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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 current 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 utilizing this complex model difficult due to the many variables of the technical aspect.

Methods and Results—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 hours 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. All 14 recipients survived with the time for arterial reconstruction ranging from 4–10 minutes. Patency of the artery was confirmed by transecting the artery near the graft 2 days and 14 days after transplantation. At day 2, 5 of the 6 arteries transected were patent and at day 14, 7 of the remaining 8 were patent for an overall patency rate of 85.7%.

Conclusion—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.

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 liver¹. The arterialized mouse OLT model better mimics human OLT as compared with a non-arterialized 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. Since Qian *et al.* developed the non-arterialized model of OLT in 1991, it has been used frequently in the study of immunological rejection, transplant tolerance and novel medication development². This model does not include arterial reconstruction but does not compromise long term mouse

survival². Some researchers have challenged the relevance of non-arterialized grafts as a model of human transplantation³. Steger *et al.* concluded that the effects of arterialization were negligible⁴. Conversely, Tian *et al.* demonstrated that arterialization was critical for long-term survival³. For grafts with prolonged cold preservation time (> 16hr), arterialization improves long-term survival³. In addition, its relevance is critical in marginal fatty liver transplants. Due to the technical difficulties associated with the smaller size of mice, fewer modifications of the arterialized OLT in the mouse have been developed as compared to arterialized OLT in the rat⁴⁻⁶. 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 thrombosis³. 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 adaption of this complex model for ischemia/reperfusion and immunologic investigations of the stressed liver in a murine model.

Materials and methods

Animals

Male inbred C57BL/6 mice weighing between 23 and 30 grams 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 12 hour dark-light cycle and free access to water and food.

Surgical procedure

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

Donor Surgical Procedure

The abdomen of the donor animal was shaved, and disinfected with betadine. The abdominal cavity was entered through a transverse sub-xiphoid 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 out of the peritoneal cavity to the left and covered with saline-soaked gauze. A cholecystectomy was performed sharply. The bile duct was dissected off of the portal vein (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 portal vein 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.) for retrograde flushing the liver with 5 mL 4 °C Ringers solution. 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 towards the proper hepatic artery (Figure 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 portal vein was divided below the level of the splenic vein and the liver was then carefully dissected from the peritoneal cavity and immersed in cold UW solution for cuff preparation.

Cuff preparation

The two-cuff technique was used as previously described by Kamada⁷. Polyethylene tubes (Becton Dickson, Sparks, MD) were cut to 2.5–3 mm in length for both the PV cuff (20G Autoguard shielded iv catheters, BD Inc) and the IVC cuff (outer diameter 1.70 mm, inner diameter 1.19 mm) and secured with 8-0 silk suture.

Recipient Surgical Procedure

Anesthesia, laparotomy and exposure were performed as in the donorsurgical procedure. Counter-clockwise dissection was performed and the stomach and esophagus were dissected free of the lobes of the liver by dividing all ligaments with electrocautery. The supra-hepatic inferior vena cava was isolated and encircled with a 4-0 silk suture for retraction of the liver, the right adrenal vein was cauterized, the infrahepatic IVC was exposed to the level of the right renal vein, and the hepatic artery was cauterized.

The cold stored donor liver was flushed with 2–3 mL of Ringer's solution at 4°C via the PV cuff. The recipient PV and infrahepatic IVC were cross-clamped with micro-vascular clamps, and isoflurane anesthesia was immediately discontinued. The native liver was retracted inferiorly and a small clamp was placed on the diaphragm near the level of the atrium to control the supra-hepatic IVC. The PV and the infra-hepatic IVC were sequentially transected with scissors and the native liver was removed. The donor liver was then placed orthotopically in the recipient's abdominal cavity. The suprahepatic IVC was anastomosed with continuous 10-0 nylon suture using a one-suture anastomosis technique. The PV was anastomosed using the cuff technique by positioning the recipient PV over the cuff previously placed on the donor PV and securing it with a circumferential 8-0 silk suture. When PV anastomosis was completed, the cross-clamps on the PV and the suprahepatic inferior vena cava were released. Anhepatic time averaged 17 ± 2.5 minutes. The infra-hepatic IVC was then re-anastomosed in a similar fashion using the cuff technique. Ligation of the gastroduodenal artery and the hepatic artery was accomplished with a single suture. An oblique opening was created on the anterior wall of the common hepatic artery with micro-scissors through which the stent connecting the arterial segment was inserted and secured with 10-0 silk suture (Figures 1, 2A, and 2B) fixed from both ends. Reconstruction of the artery took 4–6 minutes. The biliary anastomosis was completed last by securing the recipient bile duct over the stent with 8-0 silk suture, fixed from both ends. The abdomen was irrigated and the viscera replaced. The abdominal incision was closed with running 5-0

nylon suture in two layers, and the animals were placed under a warming lamp with free access to soft and dry chow.

Results

Dissection and division of the arterial segment took less than 5 minutes in the donors. In about 67% of the cases, the proper hepatic artery arises (or/and accessory left hepatic artery) from the left gastric artery or from both the common hepatic artery and the left gastric artery, so the left gastric artery was kept for full hepatic artery segment in these cases. Rarely, the proper hepatic artery arises from esophageal artery, which encircles the esophagus, and these mice were excluded from the experiments. For this arterialized model, the time for dissecting arterial segment of donors is 2–3 minutes (Figure 3). Successful rearterialization was done in 4–7 minutes in almost all transplantations (10 minutes in one case) and confirmed by arterial pulse test during surgery (Figure 2C).

Two time points (2 days and 14 days after transplantation) were selected to evaluate the patency of the reconstructed artery. The prior incision was used to enter the abdominal cavity, and careful dissection was made to identify the hepatic artery and its branches. Near the graft, the artery was transected to evaluate for the presence of blood flow. This procedure converted the transplant to a non-arterialized model since the artery was transected to determine patency. Hepatic artery occlusion occurred in 1 of 6 cases at day 2 and in 1 of 8 cases on day 14 after surgery. Bile duct integrity assessed at this time showed lower occurrence of duct necrosis and leaks in the rearterialized model (Table 1). On day 14 and 30 after implantation, liver samples were taken and placed in 10% neutral formalin, embedded with paraffin, sectioned at 4 μ m, and stained with hematoxylin-eosin (Figure 4).

Discussion

The liver is central to a tremendous number of physiologic processes and as such has rightly become the focus of intensive scientific research. Research into liver transplantation required the development of a suitable animal model. Both rat and mouse models of liver transplantation have been successfully developed. The rat model has the advantage of greater technical ease while the mouse model, although technically more challenging, offers the advantages of low cost and the availability of genetically altered mice. Due to technical difficulty and poor patency rates, the mouse model has most often not included rearterialization of the liver graft. As an adequate supply of oxygen is critical for normal liver metabolism and regeneration⁸ the mouse model has been criticized as not accurately reflecting the physiology of human liver transplantation. Additionally, it has been demonstrated that transplanting steatotic livers in mice, in the absence of rearterialization, results in a prohibitive rate of primary non-function⁹. Although the previously described models of OLT in the mouse are well established, the model described here better mimics OLT in humans. The fundamental improvement is the successful rearterialization of the graft. There are several reasons for our relative success. First, the stent is micro-renalthane implantation tubing, which is the most compatible with blood and has been used extensively in thoracic surgery. Secondly, the blood pressure of the mouse is 100/60 mmHg⁸, the high blood flow efficiently hampers the formation of thrombus. In this study, one case of artery occlusion occurred 2 days after OLT. Focal pressure to obtain hemostasis in case of bleeding at the site of the liver-duodenal ligament will lead to kink of the hepatic artery and possible early thrombosis. Another case with occluded hepatic artery on 14 days after OLT was due to infection and is confirmed by autopsy. The patency rate is 85.7% compared to 100% reported in the rat model¹⁰.

We modeled this method to reconstruct the hepatic graft artery with a stent in the mouse after a technique previously described in the rat. This method of utilizing a stent in the arterial reconstruction has several advantages; it is simple and technically straightforward to insert a stent into the common hepatic artery, the anastomosis time is shorter than that of suture anastomosis¹⁰, it is unlike suture anastomosis where the back wall is difficult to suture, kinked or twisted for the longer artery segment that comprises superior mesenteric artery, aorta, celiac trunk, common hepatic artery and proper hepatic artery, blood loss and/or thrombosis that are commonly associated with suture anastomosis are not present. On the other hand, one apparent technical drawback of this model is the size of mice compared to rat and makes the procedure difficult to perform. Although utilizing this model will require the expertise of a microsurgeon, this method has a shorter learning curve that will allow a broader dissemination of the technique and thereby facilitate the use of this clinically important animal OLT model.

Stent-facilitated arterialized mouse OLT is a simpler, and more practical than other arterialized mouse OLT models, particularly in transplant models such as steatotic liver transplants involving ob/ob mice that would not tolerate the warm ischemia time associated with an “all-sewn” technique as described by Tian *et al.*¹¹ and would succumb to the reperfusion injury. Without arterialization however, this would remain a non-survivor group⁹. As such, out of necessity, the stent-facilitated arterialization model was established, proving to be suitable to our investigational aspects. The experiments presented are “proof of principle”, allowing other laboratories to produce survivor experiments, where otherwise not feasible.

Stent-facilitated arterialization for OLT should for now be limited for use in short-term follow up experiments investigating I/R injury in otherwise nonviable genetically modified mouse recipients postoperatively. Further long-term experiments, including 30-day and longer survival groups will demonstrate, whether survival and histologic changes are comparable to the established “all-sewn” vascular anastomosis model.

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Figure 1. Schematic of arterial reconstruction: (A) Ligation of both recipient hepatic proper artery and gastroduodenal artery with one knot. The common hepatic artery was cross-clamped, one 10-0 suture surgical knot was pre-placed and an oblique opening in anterior wall of the common hepatic artery was made. (B) A stent connecting donor celiac trunk artery segment was inserted into the recipient common hepatic artery, the knot was tightened to secure the stent.

Figure 2.

A) Reconnection of the artery with stent (arrow). B) Blood flow from the artery reconnection after de-clamping (arrow) and C) Evaluation of patency of the hepatic artery. The artery is transected near the graft and blood is accumulated. Transparent stent shows no evidence of clot.

Figure 3.
Time for the dissection of donor hepatic arterial segment and recipient artery reconnection.

Figure 4. Hematoxylin and Eosin staining of the hepatic grafts sample from arterialized OLT mouse 30 days after transplantation. (A) 100× magnification (B) 400× magnification, portal tract, arteriole (red circle), and bile ductulus showed normal histology of the liver acini.

Table 1

Complication of bile leakage for mouse orthotopic liver transplantation with and without the hepatic arterial connection.

Procedure	Incidence of Bile Leak		
	<30 days	30–100 days	Total
OLT without HA reconnection	35.8% (11/31)	25.8 % (8 /31)	61.4%(19/31)
OLT with HA reconnection	14.3%(3/21)	0	14.3% (3/21)3