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TNF-α, HGF AND TGF-β1 ARE INVOLVED IN LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY USING PORTAL VEIN ARTERIALIZATIONS

TNF-α, HGF I TGF-β1 UČESTVUJU U REGENERACIJI JETRE POSLE PARCIJALNE HEPATEKTOMIJE POMOĆU ARTERIJALIZACIJE PORTNE VENE

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Summary: Experiments on liver regeneration after partial hepatectomy have shown that TNF-α, HGF and TGF-β1 and other cytokines play important roles in the different stages of liver regeneration, however, the effect of portal vein arterialization (PVA) on the expressions of these cytokines during liver regeneration is not clear. Sprague Dawley rats were randomly divided into the PVA group and control groups, and blood was collected for the detection of ALT using an automatic biochemical analyzer. The expressions of TNF-α, HGF and TGF-β1 in liver tissues were detected by quantitative RT-PCR. The ALT levels in both groups in the early period after surgery were significantly higher than those before operation, and gradually returned to normal at 7 days after surgery. At 12 h and 24 h after operation, the TNF-α expression in the PVA group was significantly higher than that in the control group ($P<0.05$), but no significant difference at 7 days after surgery was observed between the two groups. At 12 h, the HGF expression in the PVA group was similar to that in the control group, but significantly higher than in the control group at 24 h ($P<0.05$). At 24 h, the TGF-β1 expression in the PVA group was significantly lower than that in the control group ($P<0.05$), but no significant difference was found at 48 h after surgery between the two groups. The promotive effects on the portal vein arterialization at the early stage of liver regeneration were associated with the changes in the expressions of TNF-α, HGF and TGF-β1.

Keywords: hepatectomy, liver regeneration, portal vein arterialization

Kratak sadržaj: Eksperimenti vezani za regeneraciju jetre posle parcijalne hepatektomije pokazali su da TNF-α, HGF i TGF-β1 kao i drugi citokini igraju važne uloge u različitim fazama regeneracije jetre, međutim, uticaj arterijalizacije portne vene (APV) na ekspresiju ovih citokina tokom regeneracije jetre nije jasan. *Sprague Dawley* pacovi nasumično su podeljeni u APV grupu i kontrolne grupe, a krv za detekciju ALT uzeta je pomoću automatskog biohemijskog analizatora. Ekspresije TNF-α, HGF i TGF-β1 u tkivima jetre detektovane su kvantitativnom RT-PCR. Nivoi ALT u obe grupe u početnom periodu posle operacije bili su značajno viši nego pre hirurškog zahvata i postepeno su se normalizovali posle 7 dana od operacije. Posle 12 h i 24 h od zahvata, ekspresija TNF-α u APV grupi bila je značajno viša nego u kontrolnoj grupi ($P<0,05$), ali posle 7 dana od operacije nije uočena značajna razlika između dve grupe. Ekspresija HGF u APV grupi posle 12 h bila je slična onoj u kontrolnoj grupi, ali je posle 24 h bila značajno viša nego u kontrolnoj grupi ($P<0,05$). Posle 24 h, ekspresija TGF-β1 u APV grupi bila je značajno niža nego u kontrolnoj grupi ($P<0,05$), ali posle 48 h od operacije između dve grupe nije otkrivena značajna razlika. Pozitivan uticaj na arterijalizaciju portne vene u ranim fazama regeneracije jetre bio je povezan s promenama u ekspresijama TNF-α, HGF i TGF-β1.

Ključne reči: hepatektomija, regeneracija jetre, arterijalizacija portne vene

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Abbreviations: ALT, alanine aminotransferase; PVA, portal vein arterialization; SD, Sprague-Dawley; GAPDH, glyceraldehyde phosphate dehydrogenase

Introduction

Portal vein arterialization (PVA) has been used in hepatobiliary surgery and liver transplants in several cases (1). Recent studies suggested PVA could promote liver regeneration in the early stage after hepatectomy (2–4). Maggi et al. (5) and Bonnet et al. (6) followed patients with PVA for 1.5–6 years and the results showed these patients had no symptoms relevant to portal hypertension and achieved favorable long-term function. These findings not only pose a challenge to the theory of liver growth factors, but also provide evidence for long-term efficacy of PVA.

The mechanism by which PVA promotes liver regeneration is less frequently reported. Studies on liver regeneration following partial hepatectomy (7, 8) have shown that tumor necrosis factor α (TNF-α), hepatocyte growth factor (HGF) and transforming growth factor β1 (TGF-β1) as well as other cytokines play important roles in the different stages of liver regeneration. The effect of PVA on the expressions of above factors during the liver regeneration is, however, unclear.

Materials and Methods

Materials

Male Sprague-Dawley (SD) rats weighing 280–350 g were purchased from Peking VITAL RIVER Laboratory Animal Technology Co., Ltd. The study was approved by the Animal Ethics Committee of Inner Mongolia Medical College and animal care was in accordance with the NIH standards. Animals were allowed to acclimatize for 1 week and given *ad libitum* access to food and water. The animals received no nourishment other than water *ad libitum* for a period of 12–24 h before surgery.

Anesthesia and surgical procedures

Animals were anesthetized by intraperitoneally injecting ketamine (100 mg/kg). If anesthesia was too shallow, ether could be used to maintain anesthesia. The operations were carried out under a microscope (magnification: 4–25×, Peking Suohong Electronics Co. Ltd, model: YZ20T4). All animals underwent right nephrectomy and 68% hepatectomy according to previous reports (7). In the control group, the portal vein was conventionally blocked for 10 min after hepatectomy. For the animals in the PVA group, the proximal end of the portal vein and the right renal artery were anastomosed with a polyethylene tube (I.D. 0.5 mm), and the blood flow was observed at a high magnification (25×). The distal end of the portal vein was anastomosed to the inferior vena cava. A total of 48 rats were randomly divided into two groups (n=24). Rats in the control group received 68% hepatectomy and those in the PVA group received 68% hepatectomy plus PVA. Six rats were sacrificed and at the same time serological and histological specimens were collected, 12 h, 24 h, 48 h and 7 days, respectively, after the operation, according to the study design.

Detection of serum alanine aminotransferase (ALT)

At 12 h, 24 h and 7 days after surgery, blood samples were collected for the detection of ALT using an automatic biochemical analyzer (Roche Modular DPP System, Germany).

Real-time quantitative RT-PCR

After the animals were sacrificed, liver tissues were collected for real-time quantitative RT-PCR. Total RNA was extracted from the liver tissue with an RNA purification kit (Boster Biological Engineering Co, Ltd, Wuhan, China). The RNA extracted was tested by spectroscopy, and the ratio of A260/280 is between 1.7––1.9. With the Agarose Gel Electrophoresis test, strips of 28 s, 18 s and 5 s were visible and clear, without any degradation. Reverse transcription was performed using the SYBR PrimeScript RT reagent kit according to the manufacturer's instructions (TaKaRa Biotechnology Co., Ltd, Dalian, China). The reaction mixture (10 μL) included 2 μL of 5×PrimeScript Buffer, 0.5 μL of PrimeScript RT Enzyme MixI, 0.5 μL of Oligo dT Primer (50 μm/L), 0.5 μL of Random 6 mers (100 μm/L), 6.50 μL of total RNA, and the RT reaction conditions were as follows: 37 °C for 15 min and 85 °C for 5 s.

Table I TNF-β, HGF, TGF and GAPDH RT-PCR primer sequence

Target	Oligonucleotide sequence	
	Primer sense (5'-3')	Primer antisense (3'-5')
TNF-α	GCCAATGGCATGGATCTCAAAG	CAGAGCAATGACTCCAAA
HGF	TGCTGTGAATGAGACTGATGT	ACTAACCATCCACCCTACTG
TGF-β1	ATGACATGAACCGACCCTTC	TGTGTTGGTTGTAGAGGGCA
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

Quantitative real-time PCR was carried out using the fluorescent dye SYBR Green I according to the procedures previously described (10, 11). The primers were designed with the primer design software (PerkinElmer Life Sciences, Waltham, MA, USA) and are shown in *Table I*. PCR was done on a real-time PCR quantitative instrument (USA MJ OPTICON-2) and the amplifications of HGF, TGF-β and TNF-α were carried out separately. GAPDH served as an internal control. The reaction mixture included: 10 μL of SYBR Premix Taq, 0.4 μL of PCR Forward Primer (10 μm/L), 0.4 μL of PCR Reverse Primer (10 μm/L), 2 μL of cDNA and dH_2O (a final volume of 20 μL). The PCR conditions were as follows: pre-denaturation at 95 °C for 60 s; 38 cycles of denaturation at 95 °C for 10 s, annealing at 64 °C for 30 s, and extension at 72 °C for 20 s, and a final extension at 72 °C for 7 min. The melting curve was measured at 72–95 °C. Each cDNA sample was tested repeatedly for 4 times, and the mean of the Ct (mean ± SD) and CV (coefficient of variation) were calculated. The relative quantitative analysis was performed using the $2^{-\triangle\triangle Ct}$ ($\triangle Ct = Ct_{HGF/TGF\text{-}\beta/TNF\text{-}\alpha} - Ct_{GAPDH}$) method (12, 13).

Statistical Analysis

Data were expressed as mean ± SEM, and statistical analysis was done using SPSS version 13.0. Comparisons between groups were conducted with the Mann-Whitney-U-test or *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

ALT level

The serum ALT levels in both groups in the early postoperative period increased significantly when compared with those before surgery. At 24 h after surgery, the serum ALT level in the PVA group was significantly lower than that in the control group ($P<0.05$). On postoperative day 7, the serum ALT levels in both groups gradually returned to normal, but no significant difference was found between the two groups ($P>0.05$) (*Table II*).

Expressions of TNF-α, HGF and TGF-β1

At 12 h and 24 h after surgery, the TNF-α expression in the PVA group was significantly higher than that in the control group ($P<0.05$); on postoperative day 7, although the TNF-α expression in the PVA group was higher than that in the control group, no statistical difference was noted ($P>0.05$) (*Figure 1A*).

At 12 h after surgery, the HGF expression in the PVA group was lower than that in the control group ($P>0.05$), but at 24 h after operation, the HGF expression in the PVA group was significantly higher than that in the control group ($P<0.05$) (*Figure 1B*).

At 24 h after surgery, the TGF-β1 expression in the PVA group was significantly lower than that in the control group ($P<0.05$), but no statistical difference was found at 48 h after surgery ($P> 0.05$) (*Figure 1C*).

Discussion

Serum ALT is a sensitive indicator in assessing liver cell injury, but its role in the promotion of liver cell regeneration following PVA is seldom reported. In the present study, the ALT level increased significantly in both groups in the early postoperative stage, and then declined to the normal level on postoperative day 7. The changes in the ALT level were consistent with those previously described (3, 14).

Liver regeneration is a complicated physiological process related to multiple accesses and a variety of cytokines. This process is associated with the secretion of cytokines and growth factors, reconstruction of the extracellular matrix, as well as some positive and negative feedback mechanisms of stimulating or inhibiting liver regeneration (15). The process of liver regeneration can be divided into three phases including initiation, proliferation and termination. Numerous regulatory factors are involved in the three phases of liver regeneration. Studies on rats undergoing 68% hepatectomy have shown that TNF-α, HGF and TGF-β1 play critical roles in different stages of liver regeneration (16, 17).

TNF-α is mainly produced by Kupffer cells in the liver, and can stimulate Kupffer cells to produce IL-6 in an autocrine manner. TNF-α and IL-6 secreted by the activated Kupffer cells participate in the priming process during the initiation phase, allowing the liver cells

Table II Postoperative ALT activity

	n	preoperative	12 h	24 h	7 days
PVA group	24	38.67±18.98	347.50±63.37	332.00±63.74*	59.00±16.82
Control group	24		312.33±92.59	426.50±61.61	58.83±19.28

Notes: compared with control group, *P <0.05

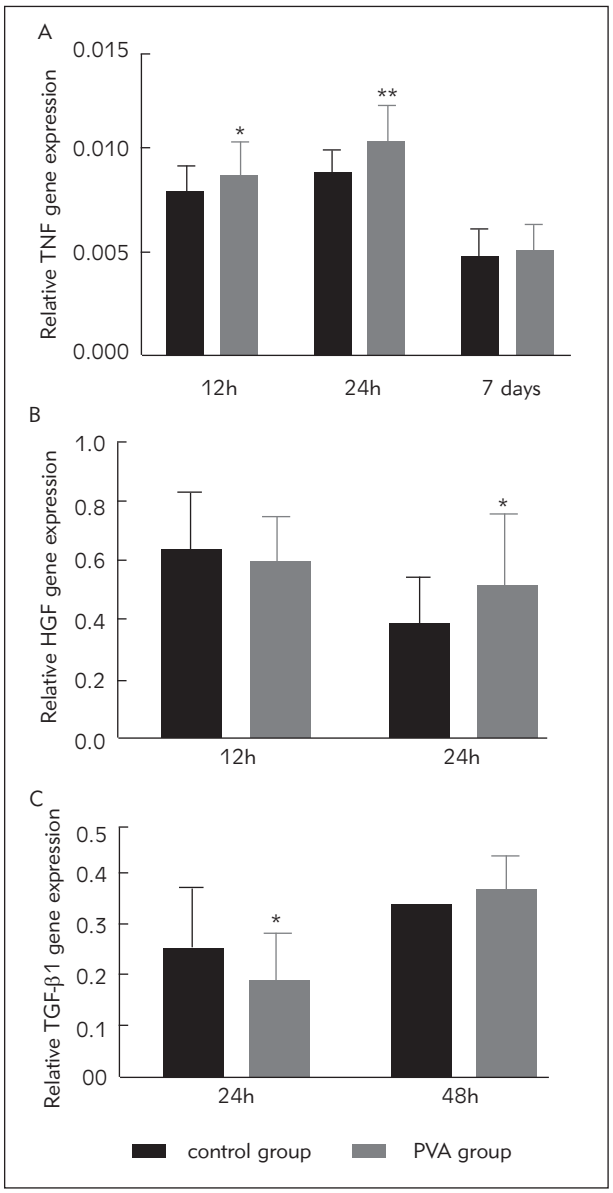

Figure I (A) TNF-α mRNA expression 12 h, 24 h and 7 days after partial liver resection in hepatic tissue (RT-PCR); (B) HGF mRNA expression at 12 and 24 h; (C) TGF-β1 mRNA expression at 24 and 48 h. *P <0.05,** P <0.01

in the G0 phase to enter the G1 phase. In addition, TNF-α can regulate the expressions of TGF-α and HGF (7, 16). Previous studies (18, 19) have confirmed that an appropriate TNF-α level in the liver contributes to the liver regeneration in an animal model of liver regeneration. In the present study, at 12 h and 24 h after surgery, the TNF-α expression in the PVA group increases significantly ($P<0.05$), suggesting that PVA promotes the initiation of liver regeneration. In addition, PVA can also increase the expressions of HGF and TGF-α. At different time points, TNF-α expression levels are not constant, significantly increasing in the early stage, reaching a peak at 24–48 h after surgery, and then decreasing gradually to normal on day 7 (17). In the present study, the changes in TNF-α expression in the control group were consistent with those previously reported.

HGF is known as a potent promoter during the liver regeneration. Also, it is an important regulatory cytokine in the proliferation phase of liver regeneration, and can promote the hepatocyte proliferation and DNA synthesis in a paracrine manner (7). The elevation of serum HGF after partial hepatectomy was followed by an increase of HGF expression in the liver. This suggested that the elevation of serum HGF might be attributed to other mechanisms. Therefore, the mRNA expression of HGF in the liver was detected in our study. Overexpression of HGF can enhance the hepatocyte proliferation and accelerate liver regeneration after partial hepatectomy (16, 20). In this study, at 24 h after surgery, HGF expression in the PVA group was significantly higher than that in the control group ($P<0.05$), suggesting PVA may be involved in the promotion of hepatocyte proliferation.

TGF-β1 is mainly produced by stellate cells and Kupffer cells in the liver. Normally, TGF-β1 is detectable in the liver, while the TGF-β1 expression in the residual liver increased significantly after partial hepatectomy. The increase in TGF-β1 inhibited the DNA replication and delayed the DNA synthesis (8). Due to the deregulation of the expression of TGF-β1 receptor during the early postoperative period, the increase in TGF-β1 cannot enhance the liver regeneration (16). In the present study, at 24 h after surgery, the TGF-β1 expression in the PVA group was significantly lower than that in the control group ($P<0.05$), despite the reduction of receptor expression. This suggests that PVA has a protective effect on the liver regeneration. At 48 h after hepatectomy, no significant difference in the TGF-β1 expression was found between the two groups ($P>0.05$), however, the number of TGF-β1 receptors increased at this time point, which might be a negative feedback to PVA promoting liver regeneration.

The promotive effect of PVA in the early stage of liver regeneration was associated with the changes in the expressions of TNF-α, HGF and TGF-β1. The specific mechanisms by which liver regeneration was promoted by PVA in liver hepatectomy remain to be elucidated.

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Conflict of interest statement

We have no conflicts of interest to declare in relation to this article.

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