

Operative Techniques, Treatment, and Precaution of Common Complications of Orthotopic Rat Liver Transplantation

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Abstract Objective This study investigates operative techniques, treatment, and precaution for common complications of orthotopic liver transplantation (OLT) in rats. **Methods** OLT was performed in 110 rats through modified two-cuff method. Operative techniques were concluded, and causes, treatment, and precaution of common complications were analyzed. **Results** In operation phase, main causes for rat death included anesthesia, airway obstruction, pneumothorax, hemorrhagic shock, long anhepatic time, and air embolism. After surgery, main causes for rat death were as follows: stoma bleeding, infection, obstruction, and necrosis of biliary tract and poor hepatic function restoration. **Conclusion** Improving operative techniques, modifying operative methods, and familiarizing with common complications can reduce occurrence of complications, heighten operative successful rate, and establish more stable models of OLT in rats.

Keywords: liver transplantation, operative technique, complication, rat

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1. Introduction

In 1973, Lee et al. first reported orthotopic liver transplantation (OLT) in rats. The researchers accomplished anastomosis of suprahepatic vena cava (SHVC), portal vein (PV), and intrahepatic VC (IHVC) by suturing and reconstructed bile duct by embedding it into duodenum. With development of operative techniques, Kamada's two-cuff technique was considered as classic technique, because it not only effectively shortens operation time but also ensures operative successful rate. However, this classic technique also underwent gradual modifications after showing several disadvantages [1,2]. Using modified two-cuff technique, we established models of OLT in rats and systematically analyzed pre- and post-operative complications. Finally, we concluded practical operative techniques and precautionary routes for common complications as reported below.

2. Materials and Methods

2.1. Animals and Materials for Trial

The present study used healthy male Sprague-Dawley (SD) rats (n = 110) weighing 250–260 g; recipients were slightly bigger than donors. All animals were provided by the experiment animal center of Sichuan University Huaxi

Medical Center. PV and IHVC comprised vinyl tubes. Stent tube of common bile duct was made with a soft pinhead of common remaining needle. For the stent tube of PV and IHVC, internal and outside diameters measured 1.5 and 2.0, 2.5 and 3.0, and 0.9 and 1.0 mm, respectively. Meanwhile, lengths measured 3.0, 3.0, and 4.0 mm, respectively. Other equipment were as follows: unit of microsurgery (made in Shanghai); vascular clamp; Satinsky clamp; 5-0, 6-0, and 8-0 medical non-damaged stylolites.

2.2. Establishing Animal Model for Rat Liver Transplantation

2.2.1. Preparation of Laboratory Animals

Donors were allowed access to water but not food 12 h prior to surgery. Recipients were provided with unlimited diet. A total of 0.03 mg atropine and 200,000 units of penicillin were injected into muscles of them. Both were under openly continuous ether anesthesia.

2.2.2. Acquisition of Donor liver

Rats were fixed and anesthetized. Abdomen of rat was shaved, prepared with rubbing alcohol, and then exposed through middle incision. Falciform ligament and left triangular ligament were cut off. Left vena phrenica was then divided and ligated. Gastrohepatic ligament was cut off. Vascular plexus between liver and esophagus was then ligated. Then, caudate lobe was freed, and right

triangular ligament was cut off. IHVC was isolated to opening of right renal vein. Right suprarenal and right renal veins were sequentially ligated and divided. After dissociation, bile duct was incised on anterior and inserted into a prepared Teflon catheter with internal diameter of 0.6 mm and secured with circumferential 5-0 silk suture. Then, pyloric, splenic, and gastroduodenal veins were ligated and divided. Importantly, hepatic artery (HA) was immediately isolated but not ligated.

Two minutes after heparinization, at a speed of 2.5 ml/min and at 4 °C, liver was perfused through abdominal aorta with 10–15 ml lactated Ringer's solution, to which 25 units per ml of heparin was previously added⁴. Meanwhile, liver surface was sprinkled with 4 °C Ringer's lactate solution to maintain low temperature. During perfusion, thoracic cavity was opened, and thoracic aorta was obstructed. SHVC was divided to let out perfusion fluid. HA was ligated and divided when liver color turned yellow. During this time, we finished perfusion of donor liver. IHVC and PV were cut off, and SHVC was then transected together, adjoining the diaphragmatic muscle. Liver was removed and placed in balanced salt solution at 4°C.

2.2.3. Trimming of Donor Liver

Trimming of donor liver was performed in balanced salt solution at 4°C. Kamada's cuff technique was applied to preparation of PV and IHVC. Vein walls were everted over the cuff and secured with circumferential 5-0 silk suture. Two 8-0 undamaged silk sutures were used to fix two sides of SHVC and acted as assistant lines. IHVC was obstructed with vascular clamp.

2.2.4. Recipient Operation

Same procedures were performed in preparation of donor operation. Peripheral ligaments of liver were cut off. Left inferior phrenic vein and vascular plexus between liver and esophagus were ligated and divided. Caudate lobe was freed. Common bile duct was freed when first porta hepatis was exposed. Left and right hepatic ducts were ligated and divided beside their junctions. Then, right suprarenal vein was ligated but was not divided. IHVC was freed up to the opening of right renal vein. Left and right branches of PV were freed. HA was then freed, ligated, and divided.

Anesthesia was then evacuated. IHVC and PV were blocked at openings of right renal vein and pyloric vein, respectively. Then, anhepatic phase was initiated. A total of 2 ml physiological saline was injected into liver at the crotch of PV to drive intrahepatic blood into systemic circulation. Then, left and right branches of PV were successively ligated with two 5-0 silk sutures. The two ligatures were reserved for later use. Liver was slightly dragged downward, and SHVC was blocked by clamping part of diaphragmatic muscle with Satinsky clamp. SHVC was cut off adjoining to liver surface. IHVC attached with part of liver tissue was divided.

Then, liver of recipient was removed. Donor liver was removed from balanced salt solution and properly placed. SHVC was anastomosed end to end using continuous suture; left and right assistants of donor were used to

suture and fix two sides of recipient SHVC. Knot was made outside the vessels. Posterior wall of SHVC should be sutured before suturing of anterior wall.

PV anastomosis was then commenced. Tension in recipient PV was maintained by traction of silk sutures on its divided right and left branches. PV was blocked at the level of pyloric vein. An incision was made at the crotch of recipient PV. Cuff of donor PV was inserted into lumen of recipient PV. Anastomosis was then completed with circumferential 6-0 silk suture. Anhepatic phase ended when blood flow of PV and SHVC were recovered.

Two 8-0 undamaged silk sutures were also used to respectively suture two sides of amputation stumps of IHVC and then acted as assistant lines. A similar method was applied to complete cuff operation. IHVC was finally opened.

A similar method was also applied to complete reconstruction of bile duct system by inserting stent tube of common bile duct of donor into bile duct of recipient and fixing it with circumferential 6-0 silk suture.

Finally, abdominal cavity was washed with warm physiological saline and desiccated with dressings. Intestinal canals were reset, and abdominal incision was closed. Approximately 1.5–2.0 ml of lactated Ringer's solution was slowly transfused via vena dorsalis penis. Rat was placed in heat preservation bag to keep it warm until the animal wake up and was able to move. A total of 200,000 units of penicillin were injected into muscle of recipient three days postoperation.

3. Results

Cutting time of donor liver lasted for 35–41 min, whereas time for liver trimming lasted for 12–15 min. Operation of recipient lasted for 40–45 min. Anhepatic phase lasted for 16–22 min. Anastomosis of SHVC lasted for 12–15 min. Anastomoses of PV and IHVC lasted for 3–4 and 4–6 min, respectively. Time of 3–4 min was needed to place stent tube of common bile duct.

After analyzing 110 models of OLT in rat, we concluded the main causes of rat death or its effects on rat survival during and after operation as follows. (1) [Table 1](#) shows main reasons for intraoperative death of rats. (2) Main reasons for postoperative 24 h death of rats include blood leakage in anastomotic stoma at SHVC (n = 3) and poor recovery of hepatic function (n = 2). Postoperative 24 h mortality was 4.5% (5/110). Including intraoperative and postoperative 24 h death of rats, total mortality of operation was 10.9% (12/110). Operative successful rate of 89.1% refers to recipient survival for over 24 h postoperation (98/110). (3) Infection primarily caused death of rats which survived for 24 h to one month (n = 4). In total, one rat died in the first week, and survival rate in one week was 88.2% (97/110). One rat died in the second week, and survival rate in two weeks was 87.3% (96/110). Finally, two rats died in the third week, and survival rate in three weeks was 85.5% (94/110). (4) Obstruction and necrosis of biliary tract primarily caused death of rats that survived for over one month (n = 5). Survival rate in one month was 80.9% (89/110).

Table 1. The main reasons for intraoperative death and corresponding counts of rats

The main reasons for intraoperative death of rats	Count
Over-deep anesthesia	1
Respiratory passages obstruction	1
Pneumothorax	1
Hemorrhagic shock	1
Over-long anhepatic phase	2
Air embolism of the vena cava and the PV	1

4. Discussion

Reasons for various complications were systematically and adequately analyzed to improve operative successful rate, to reduce occurrence of operative complication, and to establish more stable model of OLT in rats. Discussion below reports operative techniques, treatments, and precautions for common complications.

4.1. Precaution for Anesthetic Accident

Anesthetic method, which involves inhaling ether, was applied in our trial because it was convenient, controllable, and non-toxic to livers. However, this method can possibly cause anesthetic accidents, the most common condition of which is excessive deep anesthesia, which can eventually lead to cardiopulmonary arrest. Once rats were found with symptoms, such as superficial and slow respiration, sighing respiration, and cyanosis of labia oris and extremities, anesthesia should be evacuated, and cardiac compression and oxygen inhalation must be performed. Best precautionary methods for preventing anesthetic accidents include strict regulation of anesthetics, administration of proper amount of anesthetics at first dosage, and appending anesthetics only when anesthesia wears off. Anesthetic depth should be controlled during operation according to range of respiration, heart beat rate, and changes in liver color.

4.2. Securing Quality of Donor Liver

Quality of donor liver can directly affect survival time of postoperative rats. Provided ideal donor liver must possess the following characteristics: smallest mechanical injury, shortest time of hot and cold ischemia, and sufficient perfusion.

Considering that rat liver is fragile and can easily hemorrhage, operations for freeing hepatic peripheral ligaments and ligating hepatic peripheral vessels were supposed to be simultaneously finished in counterclockwise manner. HA was freed first. Ligating HA after heparinization of general blood can shorten time of hot ischemia and prevent formation of intrahepatic microthrombus. Elevating speed of liver trimming and operation of recipient can shorten the time for cold ischemia.

Suitable volume of perfusion solution, speed, and pressure should be considered to create perfect perfusion. Commonly, 10–15 ml of perfusion solution is sufficient. Our trial adopted perfusion method through abdominal aorta. In this method, a small amount of perfusion solution

was directly used for perfusion through HA, and most of perfusion solution passed gastrointestinal circulation to reach the PV for perfusion, possibly cooling donor liver under a short period and with hot ischemia time reaching approximately zero min. This method reduces chance of injury and postoperative thrombus in PV. By using this method, perfusion pressure can be successfully controlled. However, during operation, we also needed to focus on resetting all hepatic lobules and intestinal canals before we perform perfusion to ensure sufficient double perfusion. Quality of donor liver must be secured by sprinkling surface of donor liver with 4 °C balanced salt solution from start of perfusion.

4.3. Technique Used for Liver Trimming and Anastomosis

Trimming of liver was performed in 4°C balanced salt solution. Points applied for setting cuff are as follows. First, muff was kept unobstructed, and correct position and direction of ear of cannula were ensured to prevent distortion afterward. After completing the cannula, 4°C balanced salt solution from PV was injected with low pressure to check whether all vessels were unobstructed. Air embolism may be found while suturing vessels and installing cuffs of SHVC, PV, and IHVC. Therefore, lumen was douched before finishing the sutures to eliminate air and to prevent air embolism. Third, postoperative 24 h death of rats was primarily caused by blood leakage and errhysis of anastomotic stoma of SHVC. As reported [3], postoperative survival rate is affected by three key factors: amount of blood loss, operation time, and adequacy of reconstruction of each vessel. The key point for decreasing this complication includes using tunica intima-to-tunica intima continuous suture, keeping proper intervals between needles, maintaining proper tension on suture line, and mastering consummate techniques of saturation.

4.4. SHVC and Shortening Anhepatic Phase

Shortened anhepatic phase determines success of this model [4,5]. In this phase, large amounts of endotoxins, which are also termed lipopolysaccharide, were released from ectopic intestinal bacteria because of splanchnic congestion, thus harming liver graft through activated Kupffer cells and numerous mediators of inflammation [6,7]. On the other hand, prolonged anhepatic phase can also indicate prolonged ischemia of donor liver, aggravating ischemia-reperfusion injury, causing tissue injury, and even threatening donor liver function [8,9]. Regarding such complication, speed of anastomosis of SHVC is the major factor affecting anhepatic phase. We used scientific and reasonable method to cut off SHVC: liver was pushed downward to form tension on SHVC to induce a natural and extended state and cut it off easily adjoining to diaphragmatic muscle. Without retaining diaphragmatic muscle circle, we saved time for SHVC trimming. We fixed the two sides of SHVC and then used continuous suture to SHVC, benefitting fast transfer of donor liver, preventing occurrence of tearing and distortion of SHVC, and shortening anhepatic phase and time for anastomosis.

4.5. Prevention of Infection

Peritoneal cavity and lung infection were common complications in three to five-day postoperative period in rats. The former was usually caused by operative procedure, and the latter was attributed to preoperative potential focus of infection and intraoperative aspiration. Preventing these complications lie in precaution. Disinfecting surgical instruments and improving surgical condition can be applied to precautions for peritoneal cavity infection. On the other hand, suitable methods for prevention of lung infection include avoiding using rats with infection symptoms during trial, opening IHVC to recover hemoperfusion of important organs during operation, and maintaining stabilization of hemodynamics. However, one method should not be easily neglected regarding postoperative support therapies: maintaining warmth, inhaling oxygen, fluid replacement, and nutritional support are significant for decreased infection rates and satisfactory postoperative recovery. For safety, using 200,000 units of penicillin at 30 min preoperative and at three days postoperative are also important to infection prevention.

4.6. Precaution for Biliary Tract Complication

Death of rats that survived for over 30 days can be attributed to obstruction and necrosis of biliary tract. Biliary complication is the second most common cause of graft dysfunction in liver transplantation with incidence rate as high as 64% [1]. Associate operative techniques and precautions are summarized below. Stent tube of common bile duct should be cut with no burrs and unsmooth edges; otherwise, associated biliary tract complications may occur [2]. Mechanical injury of bile duct should be avoided whenever possible. Remaining rudimentary bile in bile duct of donor liver was extensively accepted to damage this anatomical structure [10]. Therefore, surgeons douche bile duct before cutting off donor liver. Steady fixation can be ensured by curving a few ditch grooves on surface of stent tube of common bile duct.

4.7. Anastomosis of HA

Whether HA should be inosculated remains controversial. As presented in reports in recent years, some scholars hold that HA reconstruction poses no significant effect on long-term survival after OLT with whole-liver graft [12,13]. Above all, anastomosis of HA plays an important role in OLT [1,11,12], and OLT with re-arterialization is more physiological than that without re-arterialization, leading to a visibly low rate of bile duct complications [9]. Nearly all blood supply of bile duct depends on HA. Hence, HA reconstruction is important for reliable grafting to maintain long-term biliary proliferation after OLT. In our study, HA reconstruction possibly partly resulted in obstruction and necrosis of biliary tract of five rats postoperative one month.

Although rebuilding HA increases total operation time of OLT, it did not lengthen anhepatic phase and affected operative successful rate. In summary, operative style of OLT with re-arterialization should be advocated when possible.

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