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• LIVER CANCER •

Effects of recombinant human growth hormone on remnant liver after hepatectomy in hepatocellular carcinoma with cirrhosis

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Abstract

AIM: To explore the effects of recombinant human growth hormone (rhGH) on the remnant liver after hepatectomy in hepatocellular carcinoma with liver cirrhosis.

METHODS: Twenty-four patients with hepatocellular carcinoma who underwent hepatectomy were randomly divided into 2 groups: parenteral nutrition (PN) group (n=12) and rhGH+PN group (n=12). Liver function, blood glucose, AFP, serum prealbumin and transferrin were detected before operation, at post-operative d 1 and d 6. Albumin (ALB) mRNA in liver biopsy specimens was detected by RT-PCR at post-operative d 6. Liver Ki67 immunohistochemical staining was studied.

RESULTS: On post-operative d 6, compared with PN group, the levels of blood glucose, serum prealbumin, transferrin, the expression of hepatic ALB mRNA and liver Ki67 labeling index were higher in rhGH+PN group.

CONCLUSION: rhGH can improve protein synthesis and liver regeneration after hepatectomy in hepatocellular carcinoma with liver cirrhosis.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors of mankind. Since 1990s HCC has become the second killer among various cancers in China[1]. Surgical resection of the tumor is considered the only potentially curative therapy, and is regarded as the first choice of treatment of HCC. However, 80% of HCC are complicated with liver cirrhosis in China. After partial hepatectomy, the risk of hemorrhage, infection, and liver failure is very high, as the liver function is usually impaired before surgery in patients with cirrhosis. For safer hepatic resection in patients with cirrhosis, the reserve of hepatic function and rapid regeneration of the remnant liver are crucial. Because liver function is usually impaired in patients with cirrhosis, and because cirrhotic livers are less able to regenerate, it is important to stimulate both the regeneration and function of the remnant cirrhotic liver after hepatectomy.

The prognosis of severe liver failure depends on the ability of the remaining hepatocytes to regenerate. Improvement of hepatic tissue repair can increase the survival rate of patients suffering from acute hepatic failure and reduce the recovery period after massive liver resection. Nutritional support is undoubtedly the most physiologic manner of treatment and it is clear that adequate nutrition should significantly speed up liver regeneration and patient recovery. The composition of parenteral nutrition (PN) for the postoperative nutritional management of patients with liver cirrhosis might be extremely important because of their metabolic disorders and hemodynamic disturbance following surgical intervention.

Considering that the provision of branched-chain amino acids (BCAA) may improve nutritional status and liver regeneration and ameliorate hepatic encephalopathy, increased provision of BCAA in patients with liver disease seems to be a reasonable approach to various forms of liver injury[2-4]. While a decline in liver protein synthesis was induced by the surgical procedure[5]. In patients given short-term PN, plasma insulin concentrations were increased 5-fold compared with control, but no effect on liver protein synthesis rates was observed[6]. Growth hormone (GH) has important direct and indirect effects on protein, carbohydrate and lipid metabolism. Potential areas of further research may include the combination of BCAA supplements with other anabolic factors (*e.g.* GH) in managing patients with catabolic disease states[7]. The aim of this study was to investigate the effects of treatment with amino acids enriched BCAA and recombinant human GH (rhGH) for 5 d on the remnant liver after hepatectomy in HCC with liver cirrhosis.

MATERIALS AND METHODS

Patients

All the patients were from our department who underwent curative resection for HCC with liver cirrhosis between September 2002 and June 2003. Curative resection was defined as complete resection of all macroscopically detectable tumors with histological tumor clearance (the entire tumor mass was included in the surgical specimen without exposure of tumor cells on the cut edge). At the time of entry, the inclusion criteria were no evidence of endocrine disease. The study protocol conformed to the ethical guidelines and the patients were enrolled after informed consent was obtained. Twenty-four patients were recruited and were randomly using sealed envelopes divided into 2 groups: PN group (n=12) and rhGH+PN group (n=12) using sealed envelopes. Between the 2 groups, there were no differences in age, sex, body mass, operative methods, operation time, intraoperative blood loss and intraoperative blood transfusion (Table 1). At the same time, 12 patients with cholelithiasis or hemangioma who underwent operation served as normal controls. None had liver cirrhosis or endocrine disease.

Nutritional support and rhGH administration

Nutritional support and rhGH administration were started on the first day after surgery and continued until d 5. PN was

initiated through a percutaneously placed subclavian vein catheter threaded into the superior vena cava. The formula provided non-protein calorie 25 kcal/kg· d. Each patient was provided 250 mL 200 mL/L MCT/LCT lipid emulsion (Guangzhou Qiaoguang Pharmaceutical Co. Ltd) and 750 mL 100 g/L aminoplasmal Hepa (Braun Co. Ltd) containing 20 kinds of amino acids, 330 g/L branched-chain amino acids and 15.3 g/L nitrogen. Nonprotein calories were provided 310 mL/L as lipid emulsion. The formula provided nitrogen 0.19 g/kg· d. The ratio of nonprotein calories to nitrogen was 132:1. The ratio of glucose (g) to insulin (IU) was 6:1. Vitamins, trace minerals, and electrolytes were supplied according to the daily requirement. This parenteral nutrition was tolerated by all patients without complications, and no patient showed symptoms of sepsis during the treatment period.

Patients in the rhGH+PN group received 10 U rhGH (Saizen, Serono Biotech&Beyond) additionally per day subcutaneously.

Table 1 Patient characteristics

	PN group (n=12)	rhGH+PN group (n=12)
General conditions		
Age (years)	46.1±13.6	50.3±10.6
Sex (M/F)	11/1	10/2
Body weight (kg)	59.7±9.3	64.3±7.7
Operative methods		
Hepatic left lateral lobectomy	0	1
Left hemihepatectomy	2	2
Right hemihepatectomy	1	1
Tumor or segmentectomy	9	8
Intraoperative information		
Operation time (min)	250.8±126.4	203.3±73.2
Intraoperative blood loss (mL)	1 331.7±1 704.8	966.7±1031.4
Intraoperative blood transfusion (mL)	1 100.0±1 334.2	408.3±609.7

Collection of samples

Blood samples were collected from antecubital veins in the morning after an overnight fast prior to operation in the normal, PN and rhGH+PN groups, on postoperative day (POD) 1 and 6 in the PN and rhGH+PN groups. Aliquots were transferred into different tubes placed for the determination of liver function, blood glucose, prealbumin, transferrin and α-fetoprotein (AFP). Blood samples were centrifuged at 4 ℃ and then frozen at -70 ℃ until assay.

Liver specimens from PN and rhGH+PN group, including hepatocellular carcinomas and adjacent non-tumor tissues were excised at the operation and percutaneous liver biopsies were taken on d 6 after operation in the PN group and rhGH+PN group, immediately frozen with liquid nitrogen, and stored at -70 ℃ for analysis of ALB mRNA. Liver biopsy specimens were obtained with Tru-Cut biopsy needles. The needle-biopsy specimens ranged in length from 15 to 20 mm. For histological examination, some liver specimens were fixed in 40 g/L neutrally-buffered formaldehyde and embedded in paraffin.

Biochemical examinations

Liver function, including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), albumin (ALB) and blood glucose were analyzed by an autoanalyzer. Serum prealbumin and transferrin were detected quantitatively by immunoturbidity assay.Serum AFP was detected quantitatively by micropartical enzyme immunoassay. The normal values were <20 μg/L.

Measurement of ALB mRNA in liver tissue

RT-PCR was performed to measure the expression levels of ALB mRNA in liver tissue. The primers used were deduced from the cDNA sequence. The sequences of the primers for ALB sense and antisense were as 5' -CCCAAGTGTCAACTCCAACT-3' (sense) and 5' - GCAGGTCTCCTTATCGTCAG-3' (antisense), a 456-bp long fragment was amplified. The sequences of the primers for β-actin sense and antisense were as 5' -ACTCTTCCAGCCTTCCTTCCT-3' (sense) and 5' -TCACCTTCACCGTTCCAGTTT-3' (antisense), a 513-bp long fragment was amplified.

Total RNA was extracted from frozen liver specimens by the guanidinium isothiocyanate method. The RNA was quantified and checked for purity by spectrophotometry at 260 and 280 nm. Aliquots of total RNA were reversely transcribed using SperSriptII Reverse Transcriptase (Invitrogen Corp) and subsequently amplified by PCR using the Taq DNA polymerase (Promega).

The PCR was carried out in 25 mL of reaction mixture containing 0.5 μL cDNA template, 2.5 μL 10×PCR-Buffer, 1.5 μL 25 mmol/L $MgCl_2$, 0.5 μL 10 mmol/L dNTPs, 0.5 μL 10 μmol/L ALB, 0.15 μL 10 μmol/L β-actin primers, 0.5 μL 5 U/μL Taq DNA polymerase. The mixture was heated for 5 min. at 94 ℃ for initial DNA denaturation, followed by 30 cycles of denaturation at 94 ℃ for 45 s, annealing at 50 ℃ for 45 s, polymerization at 72 ℃ for 1 min. and then a final extension of 10 min at 7 ℃. PCR reactions were stored frozen until analysis by agarose gel electrophoresis.

PCR reactions were electrophoresed on 15 g/L agarose gel, stained with ethidium bromide and quantitated using the interactive build analysis system (IBAS). The band intensity of the ALB was compared with the band intensity of the β-actin, and the amount of ALB mRNA was estimated.

Immunohistochemical staining of Ki67 in liver tissue

Two-step immunohistochemical staining technique was used. Main reagents included rabbit polyclonal antibody Ki67 Ab-4 (Neomarkers) and PV-6000 PicTure™ kits. Briefly, sections were deparaffinized, rehydrated, and then immersed in 0.1 mol/L citric acid buffer (pH 6.0) and boiled for 5-10 min in a microwave oven. The slides were then rinsed gently with phosphate buffered saline (PBS) at pH 7.2-7.4, and treated with 3 mL/L hydrogen peroxide in absolute methanol for 1 h at room temperature (RT) to remove endogenous peroxidase. The sections were then incubated with the primary antibody Ki67 Ab-4 (1:200 dilution) for 30 min at 37 ℃. After rinsed with PBS for 3 times, each for 2 min, the sections were incubated with PV-6000 for 30 min at 37 ℃. They were then rinsed 3 times with PBS for 2 min each and visualized with DAB. Finally, the sections were counterstained with hematoxylin.

On each slide, Ki67-positive nuclei were estimated by means of light microscopy at 400 magnifications. At least 1 000 cells were evaluated in 5 different fields each containing a minimum of 200 cells. The Ki67 labeling index (Ki67 LI) is the percentage (%) of positive cells.

Recording doses of albumin infusions within 5 d after operation

Plasma contained 4.5 g/L albumin. Doses of plasma infusion were converted into doses of albumin.

Statistical analysis

Data were expressed as mean±SD. The statistical software SPSS 10.0 was used. Statistical significance was set at P<0.05.

RESULTS

Surgery outcome and albumin doses within 5 d after operation

There was no surgical mortality in this series. The postoperative

hospital stay showed no difference between the PN group and rhGH+PN group (14.7±6.2 d *vs* 13.5±4.5 d, $P>0.05$). In the PN group, 2 patients had right pleural effusion. In the rhGH+PN group, 1 patient had right pleural effusion, another patient had subcutaneous fat liquefaction. The postoperative complication related morbidity was not different between the 2 groups ($P>0.05$). Albumin doses within 5 d after operation were not different between the PN and rhGH+PN group (57.0±48.8 g *vs* 52.4±24.4 g, $P>0.05$).

The decreased percentage of serum AFP within 5 d after operation

The decreased percentage of serum AFP within 5 d after operation were calculated by (POD 1 serum AFP- POD 6 serum AFP)/POD 1 serum AFP. In the PN group, 6 patients had preoperative serum AFP<20 μg/L. In the rhGH+PN group, 3 patients had preoperative serum AFP<20 μg/L and at POD 6, 3 patients whose preoperative serum AFP were <50 μg/L had their serum AFP<20 μg/L. Those patients were excluded from the statistics. For the other 6 patients in the 2 groups, the decrease percentage of serum AFP within 5 d after operation was not different between the PN group and rhGH+PN group (0.536±0.182 *vs* 0.579±0.193, $P>0.05$).

The changes of liver function and blood glucose

Compared with normal control group, serum AST, ALT of HCC patients with cirrhosis were significantly increased, while serum ALP, TBIL, ALB, blood glucose were not different. Serum AST, ALT, ALP, TBIL, ALB, blood glucose on preoperative day and POD 1 were not different between the PN group and rhGH+PN group. Compared with PN group, serum AST, ALT, ALP, TBIL, ALB on POD 6 in the rhGH+PN group were not different, but blood glucose was significantly increased (Table 2).

The changes of serum prealbumin and transferrin

In HCC patients with cirrhosis, serum prealbumin and transferrin were lower than the normal control group. Serum prealbumin and transferring on preoperative day and POD 1 were not different between PN group and rhGH+PN group, but serum prealbumin and transferring on POD 6 in the rhGH+PN group were significantly increased compared with the PN group (Table 2).

The changes of hepatic ALB mRNA and liver Ki67 labeling index

In HCC patients with cirrhosis, hepatic ALB mRNA of tumor tissues was lower than in adjacent non-tumor tissues, while in adjacent non-tumor tissues it was lower than the normal liver tissues. Compared with the PN group, hepatic ALB mRNA on POD 6 was significantly increased in the rhGH+PN group. Liver Ki67 labeling index in tumor tissues was higher than adjacent non-tumor tissues. Liver Ki67 labeling index on POD 6 in the rhGH+PN group was higher than the PN group (Table 3).

DISCUSSION

The prevalence of malnutrition in patients with liver cirrhosis is high[8]. Nutritional status affects prognosis, and cirrhotic patients with malnutrition are prone to develop major complications and infections. A poor nutritional status negatively influences survival, while appropriate nutritional intervention has been found to improve liver function and survival. Because the liver is the metabolic workhouse of the body, alteration in liver function clearly affects the whole body metabolism. In addition, the goals of nutritional support should include maintenance of adequate nutrition and prevention and/or amelioration of liver damage. Major resection of portions of the liver for primary hepatic or metastatic malignancy and the repair of hepatic injury associated loss of liver tissue are performed with such a frequency as to require careful consideration of the consequences of the postoperative nutritional care of the patients. Often, the recovery from such surgery involves a prolonged period of delayed oral intake.

Table 2 Comparison of liver function, blood glucose, serum prealbumin and transferrin

	Normal control group (n=12)	PN group (n=12)			rhGH+PN group (n=12)		
		Preoperative	POD 1	POD 6	Preoperative	POD 1	POD 6
AST (IU/L)	26.0±5	56.0±27[a]	761.0±578	62.0±32	55.0±25[a]	578.0±206	56.0±25
ALT (IU/L)	27.0±17	60.0±36[a]	749.0±571	153.0±95	42.0±17[a]	590.0±273	154.0±71
ALP (IU/L)	94.0±55	151.0±87	145.0±80	148.0±65	105.0±27	100.0±33	113.0±46
TBIL (μmol/L)	17.4±6.4	22.6±9.0	52.5±48.1	43.1±37.4	20.5±9.3	42.1±41.6	30.2±21.0
ALB (g/L)	42.8±4.0	39.8±4.7	31.5±3.3	34.0±3.4	40.7±3.5	32.4±3.6	37.1±5.0
Blood glucose (mmol/L)	4.6±0.6	4.8±0.9	7.3±3.5	5.3±1.5	4.5±0.6	7.0±3.2	8.8±5.2[c]
Serum prealbumin (mg/L)	279.8±43.0	202.4±32.3[a]	158.3±25.4	127.2±13.4	204.5±31.9[a]	157.2±23.0	156.4±20.0[c]
Serum transferrin (g/L)	3.5±0.5	2.5±0.2[a]	2.1±0.3	1.8±0.2	2.5±0.2[a]	2.2±0.3	2.2±0.3[c]

[a]$P<0.05$, preoperative *vs* normal control group; [c]$P<0.05$, between rhGH+PN group and PN group.

Table 3 Comparison of hepatic ALB mRNA and liver Ki67 labeling index

	Normal control group (n=12)	PN group (n=12)			rhGH+PN group (n=12)		
		Tumor tissues	Adjacent non-tumor tissues	Liver biopsy specimens POD 6	Tumor tissue	Adjacent non-tumor tissues	Liver biopsy specimens POD 6
ALB mRNA	0.69±0.05	0.38±0.01[a]	0.50±0.06[a,c]	0.63±0.05	0.38±0.01[a]	0.50±0.06[a,c]	0.71±0.06[e]
Liver Ki67 LI(%)	0	17.4±6.1[a]	0.20±0.1[a,c]	4.60±0.5	17.2±5.9[a]	0.20±0.1[a,c]	5.50±0.5[e]

[a]$P<0.05$, tumor tissues or adjacent non-tumor tissues *vs* normal control group; [c]$P<0.05$, adjacent non-tumor tissues *vs* tumor tissues in the same group,[e]$P<0.05$, of the same tissues comparison between the PN group and rhGH+PN group.

Although the liver normally has considerable reserve function and regeneration potential, an acute reduction in hepatic mass can significantly affect liver function, its metabolic activity, and substrate metabolism. The trauma caused by moderate or large operation may result in disturbance of glucose, lipid and protein metabolism including hypermetabolism and increased catabolism, which may lead to acute protein malnutrition, decline of immunological function and dysfunction of multiple organs[9-11]. Postoperative maintenance of protein synthesis is one measure to preserve a satisfactory quality of life after hepatectomy[12].

The amino acids that comprise the various parenteral feeding formulations are the important components of these regimens, because they provide the precursors for the synthesis of numerous structural and functional body proteins. BCAA appears to act favorably in albumin metabolism. Early oral supplementation of BCAA for HCV-related cirrhosis with serum albumin level between 3.5 and 3.9 g/dL and branched-chain tyrosine ratio (BTR) less than 4.0, improves serum albumin levels and thus might improve prognosis[13].

Liver protein synthesis is usually estimated in humans by measuring the synthesis rates of major exported liver proteins. Albumin is a ubiquitous protein synthesized only by hepatocytes. Albumin is a polypeptide chain of 580 amino acids that is produced by hepatocytes[14]. The expression of ALB gene is reduced in various liver deseases and the degree of reduction in the hepatic ALB mRNA level is generally correlated with the severity of the disease[15]. This study showed that hepatic ALB mRNA in tumor tissues was lower than in adjacent non-tumor tissues, and it was lower in adjacent non-tumor tissues than normal liver tissues. Serum ALB whose long half-life of approximately 20 d makes it a late index of nutritional status, and its exclusive use may delay implementation of appropriate nutritional interventions. Serum prealbumin and transferrin have been proposed as earlier nutritional markers. Clinically significant changes in albumin can be reliably predicted by earlier changes in serum transferrin and prealbumin[16]. In this study, serum AST, ALT, ALP, TBIL, ALB on POD 6 were not different between the PN group and rhGH+PN group, but blood glucose, serum prealbumin, transferrin, hepatic ALB mRNA, and liver Ki67 labeling index were significantly increased in the rhGH+PN group. Thus, rhGH can promote liver protein synthesis and liver regeneration. Moreover, liver function recovered faster in the rhGH+PN group. It has been demonstrated that PN is not able to support protein synthesis sufficiently in patients with or without malnutrition[17]. Long-term conventional PN is unable to increase or even maintain body protein; the anabolic response to PN is often suboptical because of the concomitant presence of catabolism and/or alterations in the hormonal regulation of metabolism[18,19]. GH is anabolic in protein metabolism. Gu and Wu also demonstrated that the administration of rhGH could result in significant anabolic effects on body growth and improve the efficiency of PN[20].

An important but unresolved question is intrahepatic recurrence after resection of HCC[21-26]. Metastasis and recurrence of HCC after surgical removal is still high.The frequency of 5-year recurrence after radical resection was 61.5% overall[27]. The recurrence, especially at an early period after hepatectomy, is the major cause of poor prognosis in patients with HCC[28]. This is of relevance in determining preventive and therapeutic strategies for recurrence. Although all patients in this study underwent curative tumor resection, the potential tumor-promoting effect of GH must be addressed. This study showed that the decreased percentage of serum AFP within 5 d after operation was not different between the PN group and rhGH+PN group. Furthermore, liver function recovered faster in the rhGH+PN group. However, hepatic functional damage immediately after hepatectomy is a significant risk factor for early intrahepatic recurrence in cirrhotic HCC. Careful perioperative management of hepatic function may therefore be important in preventing early recurrence and prolonging survival[29]. Tacke *et al* demonstrated no evidence for an increased risk of tumor recurrence after rhGH treatment for a short period of time after removal of a gastrointestinal adenocarcinoma[30]. Moreover, rhGH attenuated the depression in cellular immunity following surgical stress[31]. Therefore, short-term use of rhGH in HCC patients with cirrhosis after operation may be safe.

In conclusion, rhGH+PN can promote liver recovery, liver protein synthesis and liver regeneration after hepatectomy in HCC with liver cirrhosis. It may not promote HCC recurrance, but may increase blood glucose.

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