Improved two-cuff technique for orthotopic liver transplantation in rat

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BACKGROUND: **The first orthotopic liver transplantation in rat (ROLT) was reported by Lee in 1973. Kamada innovatively applied cuff technique to ROLT in 1979. However, the operative procedures were highly demanding and the operative mortality was relatively high. The purpose of this study was to improve the model of ROLT, simplify operative procedures, and enhance the successful rate of operation.**

METHODS: **Orthotopic liver transplantation was performed in 160 Wistar rats by improved two-cuff technique. The portal vein between donor and recipient was anastomosed with the cuff technique. The same method was used to anastomose the infrahepatic vena cava. The suprahepatic vena cava and the hepatic artery were anastomosed by microvascular suturing and the bile duct was anastomosed end to end by a Teflon catheter.**

RESULTS: **The average time for donor operation, graft preparation and anhepatic phase was 31 minutes, 14 minutes and 13 minutes, respectively. The anastomosis time for the suprahepatic vena cava, portal vein, infrahepatic vena cava, hepatic artery and bile duct was 7 minutes, 2 minutes, 2 minutes, 8 minutes and 1 minute, respectively. The main causes for operative mortality were pneumothorax, anesthesia, air embolism and massive bleeding, and the successful rate of operation was 92.5%. The causes for death after operation were stoma bleeding, infection, biliary obstruction and graft failure.**

CONCLUSION: **The improved two-cuff technique can reduce operative mortality, enhance survival rate, and serve as an ideal method for the establishment of animal model of ROLT.**

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KEY WORDS: **rat; liver transplantation; animal, model**

Introduction

The first successful orthotopic liver transplantation in rat (ROLT) was reported by Lee et al in 1973.[1] But there have been only a few studies using this method owing to its difficulty in operation, especially microvascular suture. In 1979 Kamada applied the cuff technique to ROLT for the first time, and greatly reduced the operative mortality (10.3%).[2] Since the technique keeps the hepatic artery not anastomosed resulting in frequent bile duct complications, the experimental outcome is still unsatisfactory.

In this study we used microvascular suture to anastomose the hepatic artery with the assistance of magnifying glasses and the bile duct end to end by a Teflon catheter. It has been proven that this method can simplify the operative procedures, shorten the clamping time for the portal vein, and enhance the successful rate of operation. It is an ideal method for the establishment of animal model of ROLT.

Methods

One hundred and sixty male Wistar rats weighing 200 to 300 g each supplied by Chongqing Medical University, Chongqing, China were used as donors and recipients. They were fasted 12 hours before operation, but free access to water. The cuffs with two processes for the portal vein and infrahepatic vena cava (IHVC) were made of a polyethylene catheter of 1.6 mm and 2.5 mm inner diameter respectively.

Donor preparation

The abdomen of the rat was shaved, prepared with alcohol, and opened via a middle incision. At first, ligaments including the falciform ligament, triangular ligament and gastrohepatic ligament around the graft were cut. The IHVC was isolated at the level of the left renal vein, and the right adrenal vein was ligated and divided. The hepatic artery was dissociated at the portal area, and the pyloric vein and splenic vein were divided near the portal vein. After dissociation, the bile duct was incised

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on the anterior wall, and inserted into a small drainage Teflon catheter with an internal diameter of 0.6 mm, secured with a circumferential 5-0 silk ligature. After the donor's blood was heparinized, the liver was ready for perfusion and excision. The portal vein was incised at the level of the superior mesenteric vein after clamping, cannulated with a catheter at the proximal end, and infused with 10 ml ice cold Ringer's solution containing 100 units heparin under low pressure. Meanwhile, the IHVC was cut to let the perfusate escape. As soon as the liver became yellow, the diaphragm was pierced with scissors, and the suprahepatic vena cava (SHVC) was cut close to the diaphragm. The SHVC was then transected together with a small portion of the diaphragm and the liver was harvested. The graft was placed in Ringer's solution at 4 ℃, being ready for preparation.

Graft preparation

The graft was prepared in Ringer's solution at 4 ℃. The cuff was slipped over the portal vein by a microforceps, and the distal end of the vein was everted over the cuff and secured with a circumferential 5-0 silk ligature. The same was performed to finish the IHVC cuff preparation. Two 8-0 silk sutures were pierced via the two corners of the SHVC.

Recipient preparation

The ligaments around the liver were cut. The portal vein, hepatic artery and bile duct were divided at the hilum of the liver. After clamping the portal vein, the IHVC was cross-clamped at the level of the right renal vein and the SHVC including a portion of the diaphragm was clamped with a Satinsky's vascular clamp. The five vessels were then cut close to the liver, and the recipient's liver was removed.

Implantation of the graft

The donor liver was placed orthotopically in the recipient's abdominal cavity. The SHVC was anastomosed end to end using continuous 8-0 nylon suture. The cuff anastomosis of the portal vein was then commenced. After it was finished, the clamp on the portal vein and the SHVC was removed. As soon as the blood flow in the portal vein was reestablished, the liver resumed its normal color and produced bile. The IHVC was anastomosed in the same way as the portal vein. The clamp on the recipient's IHVC was subsequently removed. The hepatic artery was anastomosed end to end using 10-0 suture. After an incision was made on the anterior wall of the recipient's bile duct, a Teflon catheter, attached the donor bile duct, was inserted into the lumen of the recipient bile duct, secured with a circular ligature and covered with the great omentum.

Statistical analysis

All the values were expressed as means ± standard deviation. The data were analysed by statistical software SPSS9.0. A P value less than 0.05 was defined statistically significant.

Results

ROLT was performed in 160 rats by improved two-cuff technique. The average time for major operative procedures and the survival rate after operation are shown in Table 1 and the Figure respectively. The portal vein clamping time, operative mortality and one-week survival rate in this study were significant different from those reported by Lee[1] and Kamada[2] ($P<0.05$, Table 2).

Main factors and complications, which lead to death of the rat during and after operation, were analyzed.

Table 1. The main operative time in this study (min, means±SD)

Procedure	DO	GP	AP	SHVC	PV	IHVC	HA	BD
Time	31±0.7	14±0.4	13±0.3	7±0.2	2±0.1	2±0.1	9±0.1	1±0.1

DO: donor operation; GP: graft preparation; AP: anhepatic phase; SHVC: anastomosis time of the suprahepatic vena cava; PV: portal vein; IHVC: infrahepatic vena cava; HA: hepatic artery; and BD: bile duct.

Table 2. Comparison of anhepatic phase, operative mortality and 1-week survival rate between this study and traditional methods

Marker	This study (A)	Traditional methods	
		Lee's report (B)	Kamada's report (C)
Anhepatic phase (min)	13±2	25 *	16 *
Operative mortality	7.5%		10.3% △
One-week survival rate	90.0%	72.0% △	83.0% △

*: t test, compared with A, $P<0.05$; △: χ^2 test, compared with A, $P<0.05$.

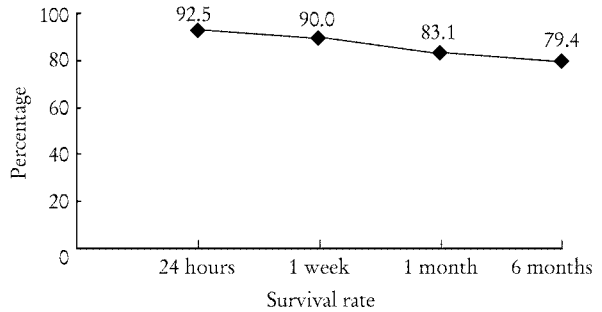

Fig. The survival rate after operation. Among the 160 rats studied, 12 rats died during operation or within 24 hours after operation (an operative successful rate of 92.5%), 144 rats survived for more than 1 week (a survival rate of 90.0%), 133 rats survived for more than 1 month (a survival rate of 83.1%), and 127 rats survived for more than 6 months (a survival rate of 79.4%).

Nine sorts of complications were encountered.

Five rats (3.1%) died intraoperatively from anesthesia accident (1 rat), pneumothorax (1), hemorrhagic shock (1), air embolism of the vena cava (1), and prolonged anhepatic phase (1).

Seven rats (4.4%) died from stoma bleeding of the suprahepatic vena cava (2 rats) and acute graft function failure (5) within 24 hours after surgery. The 5 rats with graft failure showed greyish livers with mottled black patches, which were markedly swollen or hard.

Infection, caused the death of 4 recipients (2.5%) during 1-4 weeks after operation, in which 1 died of pneumonia and 3 peritoneal sepsis.

Biliary obstruction led to the death of 5 rats (3.1%) 1 month later. Biliary sludge was formed with obstruction in 3 rats.

Discussion

Orthotopic liver transplantation in the rat is highly demanding, and the success of this procedure depends on adept skills of microsurgery.[1-3] To enhance the successful rate of the operation and prolong the survival rate, we have modified the traditional methods and put forward new operative techniques.

Cuff preparation

It is important to choose an optimal size of cuff because too long or too small a cuff might cause thrombosis.[4] To ensure blood and bile to flow smoothly, the inner diameter of the cuff should be big enough.[5] Making a circular mark around the outside wall of the cuff would benefit the fixation of the veins. In addition, to prevent biliary complications, the Teflon catheter for bile drainage should not be longer than 5 mm.[6]

Graft harvesting

Harvesting a high quality donor liver is a prerequisite for a successful operation.[2,7] Several factors should be emphasized in procurement of donor liver: First, cautions must be taken to avoid trauma to the liver and keep it moist throughout the procedure.[8,9] Dividing the ligament around the liver clockwise can minimize handling of the graft; Second, the length of the portal vein and the IHVC should be maximal for the preparation of the cuff;[1,10] Third, to avoid biliary obstruction, the upper end of the Teflon catheter inserted into the donor bile duct should be located under the confluence of the three hepatic ducts;[11] Fourth, during the perfusion, it is important to instill the perfusate under low pressure (2-3 ml/min) to avoid edema.[12] Putting broken ice or pouring cold solution around the liver is useful to reduce warm ischemic injury, which does great damage to the graft.[13-16]

Graft preparation

After the cuff was fixed to the two veins, the liver was perfused again via the portal vein to ensure the absence of twisting of the veins within the cuff.[17] Graft preparation was accomplished as quickly as possible to minimize cold ischemic injury and to shorten the time of preservation because these factors might cause great damage to the graft.[6,18-20]

Recipient operation

Several key points for this procedure must be pointed out because it is crucial to the whole operation.[5,21]

Anhepatic phase

Since occlusion of the portal blood flow for more than 26 minutes is lethal to the rat,[2,17] rapid anastomosis of the suprahepatic vena cava and the portal vein and quick reestablishment of the portal blood flow are critical to minimize warm ischemic damage to the liver and to the recipient.[22-24]

Microvascular suturing skills

Anastomosis of the SHVC and the hepatic artery is very demanding because it must be carried out expeditiously and effectively to ensure the suturing without tension to avoid stoma bleeding or stricture postoperatively.[11,25]

Anesthesia time

Ether inhalation should be removed as soon as the portal vein is clamped, because respiration is apt to cease suddenly during the anhepatic phase if the anesthesia is administered continuously.[17,26]

Cuff anastomosis techniques

The cuff method described here may shorten the total clamping time of the recipient portal vein to 13 minutes on average. Moreover, no leakage, stenosis or thrombosis may occur in the lumen of the reestablished veins. The shortened portal venous clamping time together with decreased anesthetic time probably lead to a higher one-week survival rate (90.0%) than the previous one.[1,2]

Autotransfusion

It is beneficial to transfuse 1 ml physiological saline solution via the left branch of the portal vein to the recipient, which can flush the blood stored in the hepatic sinusoid to the systemic circulation, namely autotransfusion.[9,27] This method is useful to increase blood volume, decrease the blood concentration, and prevent thrombosis postoperatively.[28]

Hepatic artery anastomosis

After reestablishing the hepatic artery flow, the graft blood supply is consistent with physiologic condi-

tion,[25,29] contributing to a higher long-term survival rate (83.1%) than the previous one (72.2%).[2]

Precaution of biliary complications

There are high incidences of bile duct necrosis, bile leakage, or ascending cholangitis when the donor bile duct is implanted into the duodenum.[1] In our study, we used a modified technique, by which the bile duct was anastomosed end to end via a Teflon catheter. We found that this method is effective to reduce these complications because the function of the sphincter of Oddi is preserved. We have not found any case of bile duct necrosis or bile leak though the possibility of sludge accumulation in the Teflon catheter still remains. We also feel that it is important to wrap the biliary anastomosis with the greater omentum to assist revascularization and also to prevent possible bile leakage.[2,12,30]

Overall, the technique we described simplifies orthotopic liver transplantation in the rat, resulting in low operative mortality, high one-week survival rate, and long-term survival with a good liver function. It seems to be suitable for establishment of animal model for study of physiology, immunological rejection, and preservation of the liver.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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