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Hepatic arterial reconstruction for orthotopic liver transplantation in the rat

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Abstract

Background—Orthotopic liver transplantation (OLT) models in rats have been investigated in many studies. The reconstruction of hepatic artery is required for reliable OLT and also requires advanced skills.

Methods—The hepatic artery reconstructions by a hand-suture technique and a new method using a micro T-tube were investigated in rats with a whole-liver syngeneic graft. Operative time and postoperative patency were compared between the hand-suture and micro T-tube techniques.

Results—Our technique using the micro T-tube shortened the operative time of recipient surgery compared with the hand-suture technique and prolonged the operative time for the donor. The patency ratio was maintained at 24 h after OLT with hand suturing but was significantly reduced with the micro T-tube, which had a patency ratio of 0.83 only up to 6 h after OLT.

Conclusion—The micro T-tube technique may have potential usefulness in the rat OLT model but requires further modification.

Keywords

Hepatic artery; Liver transplantation; Microsurgery; Surgical instruments

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Authorship: T. Hori performed the surgery and the assays and wrote this article with statistical analysis. L.B. Gardner performed the assays. F. Chen and A.M.T. Baine helped the assays. T. Hata helped the surgery. C.B. Eckman and A.R. Herdt supported this research. J.H. Nguyen and S. Uemoto supervised this research. Note that J.H. Nguyen formulated an original method by micro T-tube.

1. Introduction

The surgical technique for orthotopic liver transplantation (OLT) in a rat model was first reported using hand-suture methods in 1973 [1]. A second study in 1975 described a modified rat OLT model without reconstruction of the hepatic artery (HA) and a temporal shunt with porto-jugular veno-venous bypass [2]. However, because these models required advanced surgical skills, they were not widely adopted. The cuff method for venous reconstruction was introduced in 1973 [3], and thereafter more modified models of rat OLT without HA reconstruction became the standard for research in OLT [4,5].

Although the detailed effects of HA reconstruction on rodent OLT are still controversial, several researchers have suggested that transplanted grafts with HA reconstruction resulted in an improved function after OLT compared with grafts without HA reconstruction [6–10]. However, HA reconstruction is technically difficult and requires advanced skills [6–9,11,12]. End-to-end anastomosis of HA is the ideal method, but this requires ultramicrosurgery, including reconstruction of vessels or nerves with a diameter <0.5 mm in rodents [11–13]. HA reconstruction is very difficult in rats and may be impossible or unreliable in mice [11,12]. To simplify the procedures of HA reconstruction, many researchers have focused on modification of the technical aspect of microsurgery [4,6,11,12,14–20].

The aims of this study were to describe a novel surgical procedure for HA reconstruction using a micro T-tube and compare this method with the hand-suture method in rat OLT.

2. Materials and methods

2.1. Animals

Lewis rats (RT-1¹) were purchased from Harlan Laboratories, Inc (Indianapolis, IN). Graft donors and recipients were 8- to 10-week-old rats. The experimental protocols were approved by the Ethical Committee of our institution (Mayo Clinic, Institutional Animal Care and Use Committee, No. A19609). Rats were cared for according to the institutional guidelines for Animal Welfare.

2.2. Micro T-tube

A soft polyurethane tube (Polyurethane Catheters, Straight Tip, Hydrocoat, 3 French, 20 gauge, BC-3P, Access Technologies, Skokie, IL) was used as the micro T-tube. The inner diameter was 0.6 mm, and the outer diameter was 0.9 mm. The inner surface was hydrocoated to prevent platelet adhesion and thrombotic obstruction, to enhance the compliance and long-term tolerance and minimize bacterial aggregation. Extensions of the T-tube in each direction were all 3 mm in length with beveled ends (Fig. 1A).

2.3. Surgical procedures for OLT

Comprehensive details of the surgical procedures for rat OLT in our institution have previously been described [11,12]. In brief, the suprahepatic and infrahepatic inferior vena cavae were reconstructed by the hand-suture or cuff method, although only the cuff method was used in portal venous reconstruction to shorten the anhepatic phase [12]. The common biliary duct was reconstructed using a stent tube. A surgical microscope of ×5–20 magnification (Surgical Scope M680, Type 10445496, Leica Microsystems Inc, IL) was used. End-to-end anastomosis of the HA was performed under ×20 magnification. The sleeve technique was documented as an innovative technique for HA reconstruction [4,15,17], but we often found that the graft common HA and recipient's proper HA had similar diameters. Thus, the common and proper HAs were skeletonized from the portal

venous trunk, then the donor and recipient common HAs were anastomosed in an end-to-end fashion using the hand-suture technique. Interrupted sutures were made with monofilament nylon suture (10-0 Ethilon, BV130-3, 2820G, Ethicon Inc, Johnson & Johnson Company, NJ).

In this study, the whole-liver graft had a cold ischemic time of 2 h at 4°C in normal Ringer's preservation solution. In rat OLT, we previously demonstrated the importance of a short anhepatic phase and the exclusion of unreliable samples based on autopsy and histopathologic findings [11,12]. In this study, the anhepatic phase was kept within 20 min in each surgery and autopsy findings showing no critical complications and reasonable histopathologic findings were confirmed in each case.

2.4. Surgical procedures for HA reconstruction by the micro T-tube technique

In the Lewis rat, the left renal vein, right renal vein, and inferior mesenteric vein flow into the infrahepatic inferior vena cava. The aorta branches the celiac, right renal, left renal, superior mesenteric, and inferior mesenteric arteries until the bifurcation to common iliac artery (Fig. 1B). Note that both the aorta and the celiac artery branch the superior mesenteric artery. Several lumbar arteries are detected behind the aorta. The common HA is supplied from the celiac artery, and the common HA is divided into the proper HA and gastroduodenal artery at the left side of the portal vein. The aorta is strongly fixed to retroperitoneal tissues at the proximal level of the left renal vein, and the presence of some lumbar arteries may compromise safe procedures. Similarly, dissection of the aorta at the distal level under the inferior mesenteric vein is anatomically difficult because the aorta is placed on the infrahepatic inferior vena cava and strictly adheres to the wall of the infrahepatic inferior vena cava at this level.

In the donor operation, the aorta from the proximal side of the celiac artery to the common iliac artery was completely skeletonized (Fig. 1C). The inferior mesenteric, left renal, right renal, superior mesenteric, gastroduodenal arteries, and several lumbar arteries were all ligated using silk 7-0 sutures (Silk Suture 7-0, Braintree Scientific Inc, MA). The common and proper HAs were dissected after ligation of the gastroduodenal artery. The harvested graft was accompanied by an adequate length of aorta graft cutoff at the bifurcation (Fig. 1D). The proximal of the celiac artery was ligated using silk 7-0 suture, and complete closure of any branches was confirmed by injection of cold Ringer's solution at the bifurcation.

In the recipient operation, the aorta from the proximal level under the left renal vein to the distal level of the inferior mesenteric vein was skeletonized and the inferior mesenteric artery was isolated (Fig. 2A). The lumbar artery in this area was ligated, though ligation of the inferior mesenteric artery was not required. Two silk 7-0 sutures were set behind the skeletonized aorta beforehand, and two microclamps (Micro Vascular Clip, diameter 1 mm, 10-15 g, length 8 mm, RS-6470, Roboz Surgical Instrument Co, Inc, Gaithersburg, MD) were placed on the proximal and distal points of the aorta ($\times 5$ magnification). Thereafter, the aorta was opened widely in a cutout rather than a cutoff fashion (Fig. 2B). The micro T-tube was inserted into the aorta and was fixed to the aorta by ligation with silk 7-0 sutures (Fig. 2C). The graft aorta was fixed to the micro T-tube using silk 7-0 suture ligation (Fig. 2B), and the microclamps were released (Fig. 2D). Pulsation was confirmed in the graft aorta.

2.5. Comparison of operative time and patency between HA reconstruction by hand-suture and micro T-tube techniques

Operative times required for HA reconstruction with each technique were measured in 10 donor and recipient operations. The donor operation time included all procedures for artery

and aorta harvesting, and the recipient operation time included animal preparation before HA reconstruction and all procedures for HA reconstruction.

We previously reported that HA reconstruction in rats is quite difficult without any bleeding, thrombosis, and obstruction, even under ultramicrosurgery [11,12]. Successful hand suturing guarantees long-term patency of the anastomosed HA, but a coating with a foreign body in the micro T-tube may result in a low patency ratio in the early postoperative period. Thus we determined the patency ratio of the HAs reconstructed by the micro T-tube technique at 6, 12, and 24 h after OLT in 12 cases at each time point. Postoperative HA flow was confirmed by arterial flow at the distal of anastomosis.

2.6. Immunohistologic assessments after OLTs with HA reconstruction by both techniques

In each technique ($n = 3$), immunohistologic parameters were assessed at 0, 6, 12, and 24 h after OLT. Liver tissue was fixed in 10% neutral-buffered formalin (Protocol, 032-059, Fisher Scientific, Inc, Kalamazoo, MI), embedded in paraffin, and sliced into 4- μ m sections. Induction of apoptosis was measured by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) (ApopTag Peroxidase *In Situ* Apoptosis Detection Kit, S7100, Chemicon International, Inc, Billerica, MA). A TUNEL-positive nucleus was stained brown, and a negative nucleus was stained light blue. Slides were scanned with an automated high-throughput scanning system (Scanscope XT, Aperio Technologies, Inc, Vista, CA). To quantify the histologic findings, positive-stained nuclei were counted by Aperio Image-scope software (Aperio Technologies, Inc). All nuclei were classified into four color intensity levels, and the higher two levels were considered as positive. The ratio of positive-stained nuclei to all nuclei was calculated, and the mean ratio per square millimeter was determined.

2.7. Statistical analysis

The results were presented as mean $\pm$ standard deviation. The Student t -test and χ^2 test were used for the comparison of unpaired continuous or discontinuous variables between groups. Statistical calculations were performed using SPSS Software Version 16.0 (SPSS Inc, Chicago, IL). A P value <0.05 was considered statistically significant.

3. Results

3.1. Operative time for HA reconstruction with each technique

Operative times for HA reconstruction according to the two techniques are shown in Figure 3A. In the donor operation, the operative time for the micro T-tube technique (26.8 ± 7.1 min) was prolonged compared with that for the hand-suture technique (7.8 ± 2.2 min) ($P = 0.0001$). By contrast, the recipient operation with the micro T-tube technique (17.0 ± 2.5 min) was significantly shorter compared with that using the hand-suture technique (38.5 ± 4.7 min) ($P = 0.0001$).

3.2. Postoperative patency of reconstructed HA by each technique

The patency ratios of the reconstructed HA for each technique at 6, 12, and 24 h after OLT are shown in Figure 3B. The hand-suture technique seemed to guarantee a high patency ratio at each time point (0.92, 0.83, and 0.92, respectively), but this ratio decreased over time in OLT with the micro T-tube technique (0.83, 0.50, and 0.17, respectively). There was a significant difference in the patency ratio at 24 h after OLT between the hand-suture and micro T-tube techniques (11/12 *versus* 2/12, $P = 0.0001$), though there were no significant differences at 6 h ($P = 0.5371$) and 12 h ($P = 0.0926$).

3.3. Apoptotic induction in the graft after OLTs by each technique

Apoptotic induction was confirmed from the early postoperative period by TUNEL in each technique, and typical immunohistologic findings for TUNEL are shown in Figure 4. The HA reconstruction by T-tube technique showed a high ratio of TUNEL-positive cells compared with HA reconstruction by hand-suture technique, not at 0 h (0.001 ± 0.001 versus 0.000 ± 0.000 , $P = 0.3739$), 6 h (0.010 ± 0.005 versus 0.009 ± 0.002 , $P = 0.7749$), and 12 h (0.011 ± 0.003 versus 0.013 ± 0.005 , $P = 0.6291$) but at 24 h (0.013 ± 0.002 versus 0.022 ± 0.005 , $P = 0.0383$).

4. Discussion

Onset of graft injury related to reperfusion occurs at least several hours after portal reflow [21–23]. Patchy and/or focal necrosis is a key histopathologic finding in shear stress and reperfusion injury resulting from reduced liver volume and graft recirculation after cold ischemia, and this finding also is confirmed several hours after surgery [24,25]. Paradoxically, many researchers actually ignored the effects of HA flow in their studies using samples within 24 h after OLT [26,27]. The OLT with HA reconstruction is more physiological than OLT without HA reconstruction [15,28]. Because HA reconstruction is indispensable for reliable studies of liver regeneration, HA reconstruction by suturing has already been established, but it does involve technical difficulties [6–9,11,12]. Therefore, technical modifications that simplify the process of HA reconstruction have also been documented [4,6,14–20,28]. Although Kashfi *et al.* [4] reported faster surgical techniques for short-term survival studies and Post *et al.* [29] documented that HA reconstruction is not necessary for study of leukocyte–endothelial interaction after rat OLT, we suggest that a good physiological model with a simple but sure technique is currently needed even in short-term studies of reperfusion injury.

Although a technique using a simple tube for reconstruction of the common HA in rat OLT was reported [6], the use of a micro T-tube in rat OLT is still not established. Gregorius and Rand [30] suggested in 1976 the usefulness of a micro T-tube (silicone, manufactured by Dow Corning Co, Midland, MI) for arterial bypass via the common carotid artery in the rat. Surprisingly, however, there were no technical reports using a micro T-tube in rat OLT after this initial proposal [30], in spite of developments in antithrombosis coatings inside micro-tubes, which ensure tube patency [31,32]. If the micro T-tube can be applied in rodent OLT, HA reconstruction of a larger-sized artery, such as aorta–aorta reconstruction, appears to become possible. Thus, we hypothesized that the use of a micro T-tube may be a solution for OLT models accompanied by surgical difficulties. However, the unexpected lower patency with the micro T-tube technique at 24 h after OLT disappointed us, although we had been concerned that even a coated foreign substance might result in a low patency ratio in the early postoperative period. We strongly believe that drastic improvements and innovations are still required in our current procedures for long-term patency after OLT.

Our micro T-tube technique required an increased time to harvest the donor aorta. To compensate for this time, we already performed dissection of the aorta at the proximal level of the left renal vein in the recipient because the required length of the graft aorta can be shortened by HA reconstruction at a proximal level of recipient aorta. However, a careless retention for surgical field cannot be made after the reconstruction of other vessels because even a subtle retention will cause the anastomosis rupture. Overall, we recommend that the recipient's aorta at the level from the point under the left renal vein to the point on the inferior mesenteric vein is suitable for easy skeletonization as it is fixed more loosely in this location. The inferior mesenteric artery has no backflow, and an additional clamp on the inferior mesenteric artery is not necessary. The inferior mesenteric artery should be preserved.

The frequency of surgical issues, such as postoperative bleeding, outflow block, and anastomosis twist, was reported as approximately 10%–40% in rat OLT models, including split OLT by hand-suture method [5,11,12,33]. Many researchers recognized the surgical difficulties of this model. The OLT model with HA reconstruction is an ideal, but we also understood that the hand-suture method under ultramicrosurgery for end-to-end anastomosis of the common HAs is always very stressful, even for experienced microsurgeons. One recommended point of our new technique is its simplicity without stressful procedures in the recipient operation, although the donor operation is prolonged. Based on our experiences, HA reconstruction by micro T-tube may have an advantage in reducing stress during the recipient operation, although only at 6 h after OLT was the patency acceptable in the present study. Researchers can choose different techniques according to the defined aims of their study [4]. In this study, we considered that our novel technique by micro T-tube may be useful only in research involving early sampling. In a word, the utility of our current technique is very limited. We recognize that our technique by micro T-tube should be improved, especially regarding long-term patency. Possible resolutions for patency improvement may be the usage of drug-eluting stents or larger caliber stents [34,35], and we now consider systemic heparinization of recipients at perioperative period as a strategy to avoid the early thrombosis. We hope that this study will be informative for innovative developments in the future. The HA reconstruction is important in the rat OLT model and can provide reliable and relevant data in the liver transplantation field.

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Fig. 1.

Micro T-tube, donor operation, and back table procedure for HA reconstruction by micro T-tube technique. (A) Micro T-tube: a polyurethane micro T-tube, inner diameter 0.6 mm and outer diameter 0.9 mm, was used. The inner surface was hydrocoated. Extensions in each direction were all 3 mm with a beveled end. (B) Anatomy: left renal, right renal, and inferior mesenteric veins flow into the infrahepatic inferior vena cava. The aorta branches into celiac, superior mesenteric, right renal, left renal, and inferior mesenteric arteries until the bifurcation. Several lumbar arteries are shown behind the aorta. The common hepatic artery divides into the proper hepatic and gastroduodenal arteries. (C) Donor operation for the micro T-tube technique: the aorta from the upper point of the celiac artery to the common iliac artery was completely skeletonized in the donor operation. (D) Back table procedures for the micro T-tube technique: the harvested graft is accompanied by an adequate length of the aorta. Complete closure of branches was confirmed by injection of cold Ringer's solution from the bifurcation.

Fig. 2.

HA reconstruction by the micro T-tube technique. (A) Selected skeletonization around the aorta: the recipient's aorta from the level under the left renal vein to the level of the inferior mesenteric vein was skeletonized. (B) Insertion and fixation of the micro T-tube: two thin silk threads were set behind the skeletonized aorta beforehand and two microclamps were placed on the proximal and distal points of the recipient's aorta. Thereafter, the aorta was widely opened in cutout rather than cutoff fashion. (C) Fixation of the micro T-tube to the aorta: the micro T-tube was inserted into aorta and was fixed to the aorta by ligation with thin silk threads. (D) Fixation of the graft aorta to the micro T-tube: all reconstructions are shown. The graft aorta was fixed to the micro T-tube by thin silk thread ligation, and the microclamps were then released.

Fig. 3. Differences in operative time for HA reconstruction and the rate of postoperative patency between hand-suture and micro T-tube techniques. (A) Operative time for HA reconstruction by each technique. In the donor operation, the operative time for the micro T-tube technique was prolonged compared with that of the hand-suture technique. By contrast, the recipient operation with the micro T-tube technique had a shortened operative time compared with that of the hand-suture technique. (B) Postoperative patency of reconstructed HA by each technique. The hand-suture technique seemed to guarantee a high patency ratio at each time point but the ratio decreased with time after surgery with the micro T-tube technique. There was a significant difference in the patency ratio at 24 h after OLT between the two techniques.

Fig. 4. Immunohistologic findings for TUNEL after OLTs with HA reconstruction. HA reconstruction by hand-suture technique (A) and T-tube technique (B). TUNEL-positive nuclei are stained brown, and normal nuclei are stained light blue ($\times 50$).