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Original Article

A rat model of liver transplantation with a steatotic donor liver after cardiac death

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Abstract: This study aimed to establish a rat liver transplantation model with a steatotic donor liver after cardiac death, reflecting clinical conditions. Rats were fed a high-fat diet for 8 weeks to establish the fatty liver model. This model simulates liver steatosis caused by various factors before clinical donation after cardiac death. A pneumothorax was created in the donor rat to induce hypoxia and cardiac arrest before incising the liver. This simulated the processes of hypoxia and cardiac arrest caused by withdrawal of treatment in actual clinical situations. The harvested cardiac death donor liver was then transplanted using the Kamada technique. Donor operative time was 45.7 ± 4.2 min; cardiac arrest time, 9 ± 0.8 min; recipient surgery time, 40.3 ± 4.9 min; and no-liver time, 15 ± 2.5 min. Of 40 liver-transplanted rats, 2 died within 24 h, with a surgical success rate of 95%. The transaminase levels on post-transplantation days 1, 3, 5, and 7 were 835.4 ± 71.33 U/L, 1334.5 ± 102.13 U/L, 536.4 ± 65.52 U/L, and 218.2 ± 36.77 U/L, respectively. This rat liver transplantation model with a steatotic donor liver after cardiac death could improve the simulation of the pathophysiological processes of clinical donation after cardiac death, and could be used as a reliable and stable animal model.

Keywords: Rat, liver transplantation, cardiac death, steatosis

Introduction

The main problem in liver transplantation is the shortage of organ sources. To solve this problem, the Ministry of Health of the People's Republic of China had urged citizens to participate in organ donation after cardiac death (DCD) [1-3]. However, DCD livers often exhibit considerable steatosis [4, 5] and other disorders, such as ischemia and hypoxia caused by the withdrawal of treatment before organ procurement [6, 7]. These conditions greatly affect the quality of the donated organs, thus increasing the incidence of complications, such as the absence of graft function after transplantation, biliary complications, and rejection [8, 9]. Thus, basic research on these issues is needed in corresponding animal models. The currently reported [10] rat liver transplantation models have all failed to reflect the pathophysiological process of clinical organ DCD. To prepare animals for Kamada's two-cuff technique [11, 12], we fed rats a high-fat diet to create a severe fatty liver model. Then, a pneumothorax was created in the donor rats to induce hypoxia and natural cardiac arrest before incising the liver. This study aimed to establish a rat liver transplantation model that could simulate the pathophysiological process of clinical organ DCD.

Materials and methods

Animals

Healthy, clean Sprague-Dawley (SD) rats (all males, weighing 100-110 g) were used. The severe fatty liver model was created by feeding the donor rats a high-fat diet, increasing their weight to about 280 g. The recipients were normal rats with a slightly higher weight than the donors. The experimental animals were purchased from the Experimental Animal Center of Fujian Medical University, and fed in the SPF Laboratory Animal Room, Division of Comparative Medicine, Fuzhou General Hospital of the Nanjing Military Region. This study was car-

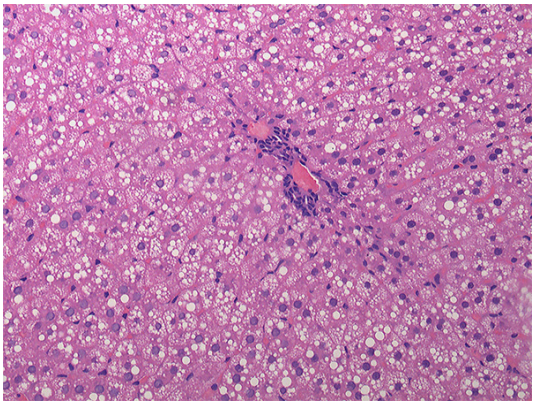

Figure 1. Pathological observation of fatty liver (HE, × 100): hepatic microbubbles-type steatosis was >60%.

ried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Fujian Medical University.

Grouping

The DCD group (n = 40) included the donor rats with induced severe fatty liver. Before incising the liver, a pneumothorax was created to induce hypoxia and natural cardiac arrest. Then, after 5 min of warm ischemia, the liver was reperfused before harvesting, and then transplanted according to the Kamada two-cuff technique.

The control group (n = 40) consisted of the normal donor rats whose livers were incised according to the Kamada technique. The cuff preparation and recipient operation in this group were the same as in the DCD group.

Outcome indicators

The postoperative 24-h survival was set as the indicator of a successful transplantation; blood was obtained from the tail vein for transaminase analysis on postoperative days 1, 3, 5, and 7, and the 1-week survival rate was calculated.

Establishment of the DCD steatotic donor liver model

Forty male SD rats, weighing 100-110 g, were fed a high-fat diet (88% normal diet, 10% lard, and 2% cholesterol) for 8 weeks [13, 14]. Five rats were randomly selected for biopsy. Severe fatty liver was diagnosed when the degree of liver microbubble-type steatosis was >60%. Rats with this condition were then used as the DCD donors (**Figures 1** and **2**).

Donor surgery

The animals were fasted for 12 h, with free access to water preoperatively. The rats were then anesthetized with ketamine (100 mg/kg, intraperitoneal injection) and a transverse incision was made over the abdomen. The liver demonstrated considerable steatosis and increased weight, and had 1.5 times the volume of the normal liver. Then, the common bile duct was freed, an oblique incision was made about 0.5 cm toward the distal end of the hepatic duct confluence region, and a prepared bile duct supporting tube (epidural catheter, 0.5 mm long) was inserted into the common bile duct. Ligation with 5-0 silk sutures was performed. The portal vein and the hepatic artery were freed; the splenic vein was ligated; the right renal artery was freed and ligated; the right renal vein was freed, ligated, and cut; the infrahepatic vena cava and abdominal aorta were freed; the femoral artery was freed; and the femoral artery was then connected to a continuous arterial blood pressure monitor (M8003A; Philips Medizin Systeme Boblingen GmbH, Boblingen, Germany). Heparin in saline 2 mL (250 U/mL) was then intravenously injected through the penile dorsal vein for systemic heparinization. A thoracotomy was performed to create a pneumothorax and induce respiratory dysfunction. The resulting hypoxia then gradually induced cardiac arrest. The arterial pressure changes during this process were closely monitored; cardiac arrest was considered to occur when the blood pressure was zero, which was observed after approximately 9 min (**Figure 3**). After a 5-min warm ischemia time, the abdominal aorta was catheterized, and 4°C lactated Ringer solution (without heparin) was used to perfuse the liver (perfusion rate, about 3 mL/min; perfusion height, 30 cm). Perfusion was performed consistently until the liver was completely pale, after about 2-3 min. The hepatic artery was cut with an electrocoagulation knife, the falciform ligament was cut, and the left inferior phrenic vein was ligated with 5-0 silk sutures. The suprahepatic vena cava was cut along the lower edge of the diaphragmatic muscular ring. The donor liver was

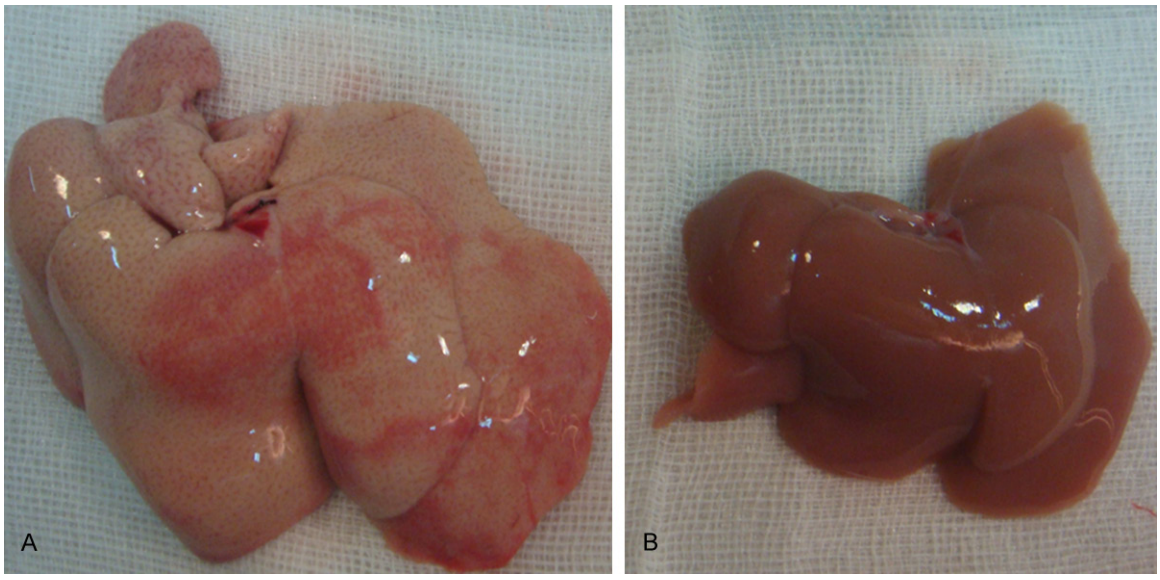

Figure 2. Livers of the same-week-old rats: the left figure showed the severe fatty liver; and the right figure showed the normal liver.

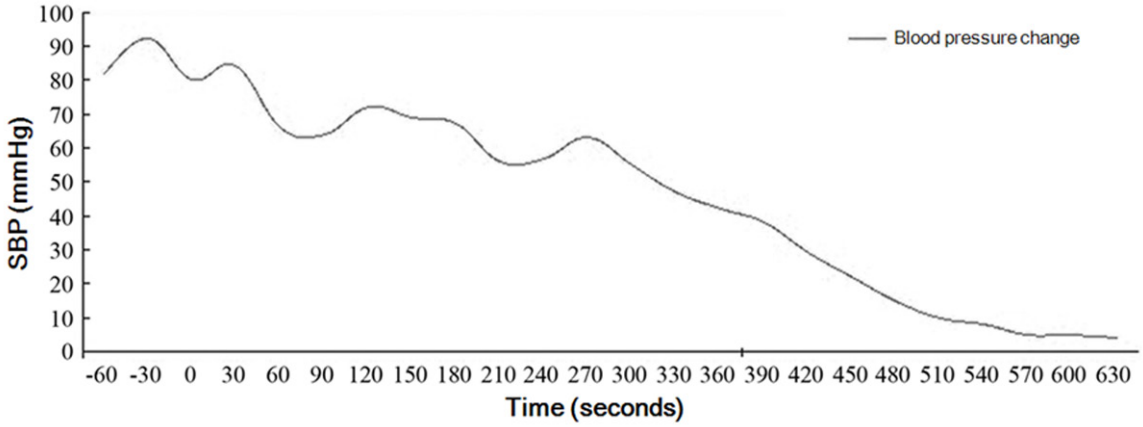

Figure 3. Blood pressure changes during the cardiac arrest after the thoracotomy.

freed from top to bottom by cutting the left hepatic triangular ligament, hepatic posterior ligament, hepatogastric ligaments, and hepatoesophageal branch; the splenic vein was ligated, and the portal vein was cut 2-4 mm below the splenic vein; the infrahepatic vena cava was bluntly dissected, the right adrenal vein was ligated, and the infrahepatic vena cava was cut at the level of the left renal vein. Then, the liver was removed and preserved in 0-4°C lactated Ringer solution.

Liver trimming

Tissues that adhered to the hepatic portal vein and inferior vena cava during the process of liver excision were removed. A catheter (polyethylene plastic tube: inner diameter 2.0 mm, outer diameter 2.5 mm) was cuffed at the portal vein, with the splenic vein ligation line as the landmark. The left or right direction of the portal vein was determined; the portal vein wall was then reversed, and the catheter was cuffed and ligated with 5-0 silk sutures. A catheter (polyethylene plastic tube: inner diameter 3.0 mm, outer diameter 3.5 mm) was cuffed at the inferior vena cava, with the renal vein ligature as the landmark for judging the direction of the inferior vena cava. The renal vein ligature point was then exposed outside the cuff, ligated, and fixed with 5-0 silk sutures. The suprahepatic

Table 1. Comparison of surgical parameters of the 2 groups

Group	Donor surgery time (min)	Donor liver weight (g)	Cuff preparation (min)	Receptor surgery time (min)	No-liver period (min)	1-week survival rate
DCD (40)	45.7 ± 4.2	16.7 ± 1.6	3.9 ± 0.48	40.3 ± 4.9	15.5 ± 2.5	90%
Control (40)	35.2 ± 3.8	11.5 ± 1.1	4.0 ± 0.52	39.4 ± 5.1	15.2 ± 2.6	95%
P	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05

Intergroup comparison, except for the donor liver weight, $P<0.05$, exhibiting the statistical significance. The other parameters showed no statistical difference.

Table 2. Transaminase changes of the 2 groups before and after the surgery

Group	Before the surgery	1 day after the surgery	3 days after the surgery	5 days after the surgery	7 days after the surgery
DCD (40)	116.7 ± 17.23	835.4 ± 71.33	1334.5 ± 102.13	536.4 ± 65.52	218.2 ± 36.77
Control (40)	110.9 ± 15.45	484.6 ± 62.69	657.7 ± 76.66	227.2 ± 49.18	148.9 ± 26.08
P	>0.05	<0.05	<0.05	<0.05	<0.05

On the postoperative 1st, 3rd, 5th and 7th day, the transaminase level of the DCD group was statistically significantly higher than the control group, $P<0.05$, while the transaminase comparison of the two groups before the transplantation had no statistically significant difference $P>0.05$.

vena cava was then trimmed to ensure the congruence of the anterior and posterior walls.

Recipient surgery

The recipients were normal SD rats subjected to preoperative 12-h fasting with free access to drinking water. Ten minutes before anesthesia induction, atropine (0.03 mg/kg) was injected intramuscularly to reduce respiratory secretions. The rat was then placed in a sealed large glass trough containing a few ether-soaked cotton balls; after a few minutes, the rat was removed from the trough and fitted with a special semi-open ether inhalation mask. After satisfactory anesthesia was achieved, an abdominal midline incision was created, from the xiphoid to the pubic symphysis. The abdominal wall was pulled toward the left and right sides with homemade retractors, and then the liver was freed in a clockwise manner. The perihepatic ligaments were cut; the left inferior phrenic vein and hepato-esophageal branch were ligated with a thin cord; the common bile duct was freed and cut at the hepatic duct confluence region; the hepatic artery was freed, ligated, and cut; the portal vein was freed to the left and right bifurcation; the inferior vena cava was freed from the right renal vein level to the diaphragmatic muscular ring level; and the right adrenal vein was ligated but not cut. Heparin in saline 0.5 mL (25 U/mL) was intravenously injected at the penile dorsal vein, which was occluded with vascular clamps. The portal vein was clamped at the level of the splenic vein, and the infrahepatic vena cava was clamped at the level of the right renal vein; this was the start of the no-liver period. The portal vein bifurcation was punctured and slowly injected with 2 mL warm saline (37.5°C) to wash out blood, until the liver was yellowish brown after about 30 s. A vena cava occlusion clamp was used, with a 3 mm diaphragm, to occlude the suprahepatic vena cava; the suprahepatic vena cava, portal vein, and infrahepatic vena cava were cut near the liver. When cutting the infrahepatic vena cava, certain tissues were retained for future catheterization. The donor liver was then removed and placed into the abdomen of the recipient; the suprahepatic vena cava of the explanted liver was continuously sutured by using 7-0 atraumatic needles (Ningbo Medical Needle Factory, Ningbo, China), with the posterior wall sutured before the anterior wall. Through an approximately 1 mm incision left in the anterior wall, saline was used to remove intravenous bubbles, and the suture was continued and tied, completing the anastomosis of the suprahepatic vena cava. The donor and recipient portal veins were rinsed with saline, and then the donor portal vein was catheterized into the recipient portal vein, ligated, and fixed with 5-0 silk sutures. Then, the portal vein and suprahepatic vena cava were opened to end the no-liver period, which lasted about 15 min. The same method

was used to catheterize the infrahepatic vena cava. An oblique incision was made on the anterior wall of the recipient's common bile duct; the donor's common bile duct supporting tube was inserted into the recipient's common bile duct, ligated with 5-0 silk sutures, and the ligature was knotted and fixed with the donor-preserved ligature. Ten milliliters of 40°C saline was used to wash and rewarm the abdominal cavity, and was extracted 1 min later. The bleeding status was checked; the hepatic hilar region was covered with the great omentum; 3 mL gentamicin (50 mg/kg) in saline was intraperitoneally injected; and the abdomen was closed. The rats were fed individually, with sugar and salt water immediately after surgery, and with a diet one night after surgery. Subcutaneous injection of gentamicin (50 mg/kg) was performed daily for 3 days postoperatively.

Statistical analysis

SPSS 13.0 statistical software (SPSS, Chicago, IL) was used for the data collection, collation, and analysis. The measurement results were expressed as mean ± standard deviation ($x \pm s$), and then subjected to a grouping t test. The rate indicator was determined by using the chi-square test. Repeated measurement data (**Table 1**) were subjected to two-factor variance analysis, with the test level set at $\alpha = 0.05$.

Results

Rat DCD steatotic donor liver transplantation model

From June 2013 to February 2014, 50 pairs of rats were subjected to pre-experimental orthotopic liver transplantation, mainly to practice microscopic surgery and evaluate pre-experimental surgical methods; however, the surgical success rate was low. The main causes of death in the formal experiment were anesthesia accident, respiratory arrest, bleeding or stenosis at the anastomotic stoma of the suprahepatic vena cava, portal vein stenosis or obstruction, cuff slipping or twisting, bleeding of the right adrenal venous plexus or lumbar venous plexus, bleeding from a liver tear, pneumothorax, biliary fistula, and others. The donor operative time of the DCD group (40 pairs) was 45.7 ± 4.2 min; the steatotic liver weight was 16.7 ± 1.6 g; the cardiac arrest time was 9 ± 0.8 min (the arterial blood pressure changes during the arrest process are shown in **Figure 3**); the warm ischemia time was 5 min; and the cuff preparation period was 3.9 ± 0.48 min. The recipient operative time was 40.3 ± 4.9 min, and the no-liver period was 15 ± 2.5 min. Within 24 h after the surgery, two rats died of bleeding from the suprahepatic vena cava; the surgical success rate was 95%. Two other rats died within 1 week, due to portal vein stenosis and twisting in one case, and biliary fistula in the other; the 1-week survival rate was 90%.

Comparison of DCD steatotic liver and normal liver transplantation

The control group (40 pairs) had one death within 24 h after the surgery, due to bleeding of the suprahepatic vena cava; the surgical success rate was 97.5%. One rat died due to a biliary fistula within 1 week, and the 1-week survival rate was 95%. The other related surgical parameters are shown in **Table 1**. **Table 1** showed that the DCD liver weight was significantly higher than the control group, and the comparison between the two groups was statistically significant. There was no statistical difference in the comparison of other parameters.

The changes in transaminase levels in the two groups after surgery are shown in **Table 2**. There were no statistically significant differences in the transaminase levels of the two groups before the transplantation; however, on postoperative days 1, 3, 5, and 7, the transaminase level of the DCD group was statistically significantly higher than that of the control group.

Discussion

Liver transplantation is the only effective treatment for end-stage liver disease. With the development of increasingly sophisticated technology and new immunosuppressive agents, liver transplantation has already achieved very satisfactory results. However, the lack of donor sources severely limits the progress of this treatment. To solve the problem of donor shortages, the Ministry of Health of the People's Republic of China urged citizens to participate in organ DCD. In recent years, DCD donor liver transplantation has shown an increasing trend and, to some extent, has alleviated the problems of supply and demand.

However, owing to the high incidence of fatty liver among populations [15-17], drug therapy before donation, and other factors, such as parenteral nutrition and hypoxia, donor livers often demonstrate considerable steatosis. Furthermore, because of the long-term warm ischemia and short perfusion time of the tissue, as well as the release of many inflammatory mediators and oxygen free radicals that could damage the liver cells, the quality of the donor liver becomes affected. Consequently, early postoperative liver function recovery in the recipient is affected, increasing the incidence of graft dysfunction, biliary complications, and chronic rejection [5-9, 18].

Therefore, basic studies on DCD donor liver transplantation need to be performed on a stable animal model. To date, there has been no reported small animal model that could simulate the pathophysiological changes of clinical DCD. The traditional model of liver transplantation in rats could not reflect the steatosis, ischemia, and hypoxia that are present in DCD livers [10]. We first fed the experimental rats to induce severe fatty liver, and simulate the steatosis of clinical DCD before donation. Before liver resection, a pneumothorax was made to induce cardiac arrest; this process took about 9 min. Therefore, this model could simulate the pathophysiological process, from withdrawal of treatment to cardiac arrest, before clinical DCD donation. Meanwhile, 5 min after cardiac arrest, an abdominal aortic perfusion was started to end the warm ischemia time, thus simulating the warm ischemia time from clinical cardiac arrest to organ perfusion. Our experimental results showed that liver function was impaired severely when transplantation was performed according to the DCD program, which was consistent with the clinical manifestations of DCD liver transplantation. Therefore, this rat model with a steatotic donor liver after cardiac death could closely simulate the pathophysiological process, and could be used for basic studies on clinical DCD liver transplantation.

If bleeding in the recipient was >2 mL during the transplantation, the postoperative survival rate was significantly reduced. In particular, because the liver volume significantly increased in rats with fatty liver, the limited operating space and the brittle texture made it possible that a slight flip would tear the liver, thus causing liver rupture and bleeding after opening [19]. This requires a much more skilled microsurgical technique. Our experience showed that the liver should not be turned before perfusion; after the liver perfusion, the suprahepatic vena cava should be cut first, then the perihepatic ligaments should be freed from top to bottom. This operation provided a better surgical field, and could minimize the chance of turning the liver. Many authors [19-21] felt that rats have a high blood viscosity, and are hypercoagulable; thus, they advocated preoperative heparinization (25 U/mL, 2 mL) to prevent blood clots. Because the liver function recovery of steatotic DCD donor liver is slow, and the blood clotting function is poor, the overuse of heparin would be much more prone to causing postoperative anastomotic and wound bleeding; however, the absolute removal of heparin would easily induce microthrombosis inside the gastrointestinal tract of the recipient during the no-liver period. Our experience showed that low-dose heparin (25 U/mL, 0.5-1 mL) should be administered. Meanwhile, before donor liver perfusion, systemic heparinization (250 U/mL, 2 mL) should be performed; the perfusion fluid should not include heparin, thus reducing the amount of heparin that enters the recipient after the donor is opened. During the recipient transplantation, heparin-free saline should be used in rinsing the vessels to avoid the abdominal absorption of heparin. Lumbar venous bleeding was also a cause of surgical failure in the initial pre-experimental stage. Because the treatment of lumbar vein bleeding was neglected, intraperitoneal bleeding appeared postoperatively; thus, the survival rate was low. The lumbar vein might be joined to the right adrenal vein and co-imported into the infrahepatic vena cava, or it might be opened separately and imported. When the right adrenal vein is ligated during surgery, the ligature should pass by the lumbar vein, which should be ligated together with the right adrenal vein.

Disclosure of conflict of interest

None.

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