Title:

Two-stage Portal Vein Ligation Facilitates Liver Regeneration Safely in Rats with Liver Cirrhosis

Abstract

Background: Portal vein (PV) embolization is performed prior to extended hepatectomy for the damaged liver to increase future remnant liver volume and prevent postoperative liver failure. This study examined whether two-stage PV ligation (PVL) increased regeneration and hypertrophy of the future remnant liver compared to conventional PVL, and whether two-stage PVL was safe for damaged liver.

Method: We produced a cirrhotic liver rat model with perioperatively maintained fibrosis. Rats were divided into: Group A (70%PVL), ligation of left branch of PV; Group B (90%PVL), ligation of right and left branches of PV; and Group C (two-stage 90%PVL), two-stage PVL with left branch ligation of PV followed by right branch ligation 7 days later. To evaluate liver regeneration, liver weight ratios, proliferating cell nuclear antigen (PCNA) labeling index (LI), mitotic index (MI), and TdT-mediated dUTP-biotin nick end labeling (TUNEL) LI in the non-ligated caudate lobe were measured.

Results: Fourteen-day survival rate was 20% in Group B but 100% in Group C. TUNEL LI differed significantly between Groups A and B at 2 and 7 days postoperatively. Weight ratios were significantly higher in Group C than in Groups A and B at 14 days postoperatively. PCNA LI and MI in the non-ligated caudate lobe decreased to preoperative levels by 7 days postoperatively in Groups A and B, but remained elevated until 14 days postoperatively in Group C.

Conclusion: In cirrhotic liver rats, two-stage PVL avoided the lethal liver failure seen with one-stage PVL, and significantly facilitated liver regeneration more than one-stage PVL.

Introduction

Malignant liver tumors can be treated with hepatectomy, and extended hepatectomy is often required for complete removal of a tumor. Extended hepatectomy is becoming safer due to improved surgical methods and perioperative management. However, liver failure without a remnant liver volume can still present a lethal complication.[1-4] Postoperative liver failure reportedly occurs in 1.2-32% of cases.[5] Liver injury such as fibrosis or cirrhosis caused by viral hepatitis and chemotherapy-associated hepatitis can compromise the hepatic regenerative capacity and lead to postoperative liver failure.[6]

To increase the future remnant liver volume and to decrease the liver volume that must be removed, portal vein (PV) embolization (PVE) is performed before extended hepatectomy. Surgical safety and therapeutic outcomes have recently been improved for PVE.[1, 7-9] However, PVE is contraindicated in some patients because of severe liver damage.[10] Most patients who undergo hepatectomy have liver damage due to hepatitis B virus, hepatitis C virus, fatty deposition, or side effects of chemotherapy. In such patients, regeneration of damaged liver after PVE and hepatectomy is reportedly poor.[11] Approximately 7-11% of PVE patients cannot undergo a subsequent hepatectomy because of insufficient hypertrophy of the future remnant liver.[1, 8, 9, 12]

Associating liver partition and PV ligation (PVL) for staged hepatectomy (ALPPS) as reported by Schnitzbauer et al[13] in 2012 induces greater hypertrophy of the future remnant liver in a shorter period in comparison with PVE, PVL, or two-stage hepatectomy. ALPPS is attracting a lot of attention, and has been performed in several high-volume centers. However, high mortality and morbidity rates have been reported after hepatectomy following ALPPS; 90-day mortality was 8-9%, and severe morbidity (Clavien-Dindo ≥IIIa) was about 40%.[14, 15] The debate about the safety of ALPPS is thus ongoing, and this method has yet to become the standard for extended hepatectomy.

In our laboratory, Sugimoto et al previously reported that two-stage PVL for rats with normal livers enhances liver regeneration after PVL by inducing hemodynamic changes twice in non-ligated lobes, in comparison to conventional one-stage PVL.[1] Here, in a rat model of cirrhotic liver, we examined the features of extended hepatectomy on the damaged liver by determining whether two-stage PVL increases future remnant liver hypertrophy compared to the standard one-stage PVL. We also assessed the safety of two-stage PVL in the damaged liver.

Materials and Methods

Animals

Four-week-old male Sprague-Dawley rats were used (body weight, 110-140 g; Shizuoka Laboratory Animals Center, Shizuoka, Japan). To produce the cirrhotic liver model, we injected 50% CCl_4 (0.2 mL/rat) into rats subcutaneously twice a week for 6 weeks.[16] This 50% CCl_4 (0.2 mL) comprised 0.1 mL CCl_4 (about 1.0 mL/kg body weight) in 0.1 mL olive oil. Because the degree of liver fibrosis tended to be related to weight gain in preliminary experiments (data not shown), only rats weighing 250-350 g after the 6-week period were used as cirrhotic liver models to equalize models. Animals were kept under constant environmental conditions with a 12-h light-dark cycle with free activity and ad libitum access to ordinary water and diet.

Drug-induced hepatic cirrhosis is a reversible change, and thus, the hepatocellular damage and liver fibrosis start improving promptly after the end of drug administration.[16, 17] In a preliminary experiment for this study, to produce the rat cirrhotic liver model, 0.2 mL of 50% CCl_4 was injected subcutaneously twice a week for 6 weeks. Upon stopping the injection of CCl_4, rapid weight gain, liver hypertrophy, and improvement in liver fibrosis in rats were seen (data not shown). To maintain the cirrhotic status of the rat liver after PVL, the rats were

injected with 0.2 mL of 50% CCl$_4$ once a week, at the time of the first and second operations. Fibrosis of the cirrhotic liver model was thus maintained postoperatively (Fig. 1).

All experiments complied with the ARRIVE guidelines. They were performed in accordance with the Regulations for Animal Experiments in Gifu University and were approved by the Committee for Animal Research and Welfare of Gifu University Graduate School of Medicine, Gifu, Japan (No. 23-21). All efforts were made to minimize the number of animals used and their suffering.

Surgical Procedure for PVL (Fig. 2) and Experimental Design[1, 18]

We freed and completely ligated the right primary branch of the PV that supplied the right lobe (about 20% of the total normal liver volume) using 6-0 synthetic non-absorbable monofilament sutures. In a similar manner, we also ligated the left main branch of the PV that supplied the left lateral and median lobes (about 70% of the total normal liver volume). The hepatic artery and bile duct were carefully maintained in a patent state to prevent bleeding. The abdominal wall was then closed layer-to-layer with continuous sutures. In the 90%PVL rat model used in this study, all rats survived without complications.[19]

Three groups were established (Fig. 1).[1] For 70%PVL (Group A), on day 0, we completely ligated the left primary branch of the PV; 7 days later, laparotomy only was performed. For 90%PVL (Group B), on day 0, we completely ligated the right and left primary branches of the PV; 7 days later, laparotomy only was performed. For two-stage 90%PVL (Group C), on day 0, we completely ligated the left primary branch of the PV. For the right primary branch of the PV, a 6-0 suture was placed and knotted, but no ligation was performed at day 0. The ends of the suture remained in the abdomen near the liver. Laparotomy was then performed 7 days later, at which time the second stage of PVL was performed. The right primary branch of the PV was completely ligated by tightening the two ends of the suture. Groups A and C

were the same until 7 days after surgery. For the first 7 days postoperatively, the data of Group C were equivalent to those of Group A.

Group A and B rats were sacrificed at 2, 7, and 14 days after surgery, and Group C rats were sacrificed at 14 days after surgery. Liver and blood specimens were obtained. Six unmanipulated control rats were sacrificed for determination of baseline liver and blood data. Each group at each time point contained at least six rats, and we compared the 14-day survival rates among the groups (n = 10 each). Finally, 22 rats each were included in Groups A and B, and 10 were included in Group C. Surgery was performed while the rats were anesthetized with light ether with a clean, but not sterile, technique at ambient temperature. No microscope was used to guide the surgeon.

Biochemical Assay of Blood Samples

At the time of sacrifice, blood was obtained from the inferior vena cava, and then serum was obtained following centrifugation at 3000 rpm for 10 min. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), lactate dehydrogenase (LDH), total cholesterol, and total bile acid levels were determined according to standard laboratory methods.

Evaluation of Liver Regeneration

Livers were divided into the left lateral and median lobe, right lobe, and caudate lobe, and the weights of each section were determined.[1]

The extent of hepatectomy and postoperative remnant liver volume are important considerations in the setting of clinical hepatectomy. Therefore, we selected the remnant liver volume for the planned hepatectomy from the ratio of the caudate lobe weight/whole liver weight according to the following formula: (caudate lobe weight/whole liver weight) × 100

(%) and the ratio of the caudate lobe weight/total body weight according to the following formula: (caudate lobe weight/body weight) × 100 (%).[1]

  The caudate lobe liver was immersed in 10% buffered formalin, paraffin embedded, and sectioned at 3 μm. Proliferative activity of the non-ligated caudate lobe was evaluated by staining with proliferating cell nuclear antigen (PCNA) and hematoxylin and eosin. The PCNA labeling index (LI) was calculated as the number of PCNA-stained nuclei per 100 hepatocyte nuclei seen in six randomly selected high-power fields (HPF, ×400) and expressed as a percentage. The mitotic index (MI) was calculated as the number of cells undergoing mitosis per 1000 cells in six randomly selected HPFs.[1]

Measurement of the LI of TdT-mediated dUTP-biotin Nick End Labeling (TUNEL) Staining in the Non-ligated Caudate Lobe

The TUNEL LI was determined as the percentage of hepatocytes staining positively for TUNEL, which was determined by counting six randomly selected HPFs.

Statistical Analysis

All values are expressed as the mean ± standard deviation. Differences among the three groups were calculated using Tukey's honestly significant difference test. Survival rates were estimated from survival curves based on the Kaplan-Meier method and compared with the Mantel-Cox log rank test. Values of p < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 22.0 software (IBM, Chicago, IL).

Results

Survival Rate after PVL for Cirrhosis Liver Models (Fig. 3)

First, we examined whether PVL for the cirrhosis liver models affected survival. Most Group

B rats (one-stage 90%PVL) died in the early postoperative period. The 7-day survival rate for Group B was 30%, and the 14-day survival rate was 20%. Conversely, all rats in Group A (one-stage 70%PVL) and Group C (two-stage 90%PVL) survived for 14 days postoperatively. Significant differences in survival were seen between Group B and the other two groups (A vs. B and B vs. C, log-rank test, $p < 0.001$ each) (Fig. 3).

To investigate the causes of death in Group B, we examined apoptosis of hepatocytes 2 and 7 days after PVL using the TUNEL LI. On postoperative day 2, the TUNEL LI was significantly higher in Group B survivors (n = 5/6; 7.2 ± 5.2%) than in Group A (0.4 ± 0.2%; $p = 0.01$). On postoperative day 7, the TUNEL LI was also significantly higher in Group B survivors (n = 4/6; 0.9 ± 0.1%) than in Group A (0.1 ± 0.1%; $p < 0.001$) (Fig. 4a).

On postoperative day 2, TUNEL-positive cells were clearly significantly more frequent in Group B than in Group A (Fig. 4b).

Liver Function Tests (Fig. 5)

We examined the influence of PVL on liver function using biochemical data. To evaluate liver damage, serum levels of AST, ALT, T-Bil, LDH, and total bile acid were examined. AST peaked on day 2 at 2393 ± 1104 IU/L for Group A and 9669 ± 5445 IU/L for Group B survivors. ALT peaked on day 2 at 1847 ± 1211 IU/L for Group A and 5692 ± 3814 IU/L for Group B survivors. Both of those were significantly higher in Group B survivors than in Group A (AST, $p = 0.01$; ALT, $p = 0.04$). On day 7 and day 14, AST and ALT improved to preoperative levels. For Group C, AST and ALT on day 14 were comparable to preoperative levels.

For Group A, T-Bil remained stable postoperatively. T-Bil peaked on day 2 at 0.30 ± 0.12 mg/dL for Group A and 1.62 ± 0.66 mg/dL for Group B survivors. T-Bil was significantly higher in Group B survivors than in Group A ($p = 0.001$). On day 7 and day 14, T-Bil

improved to preoperative levels. For Group C, T-Bil on day 14 was equivalent to the preoperative level.

LDH on day 2 was 298 ± 144 IU/L for Group A and 1235 ± 1327 IU/L for Group B survivors. LDH in Group B survivors tended to be higher than that in Group A, but no significant difference between Groups A and B was identified.

Serum levels of total cholesterol were examined, and no significant differences among Groups A, B, and C were found for any time point.

Liver Weight Ratio of the Non-ligated Caudate Lobe[1]

To evaluate liver regeneration of the non-ligated caudate lobe, weight ratios of the liver were examined (Fig. 6). Rats in Groups A and C were identical up to 7 days after surgery. For the three groups, we compared the liver weight ratio of the non-ligated caudate lobe to the whole liver (Fig. 6a). The weight ratio preoperatively was 17.0 ± 4.4% in the control group. For Group A, the weight ratio was 23.4 ± 10.6% on day 7, and increased to 27.2 ± 3.8% on day 14. For Group B survivors, weight ratios on days 7 and 14 were 28.9 ± 2.7% and 28.2 ± 12.7%, respectively. We found no significant differences between Groups A and B at any time point. On the other hand, on postoperative day 14, the liver weight ratio of the non-ligated caudate lobe to the whole liver was significantly higher in Group C (55.6 ± 10.0%) than in Groups A and B (A vs. C, p < 0.001; B vs. C, p = 0.005) (Fig. 6a).

The liver weight ratio of the non-ligated caudate lobe to the whole body was compared among groups (Fig. 6b). The weight ratio preoperatively was 0.9 ± 0.3% in the control group. For Group A, the weight ratio on day 7 was 1.0 ± 0.5%, increasing to 1.2 ± 0.2% by day 14. On the other hand, for Group B survivors, the weight ratio on day 7 was 1.0 ± 0.1%, and the ratio was unchanged on day 14 (1.0 ± 0.5%). No significant difference was seen between Groups A and B at any time point. On postoperative day 14, the liver weight ratio of the

non-ligated caudate lobe to the whole body was significantly higher in Group C (2.4 ± 0.4%) than in Groups A and B (A vs. C, p < 0.001; B vs. C, p = 0.001) (Fig. 6b).

The non-ligated caudate lobe was predominantly bigger in Group C than in Group B on POD 14 as shown in pictures of Fig. 6c. Furthermore, the ligated left lobe also tended to be morphologically atrophied in Group C more than in Group B (Fig. 6c).

PCNA LI and MI

Liver regeneration of the non-ligated caudate lobe was examined using the PCNA LI and MI (Fig. 7). The PCNA LI was 4.6 ± 2.1% preoperatively, and peaked on day 2 at 46.6 ± 18.4% for Group A and 22.2 ± 22.5% for Group B survivors. On day 7, the PCNA LI decreased to 5.9 ± 4.2% for Group A and 5.0 ± 2.9% for Group B survivors. On day 14, the PCNA LI was 3.2 ± 0.7% for Group A, 1.3 ± 0.8% for Group B survivors, and 7.3 ± 1.8% for Group C, representing a significantly higher value in Group C than in Groups A and B (A vs. C, p = 0.001; B vs. C, p < 0.001) (Fig. 7a). PCNA-positive cells were clearly more frequent in Group C than in Groups A and B (Fig. 7b).

The preoperative MI was 18.6 ± 2.8%, and peaked on day 2 at 39.1 ± 10.0% in Group A and 29.9 ± 6.9% in Group B survivors. The MI was 32.0 ± 6.2% for Group A and 27.2 ± 5.7% for Group B survivors on day 7. By day 14, the MI had decreased to 23.6 ± 4.2% for Group A and 22.7 ± 3.8% for Group B survivors; these were similar to preoperative values. For Group C, liver regeneration was maintained after the second-stage PVL on day 7, with an MI of 42.5 ± 6.8% on day 14. At day 14, the MI was significantly greater in Group C compared to Groups A and B (A vs. C, p < 0.001; B vs. C, p = 0.003) (Fig. 7c).

Discussion

Hepatocellular carcinoma, intrahepatic cholangiocarcinoma, and metastatic liver cancer can

be treated with hepatectomy, which may produce a radical cure.[1,2] Hepatectomy is now safer due to improved operative methods, instruments, and patient management. However, liver failure can still occur following extended hepatectomy and may be fatal.[3, 4]

In 1984, Makuuchi et al[7, 20] described PVE for safer extended hepatectomy, and this technique remains in wide clinical use. PVE causes atrophy in embolized lobes and compensatory hypertrophy in non-embolized lobes, thus promoting liver regeneration. Therefore, PVE results in the need for removal of less liver volume,[21] leading to a reduced frequency of liver failure after extended hepatectomy. On the other hand, hepatectomy may not be able to be performed following PVE if cancer progression occurs while waiting for hepatectomy or if insufficient hypertrophy is obtained in the future remnant liver.[8, 9, 12, 22] Methods that facilitate liver regeneration and achieve greater hypertrophy of the liver within a shorter period are necessary to allow hepatectomy in such patients.

Another method to occlude the PV is PVL. PVL and PVE are not identical, but both techniques can safely increase the remnant functional liver volume and permit resection of extensive liver tumors.[23] PVL has often been performed to occlude the PV in animal experiments.[24-26] This method was reported first by Rous and Larimore in 1920, when they found that PVL induces atrophy of the ligated lobes and hypertrophy of the non-ligated lobes in 70%PVL animal models.[26] The 90%PVL rat model that we used here has only been described by Li et al who demonstrated survival of all rats with a normal liver and no complications.[19]

The mechanisms underlying liver regeneration after PVL are thought to be as follows: PVL enhances blood flow into the non-ligated lobes and also increases shear stress to the portal wall. Expansion of the PV increases stretch stress of endothelial cells. Such stress causes the release of cytokines such as tumor necrosis factor α and interleukin 6 (IL6) from endothelial cells, which primes the hepatocytes in non-ligated lobes.[1, 24, 27-29] Cytokines and hormones

such as hepatocyte growth factor (HGF) and insulin promote liver regeneration,[30] but growth factors gradually decrease, and liver regeneration decreases over time.[31]

Sugimoto et al from our laboratory reported a new two-stage PVL method that facilitates safe and simple liver regeneration in rats with normal livers.[1] They divided PVLs for the area of planned ligation into two procedures. The second ligation was performed 7 days after the first, by which time liver regeneration had returned to the preoperative state. This produced secondary hemodynamic changes from the PV to the non-ligated lobes and the release of growth factors. Consequently, hepatocyte proliferation was maintained over a long period, and the liver weight was greatly increased in the normal rat liver.

Most patients with clinical indications for hepatectomy show liver damage such as cirrhosis, fatty liver, alcoholic liver, liver with jaundice, and drug-induced damage to the liver following chemotherapy. Liver regeneration in the damaged liver is poorer than in the normal liver.[11] In some cases, extended hepatectomy following massive PVE for a damaged liver is contraindicated due to the risk of liver failure.[10]

Accordingly, our study examined whether two-stage PVL was safe and could facilitate liver regeneration in the damaged liver using a rat model of cirrhosis. In the present cirrhotic liver model in which fibrosis was maintained, most Group B rats (one-stage 90%PVL) died in the early postoperative period, with a 14-day survival rate of 20%. In rats with a normal liver, the 14-day survival rate for one-stage 90%PVL is 100%.[1, 19] One-stage 90%PVL for rats with a normal liver is thus safe, but one-stage 90%PVL for rats with a cirrhotic liver can often be lethal. On the other hand, the 14-day survival rate for Group C rats (two-stage 90%PVL) was 100%. Therefore, when extended PVL such as 90%PVL was divided into two stages, the procedure became safe for rats with a cirrhotic liver.

The TUNEL LI showed that apoptosis in the non-ligated caudate lobe in Group B survivors was greater than that in Group A in the early postoperative period. PVL thus induced

apoptosis in Group B. Concerning the liver damage following PVL, biochemical examination such as AST, ALT, and T-Bil showed that Group B survivors had more severe liver damage than Group A rats on postoperative day 2. Because even Group B survivors had severe liver damage, the dead rats in Group B may have had more severe liver damage and apoptosis. However, Group C rats with ligation of the same PV area overall were all alive with no increases in AST, ALT, or T-Bil at this time. These results suggest that one-stage 90%PVL caused apoptosis in the non-ligated lobe and severe liver damage in the cirrhotic liver model. The rats with a cirrhotic liver could not often respond to acute changes in functional liver volume after the extended PVL. Consequently, lethal liver failure occurred with high frequency following one-stage extended PVL in the cirrhotic liver model. However, liver failure following lethal 90%PVL could be avoided using the two-stage PVL. In the cirrhotic liver model with poor functional capacity of the liver, two-stage PVL prevented acute changes in functional liver volume. Moreover, weight, PCNA LI, and MI of the non-ligated caudate lobe were higher in Group C rats than in Group B survivors. These findings showed that two-stage PVL improved the safety of extended PVL and also facilitated liver regeneration. The facilitatory effect of liver regeneration following two-stage PVL in a cirrhotic liver rat model conformed to that in rats with a normal liver as reported by Sugimoto et al.[1] Even in the cirrhotic liver rat model, two-stage PVL facilitates liver regeneration over an extended period. Two-stage PVL contributed to the safety of PVL, produced secondary hemodynamic changes from the PV to the non-ligated lobes, and may release growth factors for a long period of time. Changes of the liver related cytokines after PVL such as HGF, IL6, transforming growth factor β were more likely to happen and very interesting. However, it has been reported that the changes got up within 24 hours after PVL.[32, 33] Therefore, it was difficult to detect the peaks of cytokines and we focused on the weight gain of the liver for two weeks in this study design. Regarding the atrophic change of the ligated lobe, it's

interesting how the ligated liver changes. In Group C, two-stage PVL enlarged the non-ligated caudate lobe greatly and also tended to raise the atrophic degree of the ligated right and left lobe (Fig. 6c). The next issue is whether the second boost of liver regeneration can be related with the changes of cytokines after the first and second PVLs and whether there are the morphological and pathological differences in the ligated lobe after PVL.

On the other hand, two-stage PVL changes flow in the PV twice and causes liver damage on two occasions within a short period. However, liver damage did not become serious and quickly improved (Fig. 5). Two-stage PVL for the damaged liver can safely and effectively facilitate liver regeneration for a short period. We therefore considered that the delay in liver damage following PV occlusion such as with PVE or PVL for the cirrhotic liver will not postpone planned hepatectomy in the clinical setting.

To apply two-stage PVL or PVE clinically, two approaches to the PV are necessary and are limitations and disadvantages of two-stage PV occlusion. For PVE, the approach to the PV involves percutaneous transhepatic puncture as the most general method, an ileocolic vein approach by laparotomy, or umbilical portion puncture by round ligament bougies. For PVL, the approaches require ligation by laparotomy or laparoscopy. To complete two-stage PVE or PVL, we should choose two methods from among these approaches. Of course, we can apply the same method twice if an approach using the other method is difficult at the second approach. Our "two-stage PVL" can thus be performed using the conventional technique only and does not require use of a new technique.

In clinical settings, this study model would be applicable to cases with extensive bilateral disease in which only the caudate lobe is free of tumor, i.e., right and left PV ligatures would be made simultaneously or sequentially. However, this is an extremely invasive procedure and may be impractical in humans. Application of this rat model to the clinical situation is difficult because the liver anatomy of humans is different from that of rats. However, we

think that liver regeneration after two-stage PVL is an important and interesting phenomenon. In reality, we suppose that the occlusion of several branches of segments II, III, and IV is performed when the right PV is occluded. Occlusion of the right PV and segment IV branches is generally safe in humans,[9, 12, 22] but may be invasive for patients contraindicated for PVE because of severe cirrhosis and high PV pressure. Our study suggested that two-stage PV occlusion will contribute to the safety of extended PV occlusion in such patients. Furthermore, two-stage PV occlusion may induce liver regeneration more than the conventional one-stage PV occlusion. However, PV occlusion itself is not the goal. The final goal is to perform a hepatectomy safely after PV occlusion. In the future, we will consider whether the hypertrophic liver after two-stage PVL increases the safety of extended hepatectomy.

Our "two-stage PVL" facilitated regeneration of the future remnant liver safely and without liver failure, even if the liver was cirrhotic. Furthermore, the method was completed using only existing techniques and produced a larger future remnant liver than conventional one-stage PVL. Two-stage PVL may offer efficient pretreatment before extended hepatectomy for the damaged liver. This method may therefore improve surgical outcomes in patients undergoing extended hepatectomy for malignant liver tumors with a damaged liver.

Conclusions

In cirrhotic liver rats, lethal liver failure following one-stage extended PVL was avoided using our method of two-stage PVL. Furthermore, two-stage PVL facilitated significantly greater liver regeneration than one-stage PVL.

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Figure Captions

Fig. 1

Experimental design and histological findings of the cirrhotic liver after subcutaneously injecting 50% CCl_4. Group A: ligation of left primary branch of the portal vein (70%PVL). Group B: ligation of right and left primary branches (90%PVL). Group C: ligation of left primary branch, followed by ligation of right primary branch 7 days later (two-stage 90%PVL).

The white arrowheads show the points when rats were sacrificed to obtain liver and blood specimens.

Fig. 2

(a) Rat cirrhotic liver in situ, **(b)** Surgical procedure of portal vein ligation

The white lines show ligation points.

RL, right lobe; LML, left median lobe; LLL, left lateral lobe; CL, caudate lobe

IVC, inferior vena cava; PV, portal vein; RB, right branch; LB, left branch

Fig. 3

Survival rate after portal vein ligation

Fig. 4

(a) TUNEL labeling index of non-ligated caudate lobe after PVL

(b) TUNEL staining findings of non-ligated caudate lobe after PVL on postoperative day (POD) 2

Fig. 5

Biochemical examination of blood. AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; LDH, lactate dehydrogenase; T-chol, total cholesterol; TBA, total bile acid

Fig. 6

Liver regeneration of non-ligated lobe after PVL

(a) Weight ratio of non-ligated caudate lobe to whole liver weight

(b) Weight ratio of non-ligated caudate lobe to body weight

(c) Morphological liver findings of Group B and C on POD 14

RL, right lobe; LML, left median lobe; LLL, left lateral lobe; CL, caudate lobe

Fig. 7

(a) PCNA labeling index of non-ligated caudate lobe after PVL

(b) PCNA staining of non-ligated caudate lobe after PVL on POD 14

(c) Mitotic index of non-ligated caudate lobe after PVL