

Original Article

Selective portal vein ligation promotes liver regeneration in rats with incomplete obstructive jaundice

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Received September 28, 2016; Accepted December 21, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Selective portal vein ligation is important for hepatectomy patients. However, it is not clear that whether selected portal vein ligation is safe and feasible to patients with obstructive jaundice. In this study, the effects of selective portal vein ligation on liver regeneration were explored in rat model with incomplete obstructive jaundice. Rats were randomly divided into 3 groups: the control sham operation group (C-Sham), control obstructive jaundice group (C-OJ), and selective portal vein ligation group (PVL). Then the weight ratios of the unligated sides of liver were calculated. Ki-67 levels in the unligated sides of liver were detected by immunohistochemistry. Hematoxylin and eosin staining was performed to show the intrahepatic cholestasis. Levels of alanine aminotransferase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), and albumin (ALB) were measured. Tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) levels were detected by enzyme-linked immunosorbent assay. Results of our study showed that selective portal vein ligation promoted the liver regeneration in rats with incomplete obstructive jaundice, and did not aggravate damages to hepatic function. The underlying mechanism may involve in the increased TNF- α and IL-6 levels. Our study suggested that the selected portal vein ligation is suitable to be carried out in patients with obstructive jaundice.

Keywords: Selective portal vein ligation, liver regeneration, incomplete obstructive jaundice, tumor necrosis factor- α , interleukin-6

Introduction

Hepatectomy is a common therapy for liver diseases, such as hepatocellular carcinoma, cholangiocarcinoma and hemangioma. Hepatectomy provides another chance for patients with primary or secondary hepatobiliary tumors [1]. As the early clinical symptoms of tumors in hepatobiliary system are inconspicuous, patients are always in the advanced stage when tumors in hepatobiliary system are discovered, and the success rate of resection is only maintained at 30%.

The hepatic failure is in the risk post-hepatectomy [2, 3]. Insufficient size and function of the remnant liver usually limits the success of hepatectomy [3, 4]. Owing to the regeneration func-

tion of liver, a series of strategies are developed to solve these problems of insufficiency of the remnant liver.

Selective portal vein embolization and ligation are important options for patients who will undergo the hepatectomy [5, 6]. Portal vein embolization or ligation of the resected side of liver can induce the unblocked side of liver to atrophy, decrease the weight ratios of the blocked sides of liver, thus reducing the hepatic failure risk post large-scale hepatectomy and increasing the success rate [7, 8]. In clinic, the portal vein embolization or ligation is usually performed before hepatectomy, and leads tumors in hepatobiliary system to a "decreasing" stage to allow patients to receive a two-stage therapy [9].

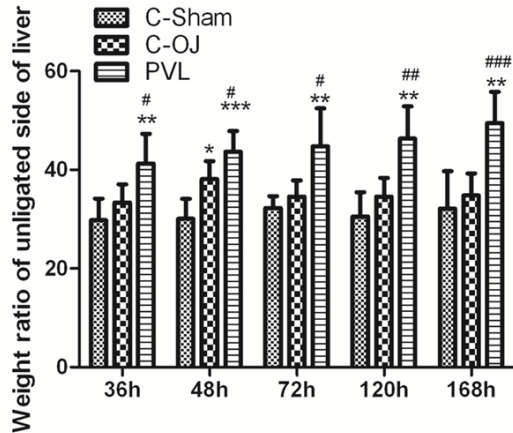


Figure 1. Selective portal vein ligation increased the weight ratios of the unligated sides of liver. After obstructive jaundice operation and selective portal vein ligation, the weight ratios of the unligated sides of liver were calculated. N = 6 for each group. The results are presented as mean $\pm$ standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the C-Sham group; #P < 0.05, ##P < 0.01, ###P < 0.001 compared with the C-OJ group.

In clinic, tumors in hepatobiliary system usually cause biliary obstruction, resulting in different degrees of obstructive jaundice, which impairs the metabolism and regeneration of livers. It is not clear that whether selected portal vein embolism or ligation in patients with obstructive jaundice is safe and feasible. In the present study, the effects of selected portal vein ligation on liver regeneration in rat model of incomplete obstructive jaundice were explored. Our study suggests that selected portal vein ligation can promote liver regeneration. It indicates that the selected portal vein ligation is suitable to be carried out in patients with obstructive jaundice.

Materials and methods

Animals

Ninth male Sprague-Dawley rats weighing 230-270 g were obtained from Experimental Animal Center of Xuzhou Medical College. Rats were housed in a controlled environment (temperature at $23 \pm 2^\circ\text{C}$, relative humidity at $50 \pm 5\%$) with 12 h light - 12 h dark cycles. Rats were permitted *ad libitum* access to standard rodent chow and water. The experiments were performed according to the Guide for the Care and Use of Laboratory Animals and were approved

by the Institutional Animal Care and Use Committee of Nanjing Medical University.

Incomplete obstructive jaundice operation

Rats were anesthetized with 1% pentobarbital sodium (40 mg/kg, intraperitoneal injection). After a midline laparotomy, the bile duct and portal vein in the left and middle lobes of liver pedicle were separated. The bile duct was ligated and disconnected in the near-end and distal-end. In the sham operation, the bile duct was dissected but not ligated. Then the abdomen was closed layer by layer.

Selective portal vein ligation operation

Rats were anesthetized with 1% pentobarbital sodium and received a midline laparotomy, and the liver was freed from its ligaments. Selective portal vein ligation was performed on the left lobes and middle lobes of liver portal vein. The ligated liver accounted for about 70% of the total liver. In the sham operation, the portal vein was dissected without ligation. Then the abdomen was closed layer by layer.

Grouping and operation

Rats were randomly divided into 3 groups: the control sham operation group (C-Sham), the control obstructive jaundice group (C-OJ), and the portal vein ligation group (PVL), n = 30 for each group. Rats in the PVL group received an incomplete obstructive jaundice operation. Thereafter, at 36 h, 48 h, 72 h, 120 h and 168 h post incomplete obstructive jaundice operation, 6 rats at each time point received selective portal vein ligation operation. 24 h after portal vein ligation, rats were sacrificed for subsequent experiments. Rats in the C-OJ group received an incomplete obstructive jaundice operation. The portal vein was separated but not ligated. Rats in the C-Sham group received a laparotomy. The bile duct and portal vein were separated but not ligated.

Evaluation of liver function

The serum of rats in each group was harvested. The levels of total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum were determined with automatic biochemical analyzer.

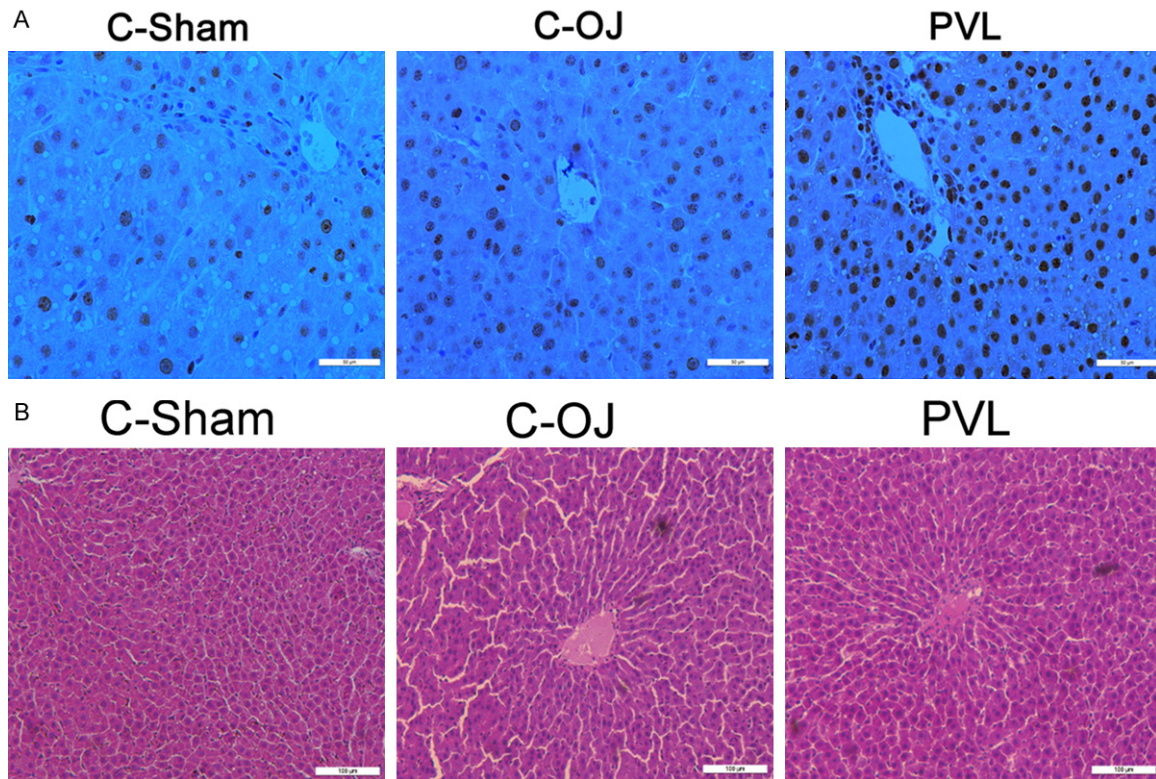


Figure 2. Selective portal vein ligation promoted liver regeneration in rats with incomplete obstructive jaundice. A. After obstructive jaundice operation and selective portal vein ligation, the expression of Ki-67 was detected by immunohistochemistry with Ki-67 antibody. Scale bar = 50 μ m. B. HE staining was performed in each group. Scale bar = 100 μ m.

Weight ratios of the unligated sides of liver

The weights of right lobe, triangle hepatic lobe, caudate lobe of liver, and weights of total liver were measured, and then the weight ratios of the unligated sides of liver was calculated according to the following formula: weight ratio of the unligated side of liver = (weight of the right lobe of liver + weights of the triangle hepatic lobe of liver + weight of the caudate lobe of liver)/total weight of liver.

Immunohistochemistry assay

The Ki-67 levels in the unligated livers were detected by immunohistochemistry. The unligated livers were harvested, fixed in 4% paraformaldehyde, embedded in paraffin and cut into 5- μ m sections. After dewaxing, rehydration and antigen retrieval, endogenous peroxidase was inactivated by 3% H_2O_2 , and nonspecific sites were blocked by goat serum. Then the sections were incubated with primary antibody against Ki-67 (Millipore, Bedford, MA, USA)

overnight at 4°C according to the manufacturer's protocol. After washing, the sections were incubated with biotin labeled-secondary antibody and horseradish peroxidase labeled-avidin. The sections were visualized with a DAB developing kit (Beyotime, Shanghai, China) and counterstained with hematoxylin. Cells were observed with an optical microscope under a 400 $\times$ magnification. Positive ratio in five random visual fields was calculated.

Hematoxylin and eosin (HE) staining

The paraffin sections were dewaxed and rehydrated, and then subjected to routine HE staining. Images of sections were captured with an optical microscope.

Enzyme-linked immunosorbent assay (ELISA)

Levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were detected using corresponding ELISA kits (Groundwork Biotechnology Diagnostic Ltd., San Diego, CA, USA) accord-

Table 1. The percentages of Ki-67 positive cells in the unligated sides of liver

	36 h	48 h	72 h	120 h	168 h
C-Sham	0.46 ± 0.17	0.48 ± 0.16	0.52 ± 0.21	0.51 ± 0.22	0.55 ± 0.18
C-OJ	15.27 ± 4.16**	28.34 ± 4.95***	23.83 ± 3.18***	20.64 ± 3.69***	21.64 ± 5.32**
PVL	39.62 ± 5.43***,##	55.75 ± 8.70***,##	45.63 ± 7.84***,#	41.82 ± 5.53***,##	37.63 ± 6.16***,#

The results are presented as mean ± standard deviation. **P < 0.01, ***P < 0.001 compared with the C-Sham group, #P < 0.05, ##P < 0.01 compared with the C-OJ group.

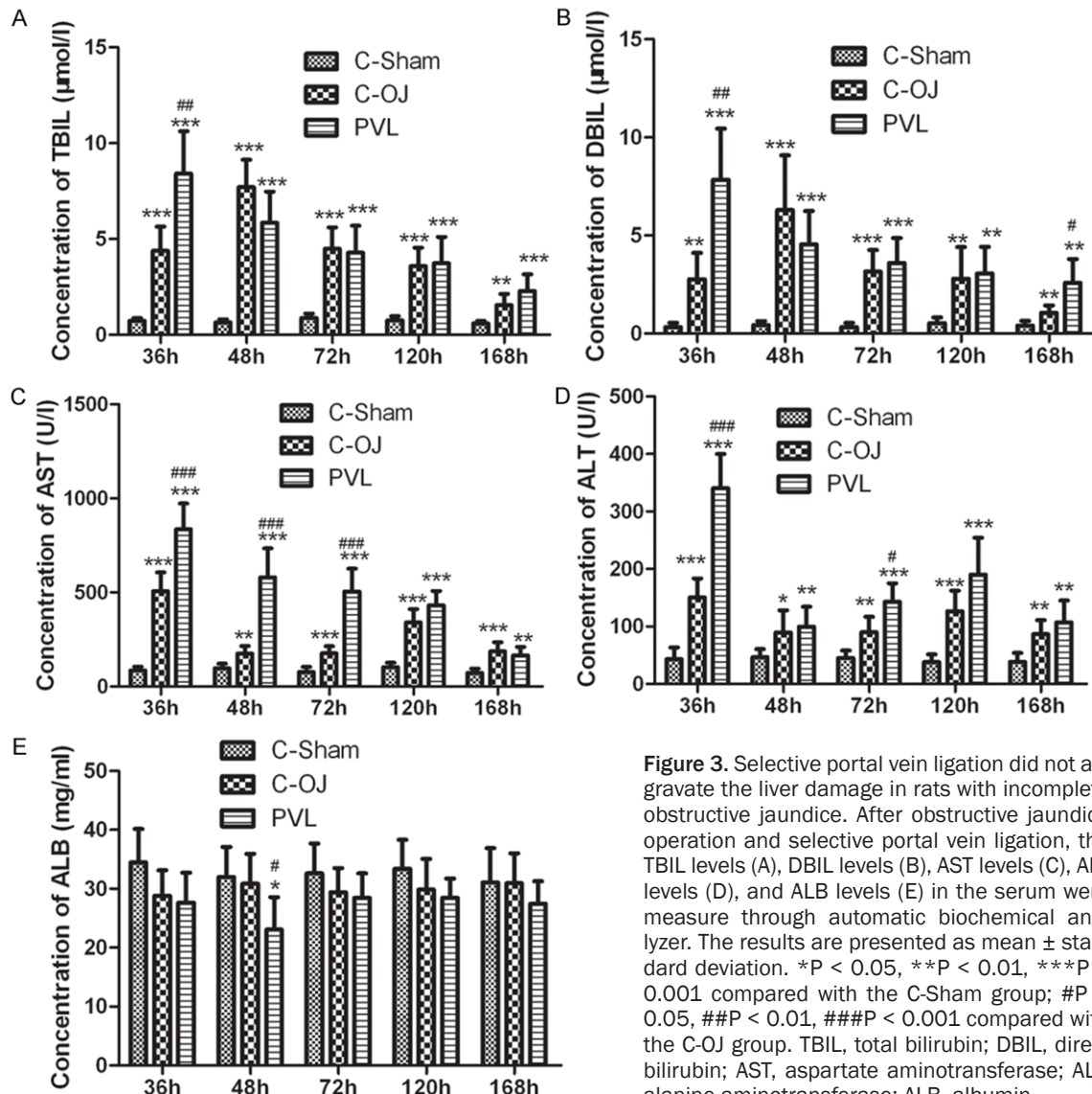


Figure 3. Selective portal vein ligation did not aggravate the liver damage in rats with incomplete obstructive jaundice. After obstructive jaundice operation and selective portal vein ligation, the TBIL levels (A), DBIL levels (B), AST levels (C), ALT levels (D), and ALB levels (E) in the serum were measure through automatic biochemical analyzer. The results are presented as mean ± standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the C-Sham group; #P < 0.05, ##P < 0.01, ###P < 0.001 compared with the C-OJ group. TBIL, total bilirubin; DBIL, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin.

ing to the manufacturer's protocol. Briefly, a standard curve was drawn using the corresponding ELISA kits, and all samples were measured in a 96-well plate with corresponding ELISA kits. The concentrations of TNF-α or IL-6 were calculated according to the standard curve.

Statistics analyze

The results are presented as mean ± standard deviation. Differences between groups were assessed by One-way Analysis of Variance and student's t test. P < 0.05 was considered to be significantly different.

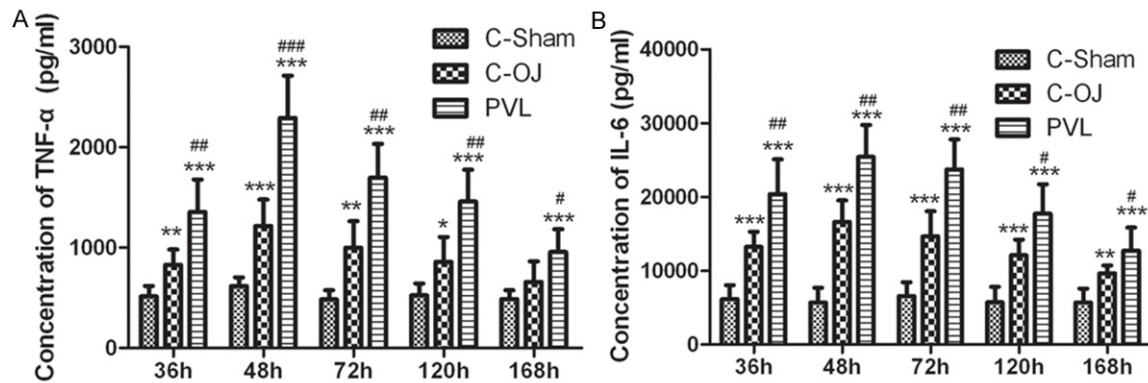


Figure 4. Selective portal vein ligation increased the TNF- α levels and IL-6 levels in rats with incomplete obstructive jaundice. After obstructive jaundice operation and selective portal vein ligation, the TNF- α levels (A) and IL-6 levels (B) were measured by enzyme-linked immunosorbent assay. The results are presented as mean $\pm$ standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the C-Sham group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ compared with the C-OJ group. TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6.

Results

Selective portal vein ligation increased the weight ratios of the unligated sides of liver in rat model with incomplete obstructive jaundice

After obstructive jaundice operation and selective portal vein ligation, the weight ratios of the unligated sides of liver were calculated. Results showed that, after obstructive jaundice operation, the weight ratios of the unligated sides of liver showed a slight increase at 48 h. No significant changes in the weight ratios of the unligated sides of liver in the C-OJ group were found at other time points. After selective portal vein ligation, the weight ratios of the unligated sides of liver were increased significantly when compared with the C-OJ group and the C-Sham group (**Figure 1**). However, in the PVL group, the weight ratios of the unligated sides of liver showed no significant difference between the five time points. These results suggested that PVL can promote liver regeneration in rats with incomplete obstructive jaundice.

Selective portal vein ligation promoted liver regeneration in rats with incomplete obstructive jaundice

Immunohistochemistry was used to detect the expression of Ki-67 in the unligated sides of liver. As shown in **Figure 2**, the Ki-67 levels in the C-OJ group were slightly higher than those of the C-Sham group (**Figure 2A**). And a marked increase in Ki-67 level was discovered in the PVL group when comparing with the C-OJ group

and the C-Sham group (**Figure 2A**). The percentages of Ki-67 positive cells were shown in **Table 1**. The percentages of Ki-67 positive cells in the PVL group were significantly higher than those in the C-Sham group and the C-OJ group. HE staining was performed to show the intrahepatic cholestasis. The results of HE staining showed that the intrahepatic cholestasis in the PVL group was remitted (**Figure 2B**).

Selective portal vein ligation did not aggravate damages to hepatic function

TBIL and DBIL levels in the C-OJ group reached a peak at 48 h, and then declined. The levels of TBIL and DBIL in the C-OJ group were higher than the C-Sham group at each time points. The TBIL and DBIL levels in the PVL group were the highest at 36 h, and were higher than those of the C-OJ group. However, at 48 h, the TBIL and DBIL levels in the PVL group were declined and were lower than those of the C-OJ group. At 72 h and 120 h, the TBIL levels and DBIL levels in the PVL group were declined to a similar level to the C-OJ group. At 168 h, the TBIL levels and DBIL levels in the PVL group were declined, but were slightly higher than those of the C-OJ group (**Figure 3A** and **3B**). These results indicated that, comparing to the obstructive jaundice group, selective portal vein ligation did not cause serious damages to hepatic function. After obstructive jaundice operation, significant increases in ALT and AST levels were observed in the C-OJ group. Thereafter, the AST and ALT levels were declined at 48 h, but reached

another peak at 120 h. The AST and ALT levels of the C-OJ group were higher than the C-Sham group at each time points (**Figure 3C** and **3D**). The AST levels in the PVL group reached the highest point at 36 h, and were higher than those in the C-OJ group at 36 h, 48 h, and 72 h. However, at 120 h and 168 h, the AST levels in the PVL group were similar to those in the C-OJ group (**Figure 3C**). The ALT levels were the highest at 36 h, and were higher than those in the C-OJ group. However, at 48 h, the ALT levels were decreased to a similar level to the C-OJ group. The ALT levels were also higher than the C-OJ group at 72 h, but declined to a similar level to the C-OJ group at 120 h and 168 h (**Figure 3D**). The ALB levels in the C-OJ group showed no significant difference compared with the C-Sham group. The ALB levels in the PVL group showed a significant decline when compared with those of the C-Sham group and the C-OJ group at 48 h (**Figure 3E**). These results demonstrated that selective portal vein ligation did not aggravate damages to hepatic function.

Selective portal vein ligation increased the TNF- α and IL-6 levels in rats with incomplete obstructive jaundice

The C-OJ group showed increased TNF- α levels compared with the C-Sham group, and peaked at 48 h. The TNF- α levels in the PVL group were higher than those in the C-OJ group at each time point (**Figure 4A**). The IL-6 levels showed a similar pattern to those of the TNF- α level. The IL-6 levels in the C-OJ group were higher than those in the C-Sham group and peaked at 48 h. In the PVL group, the IL-6 levels also peaked at 48 h, and higher than the C-OJ group at each time point (**Figure 4B**). These results indicated that the increased TNF- α and IL-6 levels may be involved in the liver regeneration promotion effects of the selective portal vein ligation in rats with incomplete obstructive jaundice.

Discussion

In the present study, the effects of selected portal vein ligation on the liver regeneration were explored under the background of incomplete obstructive jaundice. Results of our study showed that selected portal vein ligation promoted the liver regeneration in incomplete obstructive jaundice rat model.

There are double blood supply systems in the liver: the hepatic artery blood supply system and the portal vein blood supply system. Portal vein ligation or embolism takes advantages of this blood-supply characteristic of liver to block part of portal vein blood-supply in liver, resulting in atrophy, degeneration, apoptosis and necrosis of hepatocytes in the blocked sides of liver. Moreover, the proliferation in the unblocked sides of liver occurs at the early stage post-operation, and the proliferation rates of unblocked sides of liver are positively correlated to the diminishing of the blocked sides of liver, maintaining the total weights of liver and the hepatic functions. Portal vein ligation or embolization has been verified to be effective in promoting the regeneration of liver [1, 10]. In the present study, we found that selective portal vein ligation can also promote liver regeneration in rats with incomplete obstructive jaundice.

Patients with tumor in hepatobiliary system are usually accompanied with obstructive jaundice. In our study, we found that selective portal vein ligation could increase the weight ratios of the unligated sides of liver and enhanced the expression of Ki-67 in rat model of obstructive jaundice. These results demonstrated that selective portal vein ligation promoted the liver regeneration in obstructive jaundice rats. Comparative study shows that portal vein ligation is superior to portal vein embolization in inducing a regenerative response of the remnant liver [1]. Reperfusion after portal vein ligation is discovered to enhance the effect of portal vein ligation on liver regeneration [9].

Ligation of portal vein redirects portal blood to the unligated sides, resulting in hypertrophy. Portal vein ligation was reported to induce temporary impairment of total liver functions, following by rapid recovery. The main reason may be the increased function of the unligated sides of liver. Improved function in the unligated sides was reported to be more pronounced than suggested by the degree of volume gain [11]. In our study, the TBIL levels and DBIL levels in the PVL group peaked at 36 h, and no significant difference between the PVL group and C-OJ group was found at 48 h, 72 h, and 120 h. The AST levels at 120 h and 168 h, and the ALT levels at 48 h, 120 h and 168 h in the PVL group showed no significant difference to the C-OJ group. The

ALB levels showed difference to the C-OJ group only at 48 h. These results indicated that PVL in incomplete obstructive jaundice rat didn't aggravate the liver damage.

TNF- α and IL-6 play important roles in liver regeneration [12-14]. TNF- α and IL-6 are released by activated Kupffer cells and promote DNA synthesis in hepatocytes and promote the proliferation of hepatocytes [15-17]. These two cytokines also sensitize hepatocytes to growth factors. In our study, selected portal vein ligation was found to increase the levels of TNF- α and IL-6. These results prompt us to the hypothesis that TNF- α and IL-6 may be involved in the mechanism underlying the effects of selected portal vein ligation on promoting liver regeneration.

In conclusion, an incomplete obstructive jaundice rat model was built in this study. And the effects of selective portal vein ligation on liver regeneration were explored. Results of our study showed that selective portal vein ligation promoted the regeneration of the unligated sides of liver. Further study showed that the underlying mechanism may be associated with the increased TNF- α and IL-6 levels, thus promoting the regeneration of the unligated sides of liver. This study suggests that, in clinic, for patients with incompleated hepatobiliary obstruction whose hepatic failure risk is higher, selective portal vein ligation may also be taken into consideration to reduce the risk of hepatic failure and increase the success rate of secondary operation.

Acknowledgements

Thanks are giving to Junli Cao (Xuzhou Medical University) for his help in study design and animal experiments.

Disclosure of conflict of interest

None.

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