei, karyolysis, or karyorrhexis, and glycogen had almost disappeared completely on PAS stained lesions.

Electron-microscopic observation

An electron micrograph of a normal rabbit hepatocyte is shown in Figure 3, with a nucleus, mitochondria with intact cristae, glycogen, and rough endoplasmic reticulum. Immediately after insonation, the nucleus appeared irregular, but its membrane was still intact at the edge of the sonoablated lesion (Figure 4), the matrix of mitochondria was reduced and the cristae were disrupted. The ultrastructure of rough endoplasmic reticulum was disordered and some ribosome disappeared. Cavities (about 0.3-0.5 μm) were also found, with no glycogen and irregularly shaped holes in the cytoplasm. These cavities may be the result of mitochondria cavitation. Figure 5 shows that in the centre of the lesion, the nucleus was almost completely disrupted, and the integrity of the structure of mitochondria, the endoplasmic reticulum, and glycogen had disappeared. Again, there were 0.3-0.5 μm cavities in the cytoplasm. The cellular ground substance was amorphous, with many electronic deposits; this indicated typical complete disruption and destruction of the cytoplasmic ultrastructure.

DISCUSSION

In the previous study on histological changes in rabbit liver tumor

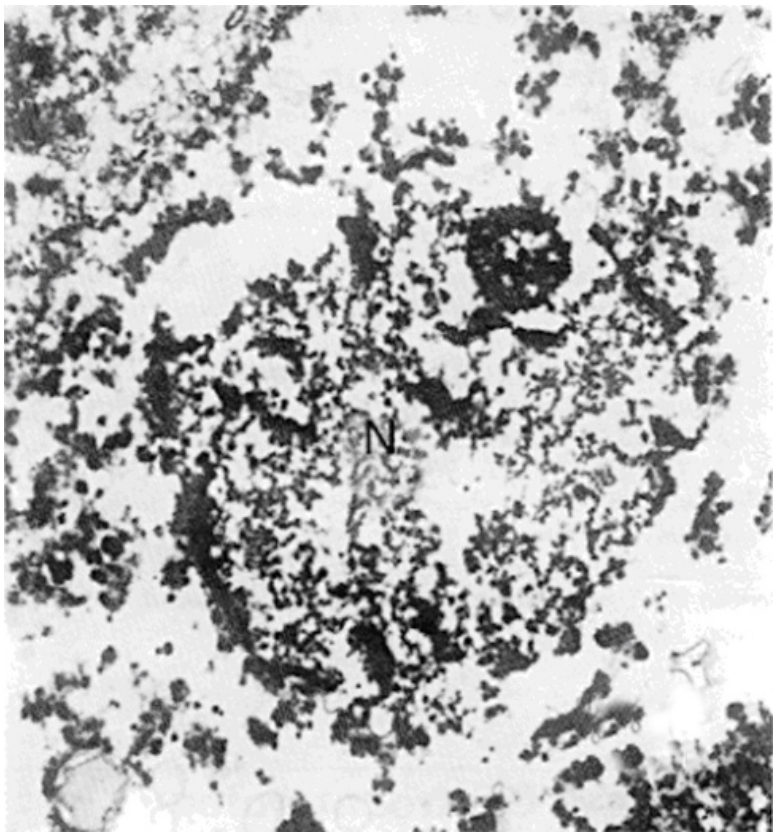

Figure 5 Electron micrography from the centre of the lesion, showing complete cellular disruption with karyorrhexis and many electronic deposits existed and cavities left.

treated with HIFU, we found that HIFU insonation at a peak intensity of 500 W/cm^2 for 20 s efficiently ablated the liver tumor, inducing coagulation necrosis 24 h later; however, evidence of prompt damage to the target area could not be clearly identified immediately after HIFU exposure[4]. With the same results, this study also revealed that the liver cells in the treated centre appeared histologically normal immediately after HIFU treatment administered at a similar intensity (1.1 MHz, 500 W/cm^2, 20 s). However, these cells exhibited irreversible degeneration with disruption of the nuclear membrane and absence of mitochondria and endoplasmic reticulum, as observed by electron microscopy. It is indicated that coagulation necrosis of the targeted cells occurred as the last stage of the cell death, and that in fact, the liver cells were significantly damaged by HIFU insonation.

Although some recent reports have examined HIFU ablation of experimental liver tumors, few of them have focused on the resultant ultrastructural changes and the underlying mechanism, which remains to be fully determined[5]. Generally, it is believed that the two main mechanisms involved in tissue sonoablation are as follows: (1) a thermal effect (rapid elevation of local tissue temperature to over 80 ℃) that promptly coagulates the cell protein and (2) a cavitation effect (generation of violently expending and collapsing gaseous bubbles) that mechanically disrupts the cellular and tissue structure. Thermal damage is usually assumed to occur in association with long exposure times of over 1 s and relatively low intensities (below 1000 W/cm^2), while cavitation occurs with high-peak intensities and brief exposure periods. There is a range of levels of intensities and exposure periods for which both phenomena may not be distinctly identified[6,7]. In our previous study[8], we found that the peak tissue temperature at the sonic focal region was > 90 ℃, with the same energy; this thermal effect could provoke tissue destruction by coagulation necrosis. Therefore, thermal necrosis may play a dominant role in HIFU tumor ablation.

ter Haar *et al*[9] used HIFU (1.7 MHz, 3000 W/cm^2, 10 s) and performed a similar study in normal rat livers. They found that the cells in the rim of the ultrasonic lesion appeared histologically normal immediately after HIFU treatment; however, these cells were functionally impaired, for their cellular glycogen was missing. These findings are in agreement with our results. Moreover, cavities (about 4 μm) could be observed by electron microscopy, but it remains to be determined whether it is due to vaporization or acoustic cavitation. In our study, we also found many 0.3-0.5 μm cavities in the cytoplasm and deduced that it may be due to the cavitation of mitochondria or other organelles. Therefore, it is suggested that the cavitation effect may also play a significant role in inducing irreversible cell death immediately after HIFU treatment when applied under a relatively low-intensity condition.

Above all, our results show that the thermal effect and cavitation due to ablation probably occur simultaneously as the HIFU is directed at the liver tissues when the intensity is relatively low. This findings will be helpful in improving the focusing and selectiveness of liver cancer ablation extracorporeally if the cavitation could be much more enhanced, which worth investigating in the future.

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ORIGINAL RESEARCH

Endoscopic monitoring in small bowel transplantation

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Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To investigate the role of endoscopic monitoring in small bowel transplantation.

METHODS: This study was conducted in two parts—an initial experimental study followed by a clinical study. In the experimental study, segmental small bowel allotransplantation was performed on white outbred pigs. Stomas were created for exteriorization of the proximal and distal ends of the intestines (Thiry-Vella loop). The grafts were monitored by endoscopy *via* stomas, with or without immunosuppressive therapy. For the clinical study, the whole small-bowel allograft of a woman with short bowel syndrome was endoscopically monitored *via* distal stoma.

RESULTS: The most common endoscopic findings of graft rejection following small bowel allotransplantation were mucosal erythema, erosion, and ulceration. Diffuse ulceration with bleeding occurred in the late phase of rejection.

CONCLUSION: Endoscopic monitoring is essential to small bowel transplantation.

Key words: Small intestine/transplantation; Graft rejection; Endoscopy; Intestinal mucosa/pathology

Li YS, Li JS, Li N, Jiang ZW, Li YX, Li XH. Endoscopic monitoring in small bowel transplantation. *World J Gastroenterol* 1997; 3(3): 137-138 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/137.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.137

INTRODUCTION

Graft rejection and infection are important causes of failure following small bowel transplantation (SBT). Endoscopy of lesions and biopsy of graft are currently the most important modalities for the detection of signs of rejection or infections such as cytomegalovirus (CMV) infection. In this study, we investigate the role of endoscopic monitoring in SBT by a combined approach of both experimental and clinical studies.

MATERIALS AND METHODS

Animals and experimental groups Twelve white outbred pigs weighing 18.5-22.5 kg were used in this study. General anesthesia was induced by administration of intravenous sodium pentobarbital. The animals were divided into two equal groups according to the presence or absence of immunosuppressive treatment: group Ⅰ (*n* = 6) with immunosuppression (cyclosporine, *Tripterygium wilfordii*, and methylprednisolone); and group Ⅱ (*n* = 6) without immunosuppression.

Operative procedure

Heterotopic segmental small bowel allotransplantation was performed as described previously[1]. The graft was perfused with and preserved in Ringer's lactate solution at 4 ℃, followed by luminal perfusion with metronidazole solution at 4 ℃. After a mean period of cold ischemia for 60 min, the segmental graft was vascularized. Both proximal and distal ends were exteriorized as stomas (Thiry-Vella loop) (Figure 1).

Endoscopic monitoring

After the transplant, the grafts were evaluated daily with endoscopic visualization and biopsy, and the biopsy specimens were examined under a standard light microscopy.

Clinical SBT

A woman with short bowel syndrome underwent whole small-bowel allotransplant (250 cm). The graft was orthotopically transplanted *via* end to side anastomosis of superior mesenteric artery aorta and end to side anastomosis of portal vein to superior mesenteric vein (Figure 2). Cyclosporine, *Tripterygium wilfordii*, and methylprednisolone were used as immunosuppressive agents. Biopsies guided or not guided by endoscopy were performed daily for 2 wk after the operation. From the 3rd to the 4th week, biopsies were performed every other or the third day. One month after the transplantation, endoscopy with biopsy was repeated. The biopsy specimens were examined under standard light microscopy.

RESULTS

Experimental study

In group Ⅰ, all animals had survived a long period of time, while those in group Ⅱ had a survival period of 17.7 ± 7.6 d. The cause of death in fatal cases was graft rejection. Thirty minutes after reperfusion, graft mucosal hyperemia and edema were seen. Mucosal erosion was seen on the first postoperative day (POD).

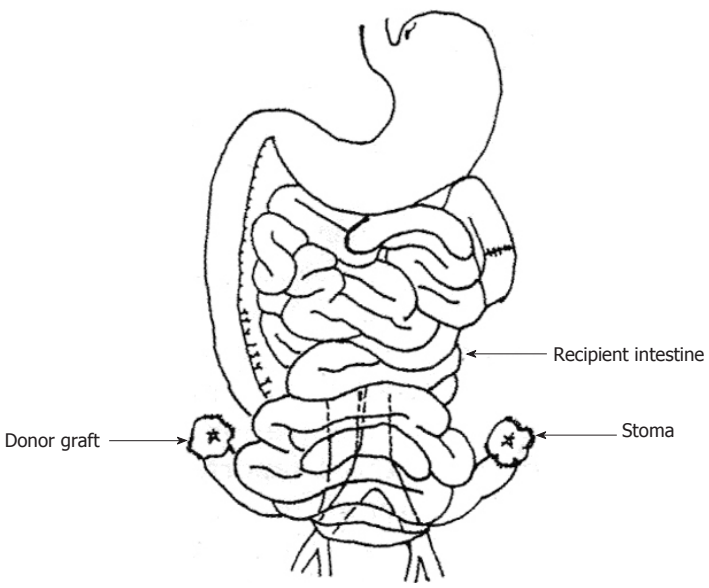

Figure 1 Heterotopic segmental small bowel allotransplantation in pigs.

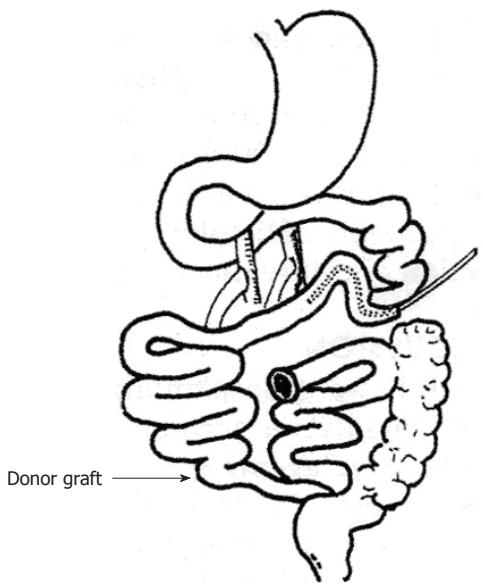

Figure 2 Whole small bowel allotransplantation in human.

Endoscopic and histologic features in the first 48 h were similar in both groups. Thereafter, in group Ⅰ, graft mucosa was restored to normal appearance, except for occasional mucosal erosion and ulceration. On the other hand, group Ⅱ showed punctate erythema on POD 4 or 5, local erosion or ulceration on POD 6 or 7, and diffuse ulceration with bleeding on POD 10. The histological features were necrosis and sloughing of villi tips, inflammatory cell infiltration, and intimal thickening or occlusion of vessels in submucosa.

Clinical study

From the 6th to the 8th month after the operation, eight endoscopic procedures with biopsies were performed. The endoscopic findings were graft mucosal hyperemia and edema on POD 6. Thereafter, endoscopic visualization showed absence of mucosal edema, glistening of the appearance of mucosa, presence of a normal vascular pattern, and increased peristalsis. Local ulceration with exudates was observed in the 3rd and the 5th month, respectively. Histological characteristics of graft rejection and infection were absent.

DISCUSSION

Small bowel transplantation has become a clinical reality mainly because of the availability of highly effective immunosuppressive agents such as cyclosporine, FK506, and OKT3. However, control of post-transplant complications such as graft rejection and infection and other complications following SBT continues to remain challenging. Signs of rejection and infection have been traditionally monitored using the absorptive function test and evaluation of motor activity, permeability of bowel, and levels of various chemical markers. These methods, however, could not entirely replace endoscopy with biopsy, and histological evaluation of biopsy continues to remain the most reliable investigation for confirming small intestinal allograft rejection[1,2].

In the clinical case of SBT, most of the operative procedures were similar to those in the experimental study (Figure 2). The graft distal stoma was used as the "window" for monitoring. Biopsy under naked eyes was slightly stimulative to the patients, but it was difficult to determine whether the specimens showed evidence of graft rejection. Specimens collected from sites adjacent to the stoma (< 10 cm) often showed signs of chronic inflammation, which are sometimes difficult to differentiate from those of rejection. Specimens obtained from the same site affected the diagnosis of rejection. In the clinical case of SBT, two biopsy samples taken from the colon did not show evidence of rejection, while two other biopsy samples observed under naked eyes showed characteristics suggestive of rejection histologically, but, endoscopically guided biopsies confirmed that rejection had not occurred. During the early stage of rejection, the mucosa shows local ulcer. Vascular characteristics were highly valuable in determining whether rejection has occurred. Therefore, it is important to perform full-thickness biopsies at several sites[1].

The rate of graft rejection was 75% in the first month and 15% for the first three months[3]. Abu-Elmagd *et al*[3] recommended that endoscopy should be performed weekly during the first three postoperative months. The results of our experimental study showed that early mucosal injury was largely related to reperfusion injury and that early definitive appearances of graft rejection were present on POD 5 or POD 6. Therefore, endoscopic visualization and biopsies of graft should commence on the 5th POD. In addition to routine regular endoscopic visualization, the endoscopic procedure should be repeated if any symptoms of rejection develop, including fever, abdominal pain, nausea, vomiting, watery diarrhea, increase in stromal output,, and dusking of mucosa. In the study by Abu Elmagd *et al*[3], the endoscopic appearances during acute rejection episode were ischemia or duskiness, local ulcer, and decrease or complete loss of peristalsis. Grafts with severe rejection showed diffuse ulceration with bleeding. Chronic graft rejection was evidenced by pseudomembrane formation, thickening of mucosal folds, and chronic ulceration. Hassanein *et al*[2] evaluated more than 220 endoscopic procedures and reported that during acute rejection mucosal features were mucosal erythema (77.8%), erosion (38%), and ulceration (33%). CMV infection appeared as local ulceration. In this study, graft rejection did not occur in the clinical case of SBT, and, the results of the experimental endoscopic visualization for signs of acute graft rejection were similar to those reported by Abu Elmagd *et al*[3] and Hassanein *et al*[2].

The findings of this study highlight the importance of endoscopic visualization with biopsy in the monitoring of cases of SBT for signs of graft rejection. However, the criteria for small intestinal graft rejection are yet to be definitively established, and the differentiation between rejection and CMV infection remains difficult. Mucosal erosion and ulceration are not characteristic of small intestinal graft rejection. However, in this study, mucosal ulceration was observed in the 3rd and 5th postoperative month in clinical SBT, but no clinical findings or histological features of graft rejection were observed. Because of the patchy distribution of the evidences of rejection, we recommend that multiple endoscopic visualization and biopsies be repeated at different sites for thorough evaluation.

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ORIGINAL RESEARCH

Construction of retroviral vectors to induce a strong expression of human class interferon gene in human hepatocellular carcinoma cells *in vitro*

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Abstract

AIM: To establish the hepatoma cell–specific expression of human interferon (IFN) gene mediated by retroviral vectors

METHODS: Human interferon α and interferon β complementary DNA (IFN cDNA) were cloned into the polylinker site of pMNSM retroviral vector to construct recombinant retroviral vectors pMNSIFNA and pMNSIFNB, with the transcription of IFN gene being driven by Simian virus 40 early region promoter (SV40) early region promoter. IFN cDNAs were also cloned into pMNAIFNA, pAMNSIFNA, and pMNAIFNB, with the transcription of IFN gene being driven by SV40 early region promoter regulated by α-fetoprotein enhancer. Next, the retroviral constructs were introduced into retroviral amphotropic packaging cells using the lipofectamine-mediated gene transfer procedure. The rate of plasmid transfection was (4-40) × 10^3 colonies/μg DNA/10^6 PA317 cells. The rate of retrovirus infection was (5-500) × 10^4 colony forming units (CFU)/mL. Further, the recombinant retroviruses were used to infect human hepatoma cells, renal carcinoma cells, and melanoma cell lines in the presence of 4 μmg/L polybrene.

RESULTS: Northern and Dot hybridization of total RNA from the neomycin-resistant colonies and IFN expression assay indicated that human α fetoprotein enhancer induced efficient and specific transcription and expression of IFN genes driven by the promoter of different origins in human hepatoma cells, leading to high production of α fetoprotein.

CONCLUSION: Cis active element of α-fetoprotein gene can drive specific expression of IFN genes in human hepatoma cells, which provides some valuable data for the hepatoma-specific immune gene therapy.

Key words: Interferon alpha; Interferon beta; Retroviridae; Carcinoma, hepatocellular; Liver neoplasms; Genes, regulatory; Gene expression; Gene therapy

Cao GW, Gao J, Du P, Qi ZT, Kong XT. Construction of retroviral vectors to induce a strong expression of human class interferon gene in human hepatocellular carcinoma cells *in vitro*. *World J Gastroenterol* 1997; 3(3): 139-142 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/139.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.139

INTRODUCTION

Class I interferon (IFN) is a potent immune-modulating factor *in vivo* that plays an important role in activating anti-tumor cytotoxic T lymphocytes and non-specific effector cells such as natural killer (NK) cells[1]. IFN genes and many other cytokine genes have been introduced into tumor cells, tumor interstitial cells, and tumor-infiltrating lymphocytes[2-4] in order to sustain an effective anti-tumor concentration of cytokine located at tumor sites. It has been shown that some cytokine-gene-modified tumor cells lost their tumorigenicity and inhibited the growth of parental tumor in animal models. Practical antitumor effects were obtained in the protocol. The morbidity of hepatoma is relatively high in China, and no effective procedure is currently available for the treatment of advanced hepatoma. In this study, hepatoma-specific IFN retroviral vectors were prepared and *in vitro* expression assays were conducted to establish an effective gene therapy against hepatoma and prevent the possible adverse effects of class I INF expression in non-tumoral tissues in addition to verifying the possibility of the enhancer of α-fetoprotein (AFP) "house-keeping gene" regulating viral promoter in retroviral vector.

MATERIALS AND METHODS

Plasmids

Plasmids pCGS261 containing human leukocyte interferon (IFN-α) cDNA were obtained from the American Type Culture Collection (ATCC), (Rockville, United States). pSPGNHIFNB10 carrying the HuIFN-β-cDNA was purchased from LMBP (Gent University,

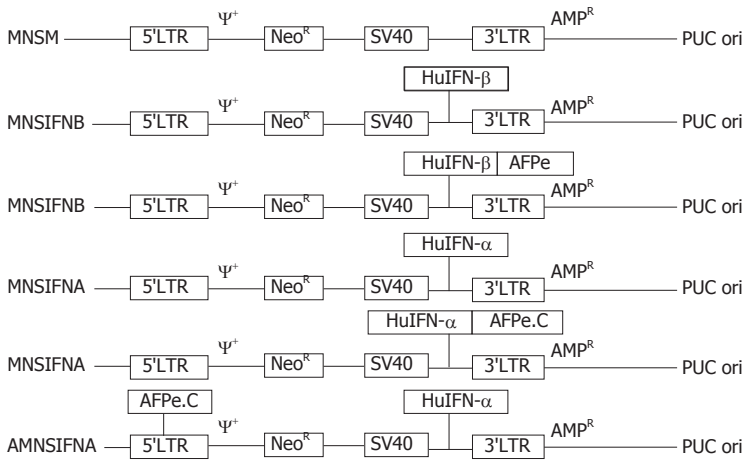

Figure 1 Structure of MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA retroviral vectors. LTR: Retroviral long-terminal repeat sequence; Neo R: Neomycin phosphotransferase gene for G418 selection; SV40: Simian virus 40 early region promoter; HuIFN-β- cDNA: Human interferon β complementary DNA; HuIFN-α cDNA, Human interferon α complementary DNA. AFPe: Human α-fetoprotein "house-keeping gene" enh ancer; AFPe.c, Human α-fetoprotein "house-keeping gene" enhancer core sequence.

Belgium). Plasmid pAF5.1-CAT containing the AFP enhancer and plasmid pGEM7z-AFPe containing human AFP enhancer core sequence were kindly provided by Professor Tamaoki (Calgary University, Canada). Retroviral vector pMNSM containing the neomycin phosphotransferase and the SV40 early region promoter was a gift from Dr. Tsuchiya (Tokyo Medical and Dental University, Japan).

Cells

Amphotropic retrovirus packaging cell line PA317, retrovirus titering cell line NIH3T3TK$^-$, human hepatoma cell lines HepG2 and Hep3B, and IFN bioassay cell line WISH were obtained from ATCC. Retroviral packaging cell Psi2 was procured from Dr. Shigeki Kuriyama. Human hepatoma cell line SMMC7721 and human melanoma cell line M21 were obtained from Chinese Academy of Sciences. Human renal carcinoma cell line RZ94602 was established at our laboratory.

Main reagents

TdR, dNTP, polybrene, PEG8000, guanidine isothiocyanate, PVP2500, and MTT were from Sigma. New generation liposome transfection reagent LIPOFECTAMINE[TM], RPMI-1640, DMEM, FCS, random primers DNA labeling system, and G418 were from Gibco. Herring sperm DNA, restriction enzymes, T4 ligase, DNA polymerase Klenow fragment, and CIP were from Promega. α ^{32}P-dCTP was from Beijing Yahui Biomedical Co. Nitrocellulose membrane was from Amersham.

Construction of retroviral vectors

Plasmids extraction with alkali lysis, enzymes digestion, ligation, isolation and harvest of DNA fragments and transformation of *Escherichia coli* was carried out according to the standard methods[5].

The procedures of construction are as follows. The plasmids pSPGNHIFNB10, pCGS261, pAF5.1-CAT, pGEM7z AFPe, and pMNSM were identified with enzyme digestion. pMNSM was linearized with *Hin*d Ⅲ/*Bam*H Ⅰ digestion. pSPGNHIFNB10 was digested with *Hin*d Ⅲ/*Bam*H Ⅰ to release 1.06-kb IFN-β cDNA. IFN-β cDNA was linked to the linearized pMNSM to construct pMNSIFNB. pAF5-1-CAT was fully digested with *Aat* Ⅱ/*Spe* Ⅰ/*Bam*H Ⅰ to release 5.5-kb human AFP enhancer sequence and blunted with Klenow. pMNSIFNB was linearized with *Eco*R I digestion and blunted with Klenow. The enhancer was linked with the linearized pMNSIFNB to construct pMNAIFNB. pMNSIFNA and pMNAIFNA were constructed using the above construction procedure with some modifications. The human IFN-β gene was replaced with human IFN-α gene, and 5.5-kb AFP enhancer sequence was replaced with 0.7-kb AFP enhancer core sequence. As for the construction of pAMNSIFNA, an 0.7-kb AFP enhancer core sequence was inserted into the long terminal repeat (LTR) region of the retroviral vector. Then, 1.1-kb human IFN-α gene was cloned into the polylinker site of pMNSM and under transcriptional control of SV40 early region promoter. The identity of all the retroviral constructs were confirmed by restricted digestion.

Lipofectamine-mediated gene transfer

Packaging cells were pretreated with 2 μmg/L TdR to induce simultaneous cell division. Psi2 cells were transfected with the retroviral constructs by using the lipofectin-mediated gene transfer procedure, which was performed as per the manufacturer's instructions. After full selection with G418, the supernatant of the modified Psi2 cells was harvested, tittered, and used to infect PA317 cells. Measurement of plasmid transfection and retrovirus infection rates was carried out according as described previously[6].

Expression assay of IFNs

Northern blot and dot hybridization were performed using a previously described method[5]. The IFN bioactivities in the supernatant of 10^6 modified cells were calculated per 24 h. IFN bioassay was performed according to the routine procedure of our laboratory.

RESULTS

Construction of the retroviral vectors MNSIFNB and MNAIFNB

All the retroviral constructs are outlined in Figure 1. IFN genes in pMNSIFN B and pMNSIFNA were controlled by the SV40 early region promoter. IFN genes derived by SV40 early region promoter in pMNAIFNB and pMNAIFNA were controlled by [WTBZ] AFP enhancer. Then, 0.7-kb AFP enhancer core sequence was inserted into the LTR region in order to enactivate the intrinsic enhancer element located in the region and diminish the intervention of the intrinsic enhancer on AFP enhancer.

Preparation of recombinant retroviruses

PA317 cells with division synchronized with TdR pretreatment were infected with the supernatants of Psi2 cells containing MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA recombinant retroviruses, and the infection rate of the retroviruses in the PA317 supernatants harvested on the third day after the infection were 5.0×10^6, 6.0×10^5, 4.5×10^4, 8.0×10^6, 1.0×10^6, and 5.0×10^6 CFU/mL, respectively. Psi2 cells were transfected with the retroviral constructs by using a lipofectamine-mediating procedure. The modified Psi2 cells were split 1/20 onto medium containing 400 μmg/L active G418. After 10 d selection, the corresponding transfection rates were as follows: 2.5×10^4, 8.5×10^3, 4.0×10^3, 2.3×10^4, 3.6×10^4, and 3.4×10^4 CFU/μg DNA/10^6 PA317 cells. The greater the genome of the vectors, the lower the infection and transfection rates. However, all the highest retroviruses producing PA317 colonies of 6 different groups could produce 10^7 CFU recombinant retroviruses per 10^6 PA317 cells per 24 h. After being frozen in liquid nitrogen for one month, the modified PA317 did not show any significant decrease in the retrovirus production ($P > 0.05$)

Transcription of IFNs cDNA in the modified tumor cells

MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA retroviruses were used to infect the tumor cells *in vitro* in the presence of 4 μg/L polybrene. The total RNA from the infected HepG2 and Hep3B hepatoma cells and M21 melanoma cells, after being fully selected with G418, were extracted by the guanidine isothiocyanate single-step methods. Total RNAs from MNSM, MNSIFNA, MNAIFNA, and AMNSIFNA modified tumor cells were electrophoresed on a 1% agarose/2.2 M formaldehyde gel and transferred onto nitrocellulose. Blots were hybridized with ^{32}P-labeled HuIFN-α-cDNA probe. Total RNAs from MNSM, MNSIFNB, and MNAIFNB modified tumor cells were subjected to 5-fold serial dilution from 10 μg, transferred onto the nitrocellulose membrane, and probed with IFN-β-cDNA. The result is shown in Figures 2 and 3. In the hepatoma cell line HepG2, which produces high levels of AFP, the transcription level of IFNs cDNA, after infection with MNAIFNB, AMNSIFNA, and MNAIFNA retroviruses, was significantly higher than that of the cells infected with MNS-IFNB and MNSIFNA retroviruses. In the Hep3B cells, which produce

Table 1 Human interferon gene expression in supernatants of gene modified human tumor cells at 20th day after infection with recombinant retroviruses ($\bar{x} \pm s$)

Cell lines	Origin	AFP[1]	Genetic alteration	G418 resistant	Interferon bioactivity (unit/10^6 cells·24 h)
HepG2	Human	2044	Parental[2]	-	0
(n = 5)	hepatocyte		MNSM	+	0
			MNSIFNB	+	218 ± 102
			MNAIFNB	+	818 ± 35
			MNSIFNA	+	128 ± 36
			MNAIFNA	+	808 ± 180
			AMNSIFNA	+	1200 ± 36
Hep3B	Human	311	Parental	-	0
(n = 4)	hepatocyte		MNSM	+	0
			MNSIFNB	+	156 ± 48
			MNAIFNB	+	462 ± 104
			MNSIFNA	+	136 ± 42
			MNAIFNA	+	548 ± 120
			AMNSIFNA	+	465 ± 204
SMMC7721	Human	ND	Parental	-	0
(n = 5)	hepatocyte		MNSM	+	0
			MNSIFNB	+	280 ± 80
			MNAIFNB	+	580 ± 56
			MNSIFNA	+	242 ± 120
			MNAIFNA	+	468 ± 84
			AMNSIFNA	+	524 ± 132
M21	Human		Parental	-	0
(n = 5)	melanocyte		MNSM	+	0
			MNSIFNB	+	324 ± 66
			MNAIFNB	+	21 ± 10
			MNSIFNA	+	226 ± 128
			MNAIFNA	+	42 ± 36
			AMNSIFNA	+	0 ± 2.4
RZ94602	Human renal		Parental	-	0
(n = 4)	cell		MNSM	+	0
			MNSIFNB	+	280 ± 64
			MNAIFNB	+	18 ± 8.4
			MNSIFNA	+	320 ± 120
			MNAIFNA	+	24 ± 48
			AMNSIFNA	+	0 ± 2.4

[1]Expressed as ng secreted per mg of cell protein per 4 d, based on historical data[8]; [2]Unmodified parental tumor cell lines; ND: Undetermined

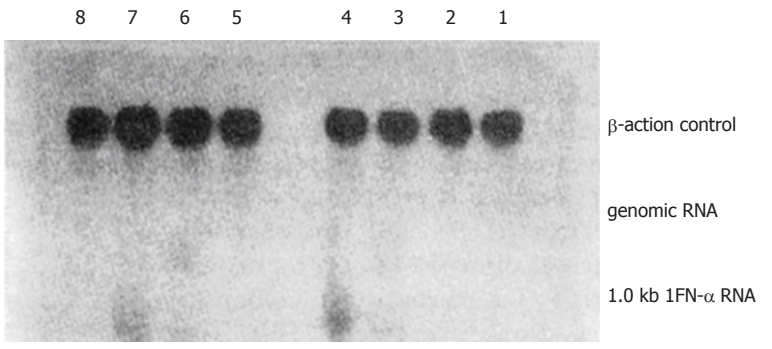

Figure 2 Northern blot of total RNA from the tumor cells modified with human interferon-α gene, probed with ^{32}P labeled human interferon-α cDNA. Lane 1: Unmodified HepG2 cells; Lane 2: MNSM modified HepG2 cells; Lane 3: MNSIFNA modified HepG2 cells; Lane 4: MNAIFNA modified HepG2 cells; Lane 5: MNSIFNB modified HepG2 cells; Lane 6: MNSIFNA modified HepG2 cells; Lane 7: AMNSIFNA modified HepG2 cells; Lane 8: MNAIFNB modified HepG2 cells.

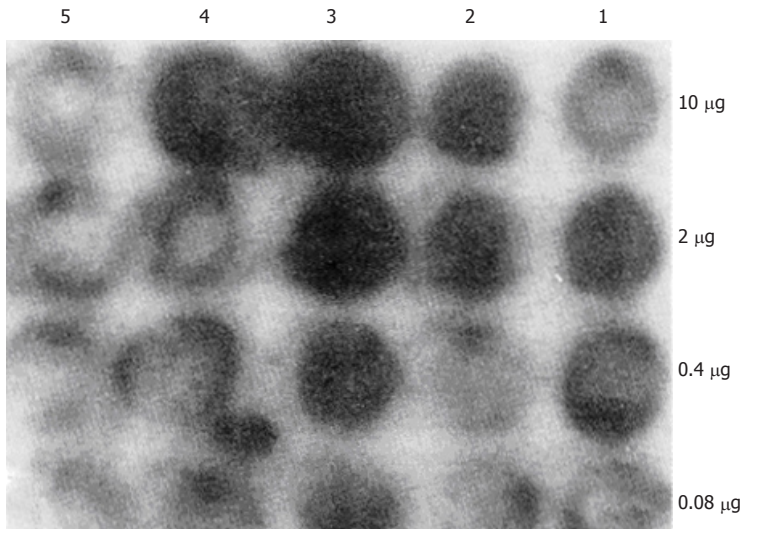

Figure 3 Dot blot of total RNA from human tumor cells modified with HuIFN-β gene, and fully selected with G418, probed with 32P labeled HuIFN-β-cDNA. Lane 1: Hep3B cells infected with MNAIFNB retroviruses; Lane 2: Hep3B cells infected with MNSIFNB retroviruses; Lane 3: HepG2 cells infected with MNAIFNB retroviruses; Lane 4: HepG2 cells infected with MNSIFNB retroviruses; Lane 5: M21 cells infected with MNSIFNB retroviruses.

intermediate levels of AFP, the difference in the IFN-β-cDNA transcription level between the two infection groups was not so significant as that in the HepG2 cells, indicating that the enhancing effect of the AFP enhancer on the SV40 early region promoter in the genome of the hepatoma cells correlated with the level of AFP produced in the hepatoma cells.

Expression of interferon in the gene-modified tumor cells

HepG2, Hep3B, SMMC7721, RZ94602, and M21 tumor cells were infected with MNSM, MNSIFNB, MNAIFNB, MNSIFNA, MNAIFNA, and AMNSIFNA retroviruses, respectively, and fully selected with G418. The bioactivity of IFNs secreted by 10^6 gene-modified tumor cells per 24 h was precisely determined with MTT measurement and $CpEI_{50}$ method. The results are shown in Table 1. In the human hepatoma cell line HepG2, which produces high levels of AFP, an AFP enhancer significantly augmented the expression of IFNs gene induced by SV40 early region promoter. Specific expression of IFNs gene in the Hep3B cell line, which produces intermediate AFP levels, was not as significant as that in HepG2 cells. IFN gene expression in the M21 and RZ94602 cell lines, which did not produce AFP, was inhibited after infection with MNAIFNB, MNAIFNA and AMNSIFNA retroviruses.

DISCUSSION

In vivo gene therapy for cancer is more practicable than the ex vivo protocol in clinical trials. As for the *in vivo* protocol, the major problem is to induce the guaranteed specific expression of the gene of interest in tumor cells. *In vivo* gene therapy can be guaranteed if the transcription regulatory sequences (TRS) of "house-keeping gene" of the tumor characteristic proteins are employed to regulate gene expression. First, high expression levels of the gene of interest can be achieved if the gene is controlled by the TRS. The

concentration of interferon or expression of IFN gene at the tumor location is correlated with the antitumor effect[1]. Second, possible microenvironmental disorders caused by the expression of Class I IFN gene in non-tumoral tissues can be prevented. This is of great significance in cancer gene therapy.

Human IFN α and IFN β bind to the same receptors and are called class I interferon. Class I IFN plays an important role *in vivo* in tumor immunosurveillance and tumor biotherapy. In the present study, human IFN α and IFN β genes were individually introduced into the established hepatoma, renal cell carcinoma, and melanoma cell lines by means of the retrovirus. Expression of IFN genes, under the transcription control of AFP enhancer, was in the hepatoma-cell-specific manner, and correlated with the AFP production levels in the hepatoma cells. Some transcription regulatory proteins existing in the nuclei of the hepatoma cells are believed to be related to the hepatoma-specific gene expression. In the AFP-producing hepatoma cells, the transcription regulatory sequence of AFP "house-keeping gene" is controlled by the tissue-specific transcription regulatory proteins. After the transcription regulatory sequence carried by the retroviruses is integrated into the genome of the hepatoma cells, the regulatory proteins could also activate AFP enhancer in the same manner. Since enhancer is not dependent on origin and orientation, AFP enhancer may transactivate the promoter of viral origin and increase the transcription rate of the promoter in certain cell types.

Promoter interference between heterologous promoter and retroviral promoter has been documented. Retroviral LTR promoter and enhancer may silence some internal heterologous promoters[7]. In this study, construction of pAMNS-IFNA was carried out by the insertion of AFP enhancer core sequence into retroviral LTR region in order to silence LTR enhancer activity and increase the hepatoma cell–specific expression of human IFN-α gene. However, the hepatoma cells–specific expression was not significantly increased after the treatment. It was demonstrated that enhancer elements in retroviral LTR region did not significantly interfere with the function of AFP "house-keeping" gene enhancer.

Tumor-tissue-specific gene therapy is carried out in the thymidine kinase prodrug transformation gene therapy, but not in cytokine gene therapy and other immune gene therapies. It has been recently demonstrated that the transcription regulatory sequences of AFP, carcinoembryonic antigen, and tyrosinase "house-keeping gene" regulated the thymidine kinase gene specifically expressed in the hepatocellular carcinoma, gastric cancer and melanoma cells[8-10].Tumor tissue specific gene therapy is considered to be an important direction of gene therapy against cancer in the near future. Probability of helper virus production, which is generated by homologous recombination, is expected to be decreased on the condition of the transcription regulatory sequences existing in the recombinant retrovirus. The future of gene therapy is subsequently guaranteed.

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ORIGINAL RESEARCH

Hepatitis G virus infection in patients with chronic non-A–E hepatitis

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Abstract

AIM: To elucidate the role of hepatitis G virus (HGV) infection in chronic non-A–E hepatitis and sequence the partial NS5 genome of HGV isolated from the serum of a Chinese patient with chronic non-A–E hepatitis

METHODS: Serum samples of patients with chronic non-A–E hepatitis were collected and total nucleic acids were extracted and subjected to reverse transcriptase-nested-polymerase chain reaction (RT-nested-PCR) using primers from the putative NS5 region of HGV genome. Then, 994bp cDNA was prepared from the positive serum, purified with electrophoresis of polyacrylamide gels, and directly sequenced using the dideoxy-mediated chain-termination method.

RESULTS: HGV-RNA was detected in 1 of the 35 patients with chronic non-A–E hepatitis. Compared with the 2 HGV isolates (PNF2161 and R10291) obtained from American patients, the HGV NS5 gene of this Beijing isolate (HG-G) showed homology of 88.0% and 89.2% respectively. On the other hand, in comparison with the West African isolate (GBV-C), the Beijing isolate showed homology of 93.5%. The patient showed persistent increase of alanine transaminase, but normal levels were achieved after interferon therapy with persistent positive HGV RNA.

CONCLUSION: HGV is one of the causes of chronic non-A–E hepatitis, but it may not be a very important cause. The nucleotide sequence of partial NS5 gene of HG-G was found to be highly homologous to the West Africa isolate.

Key words: Hepatitis G; Non-A–E hepatitis; Genes, viral; RNA, viral; DNA, viral; Polymerase chain reaction; Genome, viral

Chang JH, Wei L, Du SC, Wang H, Sun Y, Tao QM. Hepatitis G virus infection in patients with chronic non-A–E hepatitis. *World J Gastroenterol* 1997; 3(3): 143-146 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/143.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.143

INTRODUCTION

Since reliable detection of hepatitis C virus (HCV) and E virus (HEV) infections has now become possible, most of the cases of non-A, non-B hepatitis have been found to be caused by HCV and HEV. However, the etiology of some non-A, non-B hepatitis still remains unknown[1], which highlights the possibility of additional causes of human non-A, non-B, non-C, non-D, non-E (non-A–E) hepatitis. Recently, a new RNA virus genome associated with human hepatitis, termed HGV (or GBV-C), was identified simultaneously by two American laboratories[2,3]; this virus is considered to be one of the causes of human cryptogenic (non-A–E) hepatitis.

Thus far, the role of HGV infection in chronic non-A–E hepatitis and the primary structure of HGV in Chinese patients have been seldom reported. We detected HGV-RNA in the sera of 35 patients with chronic non-A–E hepatitis in Beijing by using the RT-nested PCR method and sequenced the partial NS5 gene from one isolate positive for HGV RNA.

MATERIALS AND METHODS

Subjects

Of 35 patients with chronic non-A–E hepatitis, 20 were male and 15 were female and aged at an average of 41.2 years. Six patients had history of transfusion, and the remaining 29 were sporadic cases. All were patients treated at the Institute of Hepatology, People's Hospital, BMU. The serum samples were stored at -20 ℃. They all tested negative for anti-HAV IgM, HBsAg, anti-HBc, HCV-RNA, anti-HCV, and anti-HEV.

Reagents

AMV-RT,dNTP, Taq DNA polymerase and DNA sequence kit used in this study are products of Promega.

Primers for HGV RNA detection

Oligonucleotide primers were designed as described previously[3] and were derived from the putative NS5 region of the HGV genome (Table 1). 51# and 52# (outer primers) as well as 53#, 54#(inner primers) were used for clinical detection, with the first PCR product being 400-bp in length and the second PCR product being 210-bp in length. Further, 52# and 55#(outer primers) as well as 56# and 57#(inner primers) were used for the amplification of a longer fragment as a sequence template (994 bp).

HGV RNA extraction, cDNA synthesis, amplification and sequence analysis

Total nucleic acid was extracted as described before[4]. The

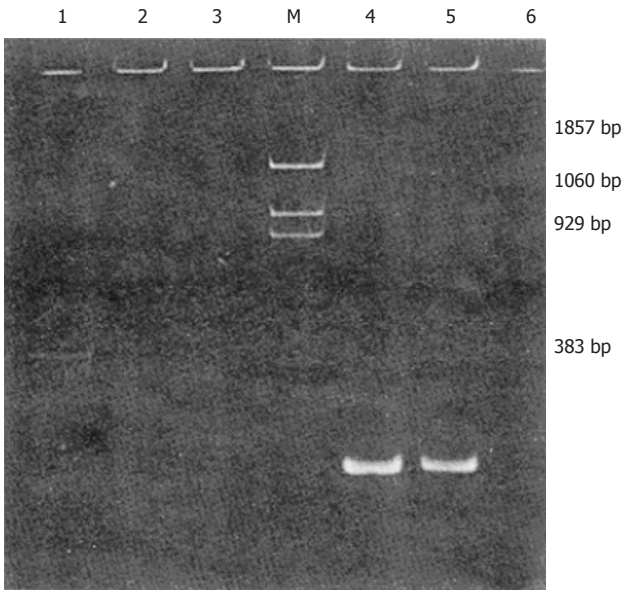

Figure 1 Electrophoretic pattern of RT-nested PCR for hepatitis G virus (HGV) RNA detection. 1, 4 were products of PCR from original sera, 2 and 5 products from 10^{-6} diluted sera, 3 and 6 were negative controls. 1, 2 and 3 were the frist PCR products, 4, 5 and 6 were the second PCR products. M was DNA marker, PBR 322/*Bst*N Ⅰ.

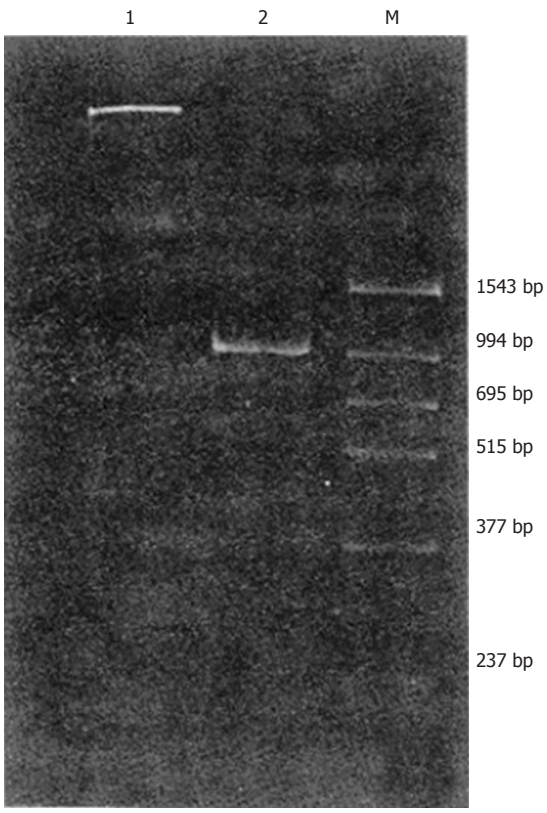

Figure 2 RT-nested PCR for amplification of long fragment cDNA of hepatitis G virus (HGV) NS5 region. 1 was negative control, 2 was positive PCR product, M was DNA and PCR marker.

extracted H GV RNA was resuspended in diethylpyrocarbonate-treated water and subjected to denaturation at 70 ℃ for 1 min, followed by a quickly chilling it on ice. Thereafter, the reverse reaction mixture (10 μL reaction-containing AMV-RT buffer, 50 pmol of primer 52#, dNTPs 200 μmol/L each, 2 units of AMV-RT) was added to synthesize cDNA at 43 ℃ for one hour. The first PCR was performed in a volume of 50 μL, containing 50 pmol primer 51# or 55#, 1.5 units of Taq P, dNTPs 200 μmol/L each. For the second PCR reaction, a volume of 5 μL was removed from the first reaction and added to a tube containing the inner primers 53# and 54# or 56# and 57#, along with dNTPs, Taq polymerase and Taq buffer, as was the case in the first reaction. The first and second PCRs were carried out for 30 cycles, consisting of 94 ℃ for 60 s, 55 ℃ for 90 s and 72 ℃ for 120 s, with a 600-s extension at 72 ℃ at the end. The second amplified product was analyzed by electrophoresis in 6% polyacrylamide gels. The positive products were purified and directly sequenced using primers 56# and 57#, as per our previously described method[5].

Table 1 Primers for the detection of hepatitis G virus (HGV) RNA with RT-nested-PCR

Primer	GBV-C position	Sequence (5'-3')
51#	7614~7632	GTTACACTTATGAGGARGC
52#	7994~8013	GCRTCCACACAGATGGCGCA
53#	7739~7758	GAGATACTTGAAGGGACTCC
54#	7930~7948	CTGGTTGGGGGTGTACTGG
55#	6885~6909	CTCTTTGTGGTAGTAGCCGAGAGAT
56#	7925~7952	CYCGCTCRTTTGGGGTGTACTGGAAGGC
57#	6959~6981	TCGGTTACTGAGTGCAGCTCAGATGAG

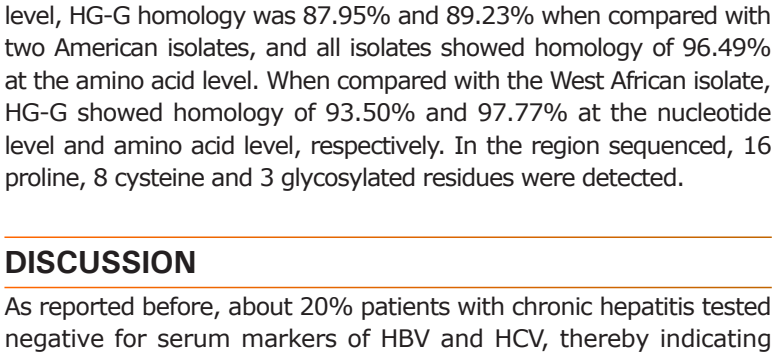

Figure 3 Clinical manifestation of the patient with chronic non-A-E hepatitis

RESULTS

RT-nested-PCR for HGV RNA detection

Of 35 patients with chronic non-A–E hepatitis, 1 tested positive for HGV RNA (Figures 1 and 2).

Clinical data of the HGV RNA positive patient with chronic non-A–E hepatitis

The patient who tested positive for HGV RNA was a 64-year-old woman who had received transfusion of 400 mL blood in 1979. She had not developed any symptoms until February 1996. She was found to have elevated serum ALT level at a physical check-up but tested negative for anti-HAV IgM, HBsAg, anti-HBc, HCV RNA, anti-HCV, and anti-HEV (Figure 3).

The primary structure of Beijing isolate (HG-G) at nucleic acid level and amino acid level in HGV NS5 region

The nucleotide sequences of HG-G in HGV NS5 as well as those of West African isolate (GBV-C) and American isolates (PNF 2161, R10291) for comparison are shown in Figure 4. The amino acid sequences of the 4 isolates are shown in Figure 5. At a nucleotide level, HG-G homology was 87.95% and 89.23% when compared with two American isolates, and all isolates showed homology of 96.49% at the amino acid level. When compared with the West African isolate, HG-G showed homology of 93.50% and 97.77% at the nucleotide level and amino acid level, respectively. In the region sequenced, 16 proline, 8 cysteine and 3 glycosylated residues were detected.

DISCUSSION

As reported before, about 20% patients with chronic hepatitis tested negative for serum markers of HBV and HCV, thereby indicating the existence of an unknown etiology in human hepatitis[1]. In 1995, HGV was found to be responsible for 9.49% of the cases of non-B non-C hepatitis and 8.33% of the cases of chronic non-A–E hepatitis[3], which indicates that HGV is one of the causes of non-A–E hepatitis. In our research, 1 (2.9%) of 35 patients with chronic non-A–E hepatitis tested positively for HGV RNA. This relatively low detection rate may be related to the insufficient exclusion of other non-virus elements and racial/ethnic and geographic differences. Consistent with other reports[3], many patients with chronic non-A–E hepatitis in our series tested negative for serum HGV RNA; this may be attributed to the lack of sufficient knowledge about the newly discovered virus in its affective factors of molecular biologic

CBV-C	tcggttacgg	agagcagctc	agatgagAAG	ACCCTGTCCG	TGACCTCCTC	GCAGGAGGAC	ACCCCGTCCT	CAGACTCATT	TGAAGTCATC	CAAGAGTGTG
R10291			...	...C.....	...T.....	.....T	.....	...T.....	C.....	.....C...
PNF2161			...	...C.....	...T.....	.....T	.....	...T.....	C...G.....	.....CC...
HG-G			...	.....	...T...T...	.....T	.....	...G.....	.....	.....C...
									7158	
CBV-C	ATACTGCTGA	ATCAGAGGAA	AGCGTCTTCA	ACGTGGCTCT	TTCCGTACTA	AAAGCCTTAT	TTCCACAGAG	CGATGCCACA	CGAAAGCTAA	CGGTTAAGAT
R10291	...G...A.....	...GG.....	...T.....	.....	.....	G.....G...	.....	T.....T	A.....T...	...C...C...G...
PNF2161	...G...A...C...	...GGG.....	...T.....	.....	.....T...	.....	.....	...C...G...C...	A...G.....T...	...C...C.....
HG-G	.....	GAGT.....T	.....	.....	.....C...	.....	.....	...C.....	...C.....T...	.....G...
									7258	
CBV-C	GTCTTGCTGT	GTTGAGAAGA	GCCTAACACG	CTTCTTTTCT	TTAGGGTTGA	CCGTGGCTGA	CGTGGCTAGC	CTGTGTGAGA	TGGAGATCCA	GAACCATACA
R10291	...AA.....	.....	.....C...G...	.....	...G.....C...	...G.....	T.....C...T	.....	.....	.....
PNF2161	...G.....C	.....A...	.....C...G...	...T...C...A	...G.....	...G.....	...T...T.....	.....	...A.....	.....
HG-G	...A.....	...G.....	.....C...G...	C	...G...AC...	...T.....	.....	.....	...A.....	.....
									7358	
CBV-C	GCCTATTGTG	ACAAGGTGCG	CACTCCGCTA	GAATTGCAAG	TTGGGTGCTT	GGTGGGCAAT	GAACTTACCT	TTGAATGTGA	CAAGTGTGAG	GCACGCCAAG
R10291	.....	.....	.....	.....	.....	.....	.....	.....	T.....	...TA...G.....
PNF2161	.....	...C.....	.....T	.....G...	.....	.....	.....	.....	T.....	...TA...G.....
HG-G	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
									7458	
CBV-C	AGACCCTTGC	CTCCTTCTCC	TACATATGGT	CCGGGGTCCC	ACTTACTCGG	GCCACTCCGG	CCAAACCACC	AGTGGTGAGG	CCGGTGGGGT	CCTTGTTGGT
R10291	.....TT...G...	.....	...T...T.....	...T.....G...	...T...G.....A...	.....A...	...T.....	T.....	.....	.....
PNF2161	...A.....TG...	.....T	.....T.....	...T...A...G...	G...G.....A.....	.....G...	.....G...T...	C.....	.....T...C...	...T.....A...
HG-G	.....T...G...	T.....T	.....C.....	...T.....	.....A.....	.....A...	.....	.....	.....	.....
									7558	
CBV-C	GGCAGACACC	ACCAAGGTCT	ACGTGACCAA	TCCGGACAAT	CTTGGGAGGA	GGGTTGACAA	GGTGACTTTC	TGGCGCGCTC	CTCGGGTACA	CGACAAGTTC
R10291	...T.....	...G...A...G...	...T...C...A...	C.....	.....A...	...A...G.....	.....C...	.....C...	...CA.....C...	T.....A...AT
PNF2161	...C.....T...	...T.....G...	...T...T.....	...A.....	...G...AC.....	.....G.....	.....C...	.....T.....	...A.....T...	T...T.....A...
HG-G	.....	.....G...	.....	.....	.....	...A.....	.....C...	.....T.....	...C.....	.....A.....
									7658	
CBV-C	CTCGTGGACT	CGATCGAGGG	CGCTCGGAGA	GCTGCTCAAG	GCTGCCTAAG	CATGGGTTAC	ACTTATGAGG	AGGCAATAAG	GACTGTTAGG	CCGCATGCTG
R10291	.....	...C.....	T...CA.....G	...G.....	C.....A.....	.....	.....	...A.....	.....	...A.....
PNF2161	.....	...T...T.....	.....AA...G	...C.....	C.....	.....	.....	...A.....	.....A...	...A.....
HG-G	.....T...	.....A...	.....G	.....	C.....	.....	.....	.....	.....	.....
									7758	
	CCATGGGCTG	GGGATCTAAG	GTGTCGGTCA	AGGACTTGGC	CACCCCTGCG	GGGAAGATGG	CTGTTCATGA	CCGGCTTCAG	GAGATACTTG	AAGGGACTCC
R10291	.....	.....	.....	.....	.....	.....	...C...C...C...	.....A.....	.....	...G.....
PNF2161	.....	.....	.....T...	.....A...	.....C.....	.....	...C...C.....	.....	.....	.....
HG-G	.....	.....	.....T.....	.....	.....	.....	...C...C...C...	...A...A...C...A	.....	.....
									7858	
CBV-C	GGTCCCTTTT	ACCCTGACTG	TCAAAAAGGA	GGTGTTCTTC	AAAGATCGTA	AGGAGGAGAA	GGCCCCCCGC	CTCATTGTGT	TCCCCCCCCT	GGACTTCCGG
R10291		TT	G	.....	C	.....	.....	.....	.....	.....
PNF2161	C	TT	G	.....	CG	.....	.....	.....	.....	.....
HG-G	C	T	.....	.....	CC	.....	.....	.....	T	T
										7924
CBV-C	ATAGCTGAAA	AGCTCATTCA	GGGAGACCCG	GGGCGGGTTG	CAAAGGCCGG	TGTTGGGGGG	GCTTACgcct	tccagtacac	ccccaaccg	cggg
R10291	.....G...	.....T...CT	.....	.....G...	...C.....G...T	GT...G.....	.....			
PNF2161	.....	.....CTT	.....A	...C.....A...	...C.....G...T	GT...G.....	.....C.....			
HG-G	.....	.....A.....TT	.....	.....	.....	.....	.....C.....			

Figure 4 Nucleotide sequence of partial hepatitis G virus (HGV) NS5 gene in the serum of a patient with chronic non-A-E hepatitis and its comparison with West African and American isolates. GBV-C is West African isolate; R10291 and PNF2161 are American isolates; HG-G is a patient with chronic non-A-E hepatitis. —denotes the same as GBV-C sequence. Capital letter is nucleotide detected, small letter is primer sequence.

CBV-C	KTLSVTSSQEDTPSSDSFEVIQECDTAESEESVFNVALSVLKALFPQSDATRKLTVKMSCCVEKSVTRFFSLGLTVADVASLCEMEIQNHTAYCDKVRTP
R10291	...P...S.....SE...G.....E.....R...N.....
PNF2161	...P...S.....SE...G.....Q.....
HG-G	.....S.....S.....D.....R.....
CBV-C	LELQVGCLVGNELTFECDKCEARQETLASFSYIWSGVPLTRATPAKPPVVRPVGSLLVADTTKVYVTNPDNVGRRVDKVTFWRAPRVHDKFLVDSIERAR
R10291	.....T.....
PNF2161	.....T.....K.....
HG-G	.....
CBV-C	RAAQGCLSMGYTYEEAIRTYEEAIRTVRPHAAMGWGSKVSVKDLATPAGKMAVHDRLQEILEGTPVPFTLTVKKEVFFKDRKEEKAPRLIVFPPLDFIAEKLIQGD
R10291	.....A...Q.....L...
PNF2161	.....A.....L...
HG-G	.....A.....F.....L...
CBV-C	PGRVAKAGVGGAY
R10291	.....VL...
PNF2161	.....VL...
HG-G	.....

Figure 5 Amino acid sequence of hepatitis G virus (HGV) NS5 region in the serum of the patient with chronic non-A-E hepatitis and its comparison with West African and American isolates.

detection. In addition, it must be recognized that there may be other causes of human hepatitis besides the known factors, that is to say, HGV may not be a very important cause of chronic non-A–E hepatitis.

The patient, who tested positive for HGV RNA in our research had a history of receiving blood transfusion 17 years before, and she did not show any symptoms and an elevated serum ALT level until February 1996. She did not respond very well to that treatment, but her serum ALT level was normalized after interferon treatment although HGV RNA continued to persist in her serum. Since we cannot find evidence of other hepatitis virus infection, we believe that HGV must be responsible for the chronic hepatitis in this case, although the clinical features are similar to those of hepatitis C.

The primary structure in HGV NS5 region in the Beijing isolate (HG-G) was also analyzed. In the region sequenced, 16 conserved proline residues and 8 conserved cysteine residues were detected; these residues may play an important role in the formation of the

spatial configuration, indicating that the product encoded by this region may be important in maintaining HGV function. With respect to the nucleic acid sequence and amino acid sequence, HG-G showed marked homology to the West African isolate. Since only 3 isolates of HGV sequences have been reported thus far, it is difficult to decide whether HG-G was of the same genotype as the West African isolate. Future studies on sequence analyses are needed for further research.

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ORIGINAL RESEARCH

Source of blood supply and embolization treatment in cavernous hemangioma and sclerosis of the liver

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Abstract

AIM: To investigate the source of the blood supply in carvenous hemangioma of liver (CHL), and provide a feasible treatment for CHL *via* thehepatic artery.

METHODS: (1) Portovenography, hepatic arteriography and portal vein staining were performed in 5 patients to determine the origin of the blood supply. Two casts of hepatic blood vessels from resected specimens were observed. (2) Clinical data from 75 patients (30 males, 45 females, aged 25-57 years, mean of 37.4) were obtained. Of these, 56 were of solitary type (44 on the right lobe, 12 on the left, with 4 having intraparenchyma), and 19 were of multiple type (9 on the right, 2 the left, 8 whole liver). Twenty-two patients were treated with sclerosis, 50 by embolization *via* hepatic artery, and 3 were excised.

RESULTS: In the 5 cases where portography was used, the contrast medium did not enter the tumor, and the tumor appeared as low density area, with the intrahepatic branches of the portal vein pushed aside. In the 5 cases with where portal vein staining was used, the normal liver parenchyma stained a deep blue; however, the tumor was not stained. The tumor area appeared as a round vacant cavity in the 2 specimen casts. For the 72 patients treated with sclerosis or embolization *via* hepatic artery or through interventional method, the tumors diminished by 10%-30% in diameter, and no tumors grew larger.

CONCLUSION: The blood supply of CHL originates from the hepatic artery. Tumors treated with sclerosis and embolization decreased in size or got fibrotic.

Key words: Liver neoplasms/blood supply; Liver neoplasms/therapy; Hemangioma, cavernous/therapy; Embolization, therapeutic; Sclerotherapy

Li GW, Zhao ZR, Li BS, Liu XG, Wang ZL, Liu QF. Source of blood supply and embolization treatment in cavernous hemangioma and sclerosis of the liver. *World J Gastroenterol* 1997; 3(3): 147-149 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/147.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.147

INTRODUCTION

Cavernous hemangioma of the liver (CHL) is a common, benign, hepatic tumor. With the development and wide application of modern imaging technics, many more cases of CHL have been diagnosed in recent years. Currently, the conventional treatment for CHL is excision of the tumor. In this report, we studied the blood supply of CHL from December 1985 to November 1992, and we found that CHL is a malformation of hepatic arterial vasculature. In this study, 22 of 72 CHL patients were treated using sclerosis, while the other 50 were treated with gelfoam microspheres with lipiodol administered either during surgery or using hepatic arterial intubation *via* the femoral artery. In all cases, the results were satisfactory.

MATERIALS AND METHODS

Portography and hepatic arteriography during operation

The portovenography and hepatic arteriography were performed in five patients as previously reported[1].

Portal vein staining during the operation

In these five cases, the portal veins were intubated *via* the right gastroepiploic vein in the right gastric vein, or *via* direct puncture of the portal vein, and 60 mg of methylene blue diluted to a final volume of 20 mL was quickly injected. The staining of the liver parenchyma and the tumor body were then observed.

Pathology and the casts of resected specimens

The two resected CHL specimens from left lobes, 10 cm and 10.5 cm in diameter, were fixed with formalin and the sections were sent for serial pathological observations. Another resected specimen, 6 cm in diameter, was made into model cast by filling the hepatic vein (yellow) and portal venous branch (blue) with methyl methacrylate after vascular lavation.

RESULTS

Radiographic observations

In the 5 cases with in which portography was used, the contrast

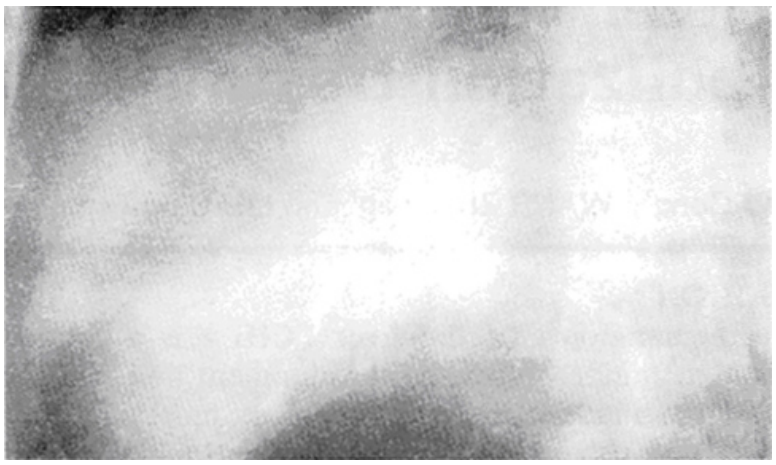

Figure 1 The tumor appeared as a filling defect.

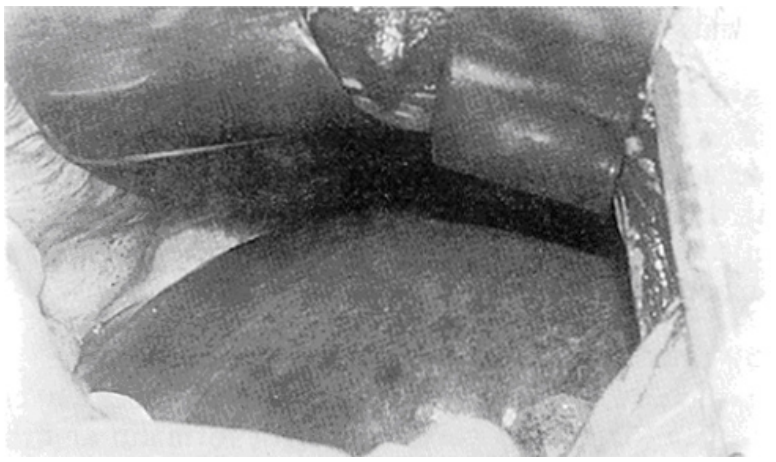

Figure 3 Methylene blue staining of the liver except the tumor area.

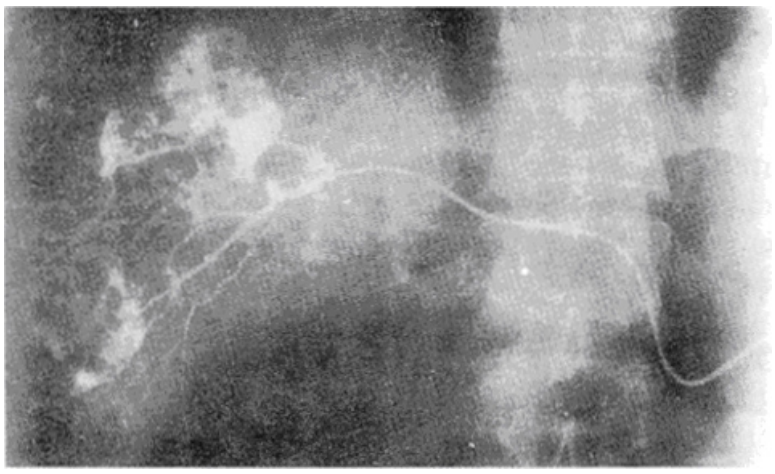

Figure 2 Radiograph by immediate visualization of tumor body after injection intubation of hepatic artery branch.

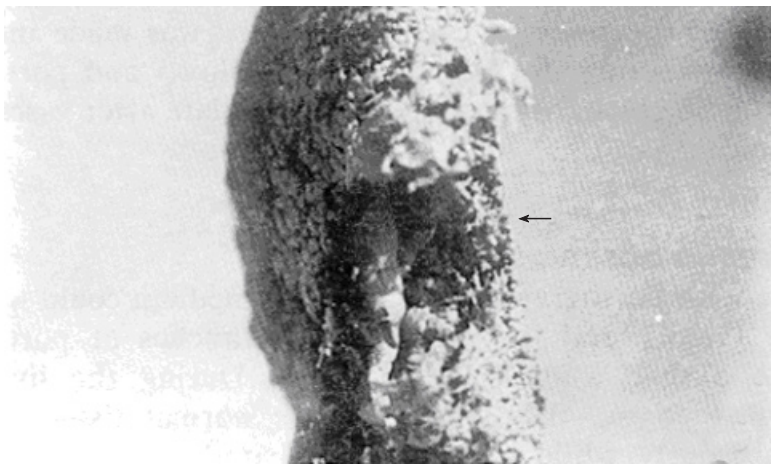

Figure 4 Specimen cast filled with methyl methacrylate *via* portal vein and hepatic vein, the tumor area appeared as a round vacant cavity.

medium could not enter the tumor, and the intrahepatic branches of portal vein were pushed aside by the tumor. During the liver parenchymal phase-contrast, the density of the normal tissue was obviously increased, while the tumor appeared to be of lower density, with sharp and clear boundaries. In 22 patients that underwent sclerosing therapy, hepatic arteriography revealed that the contrast medium immediately entered the tumor, and had a "cotton" or "popcorn" appearance. In these patients, an arteriovenous fistula was not observed (Figures 1 and 2).

Methylene blue staining of the liver

After the injection of methylene blue through portal vein, the normal liver parenchyma was stained homogenously in a deep blue; however, the tumor was not appear stained, and the boundary between the normal liver tissue and the tumor was sharp (Figure 3).

Observations on pathology and cast specimens

In the serial sections of nine biopsies and resected specimens, no normal blood vessels or bile ducts were observed under microscopy. Although CHL did not have a capsule, it did have a clear boundary with the neighboring normal liver tissues.

The casting specimens showed that the eroded tumor left behind a round vacant area in the specimens. The stained branches of the portal vein did not extend into the tumor (Figure 4).

Clinical data

Among the 75 patients included in this study, 30 were males and 45 females, with an age ranged between 25-57 years, with a mean age of 37.4 years. All patients were diagnosed by ultrasound, with 66 confirmed by CT, 34 by selective coeliac arteriography, and 20 by ECT. The diameter of the CHL ranged 6-30 cm, with only 4 of them greater than 20 cm. Among these 75 patients, 56 cases were of solitary type, with 44 were on the right lobe, 12 on the left and 4 were intraparenchymatous. Nineteen of the 75 patients had multiple type, with 9 on the right, 2 on the left and 8 in the whole liver. Of the original 75 cases, three cases were excised; the left hepatic artery was not shown on arteriography for 2 of them, however, but were confirmed during surgery. In the other 72 cases, 22 received sclerosing therapy, and 50 were treated with embolization (10 of these were interventional embolizations). Among the 75 cases, 2 were HBsAg positive, 1 HBsAb positive and none of them had cirrhosis. Twenty-one were proved to be CHL pathologically.

Surgical methods

Under direct view, the involved hepatic arterial branches were dissected and confirmed by methylene blue injection. The proximal end was ligated and intubated through the distal end, and the tube was then fixed with rubber pieces. The opening of the tube was filled with normal saline and fire sealed, before being pulled out through the abdominal wall puncture, and fixing an adhesive plaster on the skin. Through this tube, sclerosing treatment and repeated radiographic examinations were carried out[1].

For the sclerosing therapy group, the patients were divided into three subgroups. Subgroup 1 (n = 8) had 10-20 mL of 40% carbamide injected through the intubated arterial branch of the liver once daily for 20-30 d. Subgroup 2 (n = 6) had 10 mL of 49.9% ethanol injected by the same route, once every three days, for a total of 5-7 injections. Subgroup 3 (n = 8) had 2 carbamide injections and one ethanol injection administered alternatively, with usually 9 injections required.

For the embolization therapy group, 100 mg of gelfoam particles mixed with 8-16 mL of lipiodol emulsion was injected. Ten patients were given interventional embolization. Generally, one single embolization was adequate. It was only when the tumor diameter was greater than 10 cm that a second embolization was needed. In cases with multiple tumors less than 4 cm in diameter on the other lobe of the liver, anhydrated ethanol was injected into the tumor at multiple points. The injection was stopped when the tumor

appeared blanched or collapsed. After withdrawal of the needle, finger pressing was used to prevent bleeding and extravasation of ethanol.

Cessation of therapy

In the sclerosing therapy group, periodical radiography was performed. The tube was pulled out until the tumor body could not be visualized. The embolization treatment was discontinued when the tumor had shrunk by 10% based on X-ray evaluation or after two-weeks or one month. When the diameter was beyond 10 cm, a second embolization was given. Among the 28 cases, a total of 36 embolizations were performed.

Clinical criteria for CHL obliteration

(1) On intensified CT scanning, the "ring sign" and "half ring sign" reduced in size or disappeared totally after 3-6 mo. (2)The dynamic isotopic hepatic blood pool scanning showed a radioactive defect. And (3) Shrinkage of tumor on ultrasonography.

Advantages and disadvantages of sclerosing therapy and embolization therapy

The carbamide in sclerosing therapy was not irritative, and usually it took 25 d to obliterate the CHL. Ethanol injection, however, elicited intolerable pain, which lasted for about 2 h, and generally required seven injections. Interventional embolization produced lasting pain, which often required pethidine; however, only 1-2 times were needed, and the pain attenuated on the second treatment.

Follow up study

The 22 cases with sclerosing therapy were followed for 1-6 years, and CT and ultrasound examinations indicated the diameter of the tumor had shrunk by 20%-60%. In one patient who received a second operation, after one year, white and shining fibrous tissues were evident, which were confirmed pathologically. The other two female patients with tumors beyond 20 cm and who had previously had repeated skin petechiae, gained more than 5 kg of weight, and the petchiae disappeared after the treatment. The embolization group were followed for over six months, and the tumors for this group all diminished by 10%-30% in diameter, and none of the tumors grew any larger.

DISCUSSION

Since the beginning of this century, CHL has been regarded as a portal venous malformation by many researchers, but ligation of branches of portal vein was unsatisfactory as a treatment[2-5]. Yamamoto was the first to determine that the morphology of sinusoids of CHL specimens were different from that of portal and hepatic vein, but similar to that of hepatic artery using electron microscopy[6]. At present, a shunt for cavernous hemangioma has not been identified. Based on the portovenographic findings, intubational imaging of hepatic arterial branch, liver staining, and observations of the cast vessels in the resected specimens, this study has demonstrated that CHL is a malformation of the hepatic artery, and its blood supply arises completely from the hepatic artery without an arteriovenous shunt. This provides the theoretical basis for treating CHL *via* the hepatic artery. This study found that both sclerosing and embolization therapy can shrink the tumor and replace it with fibrotic tissues.

Hepatic cavernous hemangioma can grow continuously. Previously, Niemann reported 55 cases that led to diffuse hemangioma in the liver, which easily ruptured, causing a mortality rate as high as 70%[4,7]. However, excision is difficult when the tumor is of the multiple type, especially when it is near the hilum, very large in size, occupying the whole lobe of the liver, or some other contraindication exists. Our experience has found that therapy *via* intubation of hepatic artery is safe, reliable, and causes less injury, and does not require a blood transfusion.

Sclerosing agents, such as carbamide and ethanol, both require a long duration of therapy, especially the carbamide. Ethanol often produces pain for a brief period. However, Lipiodol can selectively stay for a long time in both benign and malignant hepatic tumors with rich blood supply. In this study, all 28 patients that received embolization therapy demonstrated such characteristics. Lipiodol and gelfoam microspheres are both long acting embolization agents. Gelfoam microspheres can occlude the vessels by secondary inflammation and growth of granulation tissues. The microspheres still could be identified in a few areas under microscopy after 28 d, and the hemangioma was completely filled with granulation tissues and fibrous tissues[7].

CHL is composed of cavernous blood sinusoids, without blood vessels, bile ducts or hepatocytes. Its blood supply primarily came from hepatic artery. The contrast agent and radioactive isotopes could quickly enter the periphery of the tumor, but diffused slowly and drained out after a long period, which accounts for the variegated feature of CHL. For example, the early visualization and late disappearance in hepatic angiography; the ring and half-ring signs on CT scanning, and the intensified imaging of blood pool radioactive scanning, is due to the fact that there are no hepatocytes within the tumor, making it appear that there is a radioactive defect in the static scanning. The above characteristics can also be the basis to assess the efficacy of occlusion treatment.

The variation of the hepatic artery is as high as 45%, particularly in the left lobe. This suggests that selective hepatic arteriography should be performed before intubation in order to raise the success rate of embolization therapy. Furthermore, interventional embolization therapy is only possible for certain patients. Since the hepatic artery tapers gradually, non-selective hepatic arteriography cannot show a complete picture of large and multiple hemangiomas, but rather requires superselective hepatic arteriography for the diagnosis and treatment of CHL.

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ORIGINAL RESEARCH

Survival and malignant phenotype changes of human hepatoma SMMC-7721 cell line induced by cryopreservation at -50 ℃

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Abstract

AIM: To investigate the effect of cryopreservation at -50 ℃ on the human hepatoma SMMC-7721 cell line.

METHODS: With 15% DMSO as a cryoprotectant, the SMMC-7721 cells were cryopreserved at -50 ℃, then thawed and recultured. The survival rate, mitotic index and LDH isoenzymes were compared between pre- and post-cryopreservation.

RESULTS: Thirteen hours after the thaw, the mitotic index of cryopreserved SMMC-7721 cells decreased by 1.09%. The mode scope of chromosome number (46-53) after cryopreservation tended to transfer to that of normal human cells, and the percentage of metaphases containing 46 chromosomes changed from 0% to 16%. LDH isoenzymes changed from H-like model (LDH3(29.3%) > LDH4 (26.8%) > LDH2 (25.3%) > LDH5 (14.9%) > LDH1 (3.6%) to M-like model (LDH4 (48.3%) > LDH5 (28.3%) > LDH3 (18.9%) > LDH2 (4.4%) > LDH1 (0%)). This suggests that the survival rate could reach over 95%.

CONCLUSION: Cryopreservation at -50 ℃ can be a convenient method for the cryopreservation of cell lines. However, cryopreservation at -50 ℃ is likely involved in the changes of the malignant phenotypes of the human hepatoma SMMC-7721 cell line, and may induce the differentiation of malignant cells.

Key words: Liver neoplasms; Carcinoma, hepatocellular; Cryopreservation; Tumor cells, cultured; Phenotype

Jiang SM, Xu ZH, Zhang Y, Shi XM. Survival and malignant phenotype changes of human hepatoma SMMC-7721 cell line induced by cryopreservation at -50 ℃. *World J Gastroenterol* 1997; 3(3): 150-152 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/150.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.150

INTRODUCTION

Cryopreservation and cryotherapy playimportant roles in the research and therapy of cancer. It has been reported that -50 ℃ is in the great ice crystal forming temperature region (-40 ℃ to -60 ℃), in which cells are greatly damaged, even if they incubated at this temperature for a very short period of time (10 min)[1]. This is particularly damaging to the cellular membranes and the chromatin. Therefore, how to pass this temperature region successfully is a key step in the cryopreservation of not only for maintaining cell suspensions, but also whole organs, such as heart, kidney, *etc.* Many studies have shown that some chemical reagents, such as sodium butyrate, dimethyl sulfate (DMSO), cAMP Vitamin A, and Vitamin D3 can induce the differentiation of malignant cells *in vitro*[2-4]. Nothing is known about the differentiation of cancer cells induced by the cryopreservation, especially at -50 ℃. In this report, we provide some evidence that cryopreservation at -50 ℃ may be involved in the changes of malignant phenotypes of human hepatoma SMMC-7721 cell line.

MATERIALS AND METHODS

Cell culture and cryopreservation

The human hepatoma SMMC-7721 cell line was obtained from the Shanghai Cell Bank of Academia Sinica and maintained in our laboratory. The cells were cultured in RPMI 1640 medium (Gibco Inc) supplemented with 10%-20% fetal calf serum (FCS) and incubated at 37 ℃, with 5% CO_2/95% air. The cells attached and spread well on either untreated plastic plates or untreated glass flasks. In the cryopreservation study, the cells were firstly passaged in glass flasks. When the cells grew to exponent stages, the culture medium was removed, and the cells were released from the flask using 0.25% trypsin (in D-Hank's solution, pH 7.2) for 1 min. The cells were then re suspended in RPMI 1640 medium. The cells were counted using a Bueker's chamber. Cell viability was detected by mixing the cell suspension with 0.5% aqueous solution of trypan blue (1:1). The cell suspension was adjusted to the concentration of 1-5 × 10^6/mL with 85% RPMI-1640 medium and 15% ice cold DMSO before being

Table 1 The effects of dimethyl sulfate concentration on the survival and the rate of attachment of SMMC-7721 cells after cryopreservation at -50 ℃

5 0	5 10	10 0	10 10	15 0	15 10	20 0	20 10	30 0	30 10	40 0	40 10	DMSO (%) FCS (%)
90 ± 2	90 ± 3	95 ± 2	98 ± 2	98 ± 1	99 ± 1	93 ± 4	95 ± 2	87 ± 4	98 ± 2	80 ± 4	83 ± 2	SR (%)
60 ± 3	65 ± 2	85 ± 4	90 ± 3	85 ± 3	90 ± 2	55 ± 2	60 ± 4	20 ± 4	25 ± 5	1 ± 1	2 ± 1	AR (%)

SR: Survival rate; AR: Attaching rate.

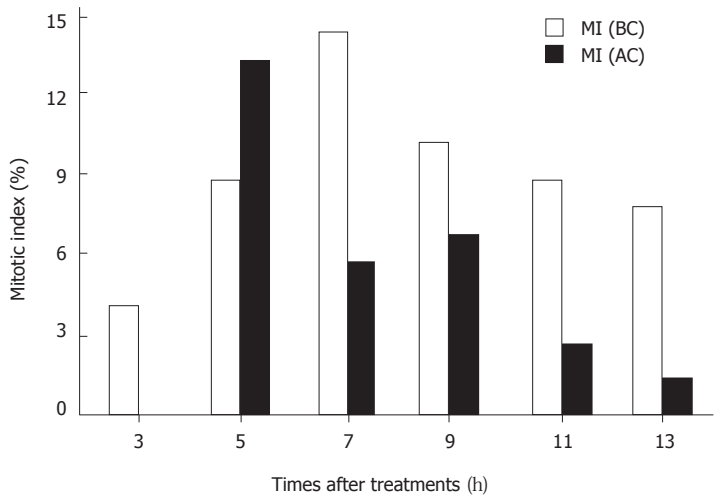

Figure 1 The comparison of mitotic index of human hepatoma SMMC-7721 cells between pre and post cryopreservation.

placed at -50 ℃ in an ultracold freezer (Sanyo, Japan).

The viability and reculture of cells after cryopreservation

After being cryopreserved at -50 ℃ for 6 mo, the cells were taken out from the freezer and thawed in a 40 ℃ water bath, swirling until the ice was dissolved. The cell suspension was transferred into the centrifugation tube, centrifuged at 500 rpm for 3 min, the preservers were removed and washed with RPMI-1640 medium for 3 times by centrifugation. The cell viability was determined as described above. The cells were recultured in RPMI-1640 medium. The rate of attachment was determined after 12 h in culture by counting the unattached cells.

The determination of mitotic index before and after cryopreservation

After being cryopreserved and treated as described above, the cells were adjusted to the concentration of 2×10^5/mL, plated in 24-well Costar tissue culture plastic dishes, and incubated at 37 ℃, with 5% CO_2/95% air for 3 h. After that the cells on cover slips in 4 parallel wells were taken out at an interval of 2 h, fixed in cooled methanol: acetic acid (3:1) and stained with Feulgen stain, and hematoxylin and eosin. The mitotic index of 1000 cells from each well were counted. The control group was treated in the same way as the experimental group but the cryopreservation step was omitted.

The analysis of the mode scope of chromosomal number

After being cultured for three days, the cryopreserved cells were given colchicine at a final concentration of 0.01 μg/mL and cultured for 6 h. The cells were then released from the flask using 0.25% trypsin and resuspended in RPMI-1640. After centrifuging the cell suspension to remove the trypsin, 0.075 mol/L KCl was added to the cells, and the cells were then incubated in a 37 ℃ water bath for 10 min. Following this, the cells were fixed with cooled methanol: acetic acid (4 ℃) for three times. The chromosomes were spread onto slides and the routine method for Giemsa stain was used to examine the chromosome sample. The mode scope of chromosomal number of 100 metaphase figures was examined under a microscope.

LDH isoenzyme analysis

After being cultured to the exponent stage, the cryopreserved cells were collected and homogenized. The supernatant of the cells was collected and LDH isoenzyme analysis was done using PAGE electrophoresis. After staining, the LDH bands were scanned with a gel scanning detector, and the percentage content of each band of the LDH was recorded.

RESULTS

The survival and growth of cells after cryopreservation at -50 ℃

After being cryopreserved in different DMSO concentrations with RPMI-1640 medium, or different DMSO concentrations with 10% FCS and RPMI 1640 medium at -50 ℃ for 6 mo, the cells were rapidly thawed in 40 ℃ water bath and washed with RPMI-1640 medium 3 times. The survival and the rate of attachment after 12 h in culture are listed in Table 1.

In all cells frozen with DMSO, the viabilities detected by trypan blue resistant staining were all greater than 80%, which suggested that DMSO could protect cells from damage during cryopreservation at -50 ℃. However, the rates of attachment were significantly different between DMSO concentrations, with a sharp decrease in attachment when treated with 30% DMSO, and very few cells attaching on the flasks in the group treated with 40% DMSO. Rather, the cells treated with 40% DMSO tended to gather in large cell clumps, remaining suspended in the medium, with many of the cells in a bubble like form. The addition of FCS to the cryoprotectant DMSO increased both the survival and the attaching rates at all concentrations. Based on the results of our study, we recommended using 10% and 15% DMSO with 85% RPMI 1640 medium as the in the freezing medium when cryopreserving a the cell line. The results clearly indicated that cryopreservation at -50 ℃ could be used as a practical and convenient method for cryopreserving SMMC-7721 cells.

The effects of -50 ℃ cryopreservation on the mitotic index

The comparison of mitotic index between SMMC-7721 cells that were not cryopreserved with those that were cultured for 13 h after 6 mo of cryopreservation is shown in Figure 1.

The MI of the cryopreserved cells was lower than that in controls at all time points examined, except for that in the 5th hour, when the MI was higher in the cryopreserved cells. The highest MI in the cryopreserved cells (13.31%) was 1.09% lower than that in the normal cultured cells. The results suggest that the cryopreservation at -50 ℃ could decrease the MI of SMMC-7721 cells.

The effects of cryopreservation on the mode scope of chromosomal numbers

After being cryopreserved more than ten times, the mode scope of chromosomal numbers ranged from 24 to 109, the main scope being 46-53 (58%) (Figure 2), and the percentage of cells with 46 chromosomes was 16%, which was significantly different from the SMMC-7721 cells in the time of establishment[5].

The effect of cryopreservation at -50 ℃ on the patterns of LDH isoenzymes

The changes of LDH patterns after cryopreservation at -50 ℃ are shown in Table 2.

After being cryopreserved at -50 ℃, LDH2 and LDH3 contents decreased and the LDH4 and LDH5 contents increased as compared with the LDH patterns in the time of establishment of the SMMC-7721 cell line. These same differences were also evident in the cryopreserved cells when compared to the human hepatoma BEL7404 cell line. After cryopreservation, the content of LDH4 was the highest (48.3%), LDH5 the second (28.3%), with the sequence of the LDH activity in these cells being LDH4 > LDH5 > LDH3 > LDH2 > LDH1.

Table 2 The effects of -50 ℃ cryopreservation on the activity of lactate dehydrogenase (%) of human hepatoma SMMC-7721 cell line

	LDH5	LDH4	LDH3	LDH2	LDH1
SC (AC)	28.3	48.3	18.9	4.4	0
SC (E)	14.9	26.8	29.3	25.3	3.6
BEL7404 cell	1.7	22.95	37.76	27.21	10.38

SC (AC): SMMC-7721 cell after cryopreservation; SC (E): SMMC-7721 cell in the establishment; BEL7404 cell: Another human hepatoma cell line.

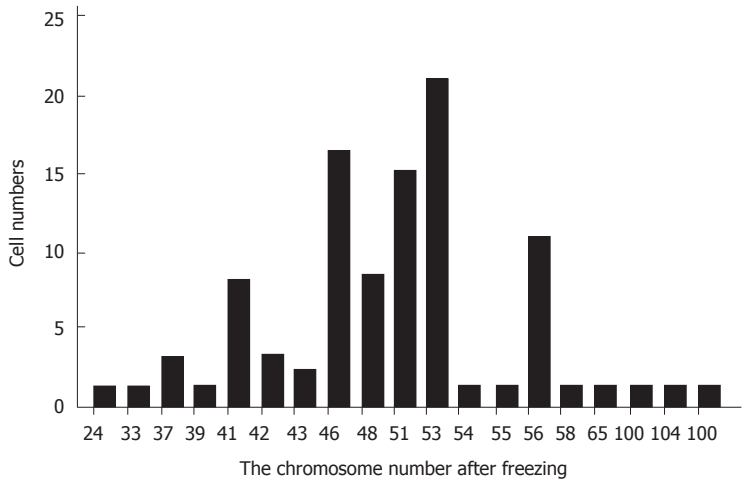

Figure 2 The effects of -50 ℃ cryopreservation on the chromosome numbers of human hepatoma SMMC-7721 cell line. Abbreviations: SC (AC): SMMC-7721 cell after cryopreservation; SC (E): SMMC-7721 cell in the establishment; and BEL7404 cell: another human hepatoma cell line

DISCUSSION

Many chemical reagents, such as sodium butyrate, DMSO, dBcAMP, vitamin A, and vitamin D3 can induce the differentiation of cultured malignant cells, which suggested that these agents might be useful in the treatment of cancer. To our knowledge, the differentiation induced by cryopreservation at -50 ℃ has not been reported. In this report, we have found that the survival rate of human hepatoma cells SMMC-7721 at -50 ℃ is 95%, as indicated by the trypan blue resistant staining. These cells also had an 85% attachment rate, spreading on the surface o f culture dishes when treated with 10%-15% DMSO as a cryoprotectant. DMSO could penetrate rapidly into the cells and decrease the great ice crystal formation, suggesting that DMSO concentration of 5%-40% could protect the cells from damage of great ice crystal at -50 ℃, and the cell survival rates were all more than 80% with trypan blue resistant staining. However; high concentration of DMSO was toxic to cells, with a lower survival rate than lower concentrations of DMSO, but more importantly, the rates of attachment on the flasks after cryopreservation was much lower at 40% DMSO. These low rates of attachment might be induced by the high osmotic pressure in cells treated with high concentration of DMSO, which caused the cells to swell up and may have destroyed the cells in the process of revitrification. It is suggested that the cryopreservation at -50 ℃ with 10%-15% DMSO as a cryoprotectant is a practical and convenient method.

It is important to remember that -50 ℃ is in the great-ice-crystal forming temperature region in which cells can suffer great damage, and the cell membrane system might be changed greatly. This damage may induce many changes in physiology and biochemistry, maybe even in chromosomal and gene level. The success of cryopreservation at -50 ℃ and the high survival rate provided may not only be a convenient method for cryopreservation, but may also offer a new means for the study of gene expression and control in response to lower temperature.

We observed a decrease in the mitotic index of cryopreserved SMMC-7721 cells during the 13 hour culture at all time points examined except for that in the 5th hour, in which the MI was higher than that in control group. This time point discrepancy might be due to the synchronism of some cells after cryopreservation, as many cells might be going to the M phase together. These results suggested that after the culture period, the growth rate in the cryopreserved group might be lower than that in the control group.

After being cryopreserved at -50 ℃ more than 10 times, the chromosomal number, the main scope of chromosomal number and LDH isoenzymes of SMMC-7721 cells were compared between cells both before and after cryopreservation. The cell line in the establishment was a superdiploid cell line, its scope of chromosomal number was 44-107, the main scope 54-58,and the percentage of cells with 46 chromosomes was 0%. After being cryopreserved, chromosomal number changed to a range of 24-109, the main scope to 46-53, the percentage of cells with 46 chromosomes to 16%. These suggested that the cells with superdiploid chromosomes had a lower tolerance to -50 ℃ than those with a normal chromosome complement, and after being cryopreserved at -50 ℃ several times, both the main scope and the scope of chromosomal number returned to a normal level. The LDH isoenzymes of SMMC-7721 cells in the establishment tended towards H pattern (LDH3 (29.3%) > LDH4 (26.8%) > LDH2 (25.3%) > LDH5 (14.9) > LDH1 (3.6%) (H pattern > M pattern). After being cryopreserved at -50 ℃ for several times, the LDH isoenzyme contents were changed to LDH4 (48.3%) > LDH5 (28.3%) > LDH3 (18.9%) > LDH2 (4.4%) > LDH1 (0%), but the normal LDH isoenzymes of human hepatocyte were of M pattern, that was LDH5 > LDH4 > LDH3 > LDH2 > LDH1. The comparison showed that the LDH pattern of SMMC-7721 cells was also returned to the normal pattern of hepatocyte cells after being cryopreserved with the distinct decreasing of LDH2 content and increasing of LDH4 and LDH5. This LDH pattern, which is different from that of human hepatoma cells, has showed that genes controlling LDH M pattern are located in the chromosome 11, and the M pattern genes are located on chromosome 12. Both the LDH pattern and the change of main scope of chromosome suggested the changes of LDH isoenzyme were induced by the change of chromosomes.

From our results, we may conclude that the cryopreservation at -50 ℃ with 10%-15% DMSO as a cryoprotectant is not only a practical and convenient method for storage, but also might induce the differentiation of human hepatocarcinoma SMMC-7721 cells, thus opening a new way for researching into the gene expression and control in response to lower temperature.

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ORIGINAL RESEARCH

Effects of Linomide on growth and metastasis of implanted human gastric cancer in nude mice

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Abstract

AIM: To elucidate the effect of angiogenesis inhibitor, Linomide, on tumor growth and metastasis in nude mice implanted with human gastric cancer.

AIM: A metastatic model of gastric cancer was established using orthotopic implantation of histologically intact tumor tissues into the gastric wall of nude mice. Linomide (0, 80, 160 mg·kg^{-1}) was given p.o. every day after the implantation, and the mice were sacrificed after 10 wk to detect tumor size and metastasis. The microvessel counts were measured by immunohistochemical staining using a monoclonal antibody against Human Factor Ⅷ related antigen.

RESULTS: Linomide treatment significantly decreased the size of the implanted tumors (control group: 1.36 ± 0.81 cm^3 *vs* Linomide treated group: 0.84 ± 0.51 cm^3 and 0.62 ± 0.35 cm^3, $P < 0.05$ and 0.01, respectively). Additionally, an antimetastatic effect of Linomide was clearly demonstrated in a dose dependent manner: mice given 80 mg·kg^{-1} Linomide developed liver metastasis in 4 of 10 cases, mice given 160 mg/kg developed metastasis in only 1 of 10 mice, while it developed in 19 of 28 mice of the control group ($P < 0.05$ and 0.01, respectively). The number of metastatic foci was also significantly less in the treated group. Furthermore, the microvessel counts in tumors of treated mice was reduced by 33%-42% as compared with the control tumors ($P < 0.01$).

CONCLUSION: Linomide has a strong inhibitory activity against *in vivo* tumor growth and metastasis of gastric cancer, effectively suppressing the growth of the primary tumor, preventing liver metastasis, and attenuating the rate of neovascularization.

Key words: Linomide; Stomach neoplasms; Neoplasm metastasis; Liver neoplasms/secondary; Neovascularization, pathologic

Tao HQ, Lin YZ, Yin HR, Gu QL, Zhu ZG, Yao M. Effects of Linomide on growth and metastasis of implanted human gastric cancer in nude mice. *World J Gastroenterol* 1997; 3(3): 153-155 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/153.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.153

INTRODUCTION

Although most gastric cancer is surgically resectable, tumor relapse and metastasis does develop in some patients. Therefore, many studies have attempted to address the control of relapse and metastasis in patients with these tumors. Neovascularization is not only the basis of tumor growth, but also the part of the complicated process of tumor metastasis, and is crucial to the development of hepatic metastasis. Since a small focus of tumor cells cannot grow at a secondary site without the induction of angiogenesis, it is expected that inhibition of angiogenesis should provide a potent form of therapy to prevent tumor recurrence and metastasis of gastric cancer.

Angiogenesis inhibitors have been reported to have inhibitory activity against both tumor growth and metastasis, and some have achieved good results in animal experiments. Specifically. Linomide, a quinoline-3-carboxamide (Figure 1), has been demonstrated to have inhibitory effects on the growth and metastasis of several rodent tumor models, and its antitumor mechanism may involve antiangiogenic effects[1-3].

Implanting human tumor cells orthotopically into the corresponding organs of nude mice results in local tumor growth, and a much higher rate of metastasis. Human gastric cancer cells injected into the stomach wall of nude mice produced tumors that eventually metastasize to the liver, demonstrating that orthotopic implantation can enhance the metastatic potential of these tumor cells[4]. Recently, a new model of human gastric cancer was developed that avoids the disruption of tumor integrity, by using the orthotopic implantation of intact tumors[5]. Such a model should better reflect the original properties of human cancer, and could be of great value in the development of new drugs and new therapy strategies. In the present study, the inhibitory effect of Linomide on the local tumor growth and hepatic metastasis and the changing of microvessel density of tumors was examined in a nude mouse model of metastatic human stomach cancer using the orthotopic implantation of histologically intact tissues.

Table 1 Inhibitory effect of Linomide in gastric cancer growth in nude mice

	n	Local tumor growth	Volume of local tumor (cm^3)
Control	28	28/28 (100%)	1.36 ± 0.81
Linomide			
80 mg/kg	10	10/10 (100%)	0.84 ± 0.51[a]
160 mg/kg	10	10/10 (100%)	0.62 ± 0.35[b]

[a]$P < 0.05$, [b]$P < 0.01$, *vs* control group.

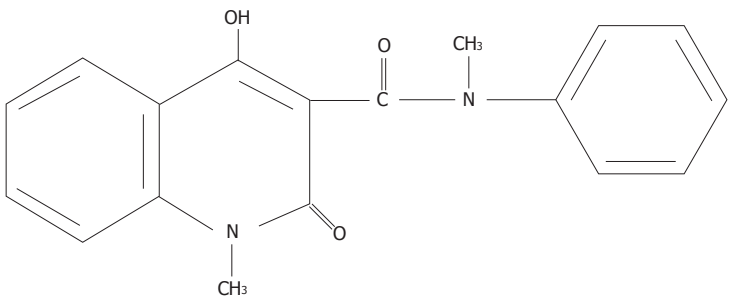

Figure 1 Structure of Linomide.

MATERIALS AND METHODS

Materials

The Linomide was a kind gift of Kabi Pharmacia Therapeutics (Helsingborg, Sweden). It was dissolved in an isotonic solution, and was administered to the mice in their drinking water. These concentrations did not affect total daily water intake of the mice. Control animals were given ordinary drinking water only.

Animals

Male BALB/c nu/nu mice were obtained from the Shanghai Cancer Institute. In this study, only animals which were 6 to 8 wk old and weighed 20 to 22 g were used.

Human gastric cancer xenografts

Human gastric adenocarcinoma cell line, SGC-7901, was kindly provided by the Shanghai Cancer Institute. Mice were inoculated s.c. in the flank with 1×10^6 SGC-7901 cells, and the xenograft was maintained by serial transplantation in nude mice. Small pieces of tissue were resected aseptically during the exponential growth phase from these tumors and then implanted into nude mice. Mice were anesthetized, and a small midline incision was made to carefully expose the stomach wall. A part of the serosal membrane, about 3 mm in diameter, in the middle of the greater curvature of the glandular stomach was removed at the site where the tumor pieces were to be implanted. A tumor piece of 5 mm in diameter was then fixed on each injured site of the serosal surface with a 7.0 Dexon transmural suture. The stomach was then returned to the peritoneal cavity, and the abdominal wall and skin were closed with 5. 0 Dexon sutures. The animals were kept in a specific-pathogen-free environment.

Assay of tumor growth and hepatic metastasis

Animals were given Linomide at a dose of 80 or 160 mg/kg daily *via* the drinking water and the same volume of normal saline was given to other mice as control. Mice were sacrificed 10 wk after implantation, or earlier if they developed signs of distress. Autopsy was performed immediately, and the tumors growing on the gastric wall were removed, and its width (a) and length (b) were measured. Tumor volumes in cm^3 were calculated by using a standard formula: $ab^2 \times 0.5236$. The ratio of tumor inhibition were calculated as:

[(Volume of control - Volume of experiment)/Volume of control] × 100%.

The liver was examined by routine histology to detect metastases.

Microvessel staining and counting

Intratumoral microvessels were identified by immunostaining using anti-human FⅧ related antigen monoclonal antibody, and microvessel density (MVD) was assessed as the count of endothelial deposits/mm^2 in the areas that were considered to be most active for neovascularization, as described previously[6].

Table 2 Inhibitory effect of Linomide in hepatic metastasis

	n	Ratio of metastasis	Metastatic foci
Control	28	19/28 (67.9%)	4.2 ± 1.4
Linomide			
80 mg/kg	10	4/10 (40.0%)[a]	2.5 ± 0.8
160 mg/kg	10	1/10 (10.0%)[b]	2

[a]$P < 0.05$, [b]$P < 0.01$, *vs* control group.

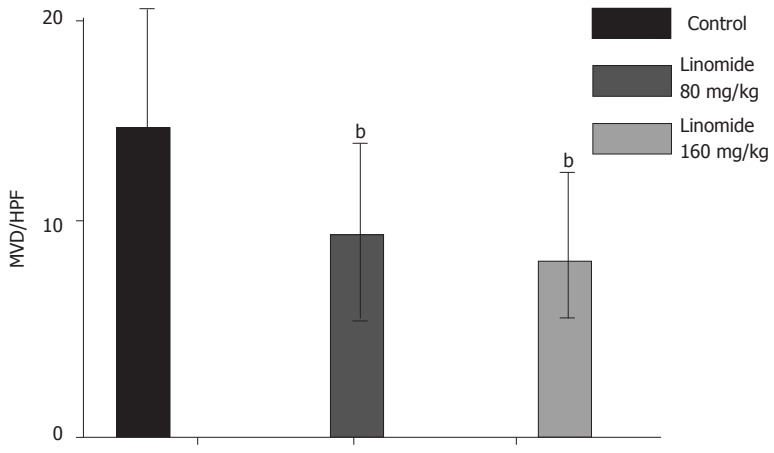

Figure 2 Blood vessel density (microvessel density; MVD) per high power field (HPF) in histological sections of gastric tumors from animals treated with different doses of Linomide. [b]$P < 0.01$ *vs* control.

Statistical analysis

Numerical values are expressed as the x- ± s. Student's *t* test and the χ^2 test were used for statistical analysis. Differences were considered significant if $P < 0.05$.

RESULTS

Tumor growth

All tumor pieces implanted in the stomach showed local orthotopic growth. Treatment of Linomide significantly decreased the tumor volume as compared to the control group, and the inhibitory effect on tumor growth was related to the dose of Linomide. The ratio of inhibition of 80 and 160 mg/kg Linomide were 38.2% and 54.4% respectively. This result indicates that the growth of gastric carcinoma can be inhibited by Linomide (Table 1).

Hepatic metastasis

Similar to human patients, the nude mouse model of metastatic human gastric cancer using orthotopic implantation of histologically intact tissue also saw extensive metastases in regional lymph nodes, peritoneum, liver, spleen and other tissues. To appraise the inhibitory effect of Linomide in gastric cancer metastasis, we examined the incidence of hepatic metastasis and the number of metastatic foci in our mouse model. We found that Linomide inhibited hepatic metastasis in a dose dependent manner. The number of metastatic foci in the liver was 2.5 ± 0.8 in the 80 $mg \cdot kg^{-1}$ group and 2 in the 160 $mg \cdot kg^{-1}$ group. In contrast, 4.2 ± 1.4 metastatic foci were found in the control group (Table 2).

Effect of Linomide on tumor angiogenesis

The capillary density in tumor of Linomide (80 and 160 mg/kg) treated mice was reduced by 33%-42% as compared with the control group. These data suggest that Linomide attenuated the rate of neovascularization, but did not completely block the initial activation of angiogenesis, nor the capability of each capillary to grow (Figure 2).

DISCUSSION

Recently, the mechanism of tumor angiogenic and angiogeneic

inhibitors have become 'hot spots' in the field of tumor research. Since tumor growth is reported to generally depend on angiogensis, angiogenic inhibitors should have an inhibitory effect on *in vivo* tumor growth. The angiogeneic inhibitor, Linomide, has been reported to have an inhibitory effect on both tumor growth and metastasis in rodent prostatic cancer and melanoma. However, its effect has not been examined in human tumors. In this study, we investigated its inhibitory effect on the local growth and hepatic metastasis of human gastric cancer in a nude mouse model, constructed using the orthotopic implantation of histologically intact tissues. Although it was previously reported that Linomide had an inhibitory effect of tumor growth, because the tumors were inoculated s.c., the model may not reflect the natural environment of tumor growth. The host organ microenvironment can profoundly influence the growth of tumor cells. The model in the present study was more appropriate than heterotopic implantation models. In this study, we found that Linomide can inhibit gastric tumor growth, the ratio of inhibition of 80 and 160 $mg \cdot kg^{-1}$ were 38.2% and 54.4%, respectively. The result is consistent with the hypothesis that the rapidly proliferating tumor is more angiogenesis dependent.

Kalland[3] had reported that continuous treatment with Linomide in mice reduced the rate of pulmonary metastasis by 85%. In the present study, we found that it inhibited hepatic metastasis in a dose-dependent manner. Hepatic metastasis was observed in only 1 or 10 (10%) of the mice treated with 160 mg/kg, and was decreased significantly in mice treated with either 80 or 160 mg/kg of Linomide as compared with the control group. This result demonstrated that Linomide has an inhibitory effect on the metastasis of human tumors.

Tumor cell metastasis occurs through a complicated process that involves five main steps: (1) angiogenesis, (2) adhesion to endothelial cell basement membrane, (3) local proteolytic destruction of the basement membrane, (4) migration into secondary site, and (5) proliferation at the secondary sites. Tumor growth and metastasis require the development of new vessels. Mature capillaries have a thickened basement membrane, but growing capillaries have fragmented basement membrane. Because growing capillaries are leaky, these new vessels also increase the opportunity for tumor cells to enter the circulation. This study found that the microvessel density of Linomide treatment group was decreased by 33%-42% as compared with control group, indicating that efficacy for hepatic metastasis by Linomide may depend on the inhibition of angiogenesis at both the first and the final step of the metastatic process. Tumor cells rarely enter circulation because of the inhibition of capillary growth by Linomide. At the same time, tumor cells, when arriving at the target organs, cannot grow without the induction of angiogenesis. The tumor cells that arrived at the liver could not grow to a detectable mass since angiogenesis was inhibited by Linomide.

Vukanovic *et al*[1] thought that Linomide could inhibit tumor associated macrophage/monocyte number and their ability to secrete TNF-α. However, the precise mechanism of Linomide on human tumor neovascularization should be investigated further. Maeda *et al*[7] have reported that tumor recurrence and metastasis are closely correlated with microvessel count in gastric carcinoma. Our study indicated that hepatic metastasis of human gastric cancer was prevented by inhibiting tumor angiogenesis. Thus, hepatic metastasis may be prevented by angiogenesis inhibitors such as Linomide through reducing the opportunities for tumor cells to enter the circulation and inhibiting the growth of tumor cells arriving in the liver.

In summary, the angiogenesis inhibitor Linomide seems to be a potent tumor growth and metastatic inhibition agent, and might serve as a clinical treatment with further studies.

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ORIGINAL RESEARCH

Loss of heterozygosity and mRNA expression at deleted in colorectal cancer gene locus in gastric cancer

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Abstract

AIM: To assess the effects of the deleted in colorectal cancer (*DCC*) gene changes on the development and progression of gastric cancer.

METHODS: The loss of heterozygosity (LOH) and mRNA expression *DCC* gastric cancer using a PCR-based detection method.

RESULTS: LOH was found in 35.3% (18/51) of the specimens, and the LOH was more frequently detected in tumors from patients with stage Ⅲ or Ⅳ cancer (50.5%) than those in stages Ⅰ or Ⅱ (14.3%) ($P < 0.05$). The occurrence of LOH was not found to correlate with the histological type, tumor size, invasion depth and lymph node metastasis of gastric cancer. The mRNA expression of the *DCC* gene was studied in 26 of the 51 cases, of which LOE was found in 30.8% (8/26). LOE was not significantly correlated to LOH or other clinicopathological parameters.

CONCLUSION: LOH and LOE of *DCC* gene are frequently encountered in gastric cancer, and the LOH of *DCC* gene is a late event associated with progression of gastric cancer.

Key words: Stomach neoplasms; *DCC* gene; Gene expression; mRNA; Heterozygosity loss

Wang DX, Fang DC, Luo YH, Liu WW. Loss of heterozygosity and mRNA expression at deleted in colorectal cancer gene locus in gastric cancer. *World J Gastroenterol* 1997; 3(3): 156-159 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/156.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.156

INTRODUCTION

Tumor suppressor genes play an important role in regulating normal cellular proliferation[1,2]. Conversely, inactivation of tumor suppressor genes at both alleles may allow a cell to lose normal growth controls and acquire a malignant phenotype. This inactivation may occur through a variety of mechanisms including deletion, rearrangement, point mutation, gene conversion, and binding of suppressor gene products with viral or cellular inactivating proteins[1,3]. The deleted in colorectal cancer (*DCC*) gene was first cloned based on frequent deletions affecting the 18q21 region in colon cancer[4]. Subsequently, loss of heterozygosity (LOH) or loss of expression (LOE) of *DCC* has been reported in several other tumor types, including breast[5,6], pancreatic[7], prostate[8] and testicular[9]carcinomas, glioblastomas[10] and hematological malignancies[11].

In a study of human gastric cancer, chromosome 18q was frequently affected by the loss of heterozygosity detectable in more than 60% of cases[12]. However, there have been no studies reported on LOE of *DCC* gene in gastric cancer. In order to investigate the effects of the *DCC* gene abnormality on the development and progression of gastric cancer, LOH and LOE of *DCC* gene were examined using a PCR based detection method.

MATERIALS AND METHODS

Tissue specimens

Tumor and corresponding noncancerous tissues were obtained from 51 patients who underwent surgical resection for gastric carcinoma between January 1993 and October 1996 at the Southwest Hospital. None of the patients had received any radiotherapy or chemotherapy preoperatively. Each pair of tumor and corresponding non-tumor tissues was stored at -80 ℃ immediately after the resection for experimental use. A 6 μm section was cut from each tissue and stained with hemotoxylin/eosin for pathological diagnosis. After diagnostic confirmation, a visual assessment was made of the approximate proportion of tumor cells *vs* normal cells in the tumor. Only the specimens in which tumor cells represented ≥ 60% of the tumor tissue were accepted for LOH and LOE analysis.

Total RNA isolation and DNA extraction

Total RNA was prepared from tumor and noncancerous tissues using the acid guanidinium thiocyanate method[13] and high molecular weight DNA was extracted using proteinase K digestion and phenol chloroform isoamyl alcohol extraction as previously described[14].

RT-PCR assay of DCC gene expression

RT-PCR was performed as described previously with some modifications[15]. *DCC* complementary DNA was amplified at 94 ℃ for 40 s; 49 ℃ for 40 s, and 72 ℃ for 1 min in a Perkin Elmer Thermocycler 2400 for 35 cycles. *DCC* primers were located on exons O and P, amplifying a 233 base pair fragment

Table 1 Relationship of loss of heterozygosity (LOH) and mRNA expression (LOE) of deleted in colorectal cancer (*DCC*) with clinicopathological parameters

Clinicopathologic parameters	LOH/informative (%)	LOE/No. examined (%)
Differentiation		
Well/moderate	4/12 (33.3)	1/6 (16.7)
Poor	12/28 (42.9)	5/14 (35.7)
Mucinous carcinoma	2/11 (18.2)	2/6 (33.3)
Tumor size		
< 5 cm	4/20 (20.0)	4/12 (33.3)
> 5 cm	14/31 (45.2)	4/14 (28.6)
Serosal invasion		
Absent	4/18 (22.2)	3/12 (25.0)
Present	12/33 (36.4)	5/14 (35.7)
Lymph node metastasis		
Absent	5/24 (20.8)	2/13 (15.4)
Present	13/27 (48.1)	6/13 (46.2)
Clinical staging		
Stages Ⅰ-Ⅱ	3/21 (14.3)	2/11 (18.2)
Stages Ⅲ-Ⅳ	15/30 (50.0)[a]	6/15 (40.0)

[a]$P < 0.05$ as compared with that of stage Ⅰ and Ⅱ.

Table 2 Relationship between loss of heterozygosity (LOH) and mRNA expression (LOE) of deleted in colorectal cancer (*DCC*) gene

Groups	LOH	LOE	No. of cases (%)
1	+	+	4 (15.4)
2	-	-	14 (53.8)
3	-	+	4 (15.4)
4	+	-	4 (15.4)

+: Positive; and -: Negative.

from the human mRNA[16]. A fragment of this size cannot be amplified from genomic DNA, for the primers were designed to frame sequences that cross an intron on the *DCC* gene. RT-PCR without RNA or without reverse transcriptase were included in each experiment as negative controls. Primers used were 5′ TTCCGCCATGGTTTTTAAATCA 3′ (*DCC* sense), and 5′ AGCCTCATTTTCAGCCACACA 3′ (*DCC* antisense).

PCR-LOH analysis

Fifty to 500 ng of genomic DNA were placed at 95 ℃ for 5 min in 20 μL buffer containing 10 mmol/L Tris (pH 8.3), 5 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 0.1 μg/μL bovine serum albumin, sense and antisense primers at 1 μm concentration. Then 2.5 units of Ampli Taq DNA polymerase was added and PCR was run at 94 ℃ for 40s; 56 ℃ for 40 s, 72 ℃ for 1 min, for 35 cycles. For M2 and M3 polymorphism[17], PCR products were digested with MspI and analyzed on 25% agarose gels. For VNTR polymorphism[18,19], PCR products were directly separated on 2.5 gels. The gel was then stained with ethidium bromide and photographed under UV light. The primers were: 5′TGCACCATGCTGAAGATTGT 3′ (M2 sense), 5′AGTACAACACAAGGTATGTG 3′ (M2 antisense); 5′ CGACTCGATCCTACAAAATC 3′ (M3 sense), 5′ TCTACCCAGGTCTCAGAG 3′ (M3 antisense); 5′ GATGACATTTTCCCTCTAG 3′ (VNTR sense), and 5′ GTGGTTATTGCCTTGAAAAG 3′ (VNTR antisense). Negative controls without genomic DNA were performed for each set of PCR reaction.

Data analysis

Photographs of thidium-stained gels were read by two observers independently. LOH and LOE was defined by a visible change in that allele: allele ratio in tumor compared to the matching normal tissues. A reduction of allelic intensity over 50% in tumor compared to the matching tissues was taken to be indicative of LOH or LOE, (Figures 1-3).

Statistical analyses

Associations between variables were made with the Chi square test, a p value of less than 0.05 was considered to be statistically significant.

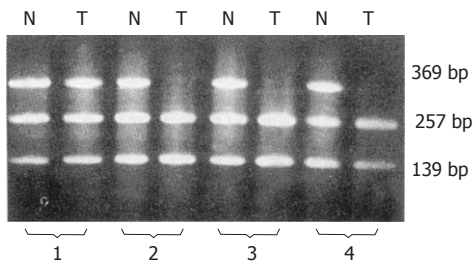

Figure 1 Heterozygosity loss of deleted in colorectal cancer (*DCC*) gene (M2) of gastric cancer. N: Normal tissue DNA; T: Tumor tissue DNA; 1: Heterozygote; 2-4: loss of heterozygosity (LOH).

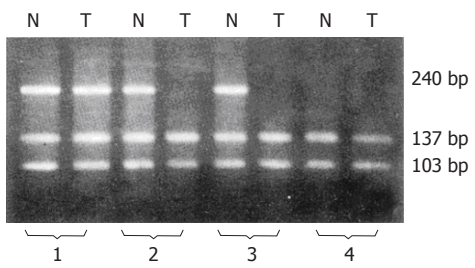

Figure 2 Heterozygosity loss of deleted in colorectal cancer (*DCC*) gene (M3) of gastric cancer. N: Normal tissue DNA; T: Tumor tissue DNA; 1: Heterozygote; 4: Homozygote; 2 and 3: loss of heterozygosity (LOH).

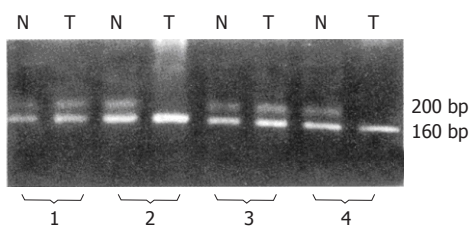

Figure 3 Heterozygosity loss of deleted in colorectal cancer (*DCC*) gene (VNTR) of gastric cancer. N: Normal tissue; T: Tumor tissue DNA; 1 and 3: Heterozygote; 2 and 4: loss of heterozygosity (LOH).

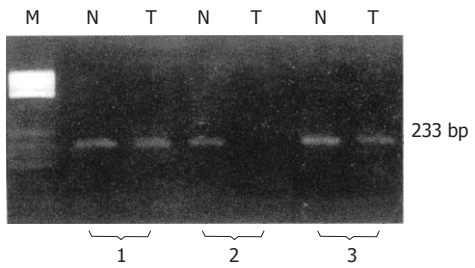

Figure 4 Loss of deleted in colorectal cancer (*DCC*) gene expression of gastric cancer. N: Normal tissue T: Tumor tissue; M: Marker of molecular weight, PBR 322/*Hae* Ⅲ; 1 and 3: Normal expression; 2: loss of heterozygosity (LOH).

RESULTS

LOH of the *DCC* gene was determined by PCR-LOH in 51 specimens of gastric cancer. In order to raise the assay sensitivity, three different sites, *i.e.*, M2, M3 and VNTR were used in this study. LOH of *DCC* was observed in 9 of 47 (19.0%) at M2, 7 of 50 (14.0%) at M3 and 3 of 26 (11.5%) at VNTR sites, respectively. If a positive allelic deletion of *DCC* was judged by LOH at one or any combination of these three sites, the incidence of LOH at the *DCC* locus was 35.3% (18/51). LOH was detected in 37.5% (6/16) of intestinal type of gastric cancer and 36.4% (12/33) of gastric type. Of the 51 cases of gastric cancer, 26 underwent the examination of expression of *DCC* mRNA, and LOE was observed in 30.8% (8/26) (Figure 4). The incidence of LOE was 44.4% (4/9) in the intestinal type and 23.5% (4/17) in gastric type. χ^2 test revealed no significant difference of the LOH and LOE between these two types of cancer ($p > 0.05$).

Correlation between LOH and LOE of *DCC* and clinicopathological data of gastric cancer are illustrated in Table 1. χ^2 test demonstrated that LOH of *DCC* was significantly higher in stages Ⅲ and Ⅳ gastric cancer than that in stage Ⅰ or Ⅱ ($P < 0.05$). Correlation between

LOH and LOE is shown in Table 2. The paired data were analyzed with χ^2 test and no significant correlation was found between LOH and LOE ($p > 0.05$).

DISCUSSION

The *DCC* gene is located on the human chromosome 18q$^{21.3}$. It was reported that the inactivation of this gene is closely related to the pathogenesis of colorectal, esophageal and pancreatic carcinoma, and this gene is considered a susceptible gene in gastrointestinal carcinomas. Uchino *et al*[12] and Ranzani *et al*[19] reported that the rate of LOH of *DCC* in gastric cancer was 61% and 42.9%, respectively; in our study, this rate was 35.5%. Together, these results suggest that *DCC* gene takes part in the pathogenesis of gastric cancer through its LOH.

To our knowledge, this is the first report on the expression of mRNA of *DCC* gene in gastric cancer, which confirmed the LOE of *DCC* mRNA in the gastric cancers with RT-PCR. This suggests that the inactivation of *DCC* in gastric cancer occurs in various patterns, and further confirms that the *DCC* gene is the susceptible gene of gastric cancer, playing an important role in the pathogenesis of gastric cancer.

The histological progression associated with the intestinal type of gastric cancer has been well documented, with apparent evolution through a sequence of superficial gastritis, intestinal metaplasia and dysplasia[20,21]. Lesions indicating an adenoma carcinoma sequence similar to that in the colorectum were also observed in the stomach[22]. In the present study we have found that the rate of LOE of *DCC* was as high as 44.4% in intestinal type of gastric cancer, which approached that of the rate seen in colorectal carcinoma[23]. However, the rate of LOE was rather low in gastric type of gastric cancer. Though there was no statistical difference between the two types of gastric cancer, similarities in genetic alterations between colorectal carcinoma and intestinal type of carcinoma may reflect a carcinogenetic pathway common to colorectal carcinoma and gastric carcinoma. It was also found in our study that the rate of LOH of intestinal type of *DCC* was similar between the two types of gastric cancer. Whether the effects of LOH and LOE of *DCC* gene are different between the two types of gastric cancer needs further studies.

The *DCC* gene encodes a molecule which shares high homology with the neural cell adhesion molecule[16]. Cell adhesion molecules are cell surface receptors that play critical roles in a number of different processes, including embryogenesis, thrombosis, wound healing, cell homing, and immunoreactivity, as well as tumor progression and metastasis. The inactivation of *DCC* gene may result in malignant degeneration of the cells and aid in the invasion and metastasis of a tumor. Kato *et al*[24] found that the incidence of LOH at *DCC* locus in colorectal carcinoma was significantly greater for patients with liver metastasis than for patients with no liver metastasis. Iino *et al*[23] and Yanoshita *et al*[13] observed that the expression of *DCC* gene in mRNA was greatly reduced or not detectable in invasive colorectal carcinoma in comparison with carcinoma in adenoma and intramucosal carcinoma. They indicated that the inactivation of the *DCC* gene was associated with the progression of early stage carcinoma to advanced stage. Itoh *et al*[25] revealed that the expression level of *DCC* mRNA was lower in liver matastasis than in primary carcinoma. These findings imply that the inactivation of the *DCC* gene occurs in the late stage of colorectal carcinoma, and was of prognostic significance. There were similar conclusions in the study of esophageal and pancreatic carcinoma[7,26]. In our study, the rate of LOH of *DCC* rose along with the increase of tumor size and the depth of invasion and the metastasis to lymph nodes, and LOE of *DCC* frequently occurred in gastric cancer of stages Ⅲ and Ⅳ with lymph node metastasis. Though these data did not find a statistical significance, they may suggest that *DCC* gene plays a definite role in the proliferation, invasion and metastasis of gastric cancer. The LOH rate of *DCC* in our study was significantly higher in the stages Ⅲ and Ⅳ than that in stages Ⅰ or Ⅱ, which indicates that LOH of *DCC* occurs in the late stage and is related to the advances of the malignancy. Ranzani *et al*[19] had similar findings as ours. Thus, it is expected that LOH and LOE of *DCC* may be potentially used as a prognostic factor for gastric cancer. However, to accurately reveal the correlation of LOH and LOE of *DCC* with clinicopathologic factors and prognosis, a larger number of cases should be examined.

The interrelation of LOH and LOE of *DCC* gene was preliminarily studied and it was found that LOH does not seem to be necessary for LOE of *DCC* mRNA, which was similar to the finding of others[14,27]. There might be some other causes, such as alterations in sequences controlling transcriptional regulation, point mutation or insertions within the *DCC* gene, or alterations in other gene controlling *DCC* gene expression. Further studies are required to examine these possibilities.

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Significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesion

Ji-Feng Wu, Yu-Lin Song, Guang-Lin Yang, Yu-Ming Dong, Dao-Bing Wang, Min-Pei Liu

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Abstract

AIM: To study the significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesions.

METHODS: Immunohistochemical staining and mucin histochemical staining were performed on paraffin-embedded sections from gastric benign and malignant lesions from 101 patients.

RESULTS: SC3A positive rate was 80.3% (57/71) in lesions of gastric carcinoma. The expression of SC3A was not related to the classification, differentiation, metastasis and or survival rates. The positive rate of SC3A in cancers secreting acid mucin (90.2%) or sulphomucin (91.3%) was higher than that in cancers without acid mucin (20.0%) or sulphomucin (60.0%) ($P < 0.01$). The positive rate of sulphomucin was higher in cases of intestinal metaplasia with cancer (88.9%) than that of cases of intestinal metaplasia with a benign lesion (35.3%) ($P < 0.01$). Additionally, the positive rate of SC3A with sulphomucin in intestinal metaplasia (60.9%) was higher than that without sulphomucin (31.3%) ($P < 0.05$).

CONCLUSION: SC3A monoclonal antibody might be helpful in the diagnosis of gastric cancer and the discernment of histogenesis.

Key words: antibodies, monoclonal/metabolism; stomach neoplasms/immunology

Wu JF, Song YL, Yang GL, Dong YM, Wang DB, Liu MP. Significance of monoclonal antibody SC3A expression in gastric carcinoma and precancerous lesion. *World J Gastroenterol* 1997; 3(3): 159 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/159.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.159

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ORIGINAL RESEARCH

Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers

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Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To evaluate the role of *p*53 in the development and progression of colorectal cancer and gastric carcinoma by analyzing the loss of heterozygosity (LOH) at 17p13.1 and 17p13.3.

METHODS: LOH at the *p*53 gene locus and 17p13.3 were examined in 22 cases of gastric carcinoma and 14 cases of colorectal cancer by Southern blot analysis.

RESULTS: Of the 22 gastrocarcinoma cases, 12 (54%) were heterozygous and LOH was detected in 6 (50%) of the 12 informative cases. In the 14 colorectal cancer cases, 10 (71%) were heterozygous, and LOH was detected in 6 (60%) of the 10 informative cases.

CONCLUSION: LOH at the *p*53 gene locus is a frequent event in multiple step carcinogenesis progression. The high frequency of LOH at 17p13.3 suggests that there may be another tumor suppresser gene in that chromosome region.

Key words: Stomach neoplasms; Colorectal neoplasms; *p*53 gene; Heterozygosity loss; Genes, suppressor, tumor

Wu GJ, Shan XN, Li MF, Shi SL, Zheng QP, Yu L, Zhao SY. Preliminary study on the loss of heterozygosity at 17p13 in gastric and colorectal cancers. *World J Gastroenterol* 1997; 3(3): 160-162 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/160.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.160

INTRODUCTION

Recent molecular biology studies have revealed that carcinogenesis is a multiple-step process involving many factors. The activation of some oncogenes and inactivation of some tumor suppresser genes play key roles in this process[1]. *p53* is a widely related tumor suppresser gene, but its function has not been clearly defined[2,3]. In this research, LOH of the *p*53 gene in two common cancers, gastric cancer and colorectal cancer, was examined by Southern blot hybridization and RFLP analysis.

MATERIALS AND METHODS

Samples

Twenty-two cases of gastric cancer and 14 cases of colorectal cancer were acquired from the affiliated hospital of Nanjing Railway Medical College. Tumor tissues and their corresponding normal tissues were obtained during surgery. The classification of tumor and normal tissues was performed by the Department of Pathology of Nanjing Railway Medical College.

Probes

php53B is a *p*53 cDNA probe located at 17p13.1. pYNZ22 is a VNTR (variable number of tandem repeats) probe located at 17p13.3. Both probes were obtained from ATCC (American Type Culture Collection).

Southern blot

Genomic DNA was isolated from tumor and normal tissues according to standard methods[4]. It was then completely digested with specific restriction endonucleases, electrophoresed on agarose gels, denatured, and transferred to nylon filters. The filters were prehybridized for 8-12 h, hybridized for 24-36 h in 50% formamide at 42°C, washed and autoradiographed for 1-4 d at -70°C. Probes were radiolabeled using the random primer method.

LOH analysis

The hybridization results of each tumor tissue were compared to that of its normal tissue. If the normal tissue had two heterozygosity bands and its corresponding tumor tissue had only one

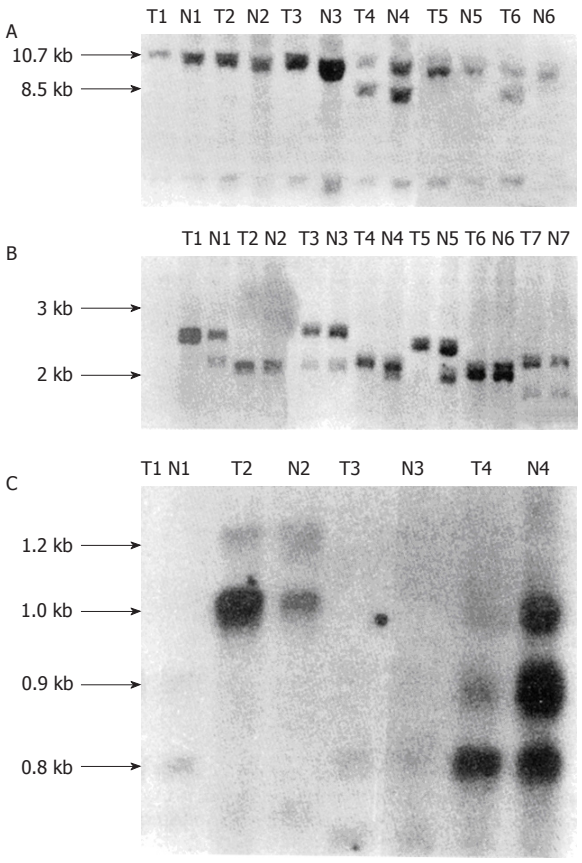

Figure 1 Loss of heterozygosity (LOH) at 17p13 in gastric carcinoma. N: Normal DNA; T: Tumor DNA. (A) LOH at the *p*53 locus. Genomic DNA was digested with ScaI. Sample 4 showed heterozygosity, and Sample 6 showed LOH. (B) LOH at YNZ22. Genomic DNA was digested with TaqI. Samples 3 and 7 showed heterozygosity. Samples 1 and 5 showed LOH. (C) LOH at YNZ22. Genomic DNA was digested with MsqI, LOH was detected in Samples 2 and 4.

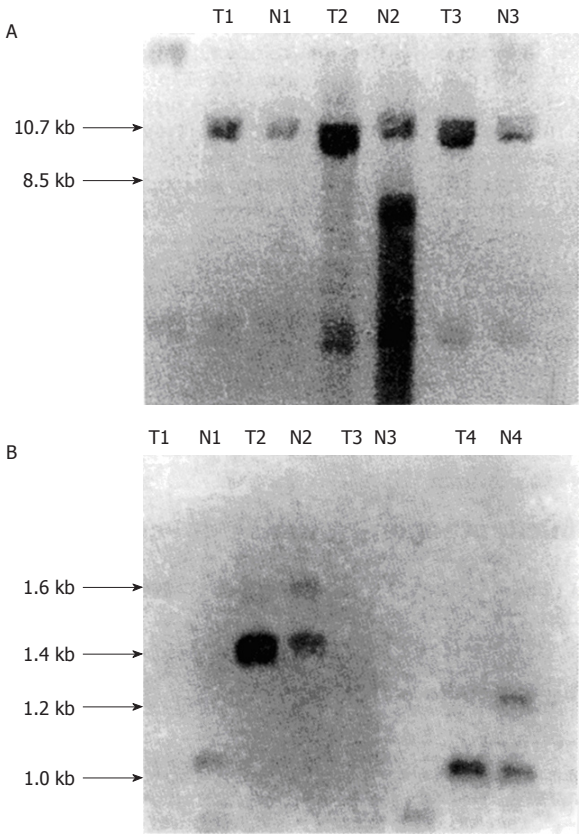

Figure 2 Loss of heterozygosity (LOH) at 17p13 in colorectal cancer. N: Normal DNA; T: Tumor DNA. (A) LOH at *p*53. Genomic DNA was digested with ScaI. LOH was detected in Sample 2. (B) LOH at YNZ22. Genomic DNA was digested with MspI. LOH was detected in Samples 2 and 4.

homozygosity band, the patient was classified as having *p*53 LOH.

RESULTS

php53B and pYNZ22 RFLP fragments

Using the ScaI restriction enzyme, we identified 8.5 kb and 10.7 kb allelic fragments with the php53B probe. After use of the MsqI restriction enzyme, we identified a group of alleles 0.5 kb to 1.3 kb with the pYNZ22 probe. While using TaqI, we identified allele fragments ranging from 2 kb to 3 kb with the pYNZ22 probe (Figures 1 and 2).

Table 1 Loss of heterozygosity (LOH) detected by the php53B and pYNZ22 probes in gastric cancer

Locus	Restriction enzyme	Number of sample	Heterozygosity and rate (%)	LOH and rate (%)
*p*53	*Sca* I	22	3 (14)*	2 (66)*
YNZ22	*Taq* I	8	4 (50)	2 (50)
	Msp I	14	6 (43)*	3 (50)*
17p13		22	12 (54)	6 (50)

*One sample showed heterozygosity and LOH detected with both probes.

Table 2 Loss of heterozygosity (LOH) detected by the php53B and pYNZ22 probes in colorectal cancer

Locus	Restriction enzyme	Number of sample	Heterozygosity and rate (%)	LOH and rate (%)
*p*53	*Sca* I	7	2 (20)	1 (50)
YNZ22	*Msp* I	14	8 (57)	5 (62)
17p13		14	10 (71)	6 (60)

The number in bracket shows the rate of heterozygosity or LOH.

LOH of 17p13 in gastric and colorectal cancer

We identified heterozygosity in all of the normal tissues from the 12 gastric cancer cases and ten colorectal cancer cases. They all showed hybridization bands of different lengths. We determined that six gastric cancer cases and six colorectal cancer cases exhibited LOH. The rate of detection was 50% and 60%, respectively. These details are shown in Tables 1 and 2.

DISCUSSION

In his "two hit" theory, Knudson showed that the loss of function of tumor suppressor genes generally involves at least two genetic mutation events[5]. These mutations can be detected by Southern blot hybridization and analysis of the LOH occurrence rate. The closely linked polymorphic gene probes located nearby or inside the possible tumor suppressor gene are used to examine the tumor tissue and its normal adjacent tissue. Detection of LOH suggests that there is a tumor suppressor gene located in the region covered by the gene probe, and two mutation events may have occurred in the tumor suppressor gene.

In this study, we detected LOH in two gastric cancer cases and one colorectal cancer case using the php53B probe. As php53B is a *p*53 cDNA probe, our data suggest that two mutation events occurred in the *p*53 gene of some gastric cancer and colorectal cancer patients, and that the normal function of the wild type *p*53 gene was lost. We also detected LOH in five gastric and colorectal cancer cases using the pYNZ22 probe, which is located at 17p13.3 and is tightly linked with p53. These results further suggest that the inactivation of *p*53 at 17p13.1 is involved in gastric and colorectal carcinogenesis.

To date, *p*53 is the only tumor suppresser gene that has been assigned to chromosome 17p13. There are conflicting reports on whether the LOH detected by the pYNZ22 probe only reflects *p*53 inactivation. Studies in breast cancer have shown that LOH detected by pYNZ22 primarily represents *p*53 inactivation[6]. However, Coles *et al*[7,8]suggested that there may be certain regulatory genes located between YNZ22 and *p*53 that control *p*53 gene expression. Damage to this gene, together with *p*53 inactivation supposedly contribute to breast cancer carcinogenesis. In our research, only one case of gastric cancer showed LOH in both *p*53 and the YNZ22 region, while the other cases did not demonstrate LOH in both *p*53 and the YNZ22 locus. Therefore, further studies are necessary, including collecting more cases and using more restriction endonucleases, to determine whether LOH detected within pYNZ22 in gastric and colorectal cancers represents *p*53 inactivation or whether there is an additional regulatory gene near the YNZ22 locus that is mutated in

gastric and colorectal cancers.

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Clinicopathogenic studies of acute diarrhea in children

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Abstract

AIM: To identify etiologic pathogens of acute diarrhea in children and to determine the diagnostic value of stool pH.

METHODS: From May 1988 to April 1992, 368 children with acute diarrhea were studied. Fresh stools were routinely examined, and stool pH was tested with pH paper. Samples were placed in Cary-Blair culture medium and were sent to the lab for bacterial isolation and identification. Rotavirus was identified in the supernatant by ELISA.

RESULTS: Thirty-one pathogens and 385 bacterial strains were found in the 368 samples, with a detection rate of 67.7%, including 37.8% of mixed infections. Among the bacteria families, vibrionaceae was the most common (39.7%), and among bacteria genera, aeromonas was the most common (26.8%). In bacterial diarrhea, stool pH tended to be basic, while in viral diarrhea it tended to be acidic.

CONCLUSION: There are 31 pathogens for children's acute diarrhea in this area. It is quite difficult to make an etiologic diagnosis only by clinical signs. However, stool pH is of some value for early disease diagnosis.

Key words: Diarrhea/etiology; Acute diseases; Diarrhea/diagnosis

Cai LM, Zhang C, Chen H, Jiang WP, Mao WX. Clinicopathogenic studies of acute diarrhea in children. *World J Gastroenterol* 1997; 3(3): 162 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/162.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.162

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ORIGINAL RESEARCH

P21 and CEA expression and AgNOR counts in dimethylhydrazine-induced colon carcinoma in rats

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Abstract

AIM: To study P21 and carcinoembryonic antigen (CEA) expression and to measure argyrophilic nucleolar organizer region (AgNOR) counts in various lesions of colonic mucosa and the mechanism of carcinogenesis.

METHODS: Thirty-eight male Wistar rats were injected with dimethylhydrazine (DMH) once a week for 25 wk. P21 and CEA expression was detected by immunohistochemical methods, and AgNOR was counted by silver staining paraffin sections from various colonic lesions.

RESULTS: The incidence of colonic carcinoma in DMH-treated rats was 71.05%(27/38), and lymph node metastasis occurred in six rats. Immunohistochemical studies showed that P21 was primarily expressed in dysplasia and carcinomas, while CEA was expressed in carcinomas and metastatic tumors. AgNOR counts were higher in dysplasia and carcinomas. There were significant differences in P21 and CEA expression between benign and malignant lesions ($P < 0.05$). The difference in AgNOR counts was also significant between normal and dysplastic tissues, and between dysplasia and malignant lesions ($P < 0.05$).

CONCLUSION: Dysplasia is a premalignant change of colonic carcinoma. The detection of P21 *via* immunohistochemistry and AgNOR counting may be an important clinical screening technique for colon carcinoma and premalignant lesions.

Key words: Colonic neoplasms; Carcinoembryonic antigen; Ras oncogene; Nucleolus organizer region; P21 oncogene

Zhang ZG, Wu JY, Fu XD, Gu DK, Fang F. P21 and CEA expression and AgNOR counts in dimethylhydrazine-induced colon carcinoma in rats. *World J Gastroenterol* 1997; 3(3): 163-165 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/163.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.163

INTRODUCTION

The transformation-inducing genes most frequently detected in solid human tumors are members of the Ras family of oncogenes. It has been suggested that P21 overexpression in the early neoplasm stage may play an important role in carcinogenesis[1,2]. In this study, we examined P21 and CEA expression and quantified nuclear silver stain of the granules of nucleolus organizer region (AgNOR) in 1.2 dimethylhydrazine (DMH)-induced colonic carcinoma in rats to study the carcinogenesis mechanism.

MATERIALS AND METHODS

Animals

Fifty male Wistar rats weighing approximately 170 g were randomly divided into a test group (T group, $n = 40$) and control group (C group, $n = 10$). Rats in the T group were subcutaneously injected with 20 mg/kg body weight DMH once a week. Rats in the C group rats were given 0.9% NaCl solution at the same time. DMH administration lasted 20 wk. At the 10^{th}, 15^{th}, and 18^{th} week, one rat was sacrificed in each group, and 10 were sacrificed at the 20^{th} week. The rest were continually fed with standard diet until the 25^{th} week, when they were all sacrificed. Intestinal tissues were fixed in 10% neutral formalin.

Pathological and immunohistochemical examination

Tissue specimens were collected from the tumor, adjacent tissues and normal colonic mucosa in T group rats, and normal colon mucosa were collected from the C group. Serial paraffin sections were cut at 4 μm thickness and were stained with hematoxylin and eosin (HE). The ABC immunohistochemical method was used for P21 staining, and the PAP method was used for CAE staining. The working dilution of the anti-P21 monoclonal antibody (Huamei Company) was 1:40, and 1:100 for the anti-CEA antibody (DAKO Company). PBS was used as a negative control.

AgNOR staining technique

A one-step silver staining method was used for AgNOR detection. The observation method and statistical standard were performed as

Table 1 DMH (dimethylhydrazine)-induced carcinogenesis in rats

Lesions	*n*	Incidence (%)	Number of mass
Well-differentiated adenocarcinoma	17	44.74	20
Undifferentiated adenocarcinoma	3	7.89	5
Mucinous adenocarcinoma	7	18.42	9
No tumor	11		
Total	38	71.05	34

Table 2 Histological changes in colons of DMH (dimethylhydrazine)-treated rats

Histological types	T group	C group
Normal epithelial cell	0	6*
Inflammation	10	4
Dysplasia	10	0
Adenoma	3	0
Carcinoma	34	0

*With normal mucosa for all intestines.

Table 3 The distribution of positive P21 and Carcino Embryonie Antigen (CEA) immunohistochemical staining in rat colonic lesions

Histological types	*n*	p21 (%)	CEA (%)
Benign			
Normal epithelial cell	10	0 (0.0)	0 (0.0)
Adenoma	3	1 (33.3)	0 (0.0)
Dysplasia	10	6 (60.0)	3 (30.0)
Malignant			
Well-differentiated adenocarcinoma	20	14 (70.0)	16 (80.0)
Undifferentiated adenocarcinoma	5	1 (20.0)	4 (80.0)
Mucinous adenocarcinoma	9	7 (77.8)	6 (66.7)

$P < 0.05$ for p21 and CEA between benign and malignant lessions.

described previously[3].

RESULTS

Animal experiments

Of the 38 rats in the T group (two rats unexpectedly died during the experiment and were excluded), 27 developed colonic carcinoma with a total of 34 masses. The incidence of carcinogenesis was 71.05% (27/38). Various colonic mucosal lesions are summarized in Table 1. The tumors were 1-13 mm in diameter and more frequently occurred in the distal colon. Most tumors appeared nodular in shape. Six rats developed mesenteric lymph node metastasis, and two developed gastric and pulmonary metastases.

Histopathological changes

T group rats exhibited inflammation and atypical dysplasia of the colonic mucosa at the 10^{th}, 15^{th}, and 18^{th} week (Figures 1 and 2). We observed adenomas and adenocarcinomas after 20 wk with metastasis (Table 2). We never observed dysplasia or tumor development in the control group.

Immunohistochemical staining

P21 and CEA staining of colonic lesions is summarized in Table 3. Normal and inflammatory mucosa were negative for expression. P21 was weakly expressed in adenomas and primarily expressed in dysplasia and carcinoma (Figure 3), while CEA was expressed in dysplasia, adenocarcinoma and metastasis (Figure 4). The difference in P21 and CEA expression between benign and malignant colonic mucosal lesions in DMH-treated rats was statistically significant ($P < 0.05$).

AgNOR count

The AgNOR counts in various colonic mucosa lesions were as follows: adenoma 2.7 ± 0.18, dysplasia 3.2 ± 0.21, and adenocarcinoma 4.6 ± 0.26, and normal epithelial cell 1.45 ± 0.08. In colonic adenocarcinoma, the AgNOR granules not only increased in number, but also became larger and irregular. The AgNOR counts were statistically significantly different between normal mucosa and dysplasia, as well as dysplasia and malignant tissue ($P < 0.05$). However, among the various colonic carcinomas, the differences were not statistically significant ($P > 0.05$).

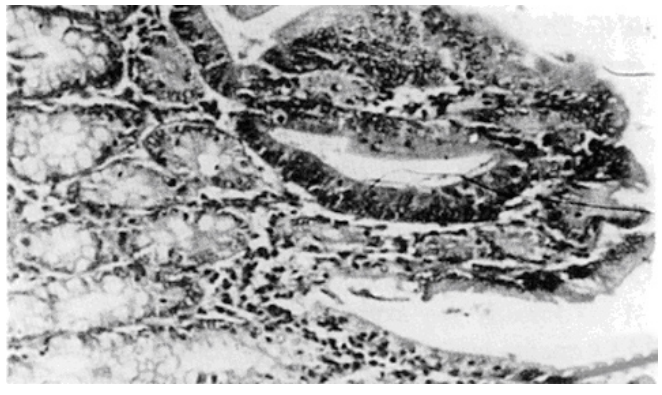

Figure 1 Dimethylhydrazine (DMH)-treated rats, dysplasia localized to the upper 1/3 of the colonic mucosa. HE × 100

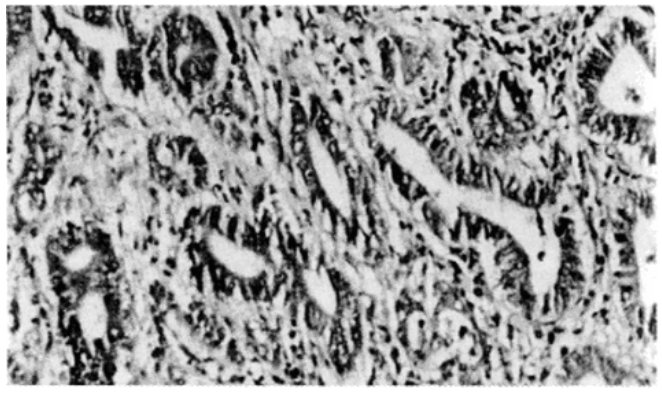

Figure 2 Dimethylhydrazine (DMH)-treated rat, colonic adenocarcinoma. HE × 100

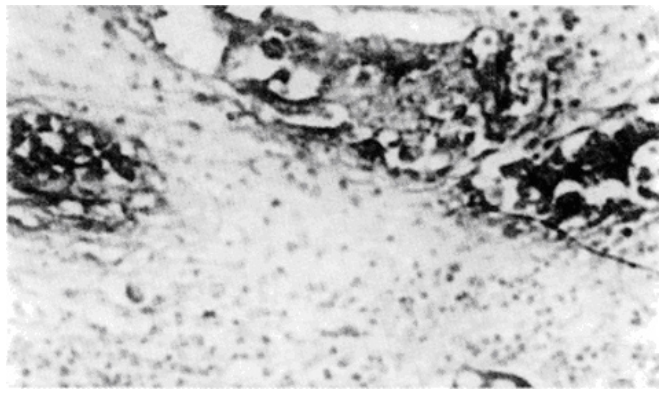

Figure 3 Colonic adenocarcinoma, positive P21 expression in the invasive carcinoma nest. ABC method × 200

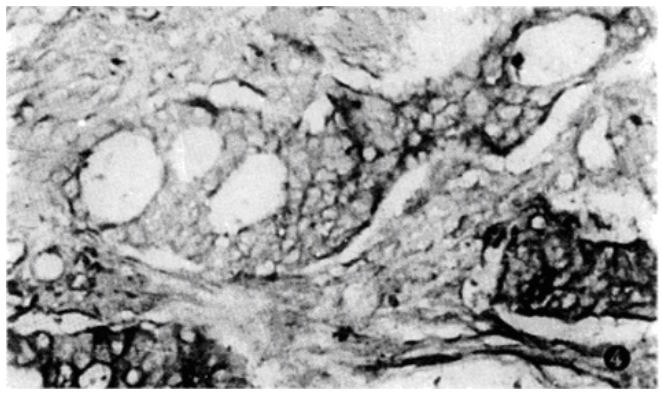

Figure 4 Colonic adenocarcinoma, positive CEA expression in the invasive carcinoma nest. PAP method × 200

DISCUSSION

We treated rats with DMH to induce colonic carcinoma and observed the sequential changes in the colonic mucosa. The marked dysplasia

in the epithelial cells initially involved one or two glands and was localized to the upper 1/3 of the mucosal layer (Figure 1). As the experiment progressed, the number of atypical glands increased, and mucosal noduli formed. Cell heterogeneity also increased, and ultimately led to colonic adenocarcinoma with invasion and metastasis, strongly suggesting that dysplasia is a premalignant lesion for colonic carcinoma.

The activation of oncogenes and P21 overexpression might be critical for malignant cell transformation[4]. P21 can inhibit the intercellular signal transmission, leading to loss of controlled cell mitosis. Therefore, P21 overexpression is an important sign of carcinogenesis[5]. In our study, we found that P21 protein was expressed in both dysplasia and adenocarcinoma. These data suggest that Ras may exert its effects on cell growth as early as the dysplasia stage to further promote cell transformation. The results support the hypothesis that dysplasia is a premalignant lesion of colonic carcinoma; therefore, ras P21 expression could be an early diagnostic indicator for premalignant lesions. CEA expression is another major marker in colonic carcinoma diagnosis. In DMH-induced colon carcinoma, dysplasia presented with P21 overexpression and increased AgNOR counts, while CEA was only expressed in carcinoma and metastasis. In metastasis, the AgNOR granules increased and clustered, which is a typical indicator of the malignant colonic carcinoma phenotype. We suggest that P21 and CEA are two proteins expressed in colon carcinoma at different stages of differentiation. In conclusion, P21 immunohistochemical detection and AgNOR quantification important for clinical screening of colon carcinoma and premalignant lesions.

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Double-bullet radioimmunotargeting therapy in 31 primary liver cancer patients

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Abstract

AIM: To observe the effect of double bullet immunotargeting therapy with chemotherapy and internal radiotherapy on primary liver cancer.

METHODS: The polyclonal horse antibody against human AFP (anti-AFPAb) and the monoclonal murine antibody against human AFP (anti-AFPMcAb) were used as carriers, and ^{131}I and mitomycin C (MMC) were used as warheads to form double bullet, *i.e.* ^{131}I anti-AFPMcAb-MMC (double bullet 1) and ^{131}I anti-AFPAb-MMC (double bullet 2) prepared using the modified chloramine T method. Double bullet targeting therapy was administered by intravenous drip once a month in 31 patients (treatment group) with unresectable primary liver cancer. Among them, 4, 17 and 10 patients were administered 1, 2 and 3 times, and the median radiation dose (MBq/case) was 193.5 ± 37.74; 651.9 ± 232.4, and 992.0 ± 230.5 respectively.

METHODS: Tumor shrinkage, decrease in AFP, and 1 and 2 -year survival rates were significantly higher than the control groups who received transarterial infusion (TAI) or transarterial chemoembolization (TACE) at the same time (50.0%, 15/30 *vs* 30.0%, 9/30, $P < 0.05$; 66.7%, 18/27 *vs* 28.0%, 7/25, $P < 0.01$ and 50.0%, 34.0% *vs* 33.0%, 3.3%, $P < 0.01$, respectively). Furthermore, the tumor progression rate (10%) in the treatment group was significantly lower than that of the control group (40.0%, $P < 0.01$).

CONCLUSION: Double bullet target therapy is more effective than traditional therapies due to the synergistic effects of the antibody, radioisotope, and anticancer agents, which together, enhance tumor killing.

Key words: Liver neoplasms/therapy; Immunotherapy; Alpha fetoproteins; Antibodies, monoclonal

Wu YD, Zhou DN, Gang YQ, Hu XH, Li ZG, Song XQ, He HP, Yang KZ, Huang BY. Double-bullet radioimmunotargeting therapy in 31 primary liver cancer patients. *World J Gastroenterol* 1997; **3(3): 165 Available from: URL: http://** www.wjgnet.com/1007-9327/full/v3/i3/165.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.165

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ORIGINAL RESEARCH

Diagnostic value of occult fecal blood testing for colorectal cancer screening

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Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To evaluate the diagnostic value of occult fecal blood testing in mass colorectal cancer screening.

METHODS: A reverse passive hemagglutination reaction fecal occult blood test (RPHA-FOBT) and colorectal cancer risk factor quantitative method were used as preliminary screening for colorectal cancer. A 60-cm fiber optic colonoscopy was used to validate the preliminary screen and was used to detect colorectal cancer in a community of 75813 subjects.

RESULTS: Compared to the 60-cm fiber optic colonoscopy as a standard reference, FOBT has a sensitivity of 41.9%, specificity of 95.8%, Youden's index of 0.38, and positive predictive value of 0.68%. These results increased with subject age from the first detection. A 3-year follow up in the target mass showed that all new cases had initially been FOBT-negative.

CONCLUSION: The value of FOBT as an indicator of colorectal cancer in mass screening is limited.

Key words: Colorectal neoplasms/diagnosis; Occult blood; Mass screening; Risk factors; Colonoscopy

Chen K, Jiao DA, Zheng S, Zhou L, Yu H, Yuan YC, Yao KY, Ma XY, Zhang Y. Diagnostic value of occult fecal blood testing for colorectal cancer screening. *World J Gastroenterol* 1997; 3(3): 166-168 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/166.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.166

INTRODUCTION

Fecal occult blood testing (FOBT) was first reported by Greegor in 1967 as a useful index in mass colorectal cancer screening[1]. Immunochemical FOBT is currently used worldwide[2-5] to detect early colorectal cancer, but the sensitivity, specificity, and positive predictive value of this method varies greatly with the differences in the selected masses. Reverse passive hemagglutination reaction fecal occult blood testing (RPHA-FOBT) was established by Zhou *et al*[4] in 1987. Since then, this method has been used among patients and colorectal cancer high risk populations with histories of rectal polyps or ulcers. The sensitivity for the two groups was 89% and 64%, respectively, and the positive predictive values were 100% and 1.5%, respectively. These results were statistically significantly different[6]. The value of RPHA-FOBT as a mass screening indicator and its relationship with a 3-year cumulative incidence rate (CIR) of colorectal cancer in a population aged ≥30 years is reported in this study.

MATERIALS AND METHODS

RPHA-FOBT and colorectal cancer risk factor quantitative methods[7] were implemented as a preliminary screening procedure, and a 60-cm fiber optic colonoscopy was performed as an accurate screening from May 1989 to May 1990 in Jianshan County, an area of the highest colorectal cancer incidence rate in China[8]. In this study, 75813 subjects were randomly selected from ten towns in Jianshan County. Of the 62611 subjects tested (82.6%), 43 colorectal cancer cases were identified, with a total detection rate of 68.7/10.5. A total of 70% of the 43 cases were classified as either Dukes A or B, the early stages of colorectal cancer.

The entire population studied was surveyed for colorectal cancer incidence. Fifty-three new cases were identified from May 1990 to May 1992, totaling 96 cases within 3 years from May 1989 to May 1992, making the CIR 153.3 per 10^5 people.

RPHA-FOBT kits were purchased from the Basic Medical Sciences Institute of Zhejiang Medical University[3,4]. Fecal samples were sent to the local hospital by the examiners, smeared on slides, and transferred to the Lab of Cancer Research Institute of Zhejiang

Table 1 Diagnostic value of PRHA-FOBT (passive hemagglutination reaction fecal occult blood testing) by age group

Sex	*n*	Sensitivity (%)	Specificity (%)	J	PV (+)
Men	30177	43.5	95.9	0.39	0.81
Women	32434	40	95.7	0.36	0.56
Total	62611	41.9	95.8	0.38	0.68

Table 2 Diagnostic value of PRHA-FOBT (reversed passive hemagglutination reaction fecal occult blood testing) by age group

Age (yrs)	Sex	Case/mass	Sensitivity (%)	Specificity (%)	PV(+) (%)
30-39	Men	23/30177	43.5	95.9	0.81
	Women	20/32434	40	95.7	0.56
	Total	43/62611	41.9	95.8	0.68
40-49	Men	22/19026	40.9	95.9	1.15
	Women	18/20517	38.9	95.6	0.77
	Total	40/39543	40	95.8	0.94
50-59	Men	19/10891	47.4	95.9	1.96
	Women	15/12497	46.7	95.3	1.18
	Total	34/23388	47.1	95.6	1.52
≥ 60	Men	8/4856	50	95.2	1.68
	Women	9/6219	55.6	94.8	1.53
	Total	17/11075	52.9	95	1.6

Table 3 CIR1 and CIR3 (One-year cumulative incidence rate and three-year cumulative incidence rate) by sex and RPHA-FOBT (reversed passive hemagglutination reaction fecal occult blood testing) Results

Sex	FOBT (+)				FOBT (-)			
	n	CIR1	CIR3	u^1	*n*	CIR1	CIR3	u^1
Men	1236	809.1	809.1	0	28941	44.9	148.6	4.01[b]
Women	1417	564.6	564.6	0	31017	38.7	112.3	3.35[b]
Total	2653	678.5	678.5	0	59958	41.7	130.1	5.55[b]

[b]$P < 0.01$; [1]$u = (|x1 - x2|)/\sqrt{x1 + x2}$.

Medical University.

RESULTS

Diagnostic value of RPHA-FOBT in mass colorectal cancer screening

FOBT sensitivities in this natural community were 43.5% in males and 40.0% and females, but the specificity was over 95%. There was no statistical significance in Youden's index (J) between males and females (0.39 *vs* 0.36, $u = 0.199$, $P > 0.05$, Table 1).

The total positive predictive value (PV) of RPHA-FOBT was 0.68% (Table 1). This indicated that endoscopic screening is too large of a scale for epidemiologists, as only approximately seven subjects among 1000 that screened positive with RPHA-FOBT. There was no statistical significance in the positive predictive value (0.81% *vs* 0.56%, $\chi^2 = 0.583$, $P > 0.05$) between men and women.

Relationship between age and RPHA-FOBT

Colorectal cancer prevalence rates vary with age, and the mass screening results correlated well with the prevalence rate. As the American Association of Cancer has suggested, people over the age of 40 should take the FOBT annually[9]. As shown in Table 2, the diagnostic index increased with the initial age of screening; *i.e.*, the sensitivity rose from 43.5% to 50.0% in men, and from 40.0% to 55.6% in women, and the positive predictive value (PV+) increased by 1%.

Follow-up of the target population

We surveyed 62611 subjects for three years to observe the long-term values of the RPHA-FOBT results for colorectal cancer. We compared the one-year CIR (CIR1) and three-year CIR (CIR3) in FOBT-positive and -negative subjects in females and males. Because FOBT-positive subjects underwent endoscopic screening, there was no change between CIR1 and CIR3 of the FOBT-positive subjects ($u = 0$), while there were statistically significant differences between CIR1 and CIR3 of FOBT-negative females and males. In FOBT-negative males and females, the CIR3 was 2.3 and 1.9 times higher than CIR1, respectively. These results demonstrate that many colorectal cancer patients were misdiagnosed because of their negative FOBT results (Table 3), and the false negative rate was very high.

DISCUSSION

We used RPHA-FOBT and the colorectal cancer risk factor quantitative method as preliminary screening procedures, and a 60-cm fiber optic colonoscopy, which could reach the splenocolic curve, as the accuracy screening procedure in FOBT-positive subjects and the high risk population indicated by the quantitative method. We determined the FOBT diagnostic values by comparing the results from FOBT to the results of the 60-cm fiberoptic colonoscopy. It was previously reported[10] that 82% of 3147 Chinese colorectal cancer patients had their cancer initiate in the colon below the splenocolic curve, suggesting that approximately 20% of cases cannot be identified by this method. However, because of its ease of use and simple preparation, it is still considered a relatively reliable and practical method for reference standard. We examined more than 3000 subjects by 60-cm fiberoptic colonoscopy in our study, and no new cases were identified during a 3-year follow up.

There have not been any similar reports in China about the application of RPHA-FOBT and its value in mass screening. In some studies abroad[2,5,6,11], the sensitivity of immunochemical FOBT ranged from 33% to 50%, which was similar to our results (Table 1). However, our results differed from the previous studies among a special population using the same materials and methods[3,4], suggesting that more than 50% of cases in a normal population cannot be detected using FOBT. The specificity of RPHA-FOBT is over 95%, but when estimated together with other diagnostic indexes, such as the Youden's index (0.39 in males, 0.36 in females in this study), its practical value for screening colorectal cancer was limited.

PV(+), the percentage of patients among FOBT-positive subjects, is another useful index to estimate the diagnostic value of FOBT. Hardcastle *et al*[12] identified 618 FOBT-positive subjects in 27651 individuals, among which 65 subjects were colorectal cancer patients, giving a PV(+) of 10.5%, which is higher than results (PV(+) 4.8%) reported by Kewenter *et al*[13]. The PV(+) reported by Gregorio *et al*[6] was 7.5%, while the PV(+) in our study (0.68%), which was significantly lower than previous reports.

As Gregorio *et al*[6] reported, the PV(+)s of FOBT were 3% and 9%, respectively, in population less than or more than 60 years old. The difference was significant, but the difference in PV(+) between sexes was not significant (male 7%, female 10%), similar to our results (Table 2). Results in our study suggested that the older the initial age of screening, the higher the rate of PV(+). The PV(+) in our study was lower than in previous other reports. The PV(+) would increase if we defined older initial ages of the screened population to reduce the workload in the screening.

The long-term value of FOBT as a colorectal cancer diagnostic index is not perfect. The CIR1 and CIR3 values were similar in FOBT-positive male and female subjects, but compared to CIR1, the CIR3 in FOBT-negative males and females was approximately 3.3 and 2.9 times higher ($P < 0.01$). The results in Table 3 show that these FOBT-negative subjects should also be monitored to quickly identify and treat new cases.

In summary, when used alone, FOBT is not a satisfactory indicator in mass colorectal cancer screening. Therefore, it is necessary to search for and develop new testing methods, or "concentrate" the target mass. We recommend that the initial age of screening should serve as an important factor in mass colorectal cancer screening.

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Difference between periportal and pericentral Kupffer cells in lipopolysaccharide uptake in rats

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Abstract

AIM: To reveal the difference in the ability of Kupffer cells in the periportal and pericentral regions of the liver to uptake lipopolysaccharides (LPS) injected into the portal vein.

METHODS: Male Wistar rats were divided into two groups: normal control group (n = 6) and $GdCl_3$-treated group (n = 8). Sixteen hours before the experiment, rats in the $GdCl_3$-treated group were injected with $GdCl_3$ *via* the tail vein to eliminate Kupffer cell function specifically in the periportal region. LPS at a dose of 20 μg/100 g body weight was injected into rats of both groups *via* the portal vein. Zero, 2, 5, 10, 30, and 60 min after LPS injection, liver samples were obtained and the distribution of LPS in Kupffer cells was observed by immunofluorescence imaging of monoclonal antibody-specific LPS staining using a confocal laser scanning microscope.

RESULTS: In the normal control group, positive reactions to LPS were found in Kupffer cells in the periportal region with the peak at two minutes after LPS injection. Kupffer cells in the pericentral region showed the peak at five minutes after LPS injection, but its fluorescent intensity to LPS at the peak time in the cytoplasm was significantly lower than that of Kupffer cells in the pericentral region. In the $GdCl_3$-treated group, Kupffer cells in the pericentral region showed the peak at two minutes following LPS injection, and the LPS fluorescent intensity showed no significant difference from that of the normal control rats at the peak point. No significant changes of LPS fluorescent intensities were found in Kupffer cells in the periportal region at various time points following LPS injection in $GdCl_3$-treated rats.

CONCLUSION: Kupffer cells in the periportal and pericentral regions showed differences in LPS uptake *via* the portal vein.

Key words: Liver/metabolism; Kupffer cells; Lipopolysaccharides/metabolism; Portal vein; Endotoxins; *Escherichia coli*

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ORIGINAL RESEARCH

Effects of metoclopramide on gastrointestinal myoelectric activity in rats

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Abstract

AIM: To investigate the effects of metoclopramide (MCP) action on myoelectric activity in the antrum and small intestine.

METHODS: Ten healthy male Wistar rats, weighing 250-350 g, were anesthetized with ketamine hydrochloride (100 mg/kg, intramuscularly). Four pairs of bipolar stainless steel electrodes 3 mm apart were implanted on the serosal surface of the antrum at one, 10 and 20 cm distal to the pylorus. Five to ten days following the operation, the gastrointestinal myoelectric activity of fasted rats after intramuscular injection of 2.5, six and 12 mg/kg MCP was recorded using a 8-channel EEG machine, and these values were quantitatively compared with the myoelectric activity after saline injection.

RESULTS: In fasted rats, 2.5 mg/kg MCP increased the amplitude of spike activity (402.0 ± 138.4 μV, *vs* 345 ± 163.4 μV, $P < 0.05$) and the percentage of the slow wave-containing spike bursts (60.4% ± 22.0% *vs* 47.4% ± 22.5%, $P < 0.01$) of small intestine (1 cm distal to the pylorus), but did not affect the myoelectric activity of the antrum. Six and 12 mg/kg MCP increased the amplitude of both the slow wave (332.8 ± 200.1 μV *vs* 191.2 ± 143.9 μV, $P < 0.01$; 330.0 ± 197.1 μV *vs* 191.2 ± 143.9 μV, $P < 0.05$) and the spike activity of the antrum (180.5 ± 69.7 μV vs 121.8 ± 63.3 μV, $P < 0.05$; 174.5 ± 71.7 μ V *vs* 123.8 ± 63.3 μV, $P < 0.05$), while in small intestine (1 cm distal to the pylorus) only the amplitude of spike activity (407.3 ± 179.0 μV *vs* 345.0 ± 163.4 μV, $P < 0.05$; 456.0 ± 145.4 μV *vs* 345.0 ± 163.4 μV, $P < 0.05$) and the percentage of the slow wave containing spike bursts (61.7% ± 26.5% *vs* 47.4% ± 22.5%, $P < 0.01$; 59.1% ± 17.3% *vs* 47.4% ± 22.5%, $P < 0.01$) was increased and the latent period significantly prolonged (2.5 ± 0.35 min *vs* 0.77 ± 0.18 min, $P < 0.01$).

CONCLUSION: Different mechanisms may be involved in enhancing the myoelectric activity of the antrum and small intestine following MCP administration.

Key words: Metoclopramide; Stomach; Intestine; Myoelectric activity

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INTRODUCTION

Metoclopramide (MCP) improves gastroduodenal coordination[1], relieves postsurgical and diabetic gastroparesis[2,3], and increases the spike activity during migrating motor complexes (MMC) of small intestine without disrupting the fasting pattern in dogs[4]. While these studies suggest that MCP complicates pharmacological action, few have studied the effects of MCP on gastrointestinal myoelectric activity. It is important to compare how different doses impact MCP's effect on the myoelectric activity of the antrum and small intestine. Such studies might be useful both for investigating the side effects of MCP and for further understanding the mechanisms controlling gastrointestinal motility.

MATERIALS AND METHODS

Ten healthy male Wistar rats, weighing 250-350 g, were anesthetized with ketamine hydrochloride (100 mg/kg, intramuscularly). Four pairs of bipolar stainless steel electrodes (3 mm apart) were implanted onto the serosal surface of the gastrointestinal tract. The first pair was placed on the antrum 0.5 cm from the pylorus and the others on the small intestine at one, 10 and 20 cm distal to the pylorus. The free ends of the electrodes were moved subcutaneously to the back of the neck. Recordings began five to ten days after operation. Electric activity was registered with a 8-channel EEG machine (ND-82B, Shanghai) with a time constant of 0.1 s and a high cutoff frequency of 30 Hz.

In the first, second and third series of experiments, MCP (The Seventh Pharmaceutical Factory of Wuxi) doses were 2.5, six and 12 mg/kg. Each experiment lasted one week and the myoelectric activities were recorded every other day.

The frequency and amplitude of the slow wave and spike activity from the antrum and small intestine were recorded and analyzed 30min following MCP administration, and the number of slow wave-containing spike bursts was also quantified. The results were represented as mean ± SD and analyzed statistically by Student's *t*

Table 1 Effects of metoclopramide on myoelectric activity in the antrum of rats (x- ± s)

	Slow wave		Spike activity
	Frequency (num/min)	Amplitude (V)	Amplitude (V)
Control	4.6 ± 0.3	191.2 ± 143.9	121.8 ± 63.3
2.3 mg/kg	4.5 ± 0.3	192.4 ± 140.1	128.8 ± 54.9
6 mg/kg	4.6 ± 0.3	332.8 ± 200.1[b]	180.5 ± 69.7[a]
12 mg/kg	4.5 ± 0.2	330.0 ± 197.1[b]	174.5 ± 71.7[a]

Values represent mean ± SD (n = 8); [a]P < 0.05, [b]P < 0.01 *vs* control.

Table 2 Effects of metoclopramide on myoelectric activity in the small intestine of rats (x- ± s)

Sit	Groups	Slow wave		Spike activity	
		Frequency (num/min)	Amplitude (V)	Amplitude (V)	Spike bursts (%)
1 cm	Control	38.2 ± 1.4	172.0 ± 101.9	345.0 ± 163.4	47.4 ± 22.5
	2.5 mg/kg	28.2 ± 1.6	175.0 ± 81.9	402.0 ± 138.4[a]	60.4 ± 22.0[b]
	6 mg/kg	38.0 ± 1.3	173.7 ± 110.1	407.3 ± 179.0[a]	61.7 ± 26.5[b]
	12 mg/kg	37.6 ± 1.1	179.0 ± 116.4	456.0 ± 145.4[b]	59.1 ± 17.3[b]
10 cm	Control	37.3 ± 1.4	201.2 ± 110.1	335.8 ± 138.6	38.1 ± 12.9
	2.5 mg/kg	36.9 ± 1.6	201.7 ± 113.3	383.6 ± 150.5	55.4 ± 16.1[b]
	6 mg/kg	37.0 ± 1.2	210.7 ± 105.2	412.5 ± 158.8[b]	54.3 ± 25.1[b]
	12 mg/kg	37.2 ± 1.3	202.5 ± 103.8	452.6 ± 144.3[b]	58.8 ± 15.6[b]
20 cm	Control	35.9 ± 1.1	153.2 ± 94.7	322.0 ± 93.1	37.9 ± 14.5
	2.5 mg/kg	36.6 ± 1.2	149.1 ± 69.1	338.2 ± 162.7	46.3 ± 12.1[a]
	6 mg/kg	36.2 ± 1.2	150.2 ± 50.2	356.4 ± 117.9	60.8 ± 22.6[e]
	12 mg/kg	35.6 ± 1.4	152.9 ± 73.4	347.3 ± 159.6	59.7 ± 24.2[b]

Values represent mean ± SD (n = 28); [a]P < 0.05, [b]P < 0.01, [e]P < 0.001 *vs* control.

test, with P < 0.05 considered significant.

RESULTS

After control recordings following saline injection were obtained at 40 min and 60 min, rats that were fasted for 16-18 h were injected intramuscularly with MCP. The lowest dose of 2.5 mg/kg MCP significantly increased the amplitude of spike activity and the percentage of slow wave-containing spike bursts in the small intestine, however did not affect the myoelectric activity in the gastric antrum (Tables 1 and 2). Nevertheless, the intermediate dose of 6 mg/kg and the highest dose of 12 mg/kg increased the amplitude of spike activity and the percentage of slow wave-containing spike bursts in the small intestine, while also raising the amplitude of both the slow wave and spike activities in the antrum. MCP did not affect the amplitude of the slow wave at any of the four electrode sites (Tables 1 and 2). MCP effects on the electric activity in the small intestine were dose-independent (Table 2, Figure 1).

The latency times of MCP's effect on the gastric antrum *vs* the small intestine were significantly different, with 0.7 ± 0.18 min in the antrum and 2.5 ± 0.35 min in the small intestine at 1 cm distal to the pylorus.

DISCUSSION

Gastrointestinal myoelectric activities are divided into slow wave and spike activity (fast wave). Slow wave pace and direct gastric contraction. Thus, the alteration of slow wave would influence the mechanical contraction[4]. The spike activity can improve gastrointestinal motility[5]. The gastrointestinal myoelectric activity is a sensitivity index of gastrointestinal motility.

In this study, we find that MCP enhances gastrointestinal myoelectric activity, which is consistent with results reported by previous investigations[6]. Our results, however, also show significant differences in drug responses between the antrum and small intestine. Firstly, the drug only increased the spike activity in the small intestine, while both the slow wave and the spike activity in the antrum increased. Secondly, MCP's effect on the myoelectric activity of the small intestine was not dose-dependent, as reported by 6 Wingate, *et al*[6] and 7 Achem-Karam, *et al*[7] in dogs. Nevertheless, the minimal effective doses (or threshold dose) of this drug varied between the antrum and small intestine. For example, a dosage as small as 2.5 mg/kg increased myoelectric activity in only the small intestine, but did not have any effect on the antrum. Thirdly, the latentcy periods of this drug between the antrum and small intestine were markedly different (*i.e.* 0.70 ± 0.18 min *vs* 2.50 ± 0.35 min, respectively). These results suggest that the biochemical control of motor activity is not consistent along the gastrointestinal tract. MCP, an analogue of procainamide[8,9], could directly act on the smooth muscle cholinergic M receptor, while also causing acetylcholine release from the postganlionic cholinergic nerve endings[10], as well as increased myoelectric activity in the antrum. However, this increased spiking activity in the small intestine may not be the result of direct action, as other mechanisms are also likely to be involved.

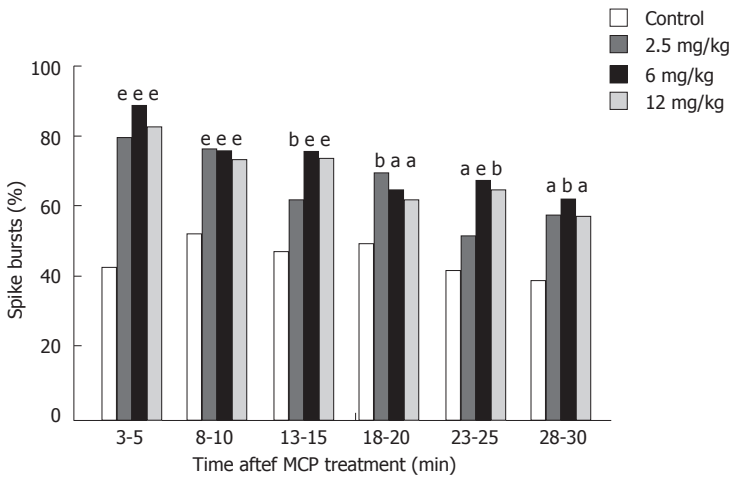

Figure 1 Dose-dependent effects of MCP (metoclopramide) on the percentage of slow wave-containing spike bursts at 1 cm distal to the pylorus of the small intestine. Note the significant increase in spike bursts induced by MCP. [a]P < 0.05, [b]P < 0.01, [e]P < 0.001 *vs* control.

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ORIGINAL RESEARCH

Characteristics of upper digestive tract diseases in Bohai Bay fishermen

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Abstract

AIM: To study the characteristics of upper digestive tract diseases (UDTDs) in fishermen who live in Bohai Bay.

METHODS: An investigation was carried out in 1488 fishermen with symptoms of UDTDs (aside from liver, biliary and pancreatic diseases) during the time period between December 1991 and February 1995. This investigation included medical history evaluations, physical, gastroscopic and pathological examinations, tests for *Helicobacter pylori* (*H. pylori*) infection, and analysis of the nitrate content in their drinking water.

RESULTS: Among the 1488 subjects investigated, 1467 suffered from one or more of the 14 UDTD diseases, most of which were chronic atrophic gastritis (CAG, 1103 cases), peptic ulcers (268 cases), and cancer of the upper digestive tract (25 cases).

CONCLUSION: The incidence rate of UDTDs tends to be high among fishermen due to their particular living habits, the high nitrate content of their drinking water, *etc*. In addition, the clinical manifestations of UDTDs in fishermen are significantly different from those of the inland residents.

Key words: Digestive tract disease; Gastroscopy; nitrate; *Helicobacter pylori*; Gastritis, atrophic; Peptic ulcer; Digestive system neoplasms

Wang YB, Wang YP, Zou J, Bai BJ, Ren GC, Cai BQ. Characteristics of upper digestive tract diseases in Bohai Bay fishermen. *World J Gastroenterol* 1997; 3(3): 171-173 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/171.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.171

INTRODUCTION

Owing to their special living environment and habits, the incidence and clinical manifestations of the UDTDs found in fishermen may be different from those of inland residents. To gain a clear understanding of the characteristics that are specific to the UDTDs in fishermen, an investigation was carried out on fishermen and their family members from a fishing village near Bo hai Bay, Qikou town, Hebei Province, from December 1991 to February 1995. These studies will provide important insight into developing improved methods for disease prevention and treatment.

MATERIALS AND METHODS

Subjects

1488 subjects who required medication for the symptoms of upper digestive tract diseases were recruited for the investigation, which accounted for 6.62% of the 22479 individuals in the town (the annual mean population), and 2.08% of the 71479 individuals who had visits to the doctor due to upper digestive tract symptoms over the three years. There were 830 men and 658 women ranging in age from 15 to 78 years who were involved in this study, with 879 patients aged 21-40 years, accounting for 59.07% of the total number of cases. The ratio of men to women was 1.26 to 1. Patients with liver, biliary or pancreatic diseases were excluded from the study. 1430 cases had a detailed record of disease course, which ranged from one month to 30 years, however the majority of cases (1108, 77.48%) covered less than five years. The primary signs and symptoms included slight epigastric pain that was either regular or irregular in 1039 (72.65%) cases, heartburn or acid regurgitation in 801 (56.01%), anorexia in 727 (50.83%), epigastric distension after meals in 563 (39.37%), epigastric tenderness in 883 (61.74%) and other rare symptoms such as belching, nausea and/or vomiting, hematemesis and melena.

Methods

A physical examination as well as a detailed inquiry into each patient's medical history was carried out. All patients who were suspected of having liver, biliary or pancreatic disease received liver function tests and B-mode ultrasonographies. 1488 patients without any liver, biliary or pancreatic diseases were enrolled into the study and underwent complete examinations of the esophagus and stomach using GIF-XQ 20 gastroscopy. The gastroscope reached to the duodenal papilla and beyond in 1463 patients, however failed to pass through the obstructed site in 25 patients with cancer. Photographs of recurring phenotypes were taken for 131 cases. Gastritis was diagnosed based on the Criteria and Schemes of Chronic Gastritis by Fibroscopy constructed by the National Seminar on Fibroscopy in 1978. Peptic ulcer, on the other hand, was diagnosed based on the diagnostic and staging criteria proposed by Sakia T, and other diseases were diagnosed based on the corresponding criteria in China. Biopsy specimens were obtained from 503 patients at two to four random sites and

subjected to pathological examination. Subsequent diagnoses were made according to the Pathologic Diagnostic Criteria for Abnormal Gastric Mucosa proposed by the National Conference on Pathology in Zhengzhou in 1978. Investigations into these patient's life habits included smoking history, alcohol consumption, eating habits (consuming very sweet or salty foods), ingesting non-steroid anti-inflammatory agents and eating schedules. The nitrate content of the resident's drinking water was measured by the Tianjin Detection Center for Environment Protection, and the shrimp pastes were cultured for fungus. In the late stage cancers, biopsy specimens were acquired from 171 patients to test for *H. pylori* using the rapid urease test.

RESULTS

Gastroscopy

Of the 1488 patients undergoing gastroscopy, 21 showed no obvious signs of abnormalities. The remaining 1467 cases were diagnosed as simple atrophic gastritis (692, 47.17%), simple atrophic gastritis (163, 11.11%), simple bulbar duodenitis (232, 15.82%), peptic ulcer (268, 18.26%), esophagus cardia gastric fundus peptic ulcer cancer (15, 1.02%), gastric cancer (10, 0.68%), or other diseases (87, 5.93%). These non-cancer diagnoses included reflux esophagitis, esophagus and stomach polyps, diverticulum of the esophagus, stomach or duodenum, inflammation of the stomach's cardiac region, achalasia, hiatus hernia, gastrolithiasis, acute gastric mucosal disease, and gastroduodenal ascariasis. Fourteen different diseases types were detected by gastroscopy. There were 1103 (75.18%) cases of CAG found in this study, including 411 cases of atrophic gastritis that accompanied by other diseases, 268 cases of peptic ulcers (including 91 gastric ulcers), 31 pyloric ulcers, 117 duodenal bulbar ulcers and 29 postbulbar ulcers. Among them, 60 patients had complex ulcers, including multiple active gastric ulcers and duodenal ulcers. Kissing ulcers were also seen frequently. In this series, 625 (42.60%) were identified as having two to five different kinds of disease by gastroscopy.

Pathological examination

Biopsy specimens from 503 cases were pathologically examined; 121 were diagnosed as superficial gastritis, peptic ulcers or cancers of the upper digestive tract, and 382 were identified as atrophic gastritis. The latter included 152 mild (39.79%), 216 moderate (56.54%), and 14 severe (3.66%) cases, with the moderate and severe CAG (230) making up 60.20% of the total cases of atrophic gastritis. Four patients were under 20 years old, of whom one had mild and three had moderate atrophic gastritis.

Living habits

The medical histories of the 1414 patients revealed that 836 (59.12%) used to smoke 20 cigarettes per day for over one year, 476 (33.66%) drank 150 g or more of spirits per day for over one year, 746 (52.75%) consumed pungent and irritant foods regularly, and 404 (28.57%) drank sugar-water on a frequent basis. All patients ate dried salt fish and shrimp paste almost daily. More than half of the patients had extremely irregular dining habits owing to their sea-based work. Only a small minority of patients ingested non-steroid anti-inflammatory agents. All patients had more than three types of the life-habits specified above. The nitrate content in the drinking water was 400 g/L (the permissible content is less than 250 g/L). Additionally, rugose colony of candida was observed in the shrimp paste cultures.

Detection of H. pylori

One hundred and seventy-one patients tested positive for *H. pylori* using the rapid urease test, of which 112 (65.48%) were positive and 59 negative (34.50%).

DISCUSSION

UDTDs are common diseases with complicated etiologies, however their incidence rates have not been reported to date. The incidence of UDTSs in the population under investigation was as high as 98.59%, however this does not represent the true rates of UDTD detection and incidence in fishermen because they make up only 6.62% of the local population. Nevertheless, our results may suggest a potential high USTD incidence rate among the fishermen in Bohai Bay. The main categories of UDTDs detected in this population included CAG, peptic ulcers, cancer of the esophagus-cardia-gastric fundus and gastric cancer. The manifestations of these three diseases were not in agreement with those previously reported in the Chinese literature. According to the data reported by Li SN, the detection rate of atrophic gastritis was 6.58% in a 15-25 year old group studied and 34.62% in a separate group ages 65 years and older in regions with high incidences of gastric cancer, while 0% and 6.90% rates, respectively, were found in low incidence regions. In comparison, the reported detection rate in China ranged from 3.20% to 34.62%. In this study, the detection rate of CAG was 74.12% using gastroscopy and 75.94% *via* pathological examination, which are markedly higher values than those mentioned above. This suggests that the incidence of CAG was significantly higher in Chinese fishermen than in the inland residents ($\chi^2 > 6.63$, $P < 0.01$). Up until now, there have been no reports on the degree of atrophy in UTDT. Our results show that the mild, moderate and severe atrophies of the gastric mucosa were 39.79%, 56.54% and 3.66%, respectively, in the 382 cases diagnosed by pathological examination. The moderate and severe atrophies, however were increased up to 60.20%. Furthermore, there were three moderate atrophic cases in the four CAG patients below 20 years of age. It is clear that CAG tends to occur in younger individuals and is significantly more severe in fishermen than in inland residents. In addition, rough gastric mucosa with obvious protrusions, pitting, bleeding, erosion, cobblestone-like morphologies and pseudohypertrophy were often seen in fisherman patients with moderate to severe, but rarely found in inland patients. The incidence of peptic ulcers was reported to be 10%[2]. Huang XQ and Qian AC[3] concluded that approximately 10% of the Chinese population suffered from peptic ulcers, with the ratio of male to female ranging from 2:1 to 7.4:1[2,4]. Among peptic ulcers, 70%-80% were duodenal ulcers[5], 17.5%-20% were gastric ulcers, 5% were complex ulcers, and among the duodenal ulcers 5% were postbulbar ulcers[5]. In this series of patients, the ratio of men to women was 1.26:1, and there were 43.65% duodenal bulbar ulcers, 19.86% postbulbar ulcers, 33.95% gastric ulcers and 22.38% complex ulcers. In comparison with values reported in the literature, there were relatively higher detection rates of gastric, postbulbar, and complex ulcers and lower detection rates of duodenal bulbar ulcers ($\chi^2 > 6.63$, $P < 0.01$). In addition, pyloric ulcers and multiple ulcers were also more common in fishermen *vs* inland residents. Notably, both esophageal and gastric carcinomas are common in China. For example, there are 19 counties and towns in China where the standardized mortality rates from esophageal cancer are over 100/100000; the mortality rates of gastric cancer is 20.95/100000 for males and 10.16/100000 for females[5]. The fishermen in this study accounted for 1.02% and 0.68% of the male and female population, respectively, therefore suggesting that Qikou Town has a high incidence rate of esophageal and gastric cancers.

The characteristics of UDTDs found in Bohai Bay fishermen could be related to the several factors. Firstly, coastal residents often consume sodium-rich food such as salt fish and shrimp paste. Long-term high sodium intake can lead to increased osmotic pressure in both the stomach and duodenum and stimulate osmotic pressure receptors on the duodenal wall. As a result, gastric emptying time is prolonged leading to the build up of noxious substances from consumed food in the stomach. This increased stimulation to gastric mucosa causes severe injury, ultimately resulting in chronic gastritis. In addition, nitrates in food are often converted to nitrites by the activity of bacterium reductases, which increase gastric cancer risk. Additionally, excessive long-term smoking can stimulate gastric acid secretion, causing incompetence of the pyloric sphincter, reflux of both bile and duodenal juice into the stomach, elevation of pepsinogen levels in serum, decrease of bicarbonate levels in pancreatic juice and a decrease of prostaglandin E2 levels in serum. The increase in aggressive factors and decrease in defensive

factors could lead to injury of the gastric and duodenal mucosa, resulting in inflammation and the generation of peptic ulcers in both the stomach and duodenum. Another possible reason why UDTD incidence is higher in fishermen than in inland residents is the nitrate content of their drinking water, which was almost twice the level set by the state. Long-term ingestion of nitrate-rich water could lead to accumulation of nitrate in the body. Nitrate is a main source of nitrosamine and nitrosylamine, which are both carcinogenic substances. High nitrate content in food may therefore be an important cause of esophageal and gastric cancers. Furthermore, consuming food that is either too hot or too sweet, in addition to drinking spirits, not only enhances aggressive factors that cause injury to gastric mucosa, but also weaken the defensive factors in the stomach, ultimately resulting in an imbalance of the two factors and the onset of gastritis and gastric ulcers.

Most experts believe that Hp infection is related to the occurrence and progress of both gastritis and peptic ulcers, however its relationship to gastric cancer requires further study.

The candida in shrimp paste is an opportunistic pathogenetic fungus, but it does not cause damage to the gastric mucosa under normal conditions. However if the gastric internal environment changes due to the previously mentioned four factors, candida may act as a pathogen by contributing to UDTDs, which were more prevalent in fishermen compared with inland residents.

It has been suggested that the successful prevention and treatment of UDTDs in fishermen depends upon improving both their social environment and living habits, in addition to pharmacotherapy - lowering nitrate content of their drinking water, minimizing salt fish and shrimp paste consumption, giving up smoking and alcohol, and eating foods with minimal irritants.

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ORIGINAL RESEARCH

Relation between bile acids and myocardial damage in obstructive jaundice

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Abstract

AIM: To investigate the morphologic changes of the myocardium and its relationship to serum bile acids in obstructive jaundice.

METHODS: Part 1: 35 rats were randomly assigned to three groups: Group Ⅰ (BDL1, *n* = 11), the common bile duct (CBD) was ligated and severed and mice were then sacrificed after one week. Group Ⅰ (BDL2, *n* = 11), the CBD was ligated and severed and mice were then killed after two weeks. Group Ⅰ (SO, *n* = 13), the CBD was isolated. Hearts were collected for morphologic studies and blood was taken to determine the total serum bile acids (TAB). Part 2: 13 rats received gastric intubation of 10% 4 mL/kg sodium cholate. Their serum TBA and the heart's morphologic changes were then examined.

RESULTS: One to two weeks after the CBD was ligated and severed, damage was evident in the mitochondria within the myocardium and the serum TBA was significantly increased. When rats were administered sodium cholate to make their peak blood concentration mimic the average blood concentration in BDL2, a similar degree of myocardial damage was observed.

CONCLUSION: An increase in endogenous bile acids is one causative factor of myocardial damage in obstructive jaundice.

Key words: Cholestasis; Bile acids and salts; Jaundice; Myocardium/pathology; Mitochondria, heart

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INTRODUCTION

Patients who suffer from jaundice due to extrahepatic biliary obstruction experience high rates of both postoperative complications and death. According to a recent research review, surgery to treat obstructive jaundice has a 10%-25% mortality rate as well as a 56% rate of major complications[1]. It is generally accepted that both cardiovascular instability and predisposition to hypotention and shock are main some of the primary causes for the development of postoperative life-threatening complications such as renal failure. Nevertheless, the mechanism of these postoperative complications remains unclear.

Previous studies on isolated cholemia demonstrated that cholemia might lead to cardiac depression. This result suggested that myocardiac dysfunction causes circulatory failure in jaundice patients[2,3]. The aim of this study is to further investigate whether there is morphologic myocardial damage. Since serum bile acids have detergent properties and are elevated in obstructive jaundice, this study also investigates the relationship between bile acids and myocardial damage.

MATERIALS AND METHODS

Part Ⅰ: 35 male Spraque-Dawley (SD) rats weighing 200-250 g were randomly divided into three groups: group Ⅰ (BDL1, *n* = 11), the common bile duct (CBD) was ligated and severed, and rats were sacrificed after one week: group Ⅱ (BDL2, *n* = 11), the CBD was also ligated and severed, however the rats were sacrificed after two weeks: group Ⅲ (SO, *n* = 13), the CBD was isolated and the rats were sacrificed after one week. When the rats were sacrificed, heart specimens were collected for both light and electron microscopic examination, and blood was taken to determine the total bile acid (TBA) and to perform the biochemical liver function test.

Part Ⅱ: 13 male SD rats weighing 200-250 g received gastric intubation of 10% 4 mL/kg sodium cholate (Sigma, USA). The changes in both their serum TBA and myocardial morphologies were

Table 1 Changes in serum total bile acid (TBA) and biochemical liver function test

	SO group	BDL1 group	BDL2 group
TBA(μmol/L)	60.83 ± 14.8	243.12 ± 129.72[a]	355.30 ± 238.38[ac]
TB(μmol/L)	5.37 ± 3.16	108.24 ± 38.33[a]	80.03 ± 37.62[a]
DB(μmol/L)	1.71 ± 1.20	76.19 ± 26.43[a]	57.57 ± 33.45[a]
AKP(u/L)	29.27 ± 8.96	51.49 ± 15.05[a]	55.21 ± 18.94[a]
γ-GT(u/L)	5.77 ± 4.32	31.43 ± 20.15[a]	31.00 ± 23.87[a]
ALT(u/L)	49.38 ± 24.53	235.57 ± 235.36	153.22 ± 148.39[a]
AST(u/L)	209.46 ± 104.47	1112.86 ± 1138.88[a]	622.33 ± 536.56[a]

[a]$P < 0.05$, significant difference from sham value; [c]$P < 0.05$, significant difference from BDL1 group. TB: Total bilirubin; DB: Direct bilirubin; AKP: Alkaline phosphatase; γ-GT: γ-glutanyl transpeptidase; ALT: Alanine transaminase; AST: Asparatate transaminase.

examined one, two, three, four, and 24 h later.

Specimens for light microscopic studies were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E). Specimens for electron microscopic studies were double fixed in osmic acid and glutaraldehyde, stained with uranyl acetate, embedded in EPON-812 epoxy resin, and observed under a Philip EM 412 electron microscope.

Total bile acid (TBA) was determined enzymatically using 3-α hydroxysteroid dehydrogenase.

All results represent the mean ± SD. Means were compared using the Student's *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

Part Ⅰ: Rats subjected to BDL ligation and severing (BDL groups 1 and 2) had significantly higher serum TB and TAB than the sham operated (SO) group (Table 1).

Morphological studies using light microscopy showed that vascular dilation was only evident within the myocardial interstitium for both BDL groups 1 and 2. Using electron microscopy, however, the myocardial mitochondria appeared swollen, decreased and distorted both both groups, with cristae and outer membranes that either looked obscured or had completely disappeared. Nevertheless, the changes seen in the BDL2 group were more obvious than those in the BDL1 group (Figures 1-3).

Part Ⅱ: The serum TBA one, two, three, four, or 24 h following sodium cholate ingestion were 47.7, 132.8, 332.7, 115.0 and 64.3 μmol/L, respectively. The mitochondria in the myocardium appeared swollen and decreased, with cristae and outer membranes that either looked indistinct or had disappeared (Figure 4).

DISCUSSION

Cardiovascular predisposition and instability to both shock and hypotension have been reported in obstructive jaundice patients. Central and peripheral mechanisms seem to be responsible for increased susceptibility to shock and decreased vascular responsiveness. Recent studies on animal models of isolated cholemia that were induced using choledochocaval anastomosis demonstrated that the mean left ventricular time (LVET) decreased, whereas the mean preejection period (PEP) and mean PEP/LVET were increased. These results suggested that the performance of the left ventricle in isolated cholemia was impaired[2,3]. However, the component of bile that was responsible for this impaired performance remained unclear. It also was unclear whether bile caused any morphological changes within the myocardium.

Bile acids are the chief constituents of bile. Due to its mild detergent properties, it may prove cytotoxic at certain concentrations[4,5]. Since serum bile acids are elevated in obstructive jaundice, it is important to investigate the relationship between bile acids and the myocardial damage characteristic of obstructive jaundice.

This study demonstrates that 1-2 wk after the CBD was ligated and severed, the serum TBA raises significantly and the myocardial mitochondria decrease in number and swell, exhibiting cristae that appear distorted, curled or entirely dissolved. When rats ingested sodium cholate until their peak blood concentration

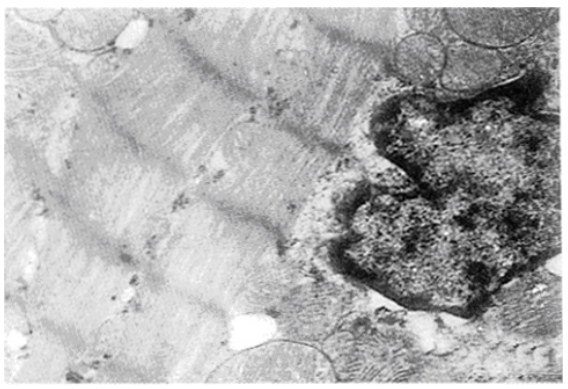

Figure 1 Electron micrograph of rat myocardium from the sham operated (SO) group (× 14000).

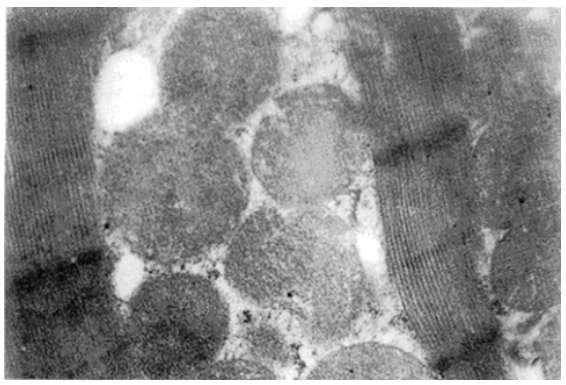

Figure 2 Electron micrograph of rat myocardium from the BDL1 group. The mitochondria appear swollen, with indistinct outer membranes and obscured cristae (× 21000).

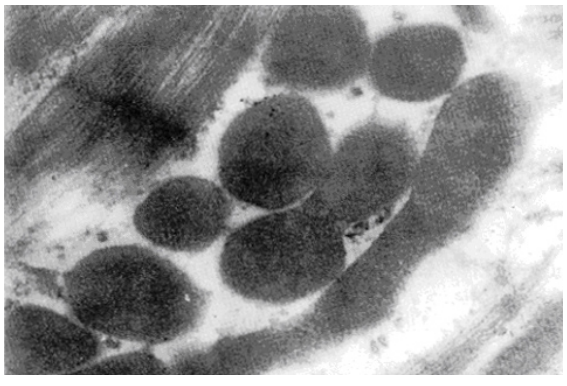

Figure 3 Electron micrograph of rat myocardium from the BDL2 group. Mitochondria appear distorted and curdled, with indistinct outer membranes and cristae (× 21000).

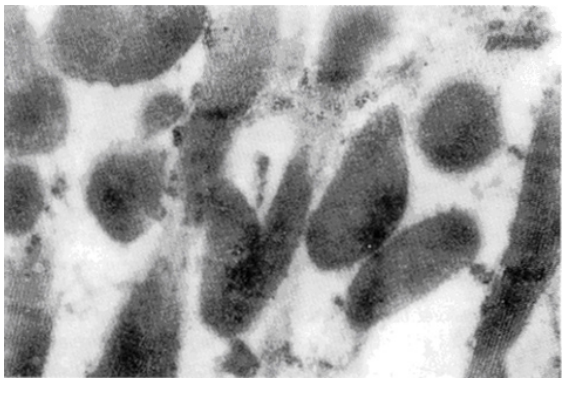

Figure 4 Electron micrograph of myocardium from sodium cholate-fed rats. The number of mitochondria are decreased and appear distorted, with indistinct outer membranes and cristae (× 21000).

mimicked the average blood concentration in obstructive jaundice, their myocardium was damaged to a similar degree. These results suggest that the myocardial mitochondria was damaged, thus indicating that endogenous bile acids have a significant deleterious effect that contributes to obstructive jaundice.

It remains unclear how the myocardium can protect itself from the damage caused by bile acids. Previous studies in rats demonstrated that ursodeoxycholic acid could limit histologic

liver alterations and portal hypertension typically induced by bile duct ligation[6]. Using the hemolysis of erythrocytes as a model of cytotoxicity, Sagawa *et al*[7] demonstrated that both liposomes and hydrophilic bile acids (such as ursodeoxycholate acid) may protect against hydrophobic bile salt-induced cell membrane damage. We anticipate that the operative mortality and morbidity rates would be decreased if perioperative measures against bile acids toxicity were taken to improve cardiac function[8].

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Immunohistochemical study on endocrine-like tumor cells in colorectal carcinomas

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Abstract

AIM: To evaluate the clinical significance of endocrine-like tumor cells in human colorectal carcinomas.

METHODS: The immunohistochemistry method (ABC) using a rabbit polyclonal antibody against human chromogranin A (CGA) was employed to determine changes in endocrine-like tumor cells from the surgically resected colorectal carcinoma tissues of patients (35 males and 27 females, aged from 19 to 78 years, with a mean age of 50.3 years). Of the 62 specimens, 44 were from rectal carcinomas, 18 from colonic carcinomas, 14 from lymph nodes and 48 from non-involvement. Dukes classification revealed 19 of the cases were in stage A, 29 cases were in stage B and 14 cases were in stage C. All of the specimens were fixed with 10% formalin, embedded with paraffin and cut into 5 μm sections. Additionally, the correlations among CGA-positive tumor cells, as well as the clinicopathologic data, age and sex of the patients, were also investigated.

RESULTS: CGA-positive tumor cells were found in 35.5% of the patients with colorectal cancers, representing 20.0% (5 of 25) and 45.9% (17 of 37) of the aged and non-aged, respectively. These differences were significant (χ^2 test, $P < 0.05$). Nevertheless, no significant correlations were found between the CGA-positive tumor cells and the sex, Dukes stages, tumor location, degree of histological differentiation or presence of lymph node metastasis.

CONCLUSION: The low incidence of endocrine-like tumor cells found in the aged patients may be a new pathological feature for colorectal carcinomas, which could explain why the aged patients usually had a better prognosis. The exact significance of these findings requires further characterization.

Key words: Colorectal neoplasms/pathology; Chromogranin A; Immunohistochemistry

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Analysis of fibronectin, fibronectin receptor and interleukin-1 in patients with cirrhosis treated by the Yanggan Jieyu decoction

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Abstract

AIM: To investigate the effects of the Yanggan Jieyu (YGJY, nourishing the liver and alleviating mental depression) decoction on the plasma concentrations of fibronectin (FN), fibronectin receptor (FNR), tumor necrosis factor alpha (TNF-α), and the activity of interleukin-1 (IL-1) in patients with cirrhosis.

METHODS: Thirty-four cases of decompensated cirrhosis were divided into the YGJY decoction treatment group and the control group (patients received standard treatment). FN, FNR and TNF-α were measured by ELISA and expressed as mg/L (FN, FNR) and ng/L (TNF-α). IL-1 was measured by mice thymocyte proliferation using a β scintillation counter and was expressed as cpm.

RESULTS: In the YGJY decoction treatment group, FN and TNF-α levels increased significantly ($P < 0.01$ and $P < 0.05$, respectively), and FNR and IL-1 levels decreased significantly ($P < 0.05$ and $P < 0.05$, respectively). In the control group, FN, FNR, TNF-α, and IL-1 levels did not significantly change.

CONCLUSION: YGJY decoction could prevent hepatic fibrosis by adjusting the plasma levels of FN, FNR, TNF-α and IL-1, which could mediate cirrhosis formation. This data is of clinical significance.

Key words: Liver cirrhosis; Yanggan Jieyu decoction; Fibronectin; Fibronectin receptors; Tumor necrosis factor; Interleukin-1; Traditional Chinese Medicine

Wu H, Gao JS, Fan JZ, Huang J, Deng JW. Analysis of fibronectin, fibronectin receptor and interleukin-1 in patients with cirrhosis treated by the Yanggan Jieyu decoction. *World J Gastroenterol* 1997; 3(3): 177-179 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/177.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.177

INTRODUCTION

Recent reports have shown that serum levels of some extracellular matrix proteins and cytokines, such as fibronectin and interleukin-1, may be biomarkers of hepatic fibrosis, as well as important molecules for initiating hepatic fibrosis[1-3]. Using the autoimmune hepatitis and cirrhotic model of C_{57} BL/6 mice, it was suggested that the Yanggan Jieyu (YGJY, nourishing the liver and alleviating mental depression) decoction could prevent liver tissue damage[4]. Clinical studies have confirmed that many cirrhotic patients were in the state of Gan Yu Xue Xu (stagnancy of the Qi and deficiency of blood in the liver). In this study, we evaluated the curative effect of the YGJY decoction in cirrhosis.

MATERIALS AND METHODS

Patients

Thirty-four patients (30 men and 4 women ranging in ages from 25 years to 68 years) diagnosed with chronic cirrhotic Hepatitis B virus (HBV) were included in this study. Their diagnoses were based on clinical and laboratory evaluations[5]. All of the cases were decompensated cirrhosis and 80% of the cases also had cirrhotic ascites).

Treatment

The patients were randomly divided into two groups. Twenty patients were treated with the YGJY decoction (35 g per day for 8 wk). The decoction is made of Bupleuri (10 g), Lycium barbarum (10 g), Rapid Angelicae Sinensis (10 g), and Radix Glycyrrhizae (5 g). The remaining 14 patients were treated by the standard methods. Twenty healthy volunteers served as controls.

Plasma FN and FNR analysis

The FN and FNR levels were assayed by ELISA. FN was detected by using a specific anti-FN rabbit serum (rabbit immunoglobulin to human fibronectin provided by Dakopatts, Denmark)[1,2]. FNR was detected by using an anti-FNR mouse antibody (Takara Biolaboratory, Japan). The results were expressed as mg/L.

IL-1 production and analysis

IL-1 was produced from human mononuclear cells (macrophages) after stimulation with 40 μg of LPS (Sigma) and 3 μg of indomethacin (Sigma). Thymocyte proliferation was analyzed using the thymocytes from 4 to 8 wk old BALB/C mice. The samples were

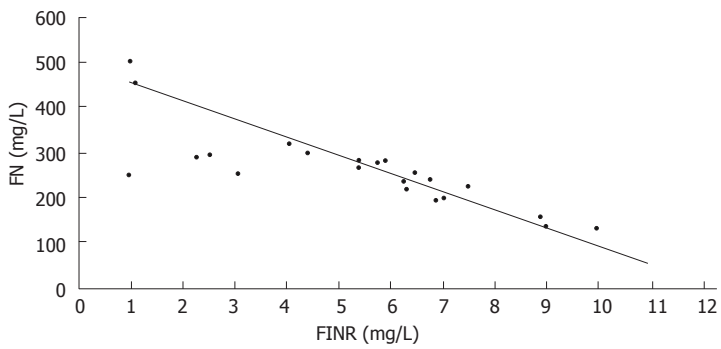

Figure 1 Serum FNR level in relation with plasma FN in 34 patients with chronic liver diseases (liver cirrhosis).

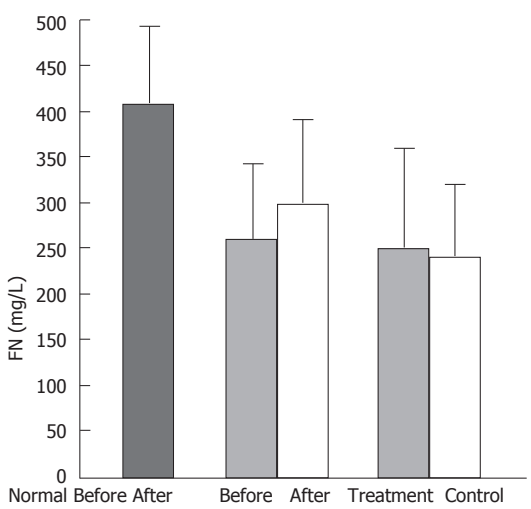

Figure 2 Serum levels of fibronectin in the hepatic fibrosis patients treated with the YGJY (Yanggan Jieyu) decoction.

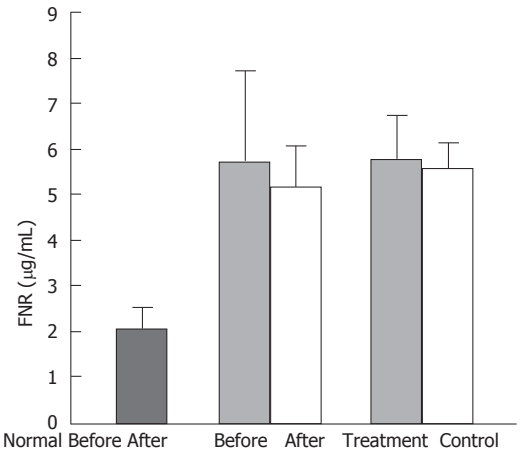

Figure 3 Serum levels of the fibronectin receptor in the hepatic fibrosis patients treated with YGJY (Yanggan Jieyu) decoction.

incubated in the presence or absence of 0.3 mg/L conA (Sigma). Sample dilutions were assayed for IL-1 activity by the incorporation of tritiated thymidine into the cells and counted with a β scintillation counter. The results were expressed as cpm[6].

Serum TNF-α analysis

The serum TNF-α concentrations were determined by the ELISA (Genzyme corporation, Cambridge, MA, United States).

Statistical analysis

The mean values in the control and cirrhotic patients before and after treatment were compared by the Student's *t* test. The correlation between FN and other parameters of hepatic fibrosis were evaluated by a linear regression analysis. Data were expressed as average ± standard deviation.

RESULTS

In the healthy control group, mean plasma FN levels were 413.0 ± 72.5 mg/L, FNR levels were 2.3 ± 0.4 mg/L, TNF-α levels were 72.3 ± 8.6 ng/L, and IL-1 activity was 1320.6 ± 419.2 cpm. In the experimental group, cirrhotic patients had significantly decreased FN levels (248 ± 97.0 mg/L, $P < 0.01$), and significantly increased FNR levels (5.5 ± 2.3 mg/L, $P < 0.01$), TNF-α levels (97.4 ± 29.4 ng/L), and IL-1 activity, (2760.8 cpm, $P < 0.05$), compared with the healthy control group. A negative correlation was observed between the serum concentrations of FN and FNR ($P < 0.01$, $r = -0.6534$) (Figure 1).

After treatment with the YGJY decoction, the FN levels significantly increased (247.9 ± 97.2 mg/L to 298.3 ± 93.2 mg/L, $P < 0.01$) (Figure 2). The FNR levels significantly decreased after YGJY treatment (5.6 ± 2.7 mg/L to 4.3 ± 2.3 mg/L, $P < 0.01$) (Figure 3). The TNF-α levels significantly increased after YGJY treatment (83.9 ± 7.1 ng/L to 93.6 ± 12.0 ng/L, $P < 0.05$) (Figure 4). The activity of IL-1 after the YGJY treatment decreased significantly (2760.8 ± 813.6 cpm to 1922.3 ± 847.0 cpm, $P < 0.01$) (Figure 5). In the standard treatment group, the FN levels, FNR levels, TNF-α levels, and the activity of IL-1 were not significantly different ($P > 0.05$, Figures 2-5).

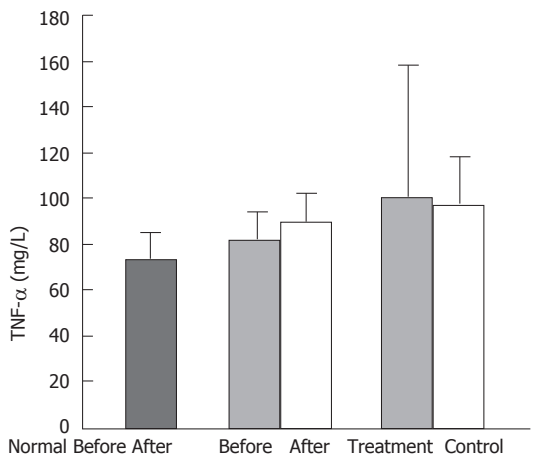

Figure 4 Tumor necrosis factor released in hepatic fibrosis patients treated with the YGJY (Yanggan Jieyu) decoction.

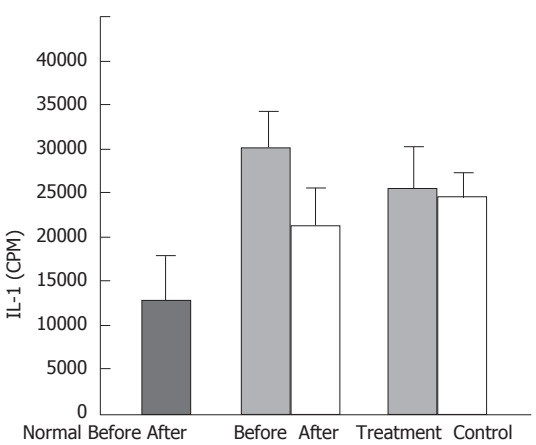

Figure 5 Interleukin-1 release in hepatic fibrosis patients treated with the YGJY (Yanggan Jieyu) decoction.

Clinical profiles

In 63.5% of patients with cirrhotic HBV, the serum globulin level was down-regulated to normal levels after treatment with YGJY decoction. However, the serum globulin level returned to normal in only 23.4% of patients treated by standard methods.

DISCUSSION

Recent studies on cirrhosis have focused on the interactions between cytokines and the extracellular matrix (ECM)[1,8,9]. Several reports have shown that serum levels of some ECM molecules, such as type III procollagen peptide, types I and IV collagen, and fibronectin, were biomarkers of hepatic fibrosis, and the accumulation of ECM molecules played a major role in liver function impairment. The FN and FNR are the main components of the

extracellular matrix. We found that in patients with decompensated liver cirrhosis, the plasma FN was significantly lower and FNR was significantly higher, with a strong negative correlation (r = -0.6534). The TNF-α levels and IL-1 activity were also increased as compared with the normal subjects. Hagiwata *et al*[10] observed that recombinant human IL-1 could increase FN in the liver of rats, and also could directly increase the transcription of type I, III and IV collagen. IL-1 may act synergistically with TNF-α to induce hepatitis[9].

These data show that serum FNR, IL-1 and TNF-α could up-regulate and FN could down-regulate the liver fibrosis process. We found that IL-1 activity and FNR levels, which could indicate increased fibrosis of the liver, were strongly down-regulated by treatment with YGJY decoction. While FN, which could indicate decreased liver fibrosis, was strongly up-regulated by YGJY decoction treatment. These changes showed that YGJY decoction could prevent liver damage and inhibit hepatic fibrosis.

TNF-α is a multifunctional cytokine, which is hypothesized to regulate inflammatory and pathological processes and orchestrate necrosis and regeneration. We detected plasma levels of TNF-α and nitrate in cirrhotic rats by reproduction with CCl_4. The TNF-α levels were significantly higher after YGJY treatment than before treatment. It has been shown that TNF-α can positively regulate liver cell regeneration and hepatocyte proliferation in rats induced by the nitrate[11]. Further studies are necessary to clarify the effect and mechanism of YGJY decoction in hepatocyte proliferation and regeneration.

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Effects of electro-acupuncture on 5-HT, NOS and the gastric mucosa of stress rats

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Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To study the effects of electro-acupuncture (EA) on 5-hydroxytryptophan (5-HT) levels, nitrous oxide (NOS) levels, nitric oxide (NO) levels, and the gastric mucosa in stress rats.

METHODS: The changes of 5-HT and NOS were measured in the gastric mucosa, and NO and 5-HT were measured in the serum by biochemical methods. The gastric mucosa was examined pathohistologically in the stress rats with gastric mucosa damage after EA. The changes before and after stress by EA were compared.

RESULTS: EA decreased the gastric mucosa damage index in the stress rats (2.71 ± 0.40 to 1.86 ± 0.69, $P < 0.01$). EA normalized NOS level in gastric mucosa to the control group. The changes before stress by EA was more obvious than that after stress. EA lowered the 5-HT levels in the gastric mucosa (μg/g wet weight, 6.91 ± 3.08 to 4.51 ± 1.62, $P < 0.01$). EA recovered the NO level in serum of the stress rats (μmol/L, 5.78 ± 1.49 to 7.91 ± 1.11, $P < 0.05$), and increased the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in serum continuously.

CONCLUSION: EA stimulation normalizes the NOS and NO levels in the gastric mucosa of stress rats. EA also lowers the high 5-HT levels and induces NO release.

Key words: Electroacupuncture; Serotonin/acu-moxibustion effects; Aminoacid oxidoreductases/acu-moxibustion effects; Gastric mucosa/Acu-moxibustion effects

Zhu SL, Xu GS, Wang ZJ, Chen QZ, Jiao J. Effects of electro-acupuncture on 5-HT, NOS and the gastric mucosa of stress rats. *World J Gastroenterol* 1997; 3(3): 179 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/179.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.179

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Alterations of erythrocyte ATPase activity and oxygen consumption in patients with liver-blood deficiency syndrome

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Author contributions: All authors contributed equally to the work.

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Abstract

AIM: To investigate the pathophysiology of erythrocyte energy metabolic changes of patients with the traditional Chinese Medicine (TCM) liver-blood deficiency syndrome (LBDS).

METHODS: Erythrocyte membrane ATPase activity and oxygen consumption rate (OCR) were determined in 66 patients with LBDS, including 35 patients with iron deficiency anemia and 31 patients with chronic aplastic anemia. Thirty healthy adults served as controls.

RESULTS: ATPase activity and OCR were decreased in patients with LBDS.

CONCLUSION: The decreased erythrocyte ATPase activity and OCR might cause the energy hypometabolism in LBDS patients.

Key words: Erythrocytes; Cell membrane; Oxygen consumption; Adenosine triphosphatase; Liver-blood deficiency syndrome; Iron-deficiency anemia; Aplastic anemia

Shi LJ, Liu JF, Zhang ZQ, Lu YQ, Shu YG, Chen GL, Xin ZH, Xu JY. Alterations of erythrocyte ATPase activity and oxygen consumption in patients with liver-blood deficiency syndrome. *World J Gastroenterol* 1997; 3(3): 180-181 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i3/180.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i3.180

INTRODUCTION

Erythrocytes, the most common blood cell, exhibit metabolic characteristics. We have conducted hemorrheologic studies in patients with liver-blood deficiency syndrome (LBDS)[1], and observed that their hematocrit (Hct) was significantly decreased. This indicated a reduced erythrocyte count. There are few reports on erythrocyte metabolic alterations in patients with LBDS. In this study, the erythrocyte membrane ATPase activity and the erythrocyte oxygen consumption rate (OCR) were determined in patients with anemia, including iron deficiency anemia and chronic aplastic anemia.

MATERIALS AND METHODS

Diagnostic criteria

General clinical data are listed in Table 1. The patients enrolled in this study all had traditional Chinese Medicine (TCM) differentiated LBDS syndrome diagnosed by two clinicians according to our certified standard[2]. The symptoms included dizziness, decreased visual acuity and/or blurred vision, numbness of the extremities, face, lips, and nails appeared pale and malnourished, tongue appeared pale, and pulse taut and/or thready. Patients presenting with decreased visual acuity and/or blurred vision and numbness of the extremities along with an additional two symptoms (excluding those with Yinxu, Yangxu and Qixu) were diagnosed with LBDS. Iron deficiency anemia (IDA) was diagnosed according to the "Diagnostic Criteria and Curative Improvement Standard of Clinical Diseases" [3]. Chronic aplastic anemia (CAA) was diagnosed according to the June 1987 Baoji Conference revised standard[4]. Healthy adult blood donors served as controls.

Instruments

Equipment used included a portable automatic balanced recorder (XWT-104, Shanghai Dahua Instrument Factory), a Clark electrode and SP-2 dissolved oxygen assay controller (China Academy Vegetal Physiology Institute), a 2219-Ⅱ thermostat circulation water bath (LKB), and a 751 spectrometer (Shanghai 3rd Analytic Instrument Factory).

Preparation of the erythrocyte membrane

Five milliliters of heparin anticoagulated fasting venous blood was centrifuged at 3000 rpm for 10 min. The buffy coat was discarded. The remainder was washed with isotonic Tris-HCl (310 mOsm, pH

Table 1 General clinical data

	n	Male/Female	Age (yr)	Diseases
Control group	30	15/15	34.2 ± 10.4 (20-46)	
Erythrocyte membrane ATPase	31	11/20	36.6 ± 13.6 (19-56)	IDA 16, CAA 15
Erythrocyte OCR	35	13/22	35.8 ± 15.2 (21-48)	IDA 19, CAA 16

Table 2 Comparison of ATPase activities

Groups	*n*	Mg^{2+}-ATPase	Na^+-K^+-ATPase	Ca^{2+}-ATPase
		(μmol Pi·mg protein·h)		
Control	30	0.300 ± 0.160	0.250 ± 0.120	0.620 ± 0.170
LBDS	31	0.130 ± 0.072[b]	0.154 ± 0.081[b]	0.530 ± 0.159[a]
CAA	15	0.132 ± 0.044[b]	0.156 ± 0.067[b]	0.562 ± 0.130
IDA	16	0.132 ± 0.091[b]	0.152 ± 0.093[b]	0.468 ± 0.215[a]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control.

Table 3 Comparison of erythrocyte OCR (oxygen consumption rate)

Groups	*n*	Oxygen consumption rate (μL 100·h·mL compressed RBC)
Control	30	107.26 ± 18.46
LBDS	35	82.25 ± 36.39[b]
CAA	16	68.83 ± 24.83[b]
IDA	19	100.17 ± 13.86[a]

[a]$P < 0.05$ *vs* CAA, [b]$P < 0.01$ *vs* control.

7.4) twice at a ratio of 1:30 with cool hypotonic Tris-HCI (20 mOsm, 2 mmol/L EDTA, pH 7.4). After centrifugation at 12000 rpm for 35 min, a pink fluid precipitant could be seen adhering to the wall of the tube. This was washed with hypotonic solution twice, and a white cell membrane preparation was obtained. The whole procedure was performed at 0 ℃ to 4 ℃, and the membrane product was stored at -20 ℃. Twenty minutes before the ATPase activity assay, the membrane was dissolved with 2 g/100 L saponin. The membrane protein content was determined by the improved Lowry method[5].

Determination of erythrocyte membrane ATPase activity

The Mg^{2+}-ATPase, Na^+-K^+-ATPase, and Ca^{2+} ATPase activities were assessed according to Reinila *et al*[6].

Determination of erythrocyte oxygen consumption rate

The erythrocyte oxygen consumption rate (OCR) was determined using the film oxygen electrode Clark technique and formula[7].

Statistics

Results were expressed as mean ± standard deviation. A *t*-test and ANVOA were used for statistical analysis.

RESULTS

The Mg^{2+}-ATPase, Na^+-K^+-ATPase, and Ca^{2+} ATPase activities in patients with LBDS were significantly decreased as compared with the healthy controls, ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively) (Table 2). The patients diagnosed with CAA did not have a significant difference in the Ca^{2+}-ATPase activity compared to normal controls.

The erythrocyte OCR was generally decreased in the LBDS patients compared with the healthy controls. The erythrocyte OCR of patients diagnosed with IDA was not significantly significant from the healthy controls ($P > 0.05$). The erythrocyte OCR of patients diagnosed with CAA was significantly decreased from the healthy controls ($P < 0.01$). The difference between the IDA and CAA patients was significant ($P < 0.01$) (Table 3).

DISCUSSION

Na^+-K^+-ATPase is responsible for the active transport of sodium and potassium across the membrane, which maintains a high intracellular concentration of potassium and a low intracellular concentration of sodium. ATP is required for the active transport of these molecules[8], If there is a decrease of Na^+-K^+-ATPase on the erythrocyte membrane, then there will be an increase of intracellular sodium concentrations, which could lead to a hypoenergetic status of the erythrocytes. Furthermore, if phosphorylation of membrane proteins is impaired in the ATPase deficient erythrocyte, then the formation of membrane protein polymers will be hindered. This affects cytoskeleton stability[9], resulting in abnormalities of the erythrocyte structure. Therefore, Na^+-K^+-ATPase is essential for the maintenance of the normal morphology, structure and function of the erythrocyte[10]. In mature erythrocytes, glucose catabolism is very active in order to provide the sodium pump with energy and to maintain the normal functioning of the erythrocytes (90% of the energy from glycolysis and 10% from the pentose phosphate pathway)[7].

We observed that the activities of the ATPases, including the Mg^{2+}-ATPase, the Na^+-K^+-ATPase and the Ca^{2+} ATPase, were significantly decreased compared to the normal controls. However, no differences were observed between the patients diagnosed with IDA and CAA. In addition, the oxygen consumption rate of the LBDS patients was decreased compared to the controls, especially the patients with CAA. Taken together, the results suggest that the erythrocyte ATPase activity and OCR are decreased in LBDS patients, which could lead to pathophysiological changes of decreased energy metabolism.

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The effects of *Astragalus membranaceus* on oxygen consumption in the intestine

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Abstract

AIM: To investigate the effects of *Astragalus membranaceus* (AM) on intestinal oxygen consumption both *in vivo* and *in vitro*.

METHODS: The oxygen consumption of the intestine was measured using an arteriovenous (A-V) O_2 difference analyzer after treatment with AM in the intestinal lumen of ten healthy, anesthetized mongrel dogs. The effects of AM on the oxygen consumption of the intestinal mucosa *in vitro* were observed using constant volume manometers.

RESULTS: The oxygen consumption of the intestine *in vivo* increased significantly ($P < 0.05$ or $P < 0.01$) after treatment with AM compared to the saline control. The oxygen consumption significantly increased after treatment with the 30% AM dilution and the 50% AM dilution compared to that of the 10% AM dilution ($P < 0.05$). There was no significant difference between the 30% AM dilution and the 50% AM dilution ($P > 0.05$). The effects of AM on oxygen consumption of the intestinal mucosa *in vivo* were similar to those *in vivo*. After treatment with the 5% AM dilution and the 1% AM dilution, the intestinal oxygen consumption increased compared to the control (Krebs Ringer phosphate buffer (KRPB)) ($P < 0.05$ and $P < 0.01$, respectively). There was no significant difference between treatment with the 10% AM dilution and the KRPB control ($P > 0.05$).

CONCLUSION: AM improved the function of intestinal oxidative metabolism.

Key words: *Astragalus membranaceus*; Oxygen consumption; Small intestine metabolism

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INTRODUCTION

It was reported that *Astragalus membranaceus* (AM) could stabilize the configuration of deoxyhemoglobin, decrease hemoglobin's affinity for oxygen, shift the oxygen hemoglobin dissociation curve to the right, increase hemoglobin's capacity for transporting oxygen, and decrease the from an oxygen supply deficiency[1,2]. AM can also decrease the oxygen consumption of cardiac muscle and maintain the balance of oxygen supply in the blood[3]. However, its effects on oxygen consumption in the intestine has not been investigated. This study observed the effects of AM on oxygen consumption in the intestine both *in vivo* and *in vitro*.

MATERIALS AND METHODS

Animal model

Ten healthy mongrel dogs (15-20 kg) of either sex were fasted for 24 h and anesthetized with pentobarbital sodium (30 mg/kg). All animals were ventilated with a positive pressure respirator (Harvard Apparatus, United States) that was adjusted to achieve normal blood pH, O_2 tension, and CO_2 tension before each experiment. A midline abdominal incision was made and a segment of jejunum (20-40 g) about 30 cm aboard to the ligament of Treitz was exteriorized. A rubber tube was placed in the lumen of the segment for introduction and withdrawal of the AM solution. After heparin sodium (500 U/kg) was administered intravenously, a vein draining in the jejunal segment and a femoral artery were cannulated for continuous measurement of intestinal oxygen consumption of the observed segment. The measured blood was directed to a reservoir, which was pumped back to the animal *via* a femoral vein at a rate equal to the total outflow in order to maintain the blood equilibrium. Both ends of the segment were ligated to the adjacent jejunum to exclude collateral flow after the rubber tube was placed into the lumen of the segment (Figure 1). The segment was covered with a plastic sheet and kept at 37 ℃ with a heat lamp and a

Table 1 Effects of *AM* (*Astragalus membranaceus*) on the intestinal oxygen consumption *in vivo* (average ± standard deviation, mL•min•100 g)

Groups	*n*	0-3 min	4-7 min	8-11 min	12-15 min
A. Normal saline	10	2.62 ± 0.51	2.71 ± 0.54	2.68 ± 0.60	2.58 ± 0.58
B. 10% concentration	10	2.96 ± 0.64	3.48 ± 0.66[a]	3.87 ± 0.69[b]	4.01 ± 0.72[b]
C. 20% concentration	10	3.18 ± 0.70	4.16 ± 0.78[bc]	4.82 ± 0.82[bc]	4.77 ± 0.83[b]
D. 30% concentration	10	3.23 ± 0.76	4.35 ± 0.79[bc]	4.74 ± 0.86[bc]	4.76 ± 0.84[b]

[a]$P < 0.05$, B,C,D *vs* A; [b]$P < 0.01$, B,C,D *vs* A; [c]$P < 0.05$, C,D *vs* B.

Table 2 Effects of *AM* (*Astragalus membranaceus*) on the oxygen consumption of the intestinal mucosa *in vitro* (average ± standard deviation, mL•min•100 g)

Groups	*n*	0-3 min	4-7 min	8-11 min	12-15 min
A. Normal saline	10	2.62 ± 0.51	2.71 ± 0.54	2.68 ± 0.60	2.58 ± 0.58
B. 10% concentration	10	2.96 ± 0.64	3.48 ± 0.66[a]	3.87 ± 0.69[b]	4.01 ± 0.72[b]
C. 20% concentration	10	3.18 ± 0.70	4.16 ± 0.78[bc]	4.82 ± 0.82[bc]	4.77 ± 0.83[b]
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[a]$P < 0.05$, B,C,D *vs* A; [b]$P < 0.01$, B,C,D *vs* A; [c]$P < 0.05$, C,D *vs* B; [d]$P < 0.01$, C,D *vs* B.

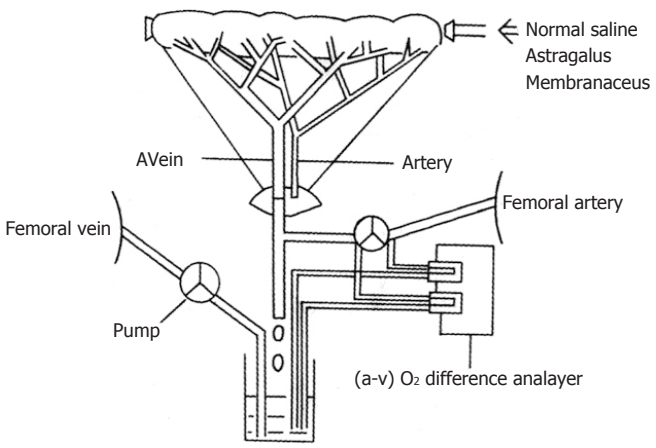

Figure 1 Representation of the animal model used to measure the oxygen consumption in the intestine after treatment with AM (*Astragalus membranaceus*).

thermoregulator (Yellow Spring Instrument, United States)[4,5].

Methods of measurement

Intestinal oxygen consumption *in vivo* was measured by an arteriovenous (A-V) O_2 difference analyzer (A-Vox. Systems, United States). After the operation was performed, normal saline was used to wash the lumen three times. Ten milliliters of normal saline was placed into the lumen while the intestinal blood flow, motility, and blood pressure were continuously measured for 15 min. This procedure was repeated every 15 min until the blood flow, motility, and blood pressure reached a steady state. Then the effects of different concentrations of AM on oxygen consumption were measured. Each dog was given each AM concentration three times. The intestinal oxygen consumption was recorded from 0-3 min, 4-7 min, 8-11 min, and 12-15 min.

The oxygen consumption of the intestinal mucosa *in vitro* was measured using constant volume manometers (Warburg Apparatus, United States). Segments of jejunum were harvested from anesthetized mongrel dogs. The tissue was immediately placed in fresh oxygenated ice-cold Krebs Ringer phosphate buffer (KRPB) solution. The container was surrounded by ice in order to keep the temperature low. Blood was removed from the tissue by perfusing KRPB *via* the local artery after the lumen was washed. The tissue was cut open along the mesenteric border, and intact mucosal sheets were obtained by gently separating the mucosa from the underlying gut wall using a microscope slide. Small pieces of mucosa, 1 mm x 2 mm, were obtained by cutting. These small pieces were transferred to the fresh, oxygenated, cold KRPB. Numerous small pieces were blotted on filter paper, weighed to the nearest 0.15 g, and placed in Warburg flasks. Each flask contained a total volume of 3 mL of incubation media that included oxygenated KRPB with 0.5 mL of one of the concentrations of AM in the experimental groups or without AM in the control group. To measure the absorption of carbon dioxide, the center well of the flask contained 0.2 mL of 10% KOH and 0.1 g of filter paper. The flasks were attached to manometers, placed in a water bath (37 ℃), and shaken at 90 cycles per minute. After a 10-15 min equilibration, manometer pressure readings were obtained every 10 min for 1 h. Additional mucosal tissue samples of 5-6 g were weighed, oven dried, and re-weighed to obtain the percentage dry weight, which was used to calculate the dry weight of the experimental samples.

Preparation of experimental solutions

AM was purchased from the Hebei Company of Medicinal Materials. Two hundred milliliters of distilled water was added to a flask containing 50 g of AM. The flask was put on an electric furnace to be decocted until the solution became 100 mL. This was labeled as the 50% concentration of AM. This solution was further diluted to 30%, 10%, 5%, 1% and 0.5% with distilled water. Fresh AM dilutions were prepared on each experimental day.

KRPB solution was prepared with NaCl (7.106 g/L), KCl (0.365 g/L), $CaCl_2$ (0.138 g/L), $MgSO_4$ (0.01 g/L), Na_2HPO_4 (2.29 g/L), and glucose (0.9 g/L). First, NaCl, KCl, $MgSO_4$ and Na_2HPO_4 were placed in distilled water and stirred. At the same time, the solution was gassed with a combination of 95% O_2 and 5% CO_2 for 10 min. $CaCl_2$ dissolved in 100 mL of distilled water was slowly added into the KRPB solution. A fresh solution was prepared on each experimental day.

Statistical methods

Analysis of variance (ANOVA) was employed to detect the differences among the four groups. The *Q*-test (Newman-Keuls test) was used to compare the differences between two groups.

RESULTS

Six different concentrations of AM (50%, 30%, 10%, 5%, 1% and 0.5%) were tested in both *in vivo* and *in vitro* experiments to determine the most effective concentration. This study reported the effects of the most effective concentration and the concentration above and below the most effective concentration.

The effects of AM on the intestinal oxygen consumption *in vivo* are reported in Table 1. After treatment with the AM solution, the intestine increased the oxygen consumption, especially after 4-7 min, compared to the saline control ($P < 0.05$ or $P < 0.01$). The intestinal oxygen consumption was different among the different AM dilutions. The oxygen consumption was significantly increased ($P < 0.05$) after treatment with the 30% and 50% AM dilutions compared to the 10% dilution. There was no significant differences in oxygen consumption between the 30% and 50% AM dilutions ($P > 0.05$).

The effects of AM on the oxygen consumption of the intestinal mucosa *in vitro* is reported in Table 2. There was no significant difference between the 10% AM dilution and the KRPB control ($P > 0.05$). The intestinal oxygen consumption was increased after treatment with the 5% and 1% AM dilutions. The oxygen consumption significantly increased ($P < 0.05$) at 50 min and 60 min after treatment with the 1% AM dilution compared to that of the KRPB control. The oxygen consumption significantly increased ($P < 0.05$ or $P < 0.01$) at 20 min, 30 min, 40 min, 50 min, and 60 min after treatment with the 5% AM dilution compared to the KRPB control, the 10% AM dilution, and the 1% AM dilution.

DISCUSSION

AM has been shown to protect cultured cardiac muscle cells during a glucose and oxygen deficient period. The proposed mechanism was that AM could stabilize the function of cardiac muscle cells, protect the mitochondria and lysosomes, and enhance the resistant capacity of oxygen deficiency[6]. In addition, AM has been shown to lower the oxygen consumption of the heart and liver mitochondria in guinea rats, increase the formation of 2, 3-diphosphoglycerate in healthy human red blood cells (*in vitro*), and improve hemoglobin's capacity for transporting oxygen[1]. After a 25% concentrated solution (1 mL/kg) of AM was injected into the empty stomachs of healthy, awakened dogs, the cycle duration of the interdigestive myoelectric

complex was changed. Phase I was shortened and phase II were prolonged in the jejunum. The cycle duration was also extended and showed an increase of action potential in phase II. Taken together, this data indicated that AM could strengthen the movement and muscle tone of the intestine[7].

Our previous study also showed that the intestinal blood flow and motility increased after treatment with the AM solution into the canine intestine *in vivo*. This revealed that AM could improve the physiological function of the intestine. This study also indicated that the pharmaceutical effects of AM on different organs were different.

Guo *et al*[8] reported that the injection of AM could directly cause peripheral vasodilatation. According to Kvietys and Granger's[9] analysis of 50 papers about the effects of vasoactive agents on oxygen uptake, vasodilators can increase oxygen uptake. Changes in oxidative metabolism, blood flow, and capillary density appeared to be the major mechanism by which vasoactive agents alter splanchnic oxygen uptake. Under *in vitro* conditions, any drug-induced changes in tissue oxygen uptake are assumed to reflect only changes in tissue oxidative metabolism. Accordingly, AM probably has the pharmaceutical action of increasing oxygen consumption.

In order to probe the effect of AM on oxygen consumption in this study, the canine intestine was treated with different dilutions of AM. Its effect on the intestinal oxygen consumption was observed. We observed that AM treatment could significantly increase the intestinal oxygen consumption *in vivo* compared to the saline control. This indicated that AM could improve the physiological function of the intestine. To eliminate the interference involved in *in vivo* experiments, the effects AM treatment on intestinal oxygen consumption *in vitro* were also measured. We observed that AM treatment could also significantly increase the intestinal oxygen consumption compared to the KRPB control.

The effects of AM treatment on the intestinal oxygen consumption varied at different dilutions. This might be explained by the use of different experimental methods. In the *in vivo* experiments, AM was placed into the intestine. This method limited the effect of the AM on the surface of the tissue. While in the *in vitro* experiments, AM was put in the flasks containing small pieces of intestinal mucosa. In this method, the surface of the tissues was in contact with the AM constantly. Therefore, different pharmaceutical responses were observed among the AM dilutions between the *in vivo* and *in vitro* experiments.

We observed that the pharmaceutical function of AM was to increase the intestinal oxygen consumption. It was consistent with the report that AM could improve the intestinal function[7]. Other studies have shown different effects of AM on oxygen consumption in different organs (*i.e.* decreased oxygen consumption in cardiac muscle cells and liver mitochondria, and increased intestinal oxygen consumption *in vivo* and *in vitro*). The mechanism, by which AM affects oxygen consumption, awaits further studies.

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Ultrastructural observation of the gastric mucosa in chronic gastritis patients treated by traditional Chinese medicine

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Abstract

AIM: To demonstrate the relationship between the ultrastructural changes of the gastric mucosa and the syndrome differentiation in chronic gastritis.

METHODS: Sixteen chronic gastritis patients with Piweixuhan (PXG, the cold of insufficiency syndrome of the spleen and the stomach) and fifteen chronic gastritis patients with Ganweibuhe (GBG, incoordination syndrome of the liver and the stomach) were treated with Jianpiwenwei decoction (JWD, invigorating the spleen and warming the stomach) or Shuganhewei decoction (SHD, dispersing the stagnated Liver Qi and regulating the stomach), respectively for three months. Before and after treatment, a gastroscopy was performed and the gastric mucosa was collected from the lesser curvature of the antrum of each patient. The ultrasections were observed and photographed under the JEM-100C X electron microscope.

RESULTS: The common ultrastructural anomalies of the two types of chronic gastritis were the plasmacyte infiltration and the lesions of the mucosal epithelial cells, chief cells and antral mucous cells. There were obvious differences between the two types. In PXG, the predominant lesion of the chief cells was swelling of the mitochondria, while in GBG the rough endoplasmic reticulum was enlarged in the chief cells and the plasmacytes. After treatment, most cases of the ultrastructural lesions reverted to normal or improved.

CONCLUSION: There was a close relationship between the ultrastructural changes of gastric mucosa and the syndrome differentiation of chronic gastritis. JWD and SHD could significantly improve the ultrastructural lesions of the gastric mucosa.

Key words: Gastritis/TCM therapy; Gastric mucosa/ultrastructure; Traditional Chinese medicine

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INTRODUCTION

Chronic gastritis is a common disease that is difficult to cure. Diagnosis and treatment of chronic gastritis by traditional Chinese medicine (TCM) has had a good therapeutic effect. In this study, we investigated the relationship between the ultrastructural changes of the gastric mucosa based on the TCM differentiation of chronic gastritis. The therapeutic effects of the TCM differentiation treatment on the gastric mucosa were also tested.

MATERIALS AND METHODS

Patients

Sixteen patients with Piweixuhan (PXG, the cold of insufficiency syndrome of the spleen and the stomach) and fifteen patients with Ganweibuhe (GBG, incoordination syndrome of the liver and the stomach) were selected from the inpatients at the First Affiliated Hospital of Lanzhou Medical College. They were diagnosed according to the TCM differentiation standard of chronic gastritis in "Guiding Principle of Clinical Research of New Drugs" by the Chinese Ministry of Public Health.

Methods

The patients with PXG were treated with a Jianpiwenwei decoction (JWD, invigorating the spleen and warming the stomach). This decoction contained 10 g of Dangshen (Radix codonopsis pilosula e), 20 g of Fuling (Poria), 15 g of Baizhu (Rhizoma atractylodis macrocephalae), 5 g of Muxiang (Radix aucklandiae), 5 g of Shenggancao (Radix glycyrrhizae), 10 g of Zhibanxia (Rhizoma pinelliae preparata), 5 g of Sharen (Fructus amomi), 10 g of Gaoliangjiang (Rhizoma alpiniae officinarum), 10 g of Yanhusuo (Rhizoma corydalis), and 10 g of Chaomaiya (Fructus hordei germinatus).

The patients with GBG were treated with a Shuganhewei

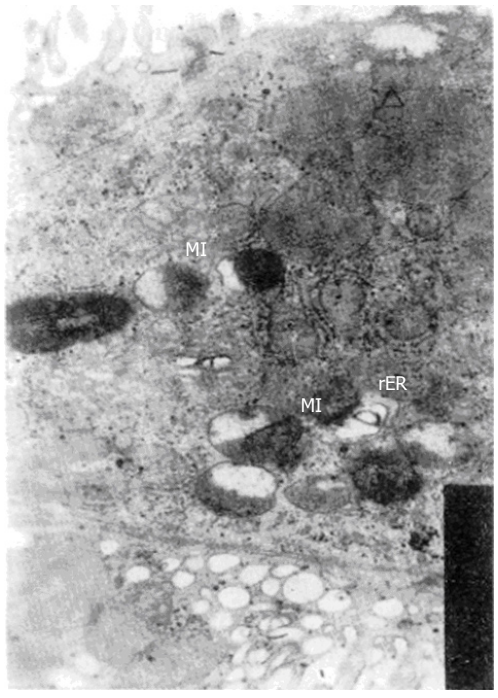

Figure 1 The mitochondria were enlarged in the chief cells in chronic gastritis patients differentiated into the PXG category.

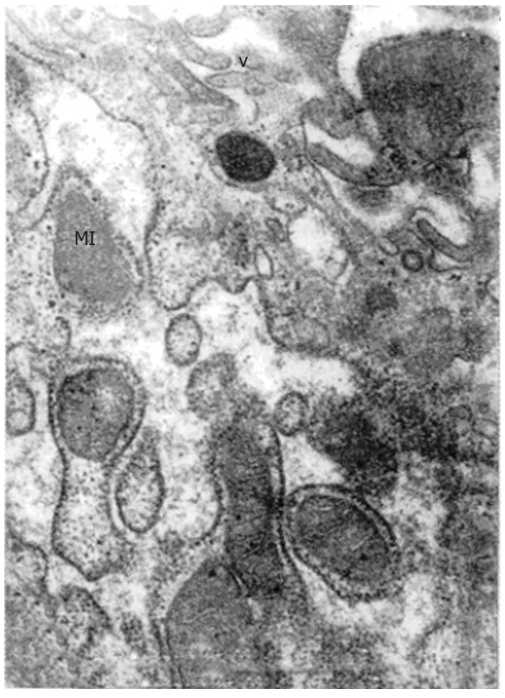

Figure 2 The rER in chief cells enlarged and some showed intracisternal sequestration in chronic gastritis patients differentiated into the GBG category.

decoction (SHD, dispersing the stagnated Liver Qi and regulating the stomach). This decoction contained 10 g of Chaihu (Radix bupleuri), 15 g of Baishao (Radix paeoniae alba), 10 g of Zhiqiao (Fructus aurantii), 5 g of Muxiang (Radix aucklandiae), 10 g of Shenggancao (Radix glycyrrhizae), 10 g of Yujin (Radix curcumae), 15 g of Yanhusuo (Rhizoma corydalis), 10 g of Baizhi (Radix angelicae dahuricae), 10 g of Danggui (Radix angelicae sinensis), and 10 g of Jineijin (Endothelium corneum galli).

Patients were given 200 mL of the respective decoctions orally for three months, twice a day. The crude content of the two prescriptions was 500 g/L. Other related medications were not given during the observation period.

Assessment of the therapeutic effects

Gastroscopy was performed before and after the treatment. The gastric mucosa was collected from the lesser curvature of the antrum and immediately immersed in a cold 3% glutaraldehyde solution. The specimens were rinsed with phosphate buffer solution, post-fixed with 1% osmium tetroxide, dehydrated in graded steps with increasing percentages of ethanol, embedded with Epon 812, sectioned with an ultratome, and double stained with uranium acetate and lead citrate. The ultrasections were observed and photographed under the JEM 100CX transmission electron microscopy.

The main ultrastructural lesions were divided into three grades: severe, moderate and mild. After treatment, the therapeutic effects were determined according to the following assessment standard: recovery (the ultrastructural lesions disappeared completely), marked effectiveness (the ultrastructural lesions lightened by two grades), effective (the ultrastructural lesions lightened by one grade), ineffective (the ultrastructural lesions had no obvious changes). All data were analyzed statistically by the χ^2 test, and significance was set at $P < 0.05$.

RESULTS

We observed that the mitochondria in the chief cells were swelled, its cristae were reduced and shortened, and some even displayed vacuolization. Some displayed pyknosis (Figure 1). The mitochondria lesions were classified into three grades: severe (the injured mitochondria exceeded 2/3 of all the mitochondria in the cytoplasm), moderate (the number of injured mitochondria was between 1/3 and 2/3 of all the mitochondria in cytoplasm), and mild (the injured mitochondria were less than 1/3 of all the mitochondria in cytoplasm).

We observed that the rough endoplasmic reticulum (rER) in the chief cells were expanded, showed vesicles and vacuoles, and some even had intracisternal sequestration[1] (Figure 2). The rER enlargement was also graded into three degrees: mild (the rER enlargement was less than 1/3 of the cytoplasm or two times less than the area of the normal rER), moderate (the rER enlargement accounted for 1/3 to 2/3 of the cytoplasm or exceeded four times of the area of the normal rER), and severe (the rER enlargement was more than 2/3 of the cytoplasm or six times more than the area of the normal rER, or formed intracisternal sequestration).

We observed that the intercellular space in the mucosal epithelial and antral mucous was widened. This change was also graded into the following three degrees: severe (the intercellular space was significantly enlarged and exceeded the width of the cells, the cell processes became slight and dispersed, and lymphocytes appeared in some spaces), moderate (the width of the intercellular space was between the severe and mild degrees), and mild (the intercellular space was small, and the cell processes became shorter and smaller). In addition, we observed that the surface microvilli were short, small and sparse, the organelles were reduced, the mucous granules decreased, the cells atrophied, and the electron density increased. In a few patients, the mucous epithelial cells became stratified epithelium.

We observed heterotypical and deformed nuclei appeared in the mucosal epithelial cells and the antral mucous cells. Many nuclei were markedly enlarged and the nucleoli increased in number and size. The nuclear membrane folded and sank, which thereby led to nucleus deformation (Figure 3). This deformation was divided into three grades: severe (the sinking depth exceeded the short radius of the nucleus), moderate (the sinking depth or scope was between the mild and the severe grades), and mild (the sinking depth was less than 1/3 of the short radius of nucleus, or the sinking scope was less than 1/3 of the nuclear circumference).

We observed that the lymphocytes and plasmacytes infiltrated into the gastric mucosa. The lymphocytes were normal. Some appeared in the enlarged mucous intercellular space or between the glandular epithelial cells. In the plasmacytes, the rER enlarged to various degrees. We utilized the same grading method as that for the chief cells (Figure 4).

In addition, we found that the capillary endothelial cells in some patients were swelled or disappeared after treatment. There were a small number of G cells scattered in the antral mucosa. The rER was enlarged in some of these cells. In our study, the parietal cells, the zymogen granules in the chief cells, the stomach body's mucous cells, and the mucigen granules were nearly normal.

The therapeutic effects on the ultrastructural lesions of the gastric mucosa are presented in Tables 1 and 2.

Table 1 The lesions and the therapeutic effects on the mitochondria and rough endoplasmic reticulum (rER) in chief cells

Differentiation	Total cases	Organelle lesions	Injured cases (%)	Markedly effective	Effective	Ineffective	Total effective rate (%)
PXG	16	Mitochondria[b]	13 (81.3)	7	4	2	84.6
		rER[6]	3 (18.8)	1	1	1	1.0
GBG	15	Mitochondria	3 (20.0)	0	2	1	66.7
		rER	12 (80.0)	8	2	2	83.3

[b]$P < 0.01$, compared with GBG.

Table 2 The therapeutic effect of the intercellular space, nuclear membrane and plasmacyte

Ultrastructural changes	Differentiation	Injured Cases (%)	Recovery	Markedly effective	Effective	Ineffective	Total effective Rate (%)
The intercellular space of mucosal epithelial cells widened	PXG	14 (87.5)	2	6	3	3	78.6
	GBG	12 (80.0)	2	5	3	2	83.3
The intercellular space of antral mucous cells widened	PXG	13 (81.3)	4	5	2	2	84.6
	GBG	13 (86.7)	3	6	3	1	92.3
The nuclear membrane of the mucosal epithelial cells and the antral mucous cells folded and sank in	PXG	10 (62.5)	3	3	2	2	80.0
	GBG	10 (66.7)	2	4	3	1	90.0
The rER in the plasmacyte enlarged	PXG	6 (37.5)[a]	1	3	0	2	66.7
	GBG	12 (80.0)	4	3	3	2	83.3

[a]$P < 0.05$, compared with GBG.

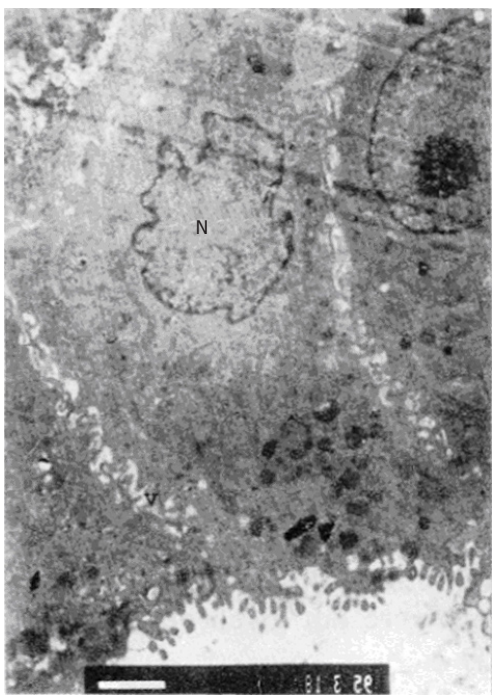

Figure 3 The intercellular space of antral mucosal epithelial cells was enlarged. N: Deformed nucleus.

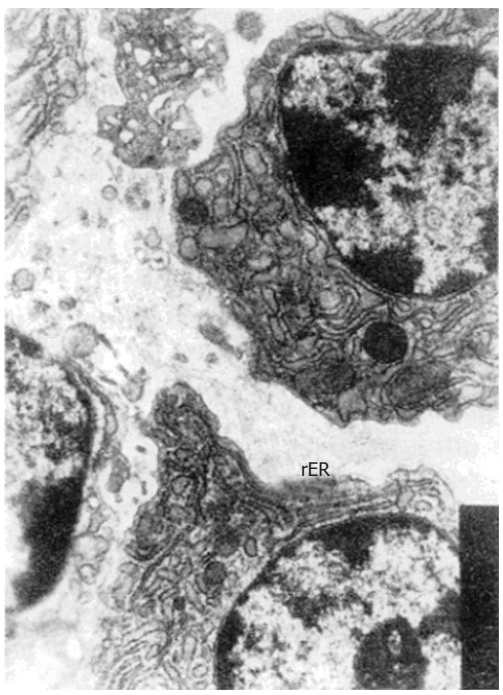

Figure 4 The plasmacytes infiltrated and the intercellular space was enlarged. rER: Enlarged rER

DISCUSSION

Optical microscopy is the primary means to diagnose the degree and the type of pathological changes in gastritis. However, it is difficult to detect the early stage lesions of organelles in the cells and to determine the nature of the lesions by optical microscopy because of its limited resolution. Moreover, the pathological grading under optical microscope is not very precise. In order to explore a more objective, reliable and precise method to determine therapeutic effects and pathological grading, we designed this study, which combined the TCM differentiation treatment and observation of the ultrastructure of the cells. Although there were some studies about the ultrastructural study of chronic gastritis[2-5], there were few studies about the relationship between the ultrastructural changes of the gastric mucosa and the TCM differentiation of chronic gastritis.

This study showed that the ultrastructural lesions in the chief cells were significantly different between the two types of gastritis. PXG was characterized by swelled mitochondria, while GBG was characterized by expanded rER. This morphological difference revealed that the TCM differentiation of chronic gastritis has a modern ultramicropathological basis. Ultrastructural research on chronic gastritis could greatly contribute to its treatment and differentiation.

This study also found that more than 80% of chronic gastritis patients had widened intercellular space of the mucosal epithelial and the antral mucous cells. This observation was consistent with the results of animal experiments reported in the literature[3,6]. On the other hand, more than 60% of the cases had deformed nuclei, the nuclear membrane was folded and sank in, and the nucleolus increased in number and size. This might be a compensated hyperfunction after the gastric mucosal barrier and the antral mucous cells were damaged. Liang *et al*[3] thought that the intercellular space enlargement was caused by mesenchyme edema. However, our results showed that the cell atrophy was a direct cause of intercellular space enlargement, which was consistent with our experimental results in a rat model[6].

The lymphocyte and plasmacyte infiltration into the gastric mucosa of chronic gastritis was reported previously, which indicated that chronic gastritis was closely related to the patient's immunity. We observed plasmacyte infiltration in 58.1% of the patients with the rER enlarged to various degrees. Interestingly, the plasmacyte infiltration appeared in 80.0% of PXG patients, but only in 37.5% of GBG patients, which indicated that the GBG may be more closely related with immune functions. This has been confirmed by our experimental research[6] and helps to explain why the lesions of chief

cells were significantly different between the two types of gastritis. We hypothesize that the rER enlargement in chief cells may be a result of immune damage. In PXG patients, the main changes of the chief cells were mitochondrial lesions. It is well known that the cAMP content in cells of PXG patients is reduced (and thus is ATP reduced), the mitochondrial permeability is increased, which causes swelling and vacuolization. As a result, energy is decreased, cell function is lowered, and cells are atrophied, which led to gastric mucosal atrophy. In this study, parietal cells were normal, which was not consistent with the literature[2-5]. This difference needs to be examined further.

We observed that the ultrastructural lesions in PXG and GBG patients were significantly improved after treatment with JWD and SHD. We hypothesized that JWD would improve the mitochondrial lesions by increasing the cAMP content in cells, while SHD would improve the rER enlargement by stabilizing the plasmacytes and inhibiting the excessive immune reaction. Both prescriptions could strengthen the resistance against diseases, regulate the patients' immune function, and accelerate blood circulation of the gastric mucosa, thus promoting the recovery of the ultrastructural lesions of the gastric mucosa.

We also observed a few G cells in the antrum, in which some rER were enlarged. But we did not observe any other changes, such as secretory granules vacuolization and reduction reported by Ren *et al*[2]. The rER enlargement of G cells mainly appeared in GBG patients, which probably was caused by the neuroendocrine disorder of G cells. SHD could make the enlarged rER in G cells recover by regulating its neuroendocrine function.

In addition, in the mesenchyme of the gastric mucosa in a few patients, the capillary endothelium swelled and the capillary cavity narrowed leading to dysfunction of the microcirculation. After treatment, these changes disappeared, suggesting that the two decoctions could significantly improve the microcirculation of the gastric mucosa. Therefore, both recovery of the mucosal epithelium and gland and the improvement of the microcirculation of gastric mucosa should be emphasized in treating chronic gastritis so as to increase the therapeutic effects.

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Characteristics of saliva secreted by patients with TCM-Piyinxu

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Abstract

AIM: To investigate various characteristics of saliva secreted by patients with TCM-Piyinxu (Spleen-yin deficiency).

METHODS: Twenty-five individuals with Piyinxu (15 males and 10 females; age range 26-70 years, mean age = 45 years) diagnosed based on criteria used in traditional Chinese medicine, were compared with 20 individuals with Shenyinxu (Kidney-yin deficiency) (11 males, 9 females; age range 35-75 years, mean age = 50) and 30 normal individuals (17 males, 13 females; age range 35-65 years, mean age = 49 years). After acid stimulation, the saliva flow in each group was measured, and the levels of amylase and protein in saliva were determined using an automatic biochemical analyzer. The resultant data were analyzed using the Kruskal-Wallis test and one-way factorial ANOVA test.

RESULTS: The flow rates of saliva and amylase in Piyinxu patients (0.27 ± 0.016 mL/min and 2134.13 ± 343.51 IU/min, respectively) were lower than those in normal subjects (0.46 ± 0.027 mL/min and 3501.63 ± 1099.63 IU/min, respectively, $P < 0.01$), but higher than those in the Shenyinxu group (0.13 ± 0.051 mL/min and 951.62 ± 383.17 IU/min, respectively, $P < 0.01$). The three groups showed no significant difference in their level of total salivary protein (Piyinxu group, 3.07 ± 0.60 g/L; Shenyinxu group, 3.01 ± 0.90 g/L, and control group, 2.94 ± 1.13 g/L, $P = 0.869$), amount of amylase per saliva volume, or their ratio of amylase to protein in secreted saliva ($P = 0.173$ and $P = 0.436$, respectively).

CONCLUSION: Piyinxu patients showed altered rates of saliva and amylase secretion when compared with those parameters in patients with Shenyinxu and normal subjects.

Key words: Spleen asthenia/physiopathology; Yin deficiency/physiopathology; Salivation

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Treatment of postoperative gastric cancer with the Fuzheng Huoxue anticancer prescription

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Abstract

AIM: To study effects of the Fuzheng Huoxue anticancer prescription (Traditional Chinese Medicine) in treatment of gastric cancer.

METHODS: Sixty-nine patients with histologically confirmed mid- or late-stage gastric cancer were assigned to two groups. The treatment group included 35 cases (26 males and 9 females; 2 patients aged 33-40 years, 18 patients aged 41-60 years, and 15 patients aged 61-75 years; mean group age = 58.4 years). The control group included 34 cases (23 males and 11 females; 4 patients aged 33-40 years, 16 patients aged 41-60 years, and 14 patients aged 61-75 years; mean group age = 56.8 years. The two groups were not significantly different in sex, age, their clinical and pathological stages of disease or operation mode. The two groups of patients were given similar treatments; however, patients in the treatment group were given the Fuzheng Huoxue anticancer prescription. In animal studies, SGC-7901 gastric cancers cells were inoculated into the backs of 30 nude mice under sterile conditions. After inoculation, the nude mice were randomly allocated to a control group, a traditional Chinese medicine group, and a chemotherapy group (n = 10 mice per group). The total weight of the 10 mice in each group was similar. Each nude mouse in the control group received 0.5 mL of saline solution each day. Mice in the traditional Chinese medicine group received 0.5 mL of the Fuzheng Huoxue anticancer prescription (containing 1.5 g crude drug) each day, while mice in the chemotherapy group were intraperitoneally injected with 1 mg of 5-Fu once a week for 8 wk.

RESULTS: Prior to treatment, the mean OKT8 percentage among gastric patients in the treatment group was 45.94% ± 8.45%, the mean OKT4/OKT8 ratio was 0.89 ± 0.19, the mean AT-Ⅲ concentration was 29.9 ± 7.9 mg/dL, the mean Fa value was 50.4% ± 24.4%, and the mean β-TG concentration was 91.0 ± 25.9 ng/dL. Prior to treatment, the mean percentage of OKT8 cells among patients in the control group was 49.21% ± 6.60%, the OKT4/OKT8 ratio was 0.94 ± 0.20, the AT-Ⅲ concentration was 32.3 ± 7.2 mg/dL, the mean Fa value was 57.3% ± 24.6%, and the mean β-TG concentration was 87.5 ± 34.2 ng/dL. After treatment, the mean OKT8 percentage among patients in the treatment group was 33.52% ± 7.80%, the mean OKT4/OKT8 ratio was 1.47 ± 0.51, the mean AT-Ⅲ concentration was 38.8 ± 5.5 mg/dL, the mean Fa value was 102.6% ± 31.6%, and the mean β-TG concentration was 62.3 ± 15.1 ng/dL. After treatment, the mean OKT8 percentage among patients in the control group was 42.22% ± 7.07%, the mean OKT4/OKT8 ratio was 1.12 ± 0.24, the mean AT-Ⅲ concentration was 30.9 ± 8.0 mg/dL, the mean Fa value was 64.6% ± 26.9%, and the mean β-TG concentration was 67.0 ± 42.1 ng/dL. These data indicate that after treatment, the immunologic function of the T lymphocytes of gastric cancer patients in the treatment group was significantly improved (P < 0.01). Additionally, the hypercoagulability in the treatment group was also improved (P< 0.001), and the mean OKT4/OKT8 ratio, antithrombin Ⅲ (AT-Ⅲ) concentration, and fibrinolytic activity, *etc.* had all beome normalized. The one-year (86%), 3-year (69%), and 5-year (40%) survival rates in the treatment group were all higher than those in the control group (P < 0.05). The mean tumor weights in the control, traditional medicine, and chemotherapy groups were 0.895 ± 0.289 g, 0.433 ± 0.177 g, and 0.357 ± 0.142 g, respectively. The tumor-inhibition rates in the traditional Chinese medicine group and chemotherapeutic group (51.6% and 60.1%, respectively) were significantly better than that in the control group (P < 0.001). The mean tumor weight in the traditional Chinese medicine group (24.68 ± 1.93 g) was significantly higher than that in both the treatment group (22.96 ± 1.87 g) and control group (22.47 ± 2.18 g).

CONCLUSION: The Fuzheng Huoxue anticancer prescription can not only replenish vital functions (Zhengqi), correct a hypercoagulatory state, improve immunologic function, and extend patient survival times, but may also directly inhibit gastric tumor growth without producing toxic side effects.

Key words: Stomach neoplasms/TCM therapy; Fuzheng Huoxue; survival rate; T lymphocyte subsets; Medicine Chinese traditional

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Table 1 Comparison of T lymphocyte subgroup levels before and after treatment (mean ± SD)

Groups		*n*	OKT3 (%)	OKT4 (%)	OKT8 (%)	OKT4/OKT8
Treatment	Before	15	56.10 ± 8.54	41.81 ± 6.18[a]	45.94 ± 8.45[b]	0.89 ± 0.19[b]
	After	20	58.68 ± 9.98	41.89 ± 8.70[a]	33.52 ± 7.80[d]	1.47 ± 0.51[d]
Control	Before	13	61.87 ± 5.01	46.61 ± 6.79	49.21 ± 6.60[b]	0.94 ± 0.20[b]
	After	12	56.11 ± 8.50	46.24 ± 6.10	42.22 ± 7.07[bd]	1.12 ± 0.24
Healthy person		15	61.66 ± 6.42	48.11 ± 8.51	31.02 ± 4.96	1.57 ± 0.32

[a]$P < 0.05$; [b]$P < 0.001$ as compared with the healthy subjects group; [c]$P < 0.05$; [d]$P < 0.001$ as compared with pretreatment.

Table 2 Changes in hypercoagulatory parameters before and after treatment in the two groups (mean ± SD)

Groups		*n*	AT-Ⅲ (mg/dl)	FA (%)	β-TG (ng/dl)
Treatment	Before	35	29.9 ± 7.9[b]	50.4 ± 24.4[b]	91.0 ± 25.9[b]
	After	35	38.8 ± 5.5[d]	102.6 ± 31.6[d]	62.3 ± 15.1[bd]
Control	Before	34	32.3 ± 7.2[a]	57.3 ± 24.6[b]	87.5 ± 34.2[b]
	After	34	30.9 ± 8.0[b]	64.6 ± 26.9[b]	67.0 ± 42.1[bc]
Healthy persons			36.4 ± 8.3 (59)	90.3 ± 24.3 (30)	25.0 ± 8.2 (103)

[a]$P < 0.05$, [b]$P < 0.01$ as compared with healthy subjects; [c]$P < 0.05$, [d]$P < 0.001$ as compared with pretreatment. The number of individuals is shown in parentheses.

INTRODUCTION

From July 1986 to July 1995, we used the Fuzheng Huoxue (strengthening the body resistance and promoting blood circulation) anticancer prescription to treat postoperative patients with mid- or late-stage gastric cancer, as well as nude mice with transplanted human gastric cancers. The treatment results are reported below.

CLINICAL INVESTIGATION

Clinical data

This study enrolled 69 patients with histologically confirmed mid- or late-stage gastric cancer, and who were being treated at Renji Hospital, affiliated with the Shanghai Second Medical University. The patients were assigned to two study groups. The treatment group included 26 males and 9 females; (two patients aged 33-40 years, 18 patients aged 41-60 years, and 15 patients aged 61-75 years; mean group age = 58.4 years). The control group consisted of 23 males and 11 females, (4 patients aged 33-40 years, 16 patients aged 41-60 years, and 14 patients aged 61-75 years; mean group age = 56.8 years). The pathological stage of each patient was determined according to the TNM classification system, as modified by the Chinese Cooperative Group in Gastric Cancer[1]. In the treatment group, 8 cases were stage Ⅱ, 15 were stage Ⅲ, and 12 were stage Ⅳ. Twenty-seven cases underwent a palliative operation and 11 cases underwent a radical operation. In the control group, 11 cases were stage Ⅱ, 11 cases were stage Ⅲ, and 12 cases were stage Ⅳ. Twenty-three cases received a palliative operation, and 11 cases received a radical operation. The two groups showed no significant difference in sex, age, clinicopathologic stage or operation mode. The healthy individuals were recruited from staff at the Shanghai Second Medical University and its affiliated hospitals.

Methods and parameters of observation

The Fuzheng Huoxue anticancer prescription contained Codonopsis pilosula (Franch.) N annf (15 g), Astragalus membranaceus (Fisch.) Bge (15 g), Atractylodes macrocephala Koidz (12 g), Poria cocos (Schw.) Wolf (12 g), Rehmannia glutinosa (Gaertn.) Lib osch (12 g), Adenophoratetraphylla (Thunb.) Fisch (15 g), Salvia miltiorrhiza Bge (15 g), and Angelica sinensis (Oliv.) Diels (12 g). One dose of this mixture was given to patients once a day. The control prescription contained Citrus tangerina Hort. et Tanaka (12 g), Magnolia officinalis Rehd. et Wils (12 g), Amomum villosum Lour (6 g), Oryza sativa L (15 g), and Hordeum vulgare L (15 g), and was also given as a single dose, once a day.

Treatment methods

Starting at one month after an operation, decoctions of the Fuzheng Huoxue anticancer prescription, the control prescription, and FT-207 manufactured by the Shanghai No.12 Pharmaceutical Factory, (100-200 mg, three times daily) were given to the designated groups for five days.

Measurements of patient survival and assay methods

Patient survival rates were calculated by the direct method. T-lymphocyte subgroups, antithrombin Ⅲ levels, fibrinolytic activity (Fa), and β-thromboglobulin (β-TG) were measured by a fluorescent immunoassay method[2], rocket electrophoresis[3], fibrolysic area[4], and radioimmunoassays, respectively.

EXPERIMENTAL INVESTIGATION

Materials and methods

Materials An oral formulation of the Fuzheng Huoxue anticancer prescription with a crude medicine concentration of 3.0 g/mL was autoclaved and then stored at low temperature. Human gastric cancer cell line SGC-7901 was purchased from the Shanghai Institute of Pharmaceutics. Female nude mice aged 6-8 wk and 5-Fu (bat. No. 930712) were purchased from the Shanghai Tumor Institute, and the Shanghai Haipu Pharmaceutic Factory, respectively.

Methods SGC-7901 cells were inoculated into the backs of nude mice under sterile conditions. The mice were weighed on the next day, and then randomly allocated to a control group, a traditional Chinese medicine group, and a chemotherapy group (n = 10 mice per group). There was no significant difference in the total body weight of the nude mice in each group. A 0.5 mL dose of saline solution was instilled into the stomach of each mouse in the control group each day, while 0.5 mL of the Fuzheng Huoxue anticancer decoction was instilled into the stomach of each mouse in the traditional Chinese medicine group. Mice in the chemotherapy group were intraperitoneally injected with 1 mg of 5 FU once a week for 8 wk.

Observation parameters Each mouse was monitored for its body weight, tumor weight, and any tumor inhibitory effects.

Experimental results

Tumor weight and tumor inhibition The mean tumor weights in the control, traditional Chinese medicine, and chemotherapy groups were 0.895 ± 0.289 g, 0.433 ± 0.177 g, and 0.357 ± 0.142 g, respectively. While compared with tumor growth in the control group, the tumor inhibition rates in the traditional Chinese medicine group and chemotherapeutic group were 51.6% and 60.1%, respectively, ($P < 0.001$).

Body weight of the nude mice The mean body weights of the nude mice in each group before and after treatment were 21.50 ± 1.51 g and 22.47 ± 2.18 g, respectively, in the control group, 21.50 ± 0.47 g and 24.68 ± 1.93 g, respectively, in the traditional Chinese medicine group, and 21.55 ± 0.64 g and 22.96 ± 1.87 g, respectively, in the chemotherapy group. None of these differences before and after treatment were statistically significant ($P > 0.05$). However, when compared with the mean pretreatment weight, the mean body weights in the traditional Chinese medicine and chemotherapy groups were significantly higher after treatment ($P < 0.05$). Furthermore, when compared with the mean body weight in the control group, the mean body weight in the traditional Chinese medicine group showed a significant increase ($P < 0.05$).

RESULTS

Comparison of survival rates between the treatment and control groups

Among the 35 patients in the treatment group, 31 survived > 0.5 years, 30 (86%) survived > 1 year, 24 (69%) survived > 3 years, and 14 (40%) survived > 5 years. Among the 34 patients in the control group, 28 (82%) survived > 0.5 years, 22 (65%) survived

> 1 year, 14 (41%) survived > 3 years, and 6 (18%)> 5 years. The 1, 3, and 5-year survival rates in the treatment group were all significantly higher than those in the control group ($P < 0.05$).

The numbers of different T lymphocyte subgroups in the peripheral blood of subjects in the two groups before and after treatment are shown in Table 1. The immunologic function of subjects in both groups showed significant improvement after treatment.

Changes in the hypercoagulatory parameters of subjects in both groups before and after treatment are shown in Table 2. While both groups were in a hypercoagulatory state before treatment, the hypercoagulatory state of the treatment group showed significant improvement after treatment. The values for AT-Ⅲ and Fa in the treatment group did not significantly differ from those in healthy individuals ($P > 0.05$). The control subjects remained in a hypercoagulatory state.

DISCUSSION

Patients with mid- or late-stage gastric cancer are usually deficient in Zheng qi, and have an impaired immunologic response[5]. The immunologic state of a human is closely correlated with tumor occurrence and development; furthermore, immune responses mediated by T lymphocytes play a major role in tumor immunity.

Our study showed that prior to treatment, the suppressor T lymphocytes of gastric cancer patients were hyperactive and the patients showed a poor immune response. Zhengxu (weakened body immunity) decreases a patient's anticancer response as well as their ability to tolerate chemotherapy. The Fuzheng method is widely used to treat tumors by practitioners of traditional Chinese medicine or combined traditional and Western medicine, and especially in Beijing, Shanghai, Tianjing, and Fujian[6,7], China.

Gastric cancer is associated with blood stasis, and the extent of blood stasis is correlated with the severity and outcome of the disease[8]. AT-Ⅲ is the most important anti-agglutination agent found in plasma. Fa directly reflects the activity of proplasminogen, and β-TG directly reflects the extent of blood stasis. The results of our study showed that prior to treatment, the patients with gastric cancer were in a state of hypercoagulation, and this was the basis for our clinical use of the Huoxue Huayu method. Zhengxu and blood stasis are basic characteristics of patients with mid- or late-stage gastric cancer. In addition to the Fuzheng method, the Huoxue method can also be used to treat such patients. The Fuzheng Huoxue anticancer prescription produced better clinical effects in treating postoperative patients with mid- or late-stage gastric cancer as compared with the effects shown in control patients. The immunologic function and hypercoagulation status of the patients improved significantly. These results suggest that the Fuzheng Huoxue anticancer prescription can not only replenish Zheng qi, enhance the anticancer response of patients, and reduce the side effects of chemotherapy, but can also improve blood stasis and circulation, as well as immunologic function, and is thus beneficial for treating tumors and extending patient survival times. Our animal studies proved that the Fuzheng Huoxue anticancer prescription inhibited tumor growth without producing any significant toxic side effects or affecting the body weights of the nude mice. Based on these results, we feel the Fuzheng Huoxue anticancer prescription has a bright future for use in cancer management. While the Fuzheng Huoxue anticancer prescription may directly inhibit or kill gastric tumor cells, these activities require further confirmation.

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Effects of tetrandrine on gastric mucosa and liver in portal hypertensive rats

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Abstract

AIM: To study the effects of tetrandrine on portal hypertensive gastric mucosal lesions.

METHODS: Portal hypertensive models were induced in Wistar rats by 60% CCl_4 3 mL/kg body weight through subcutaneous injection, once every 4 d for 56 d. The animals were randomly divided into portal hypertension, tetrandrine and propranolol groups and subsequently, treated by normal saline, tetrandrine and propranolol respectively for 15 d. Some healthy rats were used as control group. Portal venous pressure (PVP), gastric mucosal prostaglandin E_2 (PGE_2) content, gastric mucosal blood flow (GMBF), gastric adherent mucus (GAM), ALT, ALP and serum total bilirubin (STB), were measured and liver tissues were observed histologically.

RESULTS: In tetrandrine group and propranolol group, PVP was significantly lower (1.43 ± 0.13, 1.45 ± 0.12 *vs* 1.89 ± 0.18 kPa; $P < 0.01$) and gastric mucosal PGE_2 content (138.59 ± 12.68, 129.98 ± 14.31 *vs* 104.65 ± 12.97 pg/mg; $P < 0.01$), GMBF (11.80 ± 3.47, 10.54 ± 3.63 *vs* 6.61 ± 2.82 mL·h·kg; $P < 0.05$) and GAM (3.01 ± 0.15, 2.98 ± 0.21 *vs* 2.24 mg ± 0.26 mg; $P < 0.01$) was significantly higher than that in portal hypertension control group. In tetrandrine group intrahepatic proliferative fibrous tissues were reduced and serum ALT (47.67 ± 25.90 *vs* 189.33 ± 41.21 King U; $P < 0.01$), ALP (0.22 ± 0.04 *vs* 0.31 ± 0.06 μmol·s^{-1}/L;$P < 0.01$) and STB (4.75 ± 0.76 *vs* 11.12 ± 2.93 μmol/L; $P < 0.01$) were lowered as compared with those in portal hypertension control group. ALT (209.34 ± 36.91 *vs* 189.33 ± 41.21 King U; $P > 0.05$) and STB (11.63 ± 3.01 *vs* 11.12 ± 2.93 μmol/L; $P > 0.05$) in propranolol group were not different from that in portal hypertension control group, but it showed more marked hepatocellular degeneration and necrosis and elevation of ALP (0.46 ± 0.05 *vs* 0.31 ± 0.06 μmol·s^{-1}/L; $P < 0.01$).

CONCLUSION: Tetrandrine can improve the functions of gastric mucosa and liver, and facilitate the absorption of intrahepatic proliferative fibrous tissues. Propranolol can aggravate hepatosis though it may improve portal hypertensive gastric mucosal lesions.

Key words: Liver gastric mucosa; Hypertension, portal; Tetrandrine; Propranolol

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INTRODUCTION

Gastric mucosal lesions (GML) is one of common causes of upper gastrointestinal bleeding in portal hypertensive (PHT) patients with cirrhosis. So far, there have been no ideal therapy for this lesion. Although some reports have suggested that propranolol is effective for GML, attention has been paid to the side effects of this drug[1]. It has been demonstrated that tetrandrine can lower the portal venous pressure (PVP) with no significant effects on peripheral circulation[2],and decrease the serum procollagen-III-peptide concentration in cirrhotic patients[3]. Therefore, in this study we observed the effects of tetrandrine on PVP, the functions of gastric mucosa and liver, and liver histology in PHT rats with cirrhosis, and tetrandrine was compared with propranolol, in an attempt to investigate effects of tetrandrine on PHT GML.

MATERIALS AND METHODS

Animals and portal hypertension induction

A total of 148 male Wistar rats, weighing 200-300 g (254.5 ± 31.4) were used. Of them, 124 were treated by CCl_4, 24 served as normal controls. The 124 rats received 60% CCl_4 (solution in rapeseed oil) 3 mL/kg by subcutaneous injection once every 4 d for 56 d. PHT model was formed in 72 rats, which was confirmed by the histology and the PVP measurement (more than 1.47 kPa).

Experimental groups

The 72 PHT rats were randomly divided into 3 groups, 24 in each: PHT control group, received normal saline 10 mL/kg tid through peritoneal injection, for 15 d; Tetrandrine group, received NS through same administration and forage containing tetrandrine

Table 1 Portal venous pressure (PVP), gastric mucosal prostaglandin E2 (PGE2) content, gastric mucosal blood flow (GMBF) and gastric adherent mucus (GAM) ($\bar{x} \pm s$)

Groups	PVP (kPa)	PGE2 (pg/mg)	GMBF (mL·h·kg)	GAM (mg)
PHT	1.89 ± 0.18^{d}	104.65 ± 12.97^{d}	6.61 ± 2.82^{c}	2.24 ± 0.26^{d}
Propranolol	1.45 ± 0.12^{b}	129.98 ± 14.31^{b}	10.54 ± 3.63^{a}	2.98 ± 0.21^{b}
Tetrandrine	1.43 ± 0.13^{b}	138.59 ± 12.68^{b}	11.80 ± 3.47^{a}	3.01 ± 0.15^{b}
Normal	1.24 ± 0.10^{bc}	162.03 ± 13.84^{bd}	18.86 ± 4.37^{bd}	3.25 ± 0.16^{b}

$^{a}P < 0.05$, $^{b}P < 0.01$, *vs* portal hypertensive (PHT) controls group; $^{c}P < 0.05$, $^{d}P < 0.01$, *vs* propranolol group.

Table 2 Alanine aminotransferase (ALT), alkaline phosphatas (ALP) and serum total bilirubin (STB) ($\bar{x} \pm s$)

Groups	ALT (king U)	ALP ($\mu mol \cdot s^{-1}/L$)	STB ($\mu mol/L$)
PHT	189.33 ± 41.21	0.31 ± 0.06^{d}	11.12 ± 2.39
Propranolol	209.34 ± 36.91	0.46 ± 0.05^{b}	11.63 ± 3.01
Tetrandrine	47.67 ± 25.90^{bd}	0.22 ± 0.04^{ad}	4.75 ± 0.76^{bd}
Normal	36.42 ± 21.53^{bd}	0.21 ± 0.04^{ad}	4.96 ± 0.91^{bd}

$^{a}P < 0.05$, $^{b}P < 0.01$ *vs* portal hypertensive (PHT) controls group; $^{d}P < 0.01$ *vs* propranolol group.

Table 3 The liver fibrosis degree

Groups	Grade 4+++	Grade 3++	Grade 2+	Grade 1-
PHT	6	2	0	0
Propranolol	4	3	1	0
Tetrandrine^{ac}	0	4	2	2
Normal^{bd}	0	0	1	7

$^{a}P < 0.05$, $^{b}P < 0.01$, *vs* portal hypertensive (PHT) controls group; $^{c}P < 0.05$, $^{d}P < 0.01$, *vs* propranolol group.

(150 mg·kg·d) for 15 d; propranolol group, received propranolol 35 mg/kg tid peritoneal injection, for 15 d; normal group, through 24 healthy rats served as controls.

Each group was then randomly divided into 3 subgroups, 8 in each. In the 1st subgroup PVP and gastric mucosal prostaglandin E_2 (PGE_2) content were measured and liver histology and liver function were examined. Gastric mucosal blood flow (GMBF) was measured in the 2nd subgroup and gastric adherent mucus (GAM) measured in the 3rd subgroup.

Methods

PVP measurement. Laparotomy was performed under 60% urethane (5 mL/kg by peritoneal injection) anesthesia in rats fasted for 24 h. The portal vein was punctured with a needle and PVP was measured by a manometer.

Gastric mucosal PGE_2 content measurement. The stomach was excised, then put it on a piece of ice to scrape gastric mucosa. The gastric mucosal PGE_2 content was measured by radioimmunoassay[4].

Gastric mucosal blood flow measurement. The rats fasted for 24 h, under the same anesthesia, GMBF was measured by neutral red clearance test[5].

Gastric adherent mucus measurement. The rats fasted for 24 h, under the same anesthesia, the stomach was excised and opened along the lesser curvature, turned over the gastric wall. GAM was measured by alcian blue staining[6] and result was shown by adherent dye amount.

Liver function test. A blood sample 2.5 mL was taken for determing ALT, ALP and serum total bilirubin (STB). This was performed by the same staff from the laboratory department of our hospital.

Liver histology. The histological specimens were made by the pathology department of our hospital, stained with hematoxylin and eosin (HE) and observed under light microscope.

Statistical analysis

Analyses of variance among groups for data measurement were made with F test, and difference between two groups was compared with *q* test. For ranked data, multiple samples were compared with *H* test, and difference between two groups was compared with *U* test. Results were considered statistically significant at $P < 0.05$.

RESULTS

There were no significant differences in PVP between tetrandrine and propranolol groups ($P > 0.05$), PVP in both the groups was significantly lower than that in PHT control group ($P < 0.01$), but higher than that in healthy group ($P < 0.05$), (Table 1).

Gastric mucosal PGE_2 content and GMBF showed no significant difference ($P > 0.05$) between tetrandrine and propranolol groups, and were significantly higher in both the groups than that in PHT control group ($P < 0.01$; $P < 0.05$), but lower than that in healthy group ($P < 0.01$), (Table 1).

There was no significant difference in GAM between tetrandrine and propranolol groups ($P > 0.05$). The values in both the groups were significantly higher than that in PHT control group ($P < 0.01$), but without significant difference as compared with the healthy group ($P > 0.05$), (Table 1).

In ALT and STB, difference between PHT and propranolol groups was not significant ($P > 0.05$). ALT and STB were significantly higher than in tetrandrine and healthy groups ($P < 0.01$), with no significant difference ($P > 0.05$) between the latter two groups, (Table 2).

ALP in propranolol group was significantly higher than that in PHT control group ($P < 0.01$), (Table 2). It was significantly higher than that in tetrandrine and healthy groups ($P < 0.01$; $P < 0.05$), with no significant difference ($P > 0.05$) between the latter two groups.

In PHT group, pseudolobules were found in most cases, but hepatocytic degeneration and necrosis were not obvious, few red cells were found in sinusoid of the liver. In propranolol group, pseudolobules or a large quantity of proliferative fibrous tissues were found, hepatocytic degeneration and necrosis were more marked with few red cells found in sinusoid of the liver. In tetrandrine group, intrahepatic proliferative fibrous tissue was less than that in PHT group and pseudolobules disappeared, and many red cells were found in the sinusoid of the liver, no hepatocytic degeneration and necrosis were found.

According to the degree of intrahepatic proliferative fibrous tissue, four grades were established. Grade 4, pseudolobules; Grade 3, a large quantity of fibrous tissue; Grade 2, a little fibrous tissue; Grade 1, no proliferative fibrous tissues were found. Results showed that there was no significant difference between propranolol and PHT groups ($P > 0.05$). In tetrandrine group, fibrous tissue was significantly decreased as compared with that in PHT control group ($P < 0.05$). There was significant difference ($P < 0.01$) between healthy and other groups. These data are shown in Table 3.

DISCUSSION

The pathogenesis of PHT GML was the weakening of gastric mucosal protective mechanism resulting from the structural and functional impairment of mucosal microcirculation consequent upon PHT. Gastric acid and pepsin were not the main causes of this lesion[7]. Therefore, treating PHT GML by reducing PVP has been a main orientation of study in the world. Gastric mucosal PGE_2 contet, GMBF and GAM are important factors of gastric mucosal protective mechanism so that the change of these factors will reflect the therapeutic effects on PHT GML.

It has been reported that propranolol may be effective for PHT GML. Our results showed that propranolol could significantly reduce PVP and increase the gastric mucosal PGE_2 content, GMBF and GAM. Our previous electron microscopic observation showed that propranolol could significantly improve the pathological state of gastric mucosal capillaries and increase the mucigen granules of gastric mucosal epithelial cells[8]. These demonstrated that propranolol is indeed useful in improving PHT GML. Propranolol is a β adrenoreceptor-blocking agent, and its effect depends on constricting splanchnic blood vessels, decreasing blood flow into the portal venous system, reducing PVP and improving the dilatation state of gastric mucosal microcirculation. Nevertheless, in this study, ALT and STB in propranolol group were not decreased, while ALP

was increased as compared with PHT group. These suggest that propranolol could not decrease hepatocytic damage, but increase the intrahepatic obstruction on the contrary. The liver histologic observation also proved this. Because propranolol reduces PVP through decreasing portal venous blood flow and constricting blood vessel, hepatic blood supply is further reduced, resulting in marked liver damage. Moreover, further reduction of the blood flow passing through the liver causes increased blood ammonia and incidence of cerebrosis.

Effects of tetrandrine on gastric mucosa in PHT rats were almost the same as propranolol, but their mechanisms of decreasing PVP were different. Tetrandrine, a calcium-channel blocking agents, inhibits calcium ion acrossing the calcium channel of vascular smooth muscle by blocking receptors on cell membrane, leading to a decrease in intracellular calcium ion concentration, and relieving excit ation-constringent coupling. As a result, smooth muscle loosened, intra and extra-hepatic portal venous resistance decreased and PVP reduced[2]. Furthermore, it can inhibit the activity of adenylate cyclase on cell membrane, lower the intracellular cyclic adenosine monophosphate (cAMP) levels, thus disturbing the phosphorylation process of thymine, inhibiting the collagen synthesis of hepatocytes and Ito cells[9]. In this study, we also found that tetrandrine can reduce intrahepatic proliferative fibrous tissues. So PHT GML was improved because of the decreased PVP and portal venous backward blood flow. Besides, results showed that tetrandrine can significantly lower ALT, ALP and STB to the normal. From these results, it is supposed that tetrandrine alleviate hepatocytic necrosis and intrahepatic obstruction. Histologic observation also proved this. These are due to the dilatation of intra and extra-hepatic portal vein and the decrease in intrahepatic fibrous tissue and portal venous resistance, resulting in increased hepatic blood supply and better hepatic nutritional stat us.

In conclusion, tetrandrine not only can improve the function of gastric mucosa and liver, but also facilitate the absorption of intrahepatic proliferative fibrous tissues. This study suggests that tetrandrine might be a more appropriate drug for PHT GML as compared with propranolol.

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Cost-effectiveness study on treatment of duodenal ulcer

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Abstract

AIM: To compare the efficiency of therapy with a 2-week regimen of amoxicillin plus metronidazole and six weeks of Tagamet (AMT group) *vs* the efficacy of therapy with 6 wk of omeprazole plus 2 wk of amoxicillin (OA group) for ulcer healing, *Helicobacter pylori* (Hp) eradication, and decreasing the recurrence of duodenal ulcers.

METHODS: This cost-effectiveness analysis was based on results shown in a randomized controlled trial conducted in 1995 in patients with a duodenal ulcer (OA group, 46 patients; AMT group, 43 patients) and treated at class grade III A hospitals in Shanghai, China.

RESULTS: The costs of treatment in the AMT group were less than those in the OA group for ulcer healing (￥546.25 *vs* ￥1296.76 per case, $P < 0.01$), Hp eradication (￥702.32 *vs* ￥1742.53 per case, $P < 0.01$), and decreasing ulcer recurrence (￥640.39 *vs* 1424.54 per case, $P < 0.01$). Direct costs comprised the major cost involved in treatment of duodenal ulcers. The difference in the cost of treating ulcers in the two groups was primarily due to the costs of the different drugs. There was no significant difference between the two groups regarding their direct non-medical costs and indirect costs.

CONCLUSION: When based on therapeutic effectiveness and financial costs, AMT therapy was more cost-efficient than OA therapy. AMT therapy is recommended for its low cost, acceptable ulcer healing rates, ability to cure of an Hp infection, and especially when treating patients with an ulcer < 1 cm in diameter.

Key words: Duodenal ulcer; *Helicobacter pylori*; Cost-effectiveness analysis

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Endoscopic ligation for benign and malignant lesions of upper digestive tract

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INTRODUCTION

Endoscopic techniques have been used to treat variceal hemorrhage for > 50 years, and are now accepted as first line treatment for this type of esophageal bleeding. While injective sclerotherapy can control hemorrhage in 90% of cases, bleeding reoccurs and complications occur in up to 55%[1] and 40%[2] of cases, respectively. As a result of these problems, better methods for endoscopic treatment of variceal hemorrhage have been continuously studied. Banding ligation was first reported in humans in 1990[3], and has since become an important method used in endoscopic therapy. Moreover, this method has shown reliable effects when used to manage esophageal varices. Based on such reports, we used banding ligation to resect polyps and early stage cancers of the upper digestive tract, and achieved satisfactory results.

MATERIALS AND METHODS

Patients

A total of 174 patients underwent endoscopic ligation. Among these patients, 78 were treated for a variceal hemorrhage (these included 4 patients with a primary hepatic cancer, 10 patients who experienced re-bleeding after spleen resection, and 2 patients who experienced re-bleeding after a TIPS operation). Prior to undergoing ligation, these patients had bleeding frequencies that ranged from 1 to 8 times/year (mean = 2.35 times/year). Twenty-one of the patients had a polyp in the upper digestive tract (14 cases in the gastric antrum, 4 in the gastric body, 2 in the lower esophagus, and 1 in the duodenum). The polyps could be morphologically characterized as one of three types: (1) long pedunculated, (2) sub-pedunculated, and (3) thick, and had diameters ranging from 0.3 cm to 0.9 cm. Five of the polyps proved to be early stage cancers (2 cases of early esophageal cancer, and 3 cases of gastric antral cancer *in situ*).

Methods

All ligations were performed using an Olympus XQ10 XQ20 endoscope (Olympus Corporation; Tokyo, Japan), and either a plastic or stainless steel ligation device. Other equipment included a silicon rubber band (used in ligation), trip wire, inner cylinder, and an outer cylinder. A ligation device fixed at the end of the endoscope was plunged into the digestive tract, and placed in close proximity to the lesion. The lesion was then sucked into the ligation device, and a prestressed rubber band was released over the entrapped lesion by pulling the trip wire; after which, the lesion was ligated. All patients underwent endoscopic re-examinations for > 1 year. The therapeutic effectiveness of the ligation procedure was evaluated by both endoscopic observations and histopathological examinations.

RESULTS

While a majority of the 78 esophageal variceal patients who underwent ligation were cured, four patients died because the treatment did not stop the progress of their disease. The overall effectiveness of ligation was 94.8% (74/78 cases, Table 1), and the mean bleeding frequency decreased from 2.35 times/year before treatment to 0.15 times/year after treatment. While four patients reported slight dysphagia, no other complications occurred.

Table 2 shows the sloughing off times for the polyps and early stage cancers following treatment with endoscopic ligation; these times ranged from 4 to 10 d, and nearly 100% of the lesions disappeared. Furthermore, biopsy results showed no malignant cells in the resected specimens obtained from cancer patients treated with ligation. A small ulcer remained after lesion sloughing in 18 cases; however, these lesions usually healed 10 d later, and there was no subsequent bleeding or lesion recurrence. Histopathological examinations showed some infiltration of inflammatory cells at the site of the wound, but no necrosis was observed.

DISCUSSION

Banding ligation was the most important development in endoscopic

Table 1 Ligation compared with sclerotherapy for the variceal bleeding

n		Effective rate (*n*)	Bleeding times (yr)		Rebleeding (%)	Complication (*n*)	
			B-T	A-T		Dysphagia	Others
LT	78	94.8% (74/78)	2.35	0.15	3.80%	4	0
ST	32	90.6% (29/32)	2.4	1.1	30%	3	9

B-T: Before treatment; A-T: After treatment; LT: Ligation treatment; ST: Sclerotherapy

Table 2 The sloughing off time for the ligated polyp and early cancers

Lesion type	*n*	Cases with different sloughing off time		
		4-5 (d)	6-8 (d)	9-10 (d)
Polyp				
TP	8	5	3	0
SP	7	4	3	0
LP	6	1	4	1
Early cancer[1]				
Gastric CA	3	1	2	0
Esophageal Ca	2	0	2	0

[1]No cancerous cells were found in the resected specimen of 5 early stage cancers. TP: Thick polyp; SP: Sub pedunculated polyp; LP: Long pedunculated polyp.

therapy. We have used endoscopic ligation (EL) to treat esophageal variceal bleeding since 1992, and achieved satisfactory results. We also conducted a study which compared banding ligation and sclerotherapy in the management of esophageal variceal bleeding. The results indicated that EL was highly effective. Moreover, the treated patients experienced a quick recovery and had a low rebleeding rate. The 78 patients who received ligation for esophageal variceal bleeding required significantly fewer treatments, as well as treatments of shorter duration, when compared to patients treated with conventional sclerotherapy. Additionally, the patients treated with EL experienced fewer complications, as only 4 patients reported slight dysphagia, and other complications associated with sclerotherapy, (*e.g*. fever, pleura infiltration, and esophageal stricture) were not observed.

Also, when compared with a portacaval shunt operation, EL produced no effect on liver blood flow. In cases where splenic hyperfunction is not sufficiently severe to necessitate performing a splenectomy, EL can serve as an alternative to portal-azygos disconnection. Moreover, EL might be the first choice for patients who experience re-bleeding after a portacaval shunt or portal azygos disconnection, as it has the advantages of safety, convenience, and causing little or no injury. We also used EL to treat 2 patients undergoing Tips operations, and achieved satisfactory results.

Endoscopic ligation has recently been used in treatment of gastroenteric polyps and in cases requiring early cancer resection. Ligation by itself usually blocks blood flow to the polyp or cancer; this induces lesion ischemia or tissue necrosis, which causes the ligated lesion to eventually fall off. We noticed that a very small ulcer was present after a lesion fell off; however, the ligation procedure was a progressive process in which tissue damage and healing occurred almost simultaneously when the stretched silicon rubber band was recovered.

Similar to performing a microwave resection[4], different methods should be used to ligate lesions of different morphological types. When ligating sub-pedunculated and thick polyps, as well as early stage cancers, the "O" type of rubber band should be used and released at the base of the polyp, while segmental ligation should be performed when treating long pedunculated polyps. When treating polyps with a longer pedicle, we used the "U" ligation method, which allowed the ligation device to approach the juncture of the polyp base and pedicle. Next, suction was applied through the endoscope, and the rubber band was released over the entrapped polyp ("U" shape).

The authors of this paper suggest that ligation management should be selected for the resection of early stage cancers of the upper digestive tract, and especially situ cancers; because using this method can spare the patient from the risks and pain associated with an operation. In cases where a histopathological examination reveals the presence of malignant cells, ligation should be performed within 24 h after biopsy so as to guarantee the correct site for ligation. If esophageal cancer is confirmed, the tissue can be treated with Lugol's solution, and then ligated at the lightly stained site[5]. Such patients should receive regular followup examinations with endoscopy and histopathology following their ligation treatment.

The scaling time of a lesion depends on its hardness and the elasticity of the "O" rubber band. The scaling time is shorter when treating soft lesions with an "O" rubber band of better elasticity.

The current commercially available inner cylinders have a diameter of 0.9 cm, and if the polyp is too large, it cannot be sucked into the device. Furthermore, the ligation method has limited usefulness for treating colon polyps, because no currently available colonoscope has a matched ligation device. Therefore, the methods and equipment used for ligation require further development.

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Changes in mucosal permeability to lipopolysaccharide in the colon of chronic alcoholic rats

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Abstract

AIM: To evaluate the effects of chronic alcohol abuse on mucosal permeability to lipopolysaccharide (LPS) in the colon of rats.

METHODS: *Escherichia coli* LPS (20 mg/L) was injected into the colon of chronic alcoholic rats (n = 10) that had been supplied with Lieber diet every other day for six weeks. Before and 5, 10, 20, and 30 min after LPS injection, portal vein blood samples were obtained and the LPS levels in the blood were measured. The distribution of LPS in the colon tissues was observed with confocal laser scanning microscopy by immunofluorescence technique using a monoclonal antibody specific to the lipid A region of LPS. Normal rats were used as the controls (n = 6).

RESULTS: Before LPS injection, LPS levels in the portal vein blood of chronic alcoholic rats were significantly higher than that of the normal controls (3.56 ± 0.67 ng/L *vs* 2.45 ± 0.15 ng/L, $P < 0.01$). At 5, 10, 20, and 30 min after LPS injection, LPS levels were significantly higher than that before LPS injection (173.56 ± 3.45 ng/L, 154.78 ± 0.57 ng/L, 43.89 ± 0.67 ng/L, 45.38 ± 0.89 ng/L *vs* 3.56 ± 0.67 ng/L, respectively, $P < 0.01$). Most mucosal cells in the chronic alcoholic rats showed strong positive reactions to LPS, but in the normal rats, there were no significant changes in portal vein blood LPS levels and in the fluorescence reactions to LPS in the mucosal cells after LPS injection.

CONCLUSION: Chronic alcohol abuse results in a significant increase in LPS permeability in the colon mucosa cells of rats.

Key words: Colon/metabolism; Lipopolysaccharide/metabolism; *Escherichia coli*; Alcohol; Endotoxins

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Effects of Radix Rehmanniae on gastric acid secretion and gastric ulcer formation in rats

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INTRODUCTION

The traditional Chinese medicine Radix Rehmanniae (RR), or Chinese foxglove root, is the root of *Rehmannia glutinosa* Libosch, a Scrophulariaceae plant. It is divided into three types based on differences in its preparation and action: fresh (dRR), dry (dRR), or steamed (sRR). RR contains 23 glucosides (main component: iridoid glucoside), eight carbohydrates, over 20 amino acids, and inorganic ions and trace elements. Its pharmacological effects on many systems are known but not that on the gastrointestinal tract.

Table 1 Effects of dry Radix Rehmanniae (dRR) on gastric juice secretion (x- ± s)

Groups	*n*	Volume (mL)	Total acidity (μmol/mL)	Total acid output (μmol)
Control	10	2.9 ± 1.9	86.3 ± 16.1	241.3 ± 124.6
100% dRR	10	0.5 ± 0.6[a]	41.9 ± 29.1[a]	32.4 ± 56.6[a]
50% dRR	9	1.4 ± 0.9[a]	61.3 ± 33.6	104.3 ± 100.2[a]
F value		15.319	6.772	11.226

The data were tested with analysis of variance, *q* test was used in multiple comparison. [a]$P < 0.05$, as compared with control.

Table 2 Effects of steamed Radix Rehmannia (sRR) on gastric juice secretion (x- ± s)

Groups	*n*	Incidence	Number of ulcer	Inhibitory rate
Control	10	90%	10.2 ± 12.3	–
100% dRR	12	33%	3.1 ± 7.6[a]	69.60%
100% sRR	11	36%	1.1 ± 2.2[a]	89.20%
		$\chi^2 = 8.27$	F = 5.5112	

The data were analyzed with k-ws test. "0" means no acid (pH > 7), [a]$P < 0.05$, compared with control.

Table 3 Effects of 100% dRR and 100% sRR on gastric ulcer formation in pylorus ligated rats (x- ± s)

Groups	*n*	Volume (mL)	Total acidity (μmol/mL)	Total acid output (μmol)
Control	10	2.9 ± 1.9	86.3 ± 16.1	241.3 ± 124.6
100% sRR	6	< 0.1[a]	0[a]	0[a]
50% sRR	10	0.9 ± 0.6[a]	73.3 ± 29.1	72.5 ± 53.4[a]

The means were tested with analysis of variance, *q* test was used in multiple comparison. The rate was analysed with χ^2 test for contingency table data. [a]$P < 0.05$, compared with control.

MATERIALS AND METHODS

Animals

Sprague Dawley rats of both sexes, weighing 160–180 g, were supplied by our university's Centre of Experimental Animals.

Collection and measurement of gastric juice

According to Shay's method, solid food but not drinking water was withheld 48 h before the experiment for all rats. After ether anesthesia, the abdominal wall was incised and the pylorus was ligated. Two hours later, the rat was killed and its cardia was ligated. The stomach was removed and an incision was made along the greater curvature to collect and measure the gastric juice. To calculate the total acidity and total acid output, the gastric acid was titrated with 0.01 mol/L NaOH solution to pH 7 on a Type ZD$_2$ titrator.

Gastric ulcer model

The operative procedure was the same as above. The stomachs were removed, opened, washed with saline, and mucosal lesions were examined with a magnifying glass to count the number and incidence of ulcers 10–12 h after pylorus ligation. The ulcer inhibition rate was calculated as follows:

Ulcer inhibition rate = [(Average number of ulcers in treatment group – Average number of ulcers in control group)/Average number of ulcers in treatment group] × 100%

Preparation and administration of decoction

The dRR and sRR were provided by the Third Affiliated Hospital Pharmacy of Traditional Chinese Medicines. dRR or sRR (20 g) was placed in 200 mL deionized water (prepared by our university's Department of Chemistry), decocted for 30 min, filtered, and 150 mL deionized water was added to the filtrates, which were decocted for 20 min and filtered. The two filtrates were combined and concentrated to 20-mL volume, producing a 100% decoction of dRR or sRR that could be diluted to a 50% decoction with water. A 2.0-mL decoction or deionized water (control) was injected slowly into the duodenum after pylorus ligation in all the rats.

Statistical analysis

The data were analyzed statistically using computer software. Analysis of variance was used for comparing the difference among sample means; the *q* test (Newman–Keuls test) was used for multiple comparison. The data were logarithmically transformed when the homogeneity of variance was not met. If the variances were not equal after logarithmic transformation, we used the rank–sum test (k-ws test) and χ^2 test (for contingency table data) to analyze the difference in rates.

RESULTS

Effects of dRR and sRR on gastric juice secretion and ulcer

Tables 1–3 show that dRR and sRR remarkably inhibited gastric acid secretion and ulcer formation in the pylorus-ligated rats.

Dose–effect relationship

The volume of gastric juice and total acid output in the 50% dRR group were higher than that in the 100% dRR group ($P < 0.05$) (Table 1). The volume of gastric juice, total acidity, and total acid output in the 50% sRR group were also higher than that in the 100% sRR group ($P < 0.05$) (Table 2). The results suggest that the inhibitory effects of dRR and sRR on gastric juice secretion may be dose-dependent.

Comparison between dRR and sRR

The volume, total acidity, and total acid output in the 100% dRR group were all higher than that in the 100% sRR group ($P < 0.05$ or 0.01). Other differences were not statistically significant ($P > 0.05$) (Tables 1–3).

DISCUSSION

Some evidence has proven that the increased gastric juice secretion after pylorus ligation is due to excitation of the mucosal baroreceptors of the antrum and the activation of the vago-vagal reflex, and ulcer formation is related to the copious secretion of gastric juice. Therefore, the acid inhibitory effect of dRR and sRR may result from antagonization of the excitatory action of the vagus nerve. The anti-ulcer action of dRR and sRR may be in agreement with that of the acid inhibition; however, other mechanisms cannot be excluded.

The chemical components of dRR differ somewhat from that of sRR, as do the medical effects. The acid inhibitory effect of sRR is slightly stronger than that of dRR, but the relationship between the difference in activity and ingredients remains unclear.

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Detection method for peripheral venous *AFP* mRNA in hepatocellular carcinoma

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a major cause of death in patients with chronic liver disease. One of the difficulties in managing HCC is its complex character: intrahepatic metastasis, venous invasion, and distant metastasis can occur. In metastasis, tumor cells are scattered from the original site, spread hematogenously, and are arrested at the small vessels. Thus, detecting tumor cells in the circulation might predict tumor metastasis[1]. However, the number of cells in circulation could be too low to be detected morphologically.

Recently, tumor-associated genes in circulating tumor cells (predicting the presence of tumor cells in the circulation) could be detected by PCR in patients with prostate cancer with distant metastasis or in patients with neuroblastoma[2,3]. In HCC cells, the human alpha-fetoprotein (*AFP*) gene is transcribed prominently, but not in normal adult cells[4]. Thus, detecting HCC-associated gene transcription (*AFP* mRNA) in the circulation might be related to the hematogenous metastasis of HCC, even though overt metastasis might be obscure.

In the present study, a sensitive nested reverse transcription–PCR (RT-PCR) assay for detecting HCC cells in the blood was investigated.

MATERIALS AND METHODS

Cell line

The BEL-7402 HCC cell line[5] (a gift from Dr. Liu from the Shandong Medical Institute cell bank) was maintained in RPMI 1640 medium containing 15% fetal bovine serum. It served as the positive control for *AFP* mRNA expression.

AFP cDNA clone plasmid

(70 g/L, made by our laboratory).

Specimen preparation

Peripheral blood from healthy volunteers was collected in a disposable syringe containing heparin. One thousand BEL-7402 cells were added to 5 mL whole blood, and serial dilutions of tumor cells were made. Each dilution contained 1000, 100, 10, and 1 BEL-7402 cell per 5 mL whole blood.

Preparation of nuclear cells from peripheral blood

After adding an equal volume of 3% gelatin solution to the tube containing 5 mL blood, the tube was incubated for 5 min at room temperature. The supernatant was collected and centrifuged at 500 × *g* for 5 min. Residual erythrocytes were lysed by adding distilled water, and isotonicity was restored after 30 sec by adding the same volume of 1.8% NaCl solution. After 5-min centrifugation at 500 × *g*, the cells were immediately frozen using liquid nitrogen and stored until used.

Total RNA extraction, complementary DNA (cDNA) synthesis, and RT-PCR

Using an RNA extraction system (prepared by our laboratory), total RNA was extracted from the nuclear cell component of the peripheral blood. About 5 μg RNA was extracted from 5 mL blood and 1 μg RNA was extracted from 10.5 BEL-7402 cells.

RNA (1 μg), which was heated at 95 ℃ for 10 min and rapidly cooled in ice water, was mixed with 3 μL 10 × buffer (pH 8.3, Tris–HCl), 0.6 μL 10 mmol/L dNTPs, 2.5 μL 25 mmol/L $MgCl_2$, 0.5 μL each of primer #1 (upstream, 5′-ACTGAATCCAGAACACTGCATAG-3′) and primer #2 (downstream, 5′-TGCAGTCAATGCATCTTTCACCA-3′), and 2 U reverse transcriptase, and the volume was adjusted to 30 μL by adding diethylpyrocarbonate-treated water and covered with liquid paraffin. The cDNA was synthesized by incubating the mixture at 37 ℃ for 30 min. After initial denaturation at 94 ℃ for 5 min, RT-PCR was performed according to the temperature profile (94 ℃ for 45 sec, 54 ℃ for 60 sec, 72 ℃ for 45 sec) for 35 cycles. The reaction was terminated by 5-min heating at 72 ℃ and was then cooled to 4 ℃.

Nested PCR

The primers used in the nested PCR for *AFP* mRNA were #3

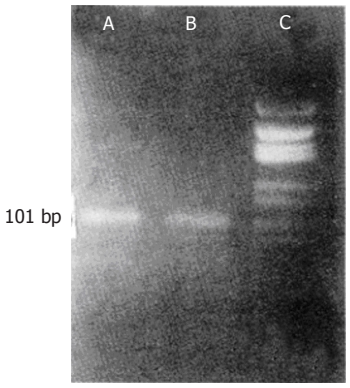

Figure 1 Detection of *AFP* mRNA in BEL-7402 cells and in alpha-fetoprotein (AFP) cDNA clone plasmid. Lane A: AFP cDNA clone plasmid; B: BEL-7402 cell; C: Marker.

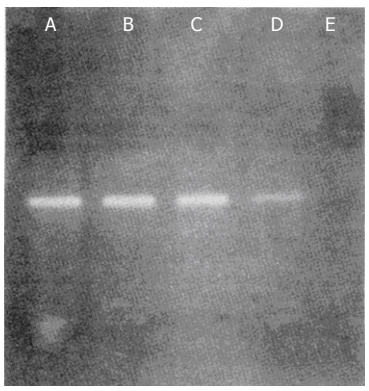

Figure 2 Detection of tumor cells in 5 mL blood. Lanes A-D: 1000, 100, 10, and 1 BEL-7402 cell, respectively. Lane E: Blank control.

(upstream, 5′-TGGAATAGCTTCCATATTGGATTC-3′) and #4 (downstream, 5′-AAGTGGCTTCTTGAACAAACTGC-3′).

The amplified RT-PCR product (2 μL) was mixed with the second PCR buffer [3 μL; 100 mmol/L Tris–HCl (pH 8.3), 10 mmol/L $MgCl_2$], 0.35 μL 42 mmol/L each primer (#3 and #4), 1.5 U *Taq* DNA polymerase, and the mixture was diluted to 30 μL with distilled water and covered with liquid paraffin. The temperature profile of the nested PCR was the same as that of the RT-PCR described above.

Gel electrophoresis

The amplified product was electrophoresed on 3.8% agarose gel and stained with ethidium bromide. The size of the amplified nested PCR product of AFP mRNA was 101 bp.

RESULTS

Absence of AFP mRNA in blood of healthy volunteers

The nested PCR did not detect *AFP* mRNA in the nuclear cell component of the peripheral blood from the healthy volunteers.

Detection of AFP mRNA in BEL-7402 cells and AFP cDNA clone

(Figure 1).

Sensitivity of nested PCR for tumor cell detection

BEL-7402 cells (10^4) were suspended in 5 mL whole blood from healthy volunteers and sequentially diluted with blood. *AFP* mRNA–specific PCR products (101 bp) could be observed in each lane, which represented 1, 10, 100, and 1000 BEL-7402 cells in 5 mL normal blood, respectively (Figure 2).

DISCUSSION

To detect tumor cells in blood, the demonstration of tumor-associated genes has been developed using PCR. In a recent study, albumin mRNA in blood was detected as a marker of circulating hepatocytes[6]. Some researchers have indicated that AFP production is increased in HCC cell lines such as BEL-7402, and *AFP* gene expression is increased during carcinogenesis[4]. Thus, we developed a nested PCR assay to detect the HCC-associated tumor gene transcript *AFP* mRNA in the nuclear cell component of blood, as the number of circulating tumor cells in patients with HCC accompanying metastasis to the distant organs might be too low. To explain the sensitivity of nested PCR for detecting HCC cells in the blood, BEL-7402 cells, established from hepatoblastoma and that produce adequate amounts of AFP, were mixed with blood[5]. The nested PCR could detect *AFP* mRNA when 1–10 tumor cells were present in 5 mL blood. Therefore, if BEL-7402 cells were circulating in a body (total volume of blood: around 5000 mL), it would be possible to detect *AFP* mRNA when more than 1000 tumor cells are present in the circulation. Although a moderate number of circulating tumor cells might be present, as assessed from the detection of the BEL-7402 cells, there might be several steps for the establishment of metastasis[1] such as tumor cell adhesion to the vascular endothelium, migration into the extracellular space, and proliferation at the metastatic foci. During this process, host organ immunological reactions take place, and some tumor cells might be damaged by immunocompetent cells, the mechanical force of the blood flow, or by platelet aggregation.

These results suggest that the highly sensitive nested PCR assay is useful for demonstrating the hematogenous spread of tumor cells. Further studies are being carried out in our laboratory, focusing particularly on predicting metastasis and post-therapeutic changes in patients with HCC by detecting circulating tumor cells.

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Clinicopathological risk factors and prognostic evaluation in hepatocellular carcinoma recurrence after surgery

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Abstract

AIM: To analyze the clinicopathological risk factors in hepatocellular carcinoma recurrence after surgery.

METHODS: We used significance testing (χ^2 and Student's *t*-test) of single and multiple factors, and Wilcoxon Cox tropic examination; a retrospective clinicopathological analysis was performed on 156 cases of hepatocellular carcinoma after hepatectomy.

RESULTS: Of the 156 cases, 68.4%, 57.3%, 46.7%, 31.5%, and 28.6% had one, two, three, four, and five postoperative tumor-free years, respectively; the total recurrence rate was 53.2% (83/156). In the 83 recurrent cases, 65 were intrahepatic subclinical, with a resection rate of 78.3% (65/83). The relevant factors involved in recurrence were: male gender, tumor number and size, capsule infiltration, and portal vein involvement. These factors were an obvious influence on the prognosis of the patients with postoperative hepatocellular carcinoma ($P < 0.05$). In the recurrent liver carcinomas, 63.1% of tumor nodes (41/65) were at the ipsilateral segment of the primary tumor nodes.

CONCLUSION: Male gender, tumor number and size, capsule infiltration, and portal vein involvement are factors for postoperative hepatocellular carcinoma recurrence. Recurrence is mainly unicentral. The right front liver lobe is the segment with a high rate of recurrence.

Key words: Liver neoplasms/surgery; Carcinoma, hepatocellular/surgery; Neoplasm recurrence, local; Prognosis; Risk factors

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Endoscopic haemoclip ligation of pedunculated polyp before polypectomy

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INTRODUCTION

Metallic haemoclips have been endoscopically placed in the gastrointestinal tract for treating bleeding lesions and closing perforations[1,2]. A further potential application is ligating pedunculated polyps prior to polypectomy as a prophylactic measure to prevent bleeding. We used metallic haemoclips to treat two patients with pedunculated colonic polyps and bloody diarrhoea successfully.

CASE REPORT

Case 1

A 58-year-old white man with a colonic polyp was referred to our unit for polypectomy. He had a history of chronic renal failure secondary to chronic glomerulonephritis for 14 years and required regular dialysis with 1 × 5000 IU heparin. Colonic examination was performed with a colonoscope (CF-IT 200, Olympus Corp., Tokyo), and three pedunculated polyps were found. Two non-bleeding polyps, both 1.5 cm, were in the sigmoid flexure; the other was 0.8 cm and had a longer stalk, measured about 0.8 cm in length and 0.4 cm in diameter, and was in the descending colon. There was active oozing (Forrest Ⅰb) over the top of the polyp with the longer stalk. A standard polypectomy snare was used to snare the polyps, and metallic clips with a delivery catheter (MD-850, HX-3L, Olympus Corp.) were used for clip ligation. Routine polypectomy was performed for the two non-bleeding polyps. In one, a small vessel fistula (Forrest Ⅱa) was seen and a clip was applied to ligate it. To prevent post-polypectomy bleeding, the stalk was ligated completely with one clip before the procedure was performed in the remaining polyp. There was no bleeding after the procedure. For the bleeding polyp, the stalk was ligated to stop the bleeding: first, the clip and delivery catheter were inserted through the endoscope and then the clip was opened, and the handle of the delivery catheter was rotated to change the direction of the clip prongs to grasp the stalk.

Thereafter, the delivery catheter was pushed and the clip was closed. Two clips were required to ligate the stalk completely. There was no massive bleeding after the polypectomy. No complications were observed by clipping and there was no recurrent bleeding during the 4-week follow-up.

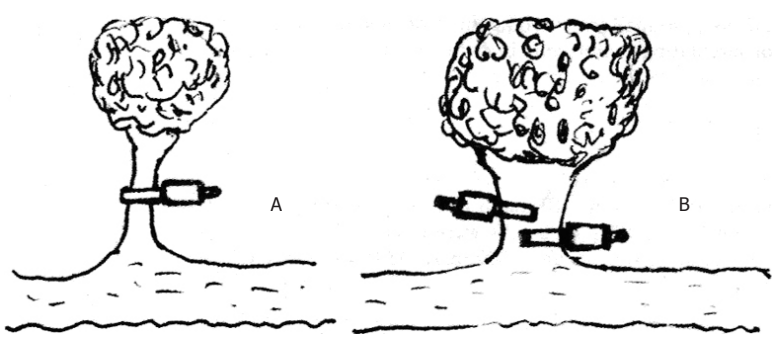

Figure 1 The principle of clip ligation for pedunculated polyps prior to polypectomy to stop bleeding or as a prophylactic measure to prevent bleeding. A: When the stalk of the polyp is thin (with a diameter **> 0.4 cm), one clip is sufficient for complete ligation of the stalk. B: When the** diameter of the stalk is **> 0.4 cm, two clips are needed for complete ligation of the stalk.**

Case 2

A 48-year-old Chinese woman with recurrent bloody diarrhoea for four years and fresh bloody diarrhoea again for four days was admitted to our hospital for colonoscopic examination. She had no history of fever, weight loss, or haemorrhoids. The total colon was examined with a colonoscope (CF-30I, Olympus Corp.), and a 2.0-cm polyp with a long stalk, about 1.0 cm in length and 0.3 cm in diameter, was found in the sigmoid colon. There was a fresh blood clot on the top of the polyp. We used the same technique as in case 1 and ligated the stalk completely with one clip.

No massive bleeding occurred after the polypectomy, but a blood vessel fistula without active bleeding was found. No complications or recurrence of bleeding were observed during the 8-week follow-up.

DISCUSSION

Ligation using suture or metallic clips is a basic surgical technique for preventing postoperative bleeding. Generally, there are nourishing blood vessels in the stalk of the pedunculated polyp, and their diameter depends on the size of the polyp and the diameter of the stalk. It is essential to ligate the vessels completely or to prevent postoperative bleeding in pedunculated polyps with or without active bleeding. The principle of clip ligation for pedunculated polyps is shown in Figure 1. The number of clips required for complete ligation of the stalk depends on the diameter of the stalk.

The improved metallic haemoclip and clip delivery system have made clips easier to apply. Clip ligation of the bleeding vessel achieves immediate haemostasis comparable to surgical ligation. A previous study has shown that endoscopic haemoclip placement is a highly effective and safe method for treating non-variceal gastrointestinal bleeding[1]. We have used the clips and succeeded in closing a 0.5-cm perforation after snare excision of a 3.7 cm × 3.4 cm gastric leiomyoma[2]. As case 1 was on dialysis with heparin and had long-stalked polyps with and without bleeding, he was at high risk for polypectomy-induced bleeding and secondary massive bleeding. We used metallic clips to ligate the stalk, arresting the bleeding immediately and avoiding massive post-polypectomy bleeding. The stalk of the pedunculated polyp should be clipped completely.

Recently, the detachable snare was introduced as a new haemostatic device for preventing postoperative bleeding in pedunculated polypectomy, and obtained good results[3]. A comparative study of haemoclips and detachable snares for ligating pedunculated polyps before polypectomy is needed in the near future.

In conclusion, as a prophylactic measure, haemoclips can be used to ligate the stalk of a polyp as a new indication either with or without bleeding before polypectomy.

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