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Takeshi Asakura
Nobuhiro Ohkohchi
Takashi Orii
Nozomi Koyamada
Shigeki Tsukamoto
Masahide Sato
Yoshitaka Enomoto
Masahiro Usuda
Susumu Satomi

Portal vein pressure is the key for successful liver transplantation of an extremely small graft in the pig model

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T. Asakura (✉) · T. Orii · N. Koyamada
S. Tsukamoto · M. Sato · Y. Enomoto
M. Usuda · S. Satomi
Division of Surgical Science
and Technology, Graduate School
of Medicine, Tohoku University,
1-1 Seiryo-machi, Aoba-ku,
980-0574 Sendai, Miyagi, Japan
E-mail: asakura@cb3.so-net.ne.jp
Tel.: +81-22-7177214
Fax: +81-22-7177217

N. Ohkohchi
Department of Surgery,
Clinical Institution of Medicine,
University of Tsukuba, Tsukuba, Japan

Abstract In partial-liver transplantation, the use of small grafts sometimes results in graft failure, usually caused by portal hypertension after transplantation (Tx). Portal hypertension after Tx can be decreased with a porto-caval shunt (PCS). The purpose of this study is to clarify the effect of the PCS on extremely reduced-size liver Tx. In a pig model, the posterior segment of 25% of a whole liver was transplanted orthotopically. The pigs were divided two groups: group A, graft with PCS ($n=7$), and group B, graft without PCS ($n=7$). The PCS was made by means of side-to-side anastomosis of the portal vein and the inferior vena cava. We examined the portal vein pressure, survival rate, regeneration rate of the graft, Ki-67 as an index of cell proliferation, and histological findings, and carried out liver-function tests. In group A, five pigs survived for more than 4 days and the remaining two died of a perforated gastric ulcer on post-operative day (POD) 2. In group B, all pigs except one died of graft failure within 24 h. Portal vein pressure after reperfusion in group A and group B was of statistically significant difference ($P<0.05$), 14.2 ± 3.2 and 18.9 ± 4.7 cmH_2O, respectively. In group A, the regeneration rate of the graft was 94%, 4 days after Tx, and Ki-67 stained remarkably in the parenchymal hepatocytes. In TEM finding, structure of the sinusoid was also well maintained after Tx. From these results we can conclude that the key to success in liver Tx with extremely small grafts lies in the control of the portal vein pressure.

Keywords Partial-liver transplantation · Portal pressure · Sinusoid · porto-caval-shunt

Introduction

The introduction of reduced-size, split liver, and living-donor liver transplantation (Tx) has widened the indication of clinical liver Tx and helped to resolve organ shortage [2, 6, 7, 20]. In general, the minimum volume for successful transplantation of a partial liver is assumed to be 30% of the standard liver volume of the recipient; however, the minimum limit of graft volume for successful liver Tx still remains unclear. In hepatic surgery, an extended hepatectomy of up to 80% of the whole liver volume is generally tolerated by the non-cirrhotic patient [15], but this does not indicate directly the minimum limit of the liver graft volume in partial-liver Tx. In experimental studies on large animals, reduced-size liver Tx of less than 30% of the whole liver in pigs have resulted in death due to graft failure within 24 h of Tx [19].

In liver transplantation, maintenance of the sinusoid structure of the graft is very important for successful

outcome [9, 13]. We assumed that graft failure after Tx of such an extremely small transplant was associated with portal hypertension after reperfusion. A porto-caval shunt can decrease portal hypertension after Tx. The purpose of this study was to clarify the effect of decreasing the portal pressure by means of porto-caval shunt (PCS) on the outcome of extremely small liver Tx.

Materials and methods

Experimental design

Animals

Landrace white pigs, weighing from 22 to 28 kg (mean ± SD, 25 ± 1.8 kg), were used for the experiment. All experiments were conducted according to the guide *Principles of Laboratory Animal Care* (NIH publication No. 86-23, revised 1985). Food was withheld for 24 h preoperatively. Anesthesia was induced by intramuscular injection of ketamine (5 mg/kg) and atropine sulfate (1.0 mg) and maintained by isoflurane.

Donor operation

After laparotomy, we cannulated the splenic vein in order to measure the portal vein pressure. At the hepatic hilus, the left hepatic artery was ligated. Using segmental vascular occlusion techniques [11, 14], we identified and ligated each of Glisson's sheathes of the left lobe and the anterior segment (Fig. 1a). After mobilization of the liver, the left and middle hepatic veins were ligated with transfixing suture with the liver (Fig. 1b). Parenchymal transection of the left lobe and the anterior segment were carried out, and then we performed left tri-segmentectomy of the liver.

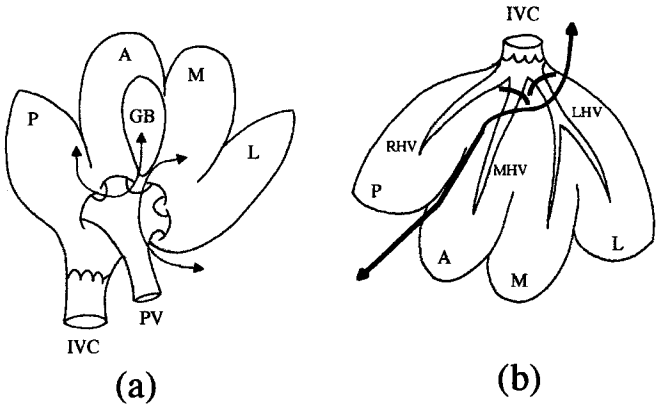

Fig. 1a, b Schematic view of left hepatic tri-segmentectomy. **a** In the hepatic hilus, the Glisson's sheathes of the left lobe and anterior segment were identified and divided (*arrow lines*). **b** The left and middle hepatic veins were ligated by transfixing suture (*thick lines*). Parenchymal transection of the left lobe and anterior segment were carried out with crush maneuver (dotted line). *L* lateral segment, *M* median segment, *A* anterior segment, *P* posterior segment, *IVC* inferior vena cava, *PV* portal vein, *GB* gallbladder, *LHV* left hepatic vein, *MHV* middle hepatic vein, *RHV* right hepatic vein

Following intravenous administration of heparin, the abdominal aorta was cannulated at the infra-renal portion. Cold University Wisconsin (UW) solution at 4 °C was flushed in from both the aorta and the splenic vein after aortic clamping. The liver graft was removed from the posterior segment and preserved in UW solution.

Back table operation

The pigs were divided into two groups: group A, graft with PCS ($n=7$), and group B, graft without shunt ($n=7$). In group A, the PCS was effected by means of side-to-side anastomosis between the portal vein and the infra-hepatic inferior vena cava (IVC) (Fig. 2). The 6-mm aperture for the PCS was made with a vascular punch.

Recipient operation

Recipient total hepatectomy was performed under ilio-porto-jugular vein bypass, by a standard technique [17]. The reduced-size grafts with and without PCS were transplanted orthotopically with vascular anastomosis performed in the usual sequence: the suprahepatic IVC, the portal vein, the infrahepatic IVC, and the hepatic artery. Before anastomosis of the portal vein, rinse solution (Torii Pharmaceutical, Tokyo, Japan) was perfused into the portal vein of the graft. Hepatic arterial reconstruction was performed under a microscope. A catheter in the common bile duct was inserted as bile drainage.

Blood was sampled before laparotomy, during the anhepatic phase, at reperfusion, at 3 h and 6 h after reperfusion, and on every post-operative day (POD). For histological examination, liver biopsies were performed before hepatectomy, after reperfusion, and on every other day up to POD 7.

Parameters examined

The levels of total bilirubin, aspartame aminotransferase (AST), and anti-thrombin III (AT III) of the serum, portal pressure, and graft volume after regeneration were measured; we measured Ki-67 as a marker of the proliferation of hepatocytes, using MIB-5 antibody (Medical and Biological Laboratories, Nagoya, Japan).Transmission electron microscope findings were examined after Tx. We measured portal vein pressure at laparotomy, after left tri-segmentectomy, and after reperfusion, using a catheter placed in the portal vein through the splenic vein.

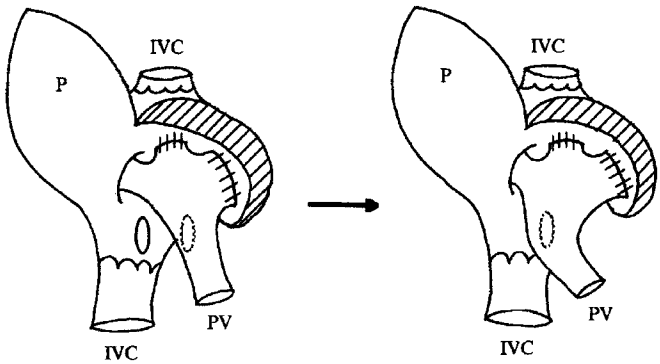

Fig. 2 Schematic view of the PCS. We made the shunt by side-to-side anastomosis between the side wall of the portal vein and the side wall of the infrahepatic IVC (*ovals*). *P* posterior segment, *IVC* inferior vena cava; *PV* portal vein

Statistical analysis

Values of parameters were expressed as mean ± SD. Statistical significance was determined by Student t test. $P < 0.05$ was regarded as significant.

Results

Recipient whole-liver weight, weight ratio of the graft to the recipient whole liver (G/R ratio), operation time, and total ischemia time (TIT) are shown in Table 1. There were no significant differences in terms of recipient whole-liver weight, G/R ratio, and operation time between the two groups . The mean TIT of group A was significantly longer than that of group B because of the time required to connect the PCS at the back table ($P < 0.01$).

Survival

The survival days and cause of death in each group are shown in Table 2. In group A, five pigs survived for more than 4 days. Causes of death were attributed to portal vein thrombosis at the anastomotic site of the PCS in one case, a perforated or hemorrhagic gastric ulcer in three cases, and biliary peritonitis in two cases.

Table 1 Recipients' whole-liver weight, G/R ratio, operation time, and TIT

Parameter	Group A ($n=7$)	Group B ($n=7$)
Recipients'whole-liver weight (g)	577 ± 52.6	537 ± 43.1
G/R ratio (%)	26.8 ± 1.9	25.0 ± 2.5
Operation time (min)	276 ± 46	274 ± 26
TIT(min)	157 ± 22*	114 ± 33

*$P < 0.01$ vs group B

Table 2 Survival and cause of death

Group	Animal no.	Survival (days)	Cause of death
A	1	2	Perforated gastric ulcer
	2	5	Portal vein thrombosis
	3	4	Biliary peritonitis
	4	2	Perforated gastric ulcer
	5	5	Bleeding gastric ulcer
	6	5	Biliary peritonitis
	7	>7	Killed
	Mean ± SD	4.0 ± 1.9*	
B	1	0	Graft failure
	2	0	Graft failure
	3	0	Graft failure
	4	0	Graft failure
	5	1	Graft failure
	6	7	Killed
	7	1	Graft failure
	Mean ± SD	1.3 ± 2.6	

*$P < 0.05$ vs group B

On the other hand, in group B, all pigs except for one died within 24 h after reperfusion. Autopsy demonstrated a large amount of bloody ascites, swelling of the liver graft, and congestion of the intestine. The mean survival times of the group-A pigs were significantly longer than those of group B ($P < 0.05$).

Liver volume after Tx

Changes of recipient liver weight are shown in Fig. 3. Group-A pigs survived for more than 4 days, the liver graft recovering to 94% of the recipient whole liver within a week. The regeneration ratio of the graft liver reached 340%.

Ki-67

Ki-67 stained protein expressed in proliferating cells. MIB-5 specifically recognizes the equivalent Ki-67 protein. Ki-67 detection after Tx is shown in Fig. 4. The Ki-67 labeling index showed only 1.0% of hepatocytes at laparotomy; it increased, however, reaching a peak value of 60% at POD 2, and decreasing to 30% at POD 4.

Portal pressure

Figure 5 shows portal pressure. There were no significant differences, in terms of mean portal vein pressures before hepatectomy and after tri-segmentectomy, between the two groups. However, after reperfusion, the values of portal vein pressure in group A, i.e., graft with PCS, were significantly lower than those in group B ($P < 0.05$).

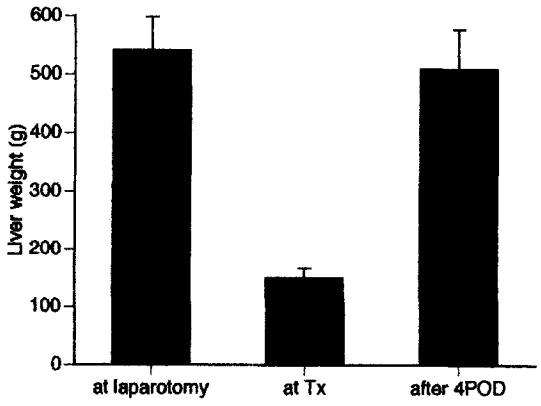

Fig. 3 Changes of recipient liver weight in the pigs that survived for more than 4 days in group A ($n=5$). The volume of the regenerated transplanted liver is almost the same as that of the native liver. Regenerated liver weight became 94% of the recipient whole liver within a week

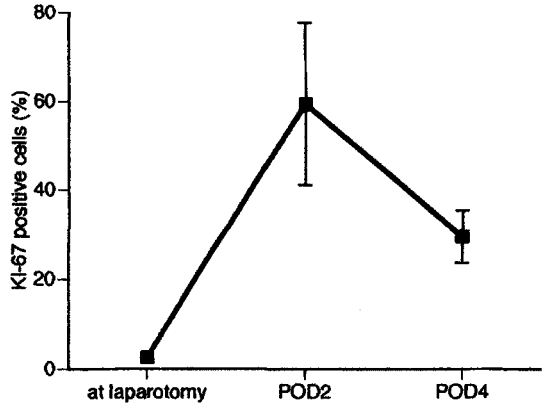

Fig. 4 Ki-67 detection after Tx in the pigs that survived for over 4 days in group A. The Ki-67 labeling index showed only 1.0% of hepatocytes positive at laparotomy; it increased abruptly, reaching a peak value of approximately 60% at POD 2, and decreased to 30% at POD 4

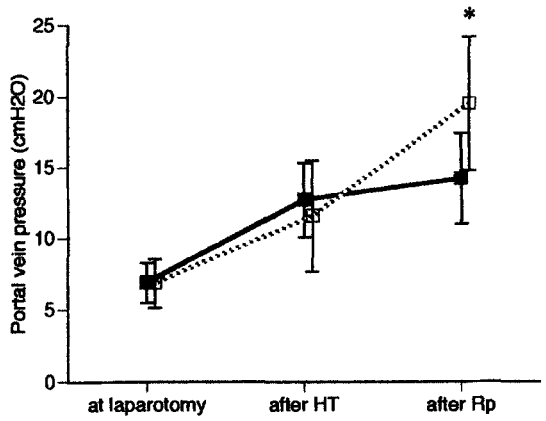

Fig. 5 Changes in portal vein pressure in both groups. *Solid squares* group A, *open squares* group B, *after HT* after tri-segmentectomy of the donor liver, *after Rp* after reperfusion of the posterior segment graft. $*P < 0.05$ vs group B

Histological findings

The histology of the graft after reperfusion is shown in Fig. 6. In group A, histopathological findings show normal structure, the same as that of the laparotomy. In group B, however, destruction of the sinusoidal lining and bleeding in the peri-portal triads were observed.

Transmission electron microphotographs of the sinusoid after reperfusion is shown in Fig. 7. In group A, the sinusoidal endothelial cells and hepatocytes are well preserved, and the structure of the endothelial lining was also perceived. In group B, however, the sinusoidal endothelial lining was completely destroyed and detached in the sinusoidal space with enlargement of Disse's spaces. Furthermore, the disruption of the sinusoidal structure and of the hepatocytes was more severe in zone 1.

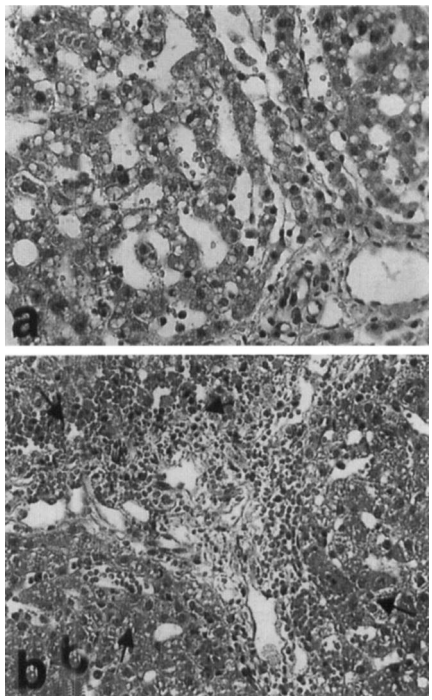

Fig. 6a, b Histological findings of hepatic tissues after reperfusion. **a** In group A, graft with PCS, remarkable changes were not observed. **b** In group B, graft without PCS, disruption of sinusoidal lining and bleeding in the portal triads were observed (*arrows*). Magnification ×50

Liver-function test

Serum AST levels and total bilirubin (T-Bil) levels until 6 h after reperfusion in both groups showed no significant differences (Table 3). In group A, the values of AST increased after reperfusion, reaching top levels at POD 2, and then decreased. However, the levels of T-Bil continued to rise until POD 4. Serum AT-III levels decreased after reperfusion in both groups. At 6 h after reperfusion, the levels of AT III in group A were significantly higher than those in group B ($P < 0.05$), but in group A, the levels of AT III reached minimal values at POD 2 and then increased.

Discussion

In clinical hepatic resections, tri-segmentectomy is performed as the standard technique in patients with a normal liver [12, 18]. In experimental studies, up to 90% of hepatic resection can be successfully performed in the rat [3]. In liver Tx, grafts are subjected to cold ischemia and reperfusion injury. In partial-liver Tx, the small graft suffers from these damages, which is why the minimum graft volume for successful liver Tx is presumably larger than the residual liver volume in the hepatic resection. In previous studies, it was reported

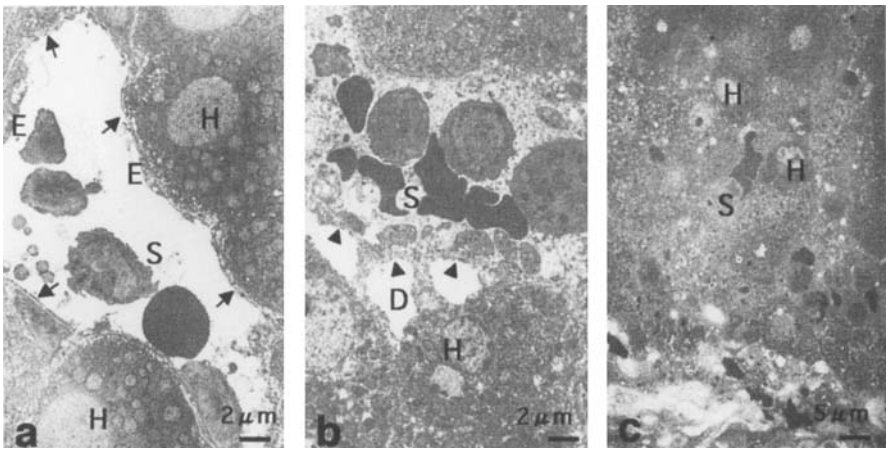

Fig. 7a–c Transmission electron microscope findings of the sinusoid after reperfusion. **a** In group A, the sinusoidal endothelial cells and hepatocytes were preserved and endothelial lining was perceived (*arrows*). **b** On the other hand, in group B, sinusoidal endothelial lining was completely disrupted and detached into the sinusoidal space with destruction of Disse's spaces (*arrow heads*). **c** In zone 1, disruption of sinusoidal structure and hepatocytes was more remarkable and the structure of the sinusoid was not identified. *D* Disse's space, *E* endothelial cell, *H* hepatocyte, *S* sinusoidal space. *Scale bar* 2 μm (**a**, **b**), 5 μm (**c**)

that Tx of less than 30% of the standard liver volume leads to graft failure after Tx in the pig [4, 5, 19]. For successful liver Tx, maintenance of the sinusoidal structure, i.e., hepatic microcirculation, and of the mitochondrial function, are essential [9, 13]. We hypothesized that portal hypertension might occur after Tx with small grafts and if the structure of the sinusoid is very sensitive to cold/ischemic injury. Furthermore, portal hypertension might be associated with graft failure after an extremely small liver Tx. Ku et al. reported that reconstruction of the porto-systemic shunt effectively reduced portal hypertension after small grafting of the liver in a canine model [10]. Hayashi et al. reported Tx with small grafts of less than 40% of the native liver as being successful with an intra-hepatic porto-systemic shunt, using a self-expanding metallic Z-stent in pigs [4, 5]. We chose portal pressure as the parameter of portal flow. Electromagnetic flowmetry assessment can measure only surface blood flow and does not reflect the whole blood flow of the liver. Furthermore, there is no physiological porto-systemic shunt system in pigs. For those reasons, we considered measurement of the portal vein pressure to be the most appropriate method of estimation of the flow in reduced-size liver graft in pigs.

In this study, we investigated the effect of the porto-systemic shunt by means of side-to-side anastomosis between the portal vein and the infra-hepatic IVC, on the result of quite small-size liver Tx. Our method is simple, and it safely decreases the portal pressure after Tx.

In our study, the TIT in group A, graft with PCS, was 43 min longer than that of group B, graft without PCS, and 43 min. of additional cold ischemia time could hardly impair liver-graft function in pigs. We excluded the cases with technical problems, i.e., early vascular thrombus, massive bleeding, and heart failure caused by bio-pump trouble. There were no technical problems in either group. The mean survival period in group A, graft with PCS, was significantly longer than that of group B, graft without PCS. We evaluated the effect of PCS on the portal hemodynamics with changes of the portal vein pressure. In our preliminary study, we assessed appropriate portal flow to decide on the size of the PCS. In cases with a large PCS, portal vein pressure decreased to a lower level, and the pig died because of poor portal flow. The findings in the large PCS model showed no regeneration in the transplanted partial liver. We can conclude that excessive decrease of portal vein pressure was found to be as harmful as high portal pressure for liver regeneration. In this study, significant elevation of

Table 3 Changes in serum levels of AST, AT III and T-Bil (*Rp-3 h* 3 h after reperfusion, *Rp-6 h* 6 h after reperfusion

Parameter	Group	At laparotomy	Rp-3 h	Rp-6 h	POD1	POD2	POD3	POD4
AST/(IU/l)	A	36 ± 28	65 ± 25	135 ± 23	238 ± 45	247 ± 78	160 ± 79	76 ± 34
	B	32 ± 17	56 ± 30	126 ± 72				
T-Bil (mg/dl)	A	0.05 ± 0.07	0.21 ± 0.17	0.31 ± 0.23	0.32 ± 0.23	0.56 ± 0.39	0.94 ± 0.81	1.14 ± 0.88
	B	0.15 ± 0.10	0.51 ± 0.28	0.41 ± 0.23				
ATIII (%)	A	118 ± 30	73 ± 19	76 ± 14*	69 ± 17	52 ± 12	63 ± 13	71 ± 19
	B	123 ± 5	53 ± 34	48 ± 24				

*$P < 0.05$ vs group B

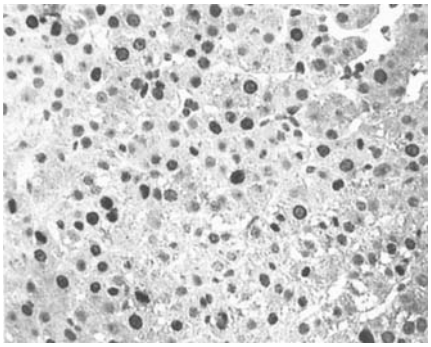

Fig. 8 Ki-67 labeling in the liver at POD 2 in the group with the PCS. Approximately 60% of hepatocytes were Ki-67 positive (magnification ×100)

the portal pressure after Tx was recognized in group B. Pathological findings clearly indicated disruption of the sinusoidal lining and disturbance of the sinusoidal microcirculation in this group. The transmission electron microphotographs clarified the disruption of sinusoidal endothelial lining, and destruction of hepatocytes was more severe in zone 1. On the other hand, such findings of microcirculatory disturbance were not found in group A, graft with PCS. Indeed, during the operation, the effect of the PCS was well reflected in the macroscopic findings after reperfusion. In group B, the graft became swollen after reperfusion, hard bowel congestion progressed, and blood oozing continued. In group A, these findings were not observed. These results of our study clearly indicate that the portal blood pressure after reperfusion rises above the level required for adequate parenchymal perfusion in the extremely small graft, and that acute portal hypertension after Tx weakens the structure of the sinusoid of the graft after reperfusion.

Ki-67 is a good index of the cell proliferation in hepatic regeneration [1]. In our study, Ki-67 staining of the graft was strongly positive on POD 2 in group A (Fig. 8). The volume of the transplanted liver became almost the same as that of native liver in this group. These results also indicate that the portal pressure in the early period after Tx is an important factor for liver regeneration in small-size liver Tx.

Changes of AST and AT-lll levels reflected recovery from cold ischemic injury and regeneration of hepatocytes. In the liver-function test, the T-Bil level increased slightly. In these cases, cholestasis occurred, due to the influence of the tube-drainage trouble of bile juice after the operation. However, transient postoperative cholestasis has been noticed following successful transplantation of small grafts in experimental models and also in clinical settings, probably reflecting the metabolic overload in the early phase during graft regeneration [8, 10, 16]. In addition to survival rate and graft regeneration, these results indicated that the Tx of liver in group A, i.e., the graft with PCS, was effective for regeneration of extremely small liver grafts.

In summary, in this study we clearly demonstrated that an extremely small graft, under 30% of the native whole liver, can be successfully transplanted with a PCS in large animals. These results suggest that portal hypertension after reperfusion is one of the most important factors aggravating microcirculatory injury of the graft, and that control of the portal vein pressure is the key to success of transplantation of extremely small grafts. We also clarified the effect of the PCS on the prevention of graft failure in the early stage. But for the clinical application of the PCS, further investigation on its long-term effect on portal hemodynamics and graft function are necessary.

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