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Combination splitting using both in situ and ex situ techniques in triple split liver transplantation in pigs

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Abstract In situ splitting of cadaver livers has been reported to reduce cold ischemic damage, to avoid biliary complications, and to result in improved graft survival. In this study, which involved a wider application of split liver transplantation (SLT), we examined the effects of a technique combining both ex situ and in situ splittings in triple SLT in pigs and compared it to ex situ splitting alone. In the combination splitting group, the splitting between the right and left lobes was done in situ with perfusion of the left lobe with cold, lactated Ringer's solution; that between the lateral and medical right lobes was done ex situ in backtable surgery. The time required for in situ splitting was 28 ± 5 min. The time for backtable surgery and the total ischemia time were significantly shorter in the combination splitting group than that in the ex situ splitting group ($P < 0.05$). One day after triple SLT, the elevations in both serum AST and LDH in the ex situ splitting group were significantly greater than those in the combination splitting group ($P < 0.05$). We conclude that combination splitting may provide a technical improvement and have a beneficial effect on the clinical application of triple SLT.

Key words Split liver · In situ split liver · Ex situ split liver · Triple split liver · Pig, liver transplantation

Introduction

Applying the technique of split liver transplantation (SLT) is an attractive strategy for resolving the donor liver shortage because it provides two recipients with organs from one donor [12]. However, SLT has been reported to pose various potential problems, such as an increased incidence of biliary complications and ischemic necrosis of the median left lobe. Inferior patient and graft survival has also been reported. There has been improvement as the result of a recently developed splitting technique, i.e., in situ splitting [7]. In situ splitting, as appliced to living-related liver transplantation (LRLT), has the beneficial effects of avoiding extended cold ischemia time and of anatomical integrity of the grafts before implantation. Its safety has been confirmed by the excellent results of LRLT [10].

We examined the potentially wider application of SLT from one donor to three recipients, i.e., triple SLT. To split one liver for three recipients, the left, right medial, and right lateral grafts each have to be drained by a hepatic vein, i.e., the right, middle, and left hepatic veins, respectively. Previous investigations have shown that small liver grafting in triple SLT in pigs is possible with the use of an inferior vena cava (IVC) patch and graft [4] and the construction of intraoperative, intrahepatic portosystemic shunt (IIPS) in backtable surgery [2, 3]. The ex situ splitting procedure in backtable surgery in the case of triple splitting takes longer than that of double splitting because of the complexity of the splitting procedure. This results in a higher risk of primary graft nonfunction (PNF) after implantation.

In this study, we established a novel technique of triple SLT using both in situ and ex situ splitting (combination splitting) to shorten the ischemia time and to de-

crease the bleeding from the raw surface of the liver graft, and we compared combination splitting with ex situ splitting in triple SLT.

Materials and methods

Animals

Landrace female pigs weighing 13–20 kg were used for donor and recipient surgery, respectively. For the experiments, the "Principles of laboratory animal care" (NIH Publication No. 86-23, revised 1985) were followed, as well as the regulations of the Animal Research Laboratory of Nagoya University School of Medicine.

Donor surgery

All pigs received ketamine-HCl for induction and were maintained on inhalation anesthesia with halothane. Experiments were carried out on four pigs in each of the two experimental groups.

Ex situ splitting

In this group, donor hepatectomy was performed according to a standard technique of multiorgan retrieval, as described previously [9]. The donor liver and intrathoracic and infrarenal inferior vena cava (IVC) were procured and preserved with University of Wisconsin (UW) solution. In backtable surgery, the vascular and biliary structures in the hilum were visualized and their lobar branches were identified. The left hepatic artery (LHA) was severed just after bifurcation of the right hepatic artery (RHA). After identification of the three main hepatic veins [right (RHV), middle (MHV), and left (LHV)] through the lumen of the upper IVC, transection lines were marked on the liver surface along both the left side of the RHV and the right side of the umbilical portion using CUSA with an electrocoagulator (USU, Olympus, Tokyo, Japan; Fig. 1). Parenchymal transection between the right and left lobes was started using USU, and the intrahepatic vessels as well as the ducts were ligated and severed. The left portal vein (LPV) and left hepatic duct (LHD) were ligated and severed. As shown in Fig. 2, the several lateral branches of the right portal vein (RPV) were identified directly from the portal vein (PV). After dividing the lateral branch of the RHA just after the bifurcation of the medial branch of the RHA, the PV was severed both above and below the orifice of the lateral branch of the RPV. Finally, parenchymal transection between the lateral and medial segments of the right lobe was carried out along the left sides of both the RHV and the intrahepatic IVC. An IVC patch and IVC graft were sutured using 5-0 prolene to reconstruct the orifice of the MHV.

Combination splitting

In this group, liver procurement proceeded as follows. After dividing the gastroduodenal artery (GDA) and the left gastric artery (LGA), the RHA and LHA were identified. The LPV, visualized beyond the bifurcation with RPV, was prepared for selective perfusion with cold, lactated Ringer's solution. An 8 Fr soft catheter was inserted from the splenic vein (SV) to the LPV, followed by selective perfusion with both the LPV and the LHA being occluded. A transection line between the right and left lobes was marked on the liver surface along the right side of the umbilical portion using USU. Parenchymal transection was carried out under the selective hepatic perfusion of cold, lactated Ringer's solution to the left hepatic lobe. The vessels and ducts of the left medial lobe were ligated and severed. Bleeding from the raw surface was minimal. After dividing the hepatic parenchyma as close to the orifice of the LHV as possible, donor hepatectomy was performed according to a standard technique of multiorgan retrieval and perfusion with UW solution [9]. The intrathoracic and infrarenal IVC were procured for reconstruction of the IVC. Backtable surgery was carried out as follows. After dividing the LHA, LPV, and left hepatic duct (LHD), the remnant parenchyma of the left lobe were transected using USU. The orifice of the LHV with a part of the IVC was fi-

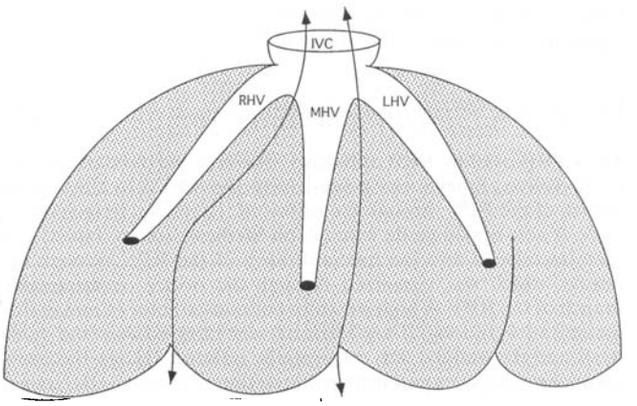

Fig. 1 Transection lines of liver parenchyma. These lines were marked on the liver surface along both the left side of the right hepatic vein *(RHV)* and the right side of the umbilical portion *(MHV* middle hepatic vein, *LHV* left hepatic vein, *IVC* inferior vena cava)

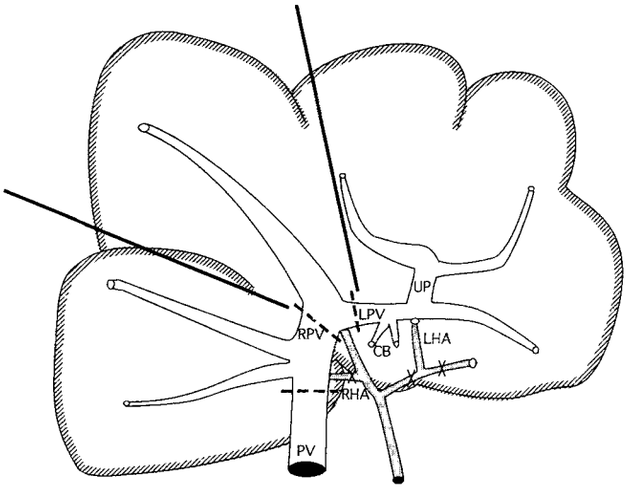

Fig. 2 Schematic view of the preparation of the hepatic hilus. *Solid lines* indicate where parenchymal transections were made, whereas *dotted lines* indicate where the portal vein system was severed (*PV* portal vein, *RPV* right portal vein, *LPV* left portal vein, *UP* umbilical portion, *CB* caudate branch, *RHA* right hepatic artery, *LHA* left hepatic artery)

nally dissected. The IVC patch and IVC graft were sutured using 5-0 prolene to reconstruct the orifice of the MHV.

Intraoperative, intrahepatic portosystemic shunt (IIPS) technique

The IIPS procedure went as follows. The metallic puncture needle (Top, Tokyo, Japan) was guided from the MHV to the medial branch of the RPV through the orifice of the medial branch of the RPV. A soft-tipped guide wire (Zeon Medical, Tokyo, Japan) was then introduced into the medial branch of the RPV through the needle, which was visualized through the orifice of the medial branch of the RPV. A high-pressure balloon catheter (4 mm in diameter and 4 cm long; Zeon Medical) was inserted over the guide wire, and the balloon was sequentially inflated against the intraluminal wall of liver parenchyma three times. Then, a self-expanding metallic Z-stent (4 mm in diameter and 4.5 cm long; Medico's Hirata, Tokyo, Japan) covered with a protective sheath was advanced directly after balloon dilation. The end of the stent was placed such that it protruded about 5–7 mm on both sides and was fixed firmly in the liver parenchyma. After the orifice of the medial branch of the RPV was anastomosed to the end of the PV using 6-0 prolene, the leakage from the raw surface of the graft was sutured by flushing UW solution through the PV and HA (Fig. 3).

Recipient surgery

Recipient hepatectomy and graft replacement were performed according to a standard technique including passive venovenous bypass a heparin-coated catheter. Before anastomosis of the PV, with the graft was perfused with rinse solution containing a protease inhibitor (nafamostat mesilate, Torii Pharma, Tokyo, Japan) [11], followed by the spread of fibrin sealant (Hoechst AG, Germany) on the raw surface of the graft. Hepatic arterial reconstruction was carried out with bifurcation of the recipient GDA, followed by cholecystoduodenostomy for biliary reconstruction.

Blood sampling

Pig sera were taken before Tx, 1 h after, and 1 day after to measure serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

Statistics

All data were expressed as mean ± SD. Statistical analysis was performed using the unpaired Student's t-test. Statistical significance was defined as a P level below 0.05.

Results

Three of the four pigs in the ex situ splitting group survived longer than 3 days and one pig died on day 1 after Tx due to PNF, whereas all of those in the combination splitting group survived longer than 3 days. Table 1 summarizes the data for the two groups of pigs. The time required for backtable surgery, as well as the total ischemia time in the combination splitting group, was significantly shorter than that in the ex situ splitting group ($P < 0.05$, Student's t-test). The graft volume of the liver in combination SLT was comparable to that in ex situ SLT. The raw surface of the graft in the combination splitting group showed no leakage of UW solution and no bleeding in backtable and recipient surgeries, whereas that in the ex situ splitting group needed suture and electrocoagulation for the leakage of UW solution and for the bleeding from the raw surface of the liver graft.

The changes in the serum AST level after reperfusion are shown in Fig. 4. One hour after reperfusion, the elevation in AST in the ex situ splitting group was greater than that in the combination group, but not sig-

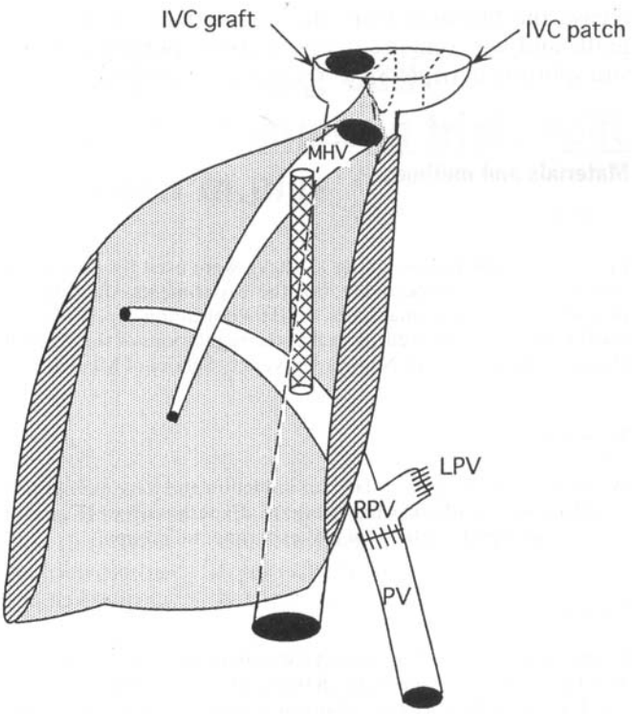

Fig. 3 Construction of the medial right lobe graft using IVC reconstruction and IIPS. IVC patch and IVC graft were sutured using 5-0 prolene to medial right lobe graft. After IIPS was inserted from MHV to the medial branch of the RPV, the orifice of the LPV was closed and that of the medial branch of the RPV was anastomosed to the PV using 6-0 prolene

Table 1 Ex situ and combination splittings in triple SLT

	Ex situ SLT ($n = 4$)	Combination SLT ($n = 4$)
Backtable surgery	2.9 ± 0.3 h	2.0 ± 0.2 h
Total ischemia time	3.6 ± 0.4 h	2.6 ± 0.2 h
In situ splitting time	0	28 ± 5 min
Graft volume	27% ± 6%	24% ± 4%

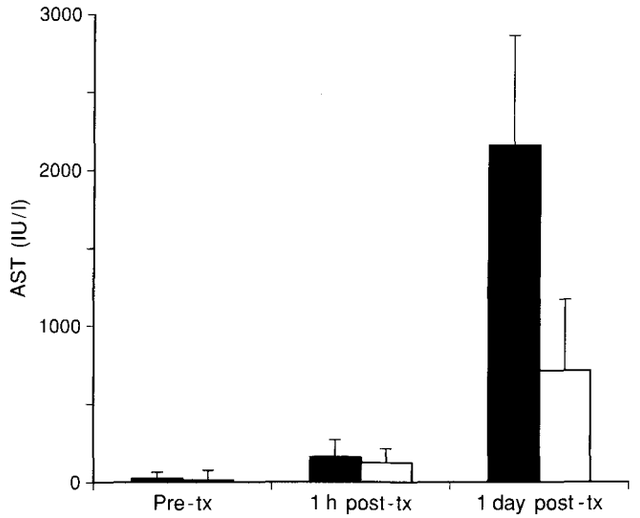

Fig. 4 Change in serum AST in the ex situ and combination splitting groups. Serum AST increased in both groups after reperfusion. One day after triple SLT, the elevation in AST in the ex situ splitting group (■) was significantly greater than that in the combination splitting group (□, $P < 0.05$)

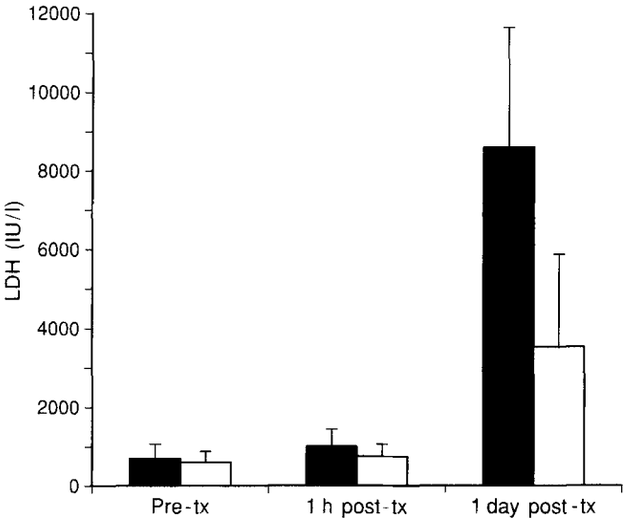

Fig. 5 Change in serum LDH in the ex situ and combination splitting groups. Serum LDH increased in both groups after reperfusion. One hour after reperfusion and 1 day after triple SLT, LDH in the ex situ splitting group (■) was significantly higher than that in the combination splitting group (□, $P < 0.05$)

nificantly so. One day after triple SLT, the elevation in AST in the ex situ splitting group was significantly greater than that in the combination splitting group ($P < 0.05$).

The changes in the serum LDH level after reperfusion are shown in Fig. 5. One hour after reperfusion, the elevation in LDH in the ex situ splitting group was significantly greater than that in the combination group ($P < 0.05$). One day after triple SLT, the difference in the elevation in LDH between the two groups was more significant ($P < 0.05$). The LDH value of one pig in the ex situ splitting group was over 10000 IU/l (12793 IU/l). This pig died just after blood sampling.

Discussion

SLT was first reported in 1988 by Pichlmayr and colleagues from Hannover, Germany [6]. Since then, there have been several reports of SLT series, especially from Europe [5, 8]. Yet, many problems, including the high incidences of both PNF and biliary complications, remain unsolved. This study clearly shows that combination splitting in triple SLT can shorten the time required for backtable surgery and render the hemostasis easy after implantation.

It has been reported that both a long ischemia time and a long benching time make tissues more susceptible to PNF and that this can be avoided by the use of in situ splitting [7]. In this study, we used the technique of cold perfusion during in situ splitting, which is an application of hepatectomy under total hepatic vascular exclusion (THVE). Cold perfusion during THVE is said to protect the viability of organs by decreasing the temperature of the tissue [1]. Given the minimum blood loss we observed during in situ splitting, it appears that cold perfusion during in situ splitting can inhibit backflow from the LHV, which may induce a protective effect in terms of graft viability. This technique seems especially feasible when dividing the lateral and medial segments of the right lobe, where there is likely to be massive bleeding from the split surface. In pigs, however, two or three right lateral branches arise directly from the main trunk of the PV, making it technically difficult to perfuse the right lobe selectively. Nonetheless, cold perfusion may be useful for clinical in situ SLT.

We previously found that small-sized liver grafts may have a deleterious effect on graft viability after implantation, but that this may be helped by IIPS [3]. These findings led to the development of the technique of triple SLT, which showed that the medial right lobe of the liver can be successfully drained by the MHV alone [2]. This latest study has demonstrated the positive effect of in situ splitting with coldk perfusion in triple SLT. Further investigations will be required to determine whether such a technique might be feasible for other organs in the setting of clinical transplantation.

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