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Research Paper

Research of combined liver-kidney transplantation model in rats

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Abstract

Objective: To set up a simple and reliable rat model of combined liver-kidney transplantation. **Methods**: SD rats served as both donors and recipients. 4℃ sodium lactate Ringer's was infused from portal veins to donated livers, and from abdominal aorta to donated kidneys, respectively. Anastomosis of the portal vein and the inferior vena cava (IVC) inferior to the right kidney between the graft and the recipient was performed by a double cuff method, then the superior hepatic vena cava with suture. A patch of donated renal artery was anastomosed to the recipient abdominal aorta. The urethra and bile duct were reconstructed with a simple inside bracket. **Results**: Among 65 cases of combined liver-kidney transplantation, the success rate in the late 40 cases was 77.5%. The function of the grafted liver and kidney remained normal. **Conclusion**: This rat model of combined liver-kidney transplantation can be established in common laboratory conditions with high success rate and meet the needs of renal transplantation experiment.

Keywords: liver; kidney; transplantation; rat

INTRODUCTION

In recent years, remarkable effect has been achieved in the treatment of end-stage liver disease with end-stage renal disease by combined liver-kidney transplantation[1-10]. However, few basic experiment researches on the liver-kidney transplantation were reported, because of its complicated operation, low success rate, and difficulty to establish an animal model . In our experiment, we established a simple and reliable animal model by modifying some Chinese and foreign methods.

MATERIALS AND METHODS

Materials

Healthy male SD rats, weighed 250-360g, were selected (provided by experimental animal center of Nanjing Medical University) and raised in a warm, ventilated room, fed with ordinary food. Operating double binocular microscope was provided by Zhenjiang Medical Optic Equipment Factory. Microsurgery instruments were purchased from Shanghai Medical Instrument Group Company, Ltd. The suture (9-0 and 6-0) was provided by Shanghai Medical Suture Factory. Reagents such as chloramine alkone, heparin sodium, lactic acid ringer's solution, UW organ maintenance fluid, and antibiotic (penicillin) were needed.

Preparation of experimental animals

We offered 12h pre-operative food but not fluid fast. The belly skin was prepared, the surgical area was disinfected with iodophor and the operation was done in clean condition. The animal was anesthetized with 20% ketamine, 100mg per kg, by intraperitoneal injection.

Donor surgery

Make a cruciform incision in the abdomen. Push the intestine to the right side or wrap it with warm wet bandage and put it outside the body. Ligate the xiphoid with silk and draw it toward the head.

Dissection of the left kidney: make the left kidney, left ureter tract, aorta and vena cava fully exposed. Cut the fat tissue with sharp dissection in the

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middle of the left ureter tract, and dissociate it until to the cross of the iliac vein. Then cut open the left ureter tract, and insert a polyethylene catheter, fix it and cut off. Isolate, ligate and cut the left adrenal vein. Isolate the superior mesenteric artery, and the aorta and inferior vena cava that is inferior and superior to the renal artery. Dissect the beginning segment of left renal artery and vein clearly.

Dissection of donor liver: Dissociate the donor liver in approximately an anti-clockwise direction. Firstly, cut the falciform ligament, ligate the left phrenic vein with 6-0 suture and cut it. Then cut the left triangle ligament and hepatogastric ligament. Ligate and cut the communicating branches between veins of diaphragm and liver. Isolate the caudate lobe, cut the right triangle ligament and post-liver ligament. Ligate the right adrenal vein with 6-0 suture and cut it. Isolate the under-liver inferior vena cava above the left renal vein, ligate and cut off the right renal artery and vein. Finally separate the hepatoduodenal ligament, dissociate the extrahepatic bile duct about 0.5cm from hilar bifurcation. Make a small incision in its front wall and from through it insert a catheter into the donor's proximal bile duct. Ligate and fix with 6-0 thread, cut off the distal bile duct, isolate the hepatic artery and do not ligate for the time being. Double ligate the pyloric vein, splenic vein, and gastroduodenal vein where they converge into the portal vein and cut them. Isolate a 1-2cm segment of portal vein trunk. Puncture the penile dorsal vein or one lumbar vein, and inject 2-3ml saline containing 50U heparin to heparinize the donor.

In situ perfusion and cutting of donor liver and kidney: Make double perfusion through the abdominal aorta and portal vein. Ligate the aorta above celiac trunk and about 1cm from below renal pedicle, respectively. Insert the catheter from the distal abdominal aorta and portal vein, and perfuse consecutively with 4 ° C lactate Ringer's solution in situ with 60cm water column gravity. Cut the inferior vena cava below the left renal vein, until the irrigating solution became clear and the left kidney turned to a yellow-white color. Then stop the renal perfusion, prune the renal artery and vein on the aorta and inferior vena cava. Cut the left kidney and put it in a box that contained storage solution and was wrapped in ice. Ligate the hepatic vein, and stop the liver perfusion. Cut the above-liver inferior vena cava together with the diaphragm, and cut the portal vein and the under-liver inferior vena cava. Take out the liver and keep it in storage solution.

Modification of the donor liver and kidney

In the storage solution, cut the superfluous diaphragm around the above-liver inferior vena cava. Prepare the portal vein, the renal vein, and the under-kidney inferior vena cava in a cuff style. Block the under-liver inferior vena cava with microvascular nips.

Recipient surgery

(1)Anesthesia as the donor surgery. Cut abdominal median incision, distract abdominal wall and appendix ensiformis by silk suture, and protect intestine with saline gauze.

(2) Liver resection of the donor. Incise and ligate left vena phrenica, liberate ambi-liver ligament and caudate lobe; incise and ligate meta-hepatic esophageal vascular support. Ligate the right adrenal vein, and liberate inferior vena cava to the vena renalis dextra level. Cut the confluence of common bile duct, incise and ligate the hepatic artery and liberate portal vein to the left and right branch. Temporarily occlude the inferior vena cava in the site of right renal vein, and ligate each branch of the portal vein. Put downward traction of liver, superior vena cava on the liver and part of diaphragmatic muscle were severed by using Satinsky clamping. Pay attention to maintaining the integrity of the diaphragm ring. Incise the inferior vena cava and hepatic portal vein close to the liver and then remove the liver.

(3) Liver transplantation. The donor liver was located into the abdominal receptors. Superior and inferior vena cava of liver were anastomosis firstly. Fix and suture the donor and receptor liver using line around the superior and inferior vena cava with the knot outside the cavity. Thread from the left corner sutured the posterior wall of the cavity continuously from left to right and stitches to the tail of thread on the right corner. Then, the same thread sutured in a row from right to left anterior corner. This anastomosis of the hepatic superior and inferior vena cava was ended before using saline irrigating tube to emit bubbles. Settle the cuff portal vein of the donor fixed in the portal vein of receptor; restore the portal venous flow and liver blood flow with Satinsky clamp to end the anhepatic phase. Then sleeve-style anastomosis of hepatic inferior vena cava proceeded.

(4) Kidney transplantation: renal vein was temporarily blocked at the site of the inferior vena cava root. Left kidney in the renal hilum resected, renal artery of donor and abdominal aortic of receptor was sutured continuously, sleeve-style anastomosis of renal vein proceeded. After recovery of renal blood

flow, urine outflow could be seen. After bile duct and ureter anastomosis completed, the liver and kidney transplanted were completely replacement, and the color of liver and kidney were crimson. Intraperitoneal washing with saline, ligation line in vitro was reserved in the renal pedicle of the receptor right renal. It would be ligated to replace nephrectomy in 2~3 days after operation.

Postoperative treatment

Keep the rats warm. Feed them with 5% glucose-physiological saline when they regained consciousness. Inject them with 100,000 u penicillin i.m.tid. Put them back into cages.

RESULTS

Before this experiment, we established a separate liver transplant [11] and renal transplantation [12] model with successful experience, which laid a foundation for the establishment of phase I model of combined liver-kidney transplantation. 65 rat liver-kidney transplants were performed, among which 25 were preliminary experiments and 40 were formal experiments. The time taken respectively is: 24~40min for donor surgery with an average of 32min; 10 to 15 min for donor liver and kidney modification; 12~22min for anhepatic phase of the recipient with an average of 16min; 35~55min for the recipient surgery with an average of 45min. The success rate of the formal experiments, defined as survival for more than two days after operation, comes to be 31/40(77.5%). Several factors caused the main death in preliminary experiments, such as pneumothorax, hemorrhage, air embolus, thrombus, and excessive duration of anhepatic phase. Through technological improvements, the mortality declines significantly.The major causes of death were infection, biliary obstruction, renal failure, liver failure, and so on. The blood level of ALT before and after transplantation was (47.8±16.9) U and (54.1±21.7)U, respectively, and that of Cr before and after the operation was (522±107) and (629±256)μmol/L respectively There was no pathological abnormality about the liver and kidney morphology and structure after transplantation.

DISCUSSION

Kamada[13] reported the building of rat liver-kidney transplantation model in 1985. The phase I rat liver-kidney transplantation is not the mere combination of separate liver or kidney transplantation. The delay of anesthesia and operation time, trauma and loss of body fluids, and resection of liver and kidney at the same time, could induce pathophysiological changes in rats, especially changes in the respiratory and circulatory system, which can directly affect the survival of the rats. This requires more skilled microsurgical techniques and anatomical knowledge, reasonable design of operation, and proper material.

Resection of the donor kidney and liver

The author advocated separate resection of the liver and kidney. The removal and isolation of fat and connective tissue at the root of the renal artery and vein should be done in vivo, as the vessels in vivo run full and clear and were easy to deal with, compared with that in vitro. In addition, ligation of several hidden vessels was particularly important: (1) The right adrenal vein is often hidden behind the right liver, and many beginners often overlook the vessels here and forget to ligate them, thus causing bleeding after surgery. (2) The left inferior phrenic vein divides directly from the above-ì liver superior vena cava. As its thin wall is closely sticked with diaphragm, improper separation can easily cause bleeding and pneumothorax; (3) There is a thicker vessel between the esophagus and liver ligaments, which is easily forgotten to ligate and cause bleeding after surgery; (4) The proper hepatic artery runs close to the portal vein and has the left adrenal branch. When operated, gentle moves are required to avoid rupture of the portal vein and massive haemorrhage.

The production of casing

Casing is the key to success, but to find a suitable one is not easy. Vessels of different diameters require casing of corresponding diameter. Casing requires a large cavity and thin wall, in order to ensure smooth flow of blood, bile and urine. The texture of catheter cannot be too soft, or it will easily collapse and narrow; or it will be hard to loop and fix. Here we give a brief introduction of our own method to produce a casing. Heat the infusion device and shape it into casing of different diameters. Immerse it into ether for about half a month. Another approach is to dilate ueteral catheter with needles of different diameters. To ensure stable ligation, branding prints can be made on the walls of catheters with heated needles, to facilitate ligation and prevent slippage of the cuff. In addition, the catheter inserted into the bile duct cannot be too long in case of cholestasis and biliary obstruction (usually <5mm) [14].

Liver and kidney transplantation

The normal sequence of transplantation is liver first and kidney next, otherwise it may affect the survival of grafted kidney. Vascular reconstruction is the most crucial step to the success of donor surgery. When clamping the above-liver inferior vena cava, the upper and lower arm of Satinsky clamp should be equal. Suture can only be done after safe fixation, or the vessel will rupture. Before anastomosis heparinized saline should be used to perfuse the catheter to evacuate the bubble, otherwise it will create air embolism. Similarly, when casing the portal vein, under-liver inferior vena cava and renal vein, remember to evacuate bubble and do the casing in saline as far as possible. After the anastomosis of the above-liver superior vena cava and the portal vein, make sure blood flow be smooth and the anhepatic phase be minimized with 26 min [15-17]. Secondly, in our model we adopted the conventional method home or abroad by resecting left kidney and placing the donor kidney on the left, as the anatomical variation of the left kidney is rare, the left renal vein is longer and the operation is convenient and simple [18-19]. As the renal artery is thin, the renal artery-renal artery anastomosis can easily lead to narrow of the casing and finally renal function failure.

In addition, we performed the delayed original renal vascular ligation in vitro[20] in place of nephrectomy. The rat combined liver-kidney transplantation can result in severe trauma, and if the grafted kidney does not function in time after resection of the original kidney (referring particularly to the contralateral kidney in our experiment), the recipient will die immediately.

With high success rate and satisfactory recovery of renal and liver function, our animal model lay a sound basis for further study on combined liver and kidney transplantation in the future.

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