

Rat liver transplantation without preservation of “phrenic ring” using double cuff method

Yong Jiang, Yu-Dong Qiu, Xiao-Ping Gu, Xin-Hua Zhu, Yi-Tao Ding

Yong Jiang, Yu-Dong Qiu, Xiao-Ping Gu, Xin-Hua Zhu, Yi-Tao Ding, Department of Hepatobiliary Surgery, Gulou Hospital, Medical Department of Nanjing University, Nanjing, 210008, Jiangsu Province, China

Yong Jiang, Department of Hepatobiliary Surgery, Changzhou 1st People's Hospital, Changzhou, 213003, Jiangsu Province, China

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Correspondence to: Dr. Yong Jiang, Department of Hepatobiliary Surgery, Changzhou 1st People's Hospital, Changzhou, 213003, Jiangsu Province, China. yyjiang8888@hotmail.com

Telephone: +86-519-6102280 **Fax:** +86-519-6621235

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Abstract

AIM: To develop a double cuff method for rat liver transplantation without preservation of “phrenic ring” to shorten the portal vein clamping time.

METHODS: “Phrenic ring” was completely excluded from the donor liver, and end to end anastomosis of suprahepatic inferior vena cava was performed.

RESULTS: The portal vein clamping time was shortened to 10.6 min, the successful rate was 83.1%.

CONCLUSION: This method can simplify the operation and shorten the portal vein clamping time.

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INTRODUCTION

The orthotopic rat liver transplantation (ORLT) model was first described by Lee *et al.* in 1973^[1], and subsequently modified by Kamada and Calne in 1979^[2]. This model has been widely accepted and used in current scientific researches^[3-13]. In order to study the ischemia/reperfusion injury after liver transplantation, we modified the ORLT model established by Jiang *et al.*^[14]. We constructed a double cuff ORLT model without preservation of “phrenic ring”. This model can simplify the operation and shorten the portal vein clamping time.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats weighing 200-250 g were used as donors and recipients respectively. The body mass of donors was generally lower than that of corresponding recipients. The rats were housed in pathogen-free conditions with a 12 h light-dark cycle and had free access to water and were fasted for 14 h before operation. All experiments were performed in compliance with the standards for animal use and care set by Institutional Animal Care Committee.

Surgical procedures

All surgical procedures were performed under the naked eye. On the basis of the procedures described by Kamada *et al.*^[2], we made the following modifications. (1) The harvested liver from the donor was placed in a 4 °C saline bath measuring 6 cm in diameter and 3 cm in depth. The whole “phrenic ring” was removed from the suprahepatic inferior vena cava (IVC), leaving the vessel wall of suprahepatic IVC. After the double-cuff preparation was finished, the liver was perfused with cold lactated Ringer's solution through the portal vein and infrahepatic IVC. The liver was preserved in 4 °C University of Wisconsin (UW) solution for 24 h (in order to study cold preservation/reperfusion injury). (2) Instead of anastomosis of the donor “phrenic ring” with the receptor's suprahepatic IVC, the donor suprahepatic IVC was anastomosed end-to-end with the receptor suprahepatic IVC using a 7/0 continuous nylon suture.

RESULTS

Seventy-two ORLTs were performed without preservation of “phrenic ring” in rats. Of which, 59 were successful (success rate 83.1%). The rats survived over 24 h. One rat had survived more than 2 mo. One-week survival rate was not obtained because specimens were taken for study. Our criteria for operative success were as follows. After ORLT, the receptor rat could turn over, and run about actively. The rats were agile to the surrounding and could drink water. The portal vein clamping time (PVCT) was shortened (10.6 ± 1.36 min), and the shortest PVCT reached 8 min. The cause of death included a number of contributing factors: anesthesia too deep and respiratory failure (4/13); thrombosis of the portal vein (1/13); wound bleeding of the right adrenal gland area (1/13); failure of the portal vein anastomosis (2/13); stenosis or obstruction of suprahepatic IVC (4/13); and failure of the infrahepatic IVC anastomosis (1/13). Failure to control ether anesthesia and narrowing or obstruction of the outflow tract was the main cause of operative failure. In the latter case, most of them occurred at the initial stage.

DISCUSSION

Original intention of establishing the model

The author has been engaged in the research of ischemia/reperfusion injury of liver transplantation. In this process, PVCT needs to be shortened in order to reduce the interference with a too long period of splanchnic congestion. PVCT should be shortened to less than 14 min, otherwise a lethal endotoxin like syndrome induced by splanchnic congestion might play the central role which might bring about a noted systemic error in research^[15]. In the beginning by following Kamada's method, we found that the repair of “phrenic ring” and eversion anastomosis were the “bottleneck” of PVCT. Thus we established the double cuff method for ORLT without preservation of “phrenic ring”. In the early stage, suprahepatic IVC was sometimes lacerated. But on the basis of improved microsurgery and vascular anastomosis technique, this method can not only avoid laceration of the suprahepatic IVC, but also shorten PVCT. A more scientific model is thus provided for

the research of ischemia-reperfusion and rejection-tolerance in liver transplantation.

Good exposure is the key point of a successful operation

As other surgical operations, good exposure is also the key point of liver transplantation. As “phrenic ring” is not preserved, anastomosis of suprahepatic IVC becomes more important. Good exposure was achieved in the following ways. Full-length median incision on the abdomen was dragged to both sides and fixed, xiphoid process was dragged cephalad with our designed apparatus, a small pillow underlied the rat chest to raise the suprahepatic IVC stoma. By this method, no suprahepatic IVC was lacerated, PVCT was shortened to nearly 10 min. Not only the disturbance of respiratory and circulation was reduced, but also splanchnic congestion induced endotoxin like syndrome was reduced, which was hard to be attained by “triple-cuff” or conventional “double-cuff” liver transplantation.

Other relevant experiences concerning anaesthesia of donor and receptor rats

Donor livers were anaesthetized by ketamine. Kamada *et al.* recommended a dosage of 100 mg/5 Kg body mass^[2], but actually 40 to 50 mg ketamine was administrated to rats weighing 180-230 g. Receptor rats were maintained by ether inhalation anaesthesia. Steadiness of this anaesthesia is also crucial to a successful ORLT. The shallow anaesthesia would result in difficulty of operation and even bleeding, but too deep anaesthesia would lead to depression of breath and difficulty in postoperative recovery or even death. Out of control of the depth of ether anaesthesia was the main reason of postoperative respiratory failure, and also one of the main causes of death of the receptor rats (30.8%, 4/13). Our experience is “deep induction, shallow maintenance”. In the induction period, a bigger dosage of ether was given, but a low density of ether in mask was maintained during operation.

Turning over of the donor liver during its harvest

We adopted the methods recommended by Wang *et al.*^[16]. After a midline abdominal incision was made, the liver was freed from surrounding ligaments, the left infraphrenic vein and para-oesophagus vein were ligated and divided. Then the right renal vein and right adrenal vein were ligated. The suprahepatic vein was freed. Hepatoportal structure was dissected and freed. The common duct was cannulated. The donor liver was thus harvested. It was turned over only once. So this method can not only protect the donor liver, but also shorten the heat ischemic time.

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