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New method of stent-facilitated arterial reconstruction for orthotopic mouse liver transplantation

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ABSTRACT

Background: Arterialized orthotopic liver transplantation (OLT) in the mouse mimics human liver transplantation physiologically and clinically. The present method of sutured anastomosis for reconstruction of the hepatic artery is complex and is associated with high incidence of complications and failure. This makes the endpoint assessment of using this complex model difficult because of the many variables of the technical aspect.

Methods: A total of 14 pairs of donors and recipients from syngeneic male mice were used for arterialized OLT. The grafts were stored in University of Wisconsin solution at 4°C for less than 4 h, and the recipients underwent OLT using a two-cuff technique. The arterial reconstruction was facilitated by the use of a single stent connecting the donor liver artery segment to the recipient common hepatic artery.

Results: All 14 recipients survived with the time for arterial reconstruction ranging from 4–10 min. Patency of the artery was confirmed by transecting the artery near the graft 2 and 14 d after transplantation. At day 2, five of the six arteries transected were patent and at day 14, seven of the remaining eight were patent for an overall patency rate of 85.7%.

Conclusions: The stent-facilitated arterial reconstruction can be done quickly with a high patency rate. This model expands the translational research efforts to address marginal livers such as steatotic livers.

1. Introduction

The liver has a dual blood supply with the hepatic artery and portal vein (PV) supplying approximately 25% and 75% of liver blood flow, respectively. Importantly, the hepatic artery flow accounts for approximately 50% of the oxygen delivery to the liver [1]. The arterialized mouse orthotopic liver transplantation (OLT) model better mimics human OLT compared with a nonarterialized graft, and clinicians and researchers therefore favor it. In clinical transplantation, the arterial reconstruction is mandatory and early hepatic artery thrombosis leads to graft failure and death unless the patient can be quickly retransplanted. Because Qian *et al.* developed the nonarterialized model of OLT in 1991, it has been used

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frequently in the study of immunologic rejection, transplant tolerance, and novel medication development [2]. This model does not include arterial reconstruction and does not compromise long-term mouse survival [2]. Some researchers have challenged the relevance of nonarterialized grafts as a model of human transplantation [3]. Steger *et al.* concluded that the effects of arterialization were negligible [4]. Conversely, Tian *et al.* demonstrated that arterialization was critical for long-term survival [3]. For grafts with prolonged cold preservation time (≥16 h), arterialization improves long-term survival [3]. In addition, its relevance is critical in marginal fatty liver transplants. Because of the technical difficulties associated with the smaller size of mice, fewer modifications of the arterialized OLT in the mouse have been developed compared with arterialized OLT in the rat [4–6]. In 2002, Tian *et al.* introduced an end-to-side suture anastomosis between the donor superior mesenteric artery and the recipient abdominal aorta. This procedure is complicated, time consuming, and results in a longer arterial segment that is more prone to kinking and subsequent thrombosis [3]. Furthermore, this adds a level of difficulty that makes it hard to reproduce.

The purpose of this report is to present a new method that simplifies the reconstruction of the hepatic artery, shortens both donor and recipient surgery times, and is less technically difficult than previously described methods. This method would facilitate the adaptation of this complex model for ischemia–reperfusion and immunologic investigations of the stressed liver in a murine model.

2. Materials and methods

2.1. Animals

Male inbred C57BL/6 mice weighing between 23 and 30 g purchased from the Jackson Laboratory (Bar Harbor, Maine) were used as donors and recipients. All animal experiments were reviewed and approved by the Medical University of South Carolina Institutional Animal Care and Use Committee, and all experimental animals were treated in accordance with the guidelines described in Public Health Service Policy on Humane Care and Use of Laboratory Animals by the Awardee Institutions (OLAW NIH, September, 1986) and the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996). Mice were housed under standard conditions with a 12-h dark–light cycle and free access to water and food.

2.2. Surgical procedure

All surgical procedures were performed under aseptic conditions by a single microsurgeon with the aid of 6–40 × magnification microscope (Wild Heerbrugg, Switzerland). Isoflurane was administered as a general inhalation anesthetic in all cases.

2.3. Donor surgical procedure

The abdomen of the donor animal was shaved and disinfected with betadine. The abdominal cavity was entered through a transverse subxiphoid incision, the falciform ligament was electrocauterized and transected with the bipolar coagulator. The xiphoid process was held up cephalad with a mosquito clamp to provide exposure, the liver was covered with saline-soaked gauze, and the hollow viscera were retracted to the left of the peritoneal cavity and covered with saline-soaked gauze. A cholecystectomy was performed sharply. The bile duct was dissected off the PV and cannulated with 4-mm Peek TM Tubing (outer diameter, 0.37 mm; inner diameter, 0.15 mm; Upchurch Scientific, Oak Harbor, WA), and secured with 8-0 silk suture tie before being divided, preserving approximately three-fourths of the bile duct. The PV was skeletonized to the level of the superior mesenteric vein by ligation of the pyloric vein and splenic vein with 8-0 silk sutures. Careful dissection was performed to expose the infrahepatic vena cava and the renal vessels. The right renal vein was ligated with 10-0 silk suture. The right adrenal vein was cauterized and transected. Hemostasis of the lumbar veins was achieved using electrocautery as necessary. The dissection of the inferior vena cava (IVC) was also completed to the level of the left renal vein. The stomach and esophagus were dissected free of the liver by dividing all attached ligaments. The abdominal aorta below the level of the left renal vein was exposed and occluded with a microclamp above the celiac trunk. The more distal aorta was pierced below the left renal artery with a needle syringe (30.5G; Becton Dickinson Inc, Sparks, MD) for retrograde flushing of the liver with 5 mL of Ringer solution at 4°C. A transverse incision was made with scissors on the anterior wall of the PV, through which a 24-gauge catheter was inserted to slowly perfuse the liver with 2–3 mL of cold University of Wisconsin solution (Viaspan; Bristol-Myers Squibb, New York, NY). The celiac trunk and common hepatic artery were carefully dissected and the splenic and gastroduodenal arteries were cauterized. A 3-mm stent was inserted into the celiac trunk and secured with 10-0 silk suture tie. The splenic and left gastric branches were tied off with 10-0 silk, leaving flow directed toward the proper hepatic artery (Fig. 1). The suprahepatic IVC was transected at the level of the diaphragm, and the infrahepatic IVC was cut at the level of the left renal veins. The PV was divided below the level of the splenic vein, and the liver was then carefully dissected from the peritoneal cavity and immersed in cold University of Wisconsin (UW) solution for cuff preparation.

2.4. Cuff preparation

The two-cuff technique was used as previously described by Kamada and Calne [7]. Polyethylene tubes (Becton Dickinson Inc) were cut to 2.5–3 mm in length for both the PV cuff (20G Autoguard shielded IV catheters; Becton Dickinson) and the IVC cuff (outer diameter, 1.70 mm; inner diameter, 1.19 mm) and secured with 8-0 silk suture.

2.5. Recipient surgical procedure

Anesthesia, laparotomy, and exposure were performed as in the donor surgical procedure. Counterclockwise dissection was performed and the stomach and esophagus were dissected free of the lobes of the liver by dividing all ligaments with electrocautery. The suprahepatic IVC was isolated and