Surgical techniques of orthotopic liver transplantation in rats by a single operator under direct vision☆

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Abstract

BACKGROUND: Rat model of orthotopic liver transplantation is a very valuable model for experimental study in liver transplantation including organ preservation, tissue ischemia-reperfusion injury, allograft rejection and immune tolerance mechanism. Stable liver transplantation animal model is the basis of the related experimental studies. However, its experimental operation is long and boring, especially performed by a single operator under direct vision.

OBJECTIVE: To investigate the operation techniques to establish a stable rat model of orthotopic liver transplantation by a single operator under direct vision.

METHODS: The orthotopic liver transplantation was performed using two-cuff method in 50 pairs of rats. We exposed the abdominal cavity fully, perfused the donor liver through abdominal aorta without flipping donor liver; suprahepatic inferior vena cava was *in vivo* cut down using one-step method, without diaphragm ring; the suprahepatic inferior vena cava was anastomosed with single-row suture, and the cuff of portal vein was installed by fixing the blood vessel forceps on rubber. Hepatic artery was not reconstructed. Fluid replacement was administered to maintain hemodynamic stability in rats after operation.

RESULTS AND CONCLUSION: The donor operative time was (36.2 ± 2.5) minutes, donor liver trimming time was (12.2 ± 1.5) minutes, receptor operative time was (45.6 ± 3.5) minutes, suprahepatic inferior vena cava anastomosis time was (10.1 ± 2.1) minutes, portal vein cuff time was (1.5 ± 0.9) minutes, infrahepatic inferior vena cave cuff time was (1.1 ± 0.6) minutes, anhepatic phase was (15.1 ± 2.2) minutes. The success ratio of the operation was 100% and the survival rates within 1 week and 1 month were all 100%. It is indicated that the key factors of a successful model were stable anesthesia, good donor liver perfusion, adequate exposure, skilled microsurgical technology and vascular anastomosis technique.

INTRODUCTION

Rat orthotopic liver transplantation (OLT) model is the most commonly used small animal models in basic researches for liver transplantation, it fits for the experimental studies addressing donor liver preservation, cold ischemia-reperfusion injury, bile duct injury, transplant immunological rejection and immune tolerance mechanisms[1-6], improving the success and stability of the model is an important prerequisite for conducting basic research. In 1973, Lee *et al*[7] for the first time established a rat model of liver transplantation, afterwards, Kamada *et al*[8] applied cuff technique, through which significantly simplified the surgical techniques and shortened the operation time, especially at the anhepatic phase, the survival rate of rat vein liver graft was significantly increased (> 90%). In recent years, there have been many domestic modified or improved models of rat orthotopic liver transplantation, but they can not go beyond the classic two-cuff technique surgical approach[9-18]. In this study, authors adopted classical Kamada two-cuff technique as the basis, fully understood the nature of this literature, combined with the pre-test results of orthotopic liver transplantation in nearly 200 pairs of rats, finally achieved a success on establishing OLT models in 50 pairs of rats.

MATERIALS AND METHODS

Design

Method modifications.

Time and setting

The experiment was performed at the Animal Experiment Center of Fuzhou General Hospital from December 2008 to March 2009.

Materials

One hundred adult male Sprague-Dawley (SD) rats, weighing 200–250 g, were purchased from Shanghai SLAC Laboratory Animal Co.Ltd (License No. SCXK-HU-2007-2003); all animals were fed in constant temperature and humidity environment and then divided into donors and receptors, taking the body weight of donor was less than the receptor's for about 20 g. Before liver transplantation, animals were subjected to fasting 8–12 hours, with free access to drink 5% glucose water. During the experiments, all animal disposal methods comply with the Animal Ethics Requirements.

Surgical instruments: micro-surgery equipment package (Shanghai Bell Surgical Instruments Factory), 5/0, 7/0, 8/0 medical non-invasive suture line (Ningbo Chenghe Microscopic Instruments Factory), gauze, cotton balls, cotton swabs, infusion set, gloves, 2 mL, 5 mL syringe (Henan Piao'an Group Co., Ltd.), saline, lactate Ringer's solution, heparin, chloral hydrate, ether (Hospital Supply Center provided). Ordinary glass tube served as a lumbar pad. Infrahepatic inferior vena cava and portal vein cuff were carved with thin-walled polyethylene pipe at the inner diameter of 2.4–2.6 mm and 1.8–2.0 mm, respectively. 1-mm oese was retained in sleeve outer diameter near the head-end notches and caudal end, sterilized by formaldehyde fumigation before use. Common bile duct stent

applied epidural catheter, about 5.0 mm long, outer diameter was about 0.8 mm, both ends were cut into a slope (Figure 1), self-abdominal retractor (Figure 2).

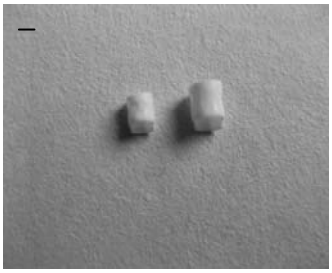

Figure 1 Portal vein and infrahepatic inferior vena cava cuff (Bar=2 mm)

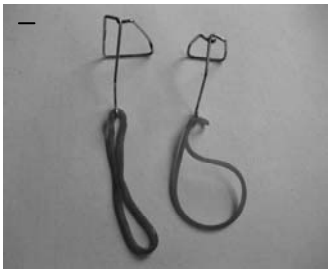

Figure 2 Self-made abdominal retractors (Bar=5 mm)

Methods

Anesthesia

Donors were anesthetized using intraperitoneal injection with chloral hydrate at a dose of 3 mL/kg; receptors were anesthetized using ether inhalation, and then semi-open ether inhalation.

Donor liver

After the success of anesthesia, donors were fixed supinely on the self-made operation board, with lumbar pad on back. A "+" shaped incision was inserted into abdomen after disinfection with iodophor and donor heparinization *via* injected with 30 U/mL heparin saline 2 mL through penile vein, with vascular clamp holding xiphoid process pulling back to isolate the falciform ligament. To break away from the left lateral lobe of liver capsule, wet saline gauze wrapped intestine and pull forwards the left lower abdomen. By use of lumbar pad, the liver was forced to fall along the diaphragm direction, fully exposed the first hepatic portal, infrahepatic inferior vena cava and abdominal aorta, freeing infraheptic inferior vena cava to the anterior left renal vein, freeing abdominal aorta abdominal segment, 1/0 ligature was pre-set in celiac trunk starting point proximal end. The abdominal aorta were clamped upon the left and right common iliac artery bifurcation, then a small incision was cut close to the lower part of the abdominal aorta blocked, placed into plastic outer catheter tube, connected with infusion at 4 ℃ lactate Ringer's solution containing 25 U/mL heparin, suspension height was about 1 m, open flow rate was about 60 drops/minute, then rapidly ligated celiac pre-ligature above abdominal aorta trunk, the infrahepatic inferior vena cava was cut off at about 5 mm below the debouchement level of inferior vena cava right and renal vein for fluid flow as the outflow tract. A little crushed ice was placed in the gap between left and right liver, to complete liver repair *in vivo*. Firstly the triangular ligaments were broken away, ligating the left phrenic vein and portal vein esophageal branch, breaking away the inferior vena cava ligament. Raising stomach, cutting greater omentum and freeing liver caudate lobe. Gently pulling right renal artery and vein, ligating and cutting right renal vein near the inferior vena cava, ligating and cutting right adrenal blood vessels close to the liver. At 6 mm away from the left and right hepatic duct confluence point, common bile duct anterior wall was cut and implanted with common bile duct common bile duct stent at the proximal end, 5/0 silk ligature fixed, pre-reserved for sewing. Freeing portal vein, ligating and cutting the pyloric vein using 9/0 silk close to the portal vein, freeing proper hepatic artery. When the liver was colored as dark yellow and became brighter using perfusion fluid, cut off at the bifurcation of portal vein splenic vein, cutting directly proper hepatic artery. Suprahepatic inferior vena cava was clamped with two wet cotton swabs and pulled parallel to the diaphragm, taut infrahepatic inferior vena cava, Suprahepatic inferior vena cava was cut using ophthalmology scissors close to the diaphragm. At this time, the entire donor liver was free and removed, preserving at 4 ℃ lactate Ringer's solution for installing sleeve.

Donor liver trimming

The trimming was performed at 4 ℃ lactate Ringer's solution. Removing excessive fat connective tissue around the portal vein and infrahepatic inferior vena cava. Non-invasive vascular clamp was subjected to the portal vein cuff and fixed on the rubber, with the ear shank towards the lower edge of the liver, then portal vein was pulled out of the casing, and covered with the casing body, the 5/0 silk tied a fixed loop. The infrahepatic inferior vena cava received the similar treatment. When casing, right renal vein ligation point and pyloric vein ligation points, respectively, were taken as positioning and directional signs to determine the direction of casing. The left and right corner of donor liver suprahepatic inferior vena cava was preset a non-invasive 8/0 suture from outside to inside, the length should be moderate, end clipped with vascular clamping. The trimmed donor liver was placed in the refrigerator (Figure 3).

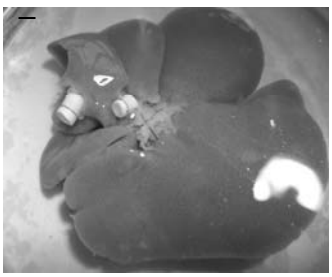

Figure 3 Donor liver was colored as dark yellow and felt soft, portal vein and infrahepatic inferior vena cava cuff and bile duct stent were installed (Bar=5 mm)

Receptor surgery

Receptors were successfully anesthetized using ether inhalation, the middle abdomen was depilated and disinfected with iodophor, the self-made ether barrel was full with many ether soaked cotton balls, regulating the distance between

ether cylinder and rat head to control the anesthesia degree. Through abdominal incision, wet gauze wrapped intestine and pushed to the left lower abdomen, anti-clockwise separating the liver, ligating the left phrenic vein near the diaphragm side, without cutting; the ligation was not available when the gap between left phrenic vein and the diaphragm was not obvious, to avoid separation bleeding. Fully freeing inferior vena cava above right renal vein level, ligating the right adrenal vein without cut, served as a donor liver support point and helping to install inferior vena cava cuff. The common bile duct was broken away in the left and right hepatic duct bifurcation. Ligation the proper hepatic artery. After the rat breathing were slowed and then cessation of ether inhalation, the receptor inferior vena cava was occluded at the level of the right renal vein, the portal vein was occluded at the level of the splenic vein, portal vein was injected with 2 mL saline to slowly drive off the liver blood. The liver was tracted downward, Satinsky clamp together with some diaphragms (about 4 mm) for ring clamp to block suprahepatic inferior vena cava and then cut off the liver without liver tissues. On the left and right portal vein bifurcation, the portal vein was cut off, retaining liver tissue to cut infrahepatic inferior vena cava.

Orthotopic liver transplantation

By use of lumbar pad, fully exposed view, small wet cotton pall placed in the right subphrenic spaces, placed the donor liver in situ, donor-receptor suprahepatic inferior vena cava openings aligned, the distance can be maintained at about 8 mm, so as to maintain a certain tension after the left and right corner suture and fixation and to expose the posterior wall (Figure 4). The Satinsky clamp was fixed on the rubber. The 8/0 non-invasive ligature pre-set on donor liver was ligated on the left and right cornerof caval vein. Suture needle penetrated cavity through left corner, from the left corner continuous suture suprahepatic inferior vena cava, needle distances were uniform at about 1 mm, distant from the edge of about 1 mm, transferred to the right corner of the cavity, continuous suture the suprahepatic inferior vena cava, needle distance was same as the former, to the left corner tightened suture line, driving bubble out of blood vessel lumen using heparin saline and knotted with the left corner thread, at a moderate tension. Flip up the liver, direct non-invasive vascular clamp biting donor liver portal vein cuff ear handles, fixed on the rubber to prevent the cuff reversed, the high should be linear with the receptor portal vein. Move down a little receptor portal vein occlusion clamp, heparin-saline solution driving out of the portal vein blood clots, wrapped the receptor portal vein into the donor liver portal vein sleeve cuff, vessel clamp fix together with the cuff ear and handle temporarily, then quickly ligation. Restoration of liver blood flow, termination anhepatic phase, the liver became red rapidly, intestinal congestion improved, mesenteric artery pulsed powerful, bile flowed a few seconds later. Moving down the inferior vena cava occlusion clip, rinsing infrahepatic inferior vena cava lumen, the donor liver infrahepatic inferior vena cava cuff was placed, pay attention to sleeve direction and ligation. Right kidney showed red color after revascularization. Slowly injected saline containing 5% sodium bicarbonate 1.0–2.0 mL *via* the tail vein if needed. Intraperitoneal perfusion of warm normal saline rewarming. Installation and ligation of common bile duct stent, the greater omentum with good blood circulation covered the common bile duct anastomosis and intestine reset, before abdominal closure intraperitoneal injection of penicillin 200 000 U. The rats turned around and stood up about a few minutes later, the limbs crawling.

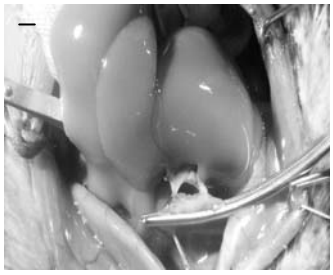

Figure 4 Suprahepatic inferior vena cava anastomosis procedure, with moderate distance and tension (Bar=5 mm)

Treatment after liver transplantation

Following liver transplantation, the rats were rewarmed by lamps 2 hours. At the day after operation, rats were given 5% glucose, and free access to eat 24 hours later, they were fed in single cage for 3 days.

Main outcome measures

Donor operative time, donor liver trimming time, receptor operative time, suprahepatic inferior vena cava anastomosis time, portal vein cuff time, infrahepatic inferior vena cava cuff time, anhepatic period time, receptor survival rate within 1 week and 1 month.

Design, enforcement and evaluation

This study was designed and implemented by the first author, assessed by the second author. All authors have undergone formal training. There is no blind evaluation used.

RESULTS

Quantitative analysis of experimental animals

In the pre-experiment, 200 pairs of rats were involved as a model training period, and not included in the results analysis. In the formal experiment, 50 pairs of rats entered the final analysis without any loss.

Results of liver transplantation

The donor operative time was (36.2 ± 2.5) minutes, donor liver trimming time (12.2 ± 1.5) minutes, receptor operative time (45.6 ± 3.5) minutes, suprahepatic inferior vena cava anastomosis time (10.1 ± 2.1) minutes, portal vein cuff time (1.5 ± 0.9) minutes, infrahepatic inferior vena cava cuff time (1.1 ± 0.6) minutes, anhepatic period (15.1 ± 2.2) minutes, the transplant success rate was 100%, 1-week survival rate was 100%, 1-month survival rate was 100%.

Adverse events and complications

Within 24-hour death was taken as operation failure, survival for more than 4 days was taken as long-term survival. The common early post-transplant complications at the pre-experimental phase included deep anesthesia, pneumothorax, suprahepatic inferior vena cava bleeding, portal

vein thrombosis, pulmonary infection, intra-abdominal infections; late complications included biliary fistula, biliary obstruction, intestinal obstruction, volvulus. The 50 receptor rats involved in the formal experiment all survived for a long term without early post-transplant complications. There were 9 rats occurred late biliary duct obstruction caused expansion, although the recipient rats survived for more than 1 month, still the malnutrition, weight loss, jaundice.

DISCUSSION

The successful establishment of rat OLT model makes some research projects difficult to perform in human be feasible[19]. But the experiment operation process is long and boring, especially by the single operator under direct vision. Beginners need to have enough patience and confidence to train, and achieve stability after a certain number of operations. The goal of many researchers is to achieve proficiency in methods of operation in the shortest possible time, while reducing the number of experimental animals to obtain the purpose of cost-effective[20]. This requires that beginners should not only fully understand the theory and experience of our predecessors, but also put into hard work and appropriate methods of training. Most beginners have no micro-operation experience, therefore the theory learning and the use of micro-devices, vascular anastomosis techniques, should be carried out as early as possible[21-22]. With regard to the use of surgical microscope or magnifying glass, the author's experience has shown that suprahepatic inferior vena cava of 200–250 g rats was approximately 0.6 cm wide, it can completely sutured under direct vision, and is not limited by the changed view of surgical microscope by the beginners. Much more important is the use of micro-devices and vascular anastomosis methods.

The stability of anesthesia is essential for rat OLT. Donor anesthesia adopted chloral hydrate 3 mL/kg through intraperitoneal injection, quick onset and maintaining a long time, not increasing the secretion of rat airway secretions. The rat's breath slowed down was taken as the control index, if the effect is not apparent the dose could increase by 0.1–0.2 mL. Receptor was anesthetized using ether inhalation, rapid onset, full fasting before transplantation is an important way to prevent an increase of digestive secretions, atropine is not necessarily required. Rats can be placed in transparent containers with a damp cotton ball, it can be conducive to observe the rat's anesthetized conditions, and will not make rat suffocation. Once agitation occurred at the time of surgery, the semi-open ether anesthesia was also used in rats, with a quiet and slow breathing as the basis. Before the start of anhepatic phase, rats were allowing more inhaled ether for a few seconds, controlled breathing slowed, basically it is safe through the anhepatic phase. Good surgical exposure is critical to the success model. By use of lumbar pad on-demand, self-made abdominal retractor, we achieve good results.

Donor rat systemic heparinization is necessary. With regard to the timing and dosage of heparin in rats, there is not a unified conclusion. In the present study, intravenous injection of heparin saline was given; according to Kamada *et al*[23] described the dose of 30 U/mL, a total injection of 2 mL, can achieve satisfactory results. A uniform texture donor liver is a prerequisite to the success of liver transplantation. Any traditional methods of flipping the liver, even if more gentle, will be localized pressure causing mechanical injury in liver tissue and micro-thrombosis, so that affects liver perfusion and the success rate of transplantation[24-25]. In this study, firstly perfusion liver through abdominal aortic, without flipping the liver, warm ischemia time was 0 minute, liver perfusion was uniform and soft. When separation, to deal with portal and hepatic inferior vena cava firstly, although flipping the liver, but no significant effect on quality of donor liver, nor worry about graft unexpected bleeding. Abdominal aortic perfusion have a dual role of perfusion, markedly improved quality of donor liver[26]. Liver perfusion method used intravenous drip hanging to an altitude of about 1 m, taking $\leqslant$ 60 drops/minute constant infusion, accurately controlling the amount, speed and pressure of perfusion to ensure the quality of donor liver. Tokunaga *et al*[27] reported that the effective perfusion is not dependent on the increase of perfusion fluid, slow and evenly infusion is an important guarantee to obtain uniform texture of donor liver. Suprahepatic inferior vena cava suture is the core technology of liver transplantation, it require skilled and fast[28-30]. Superior inferior vena cava of the donor liver was isolated by a "one-step *in vivo*" method, it was directly cut close to the diaphragm, without diaphragm ring, that can obviously save the trimming time of the liver *ex vivo*, reduce the cold ischemia time. And the superhepatic vena cava stump was regular, without diaphragm, vascular plasticity is good which is beneficial for anastomosis and does not easily cause anastomotic stenosis. The two suprahepatic vena cava should maintain a distance about 0.8 cm, it was reported that the superhepatic vena cava of donor liver could be retained approximately 0.2 cm, receptors' may retain about 0.5 cm[8]. That prevented vein posterior wall adhered and revealed unclearly after corners fixed. When suture, the needle distance should be uniform to avoid wrinkling endoleak of vascular wall, ensure a smooth lining and to reduce post-transplant thrombosis.

Anhepatic phase is also the key to the success of liver transplantation. Liu *et al*[31] reported that the receptor celiac abdominal aorta was blocked during anhepatic phase , which can increase rat tolerance, thereby enhancing the success rate of the model. We believe that on the basis of technical proficiency, such approach is not necessary. Kamada *et al*[8] proposed anhepatic phase should not exceed 26 minutes, otherwise the rats were very difficult to survive. The author's experience also shows that a direct relationship between the duration of anhepatic phase and the survival. Moreover, the skilled techniques of portal vein casing is also very important, the key of applying rubber fixation to install sleeve is that donor-receptor vein debouch were in a linear line, first posterior sets, then turned on the anterior wall of homeopathy, so that the portal vein is easy to cover into the cuff. In this experiment, about 1 minute is enough to complete the installation of the portal vein casing.

Biliary tract infection and bile leakage is an important factor for long-term survival after OLT, the length of donor liver bile duct can not be too long, the surrounding tissue can not be over-stripping. Ensure tension-free, non-reversed, drainage unobstructed. Wrapped using the greater omentum with good blood circulation is feasible. Post-operative rehydration was performed mainly in the cases of the longer anhepatic phase. It was not requird if the hemodynamics of receptor was stable

after operation. Rewarming after surgery is also very important for the recovery of rat heart and lung function.

In summary, abundant theory, good anesthesia, adequate surgical exposure, good quality of donor liver, skilled vascular suture technique is the key to establish stable rat OLT model.

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直视下单人操作大鼠原位肝移植的手术技巧☆

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摘要

背景：大鼠原位肝移植模型是一种非常有价值的模型，适用于肝移植器官保存、组织缺血再灌注损伤、移植免疫排斥反应及免疫耐受机制等方面的实验研究，稳定的肝移植动物模型是肝移植实验研究的基础。但其实验操作过程冗长而枯燥，尤其是单人直视下操作。

目的：探讨直视下单人操作建立稳定大鼠原位肝移植模型的手术操作技巧。

方法：采用“二袖套法”对50对SD大鼠行原位肝移植，充分暴露腹腔，不翻动肝脏先行经腹主动脉供肝灌注；体内一步法离断肝上下腔静脉，不带膈肌环；吻合肝上下腔静脉采用单线连续缝合；橡皮泥固定法安装门静脉袖套，肝动脉不重建。移植后充分补液维持大鼠血液动力学稳定。

结果与结论：供体手术时间为(36.2±2.5) min，供肝修整时间为(12.2±1.5) min，受体手术时间为(45.6±3.5) min，肝上下腔静脉吻合时间为(10.1±2.1) min，门静脉袖套时间为(1.5±0.9) min，肝下下腔静脉袖套时间为(1.1±0.6) min，无肝期为(15.1±2.2) min，移植成功率100%，1周存活率100%，1个月存活率100%。结果提示，稳定的麻醉、良好的供肝灌注、充分的暴露、熟练的显微外科操作及血管吻合技术是确保模型成功的关键因素。

关键词：动物模型；手术技巧；大鼠；肝移植；器官移植

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