Cause analysis of early death after liver transplantation in rhesus monkey*☆

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Abstract

BACKGROUND: Various factors contribute to the establishment of liver transplantation models in rhesus monkey, the rate of successful operation and long-term survival are very low.

OBJECTIVE: To analyze the cause of early death following liver transplantation in rhesus monkey.

METHODS: Liver transplantation models were fabricated with the classical and modified methods in rhesus monkeys. Operation of donor was performed quickly by a big crucial incision of abdomen. The improved double-cuff of the portal vein and inferior vena cava were finished, in addition to stay pipe of biliary tract in the process of repairing donor liver. Operation of the receptor was performed by classical orthotopic liver transplantation.

RESULTS AND CONCLUSION: A total of 25 pairs of rhesus monkeys were successfully for establishing liver transplantation models. Seven rhesus monkeys died within early stage of post-operation, including six out of nine monkeys died by using the classical approach and one out of sixteen monkeys died by using the improved approach. There were five of seven monkeys died of intra-abdominal hemorrhage, one died of primary graft nonfunction and one died of respiratory failure. Results indicated that, the major death cause after classical orthotopic liver transplantation in rhesus monkey is abdominal hemorrhage. The improved methods of liver transplantation apparently reduce the hemorrhage and raise early survival rate following liver transplantation.

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INTRODUCTION

Currently, ideal animal of liver transplantation preclinical studies is non-human primates[1-5]. However, many factors greatly affect the establishment of a repeatable and stable liver transplantation model in non-human primates[6-10]. Early death after liver transplantation in rhesus monkeys impedes experimental study regarding liver transplantation in rhesus monkey. This study aims to elucidate the cause for the early death after liver transplantation in rhesus monkey.

MATERIALS AND METHODS

Design

A single sample observation.

Time and setting

The experiment was performed from September 2009 to January 2010 in the Experimental Animal Center of Kunming Medical College, China.

Materials

Both donors and receptors were healthy rhesus monkeys, provided by the Experimental Animal Center of Kunming Medical College, China; donors: of either sex, weighing 5.3–8.1 kg; receptors: male, weighing 7.2–11.5 kg. All rhesus monkeys were raised in the cleaning animal room of Experimental Animal Center of Kunming Medical College, China, with free access to food and water. Donors were freely available to the water, but to limited amount of food prior to the operation; receptors were preoperatively deprived food for 12 hours and water for 6 hours; receptors were preoperatively treated with antibiotics cefazolin 0.1 g/kg. All experimental protocols on animals were in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of the People's Republic of China[11].

Methods

Donors and receptors

Liver transplantation models were established with the classical orthotopic approach in nine rhesus monkeys, and with improved approach in 16 rhesus monkeys. The classical orthotopic liver transplantation adopted direct anastomosis of the hepatic vein, portal vein and hepatic artery, as well as biliary stenting. The improved surgical procedures are summarized as below.

Donor sample and repair liver

Donor was intravenously injected with 0.5 mL/kg 30 g/L sodium pentobarbital saline at sterile condition. Operation of donor was performed quickly by a big crucial incision of abdomen. The liver was repaired with HTK solution at 4 ℃, suspicious tissues were ligated. The handle of self-made cannula was placed in the front of portal vein and inferior vena cava, respectively, and the tied ligature of spleen veins was turned inside out of the self-made cannula. Furthermore, the tied ligature was placed in the left of the self-made cannula; the same to inferior vena cava except that the tied ligature of right renal vein was placed in the right of the self-made cannula; the portal vein and inferior vena cava were washed with self-made perfusate respectively. Bile duct was implanted with 2-mm stent.

Receptor liver resection and liver transplantation[12-13]

Receptors were intravenously injected with 0.5 mL/kg 30 g/L sodium pentobarbital saline at sterile condition, then subcutaneously injected with 0.03–0.04 mg/kg APO. Operation of the receptor was

simultaneously performed when the liver began to repair. (1) After skin preparation and disinfection, a large incision was made in epigastric zone, followed by the dissociation of perihepatic ligament, suprahepatic and infrahepatic inferior vena cava; the first porta hepatis was anatomized (hepatic artery, portal vein and bile duct); excessive tissues around infrahepatic inferior vena cava was dissociated between the right renal vein and the right adrenal vein, at 0.5–1.0 cm long; right adrenal vein and lumbar veins were ligated using a 4-0 suture close to the post-hepatic inferior vena cava. (2) the blood in spleen and liver was removed according to the autotransfusion of liver transplantation in rats[14-16], and the liver was resected: portal vein and infrahepatic inferior vena cava were occluded, the portal vein was slowly injected with 60–100 mL sterile balanced solution, then the liver colored as yellowish brown, the blood was almost removed. Suprahepatic inferior vena cava was immediately blocked with portal vein clamp and neatly cut; a bell-mouth incision was trimmed at the bifurcation of the portal vein; hepatic tissues were suggested to retain in infrahepatic inferior vena cava to facilitate the cannula. (3) Donor liver implant: Classical orthotopic liver transplantation (double-cuff + stenting tube method). Suprahepatic inferior vena cava was continuously anastomosed with 5-0 prolene, portal vein cuff was utilized to connect the donor with the receptor, then the portal vein opened. After infrahepatic inferior vena cava effused blood, the cuff connected to the infrahepatic inferior vena cava, the clamps of suprahepatic and infrahepatic inferior vena cava were opened, indicating anhepatic period was over. The liver and gastrointestinal tract was rewarming with sterile 0.9% sodium chloride injection at 40–50 ℃, until the color returned to normal liver (rosy). Hepatic artery was reconstructed through donor-receptor common hepatic artery end-to-end anastomosis using 9-0 prolene suture line under 3.5 magnification; the common bile duct stent was implanted, the abdominal cavity was rinsed with warm salt, the abdomen was closed after no bleeding or bile leakage was seen.

Posttransplantation observation and treatment

The rhesus monkeys were observed for the activities, facial expression, food and water, as well as response to stimuli. Animals were dissected immediately after the death or near death, to analyze the death cause. The water drinking was available 2 hours postoperation and food available at 24 hours. Monkeys were fed in single cage at 22–25 ℃. Since the day of operation, monkeys were intramuscularly injected with 0.1 g/kg cefazolin sodium, twice per day, for 2 days. After operation, the monkeys were injected with 500–1 000 mL/d, to recruit colloid and sugar, thus maintaining electrolyte and acid-base balance in the body.

Main outcome measures

Death and its cause at early stage after transplantation (within 6 hours after the opening of occluded veins).

Statistical analysis

SPSS 17.0 statistical software was utilized for analysis of numeration data. The classical and improved methods were compared with FISHER exact probabilistic method, a level of $P < 0.05$ was considered statistically significant difference.

RESULTS

Quantitative analysis of experimental animals

Fifty rhesus monkeys served as the donors and receptors in this study, without any drop-loss, all were involved in the result analysis.

Early death after liver transplantation in rhesus monkeys

Among 25 pairs of the successful model of liver transplantation in rhesus monkeys, seven died at early stage (within 6 hours after the opening of occluded veins), with the mortality of 28% (7/25), including 6 adopted the classical approach (24%, 6/25), and 1 adopted the improved approach (4%, 1/25).

Cause of early death after liver transplantation in rhesus monkeys

Five cases died of abdominal bleeding, the mortality was up to 20% (5/25), accounting for 71% of total early deaths; in which 4 cases received the improved approach (16%, 4/25) and 1 case received improved approach (4%, 1/25). One case died of primary liver graft nonfunction, the mortality was 4% (1/25), accounting for 14% of total early deaths. One case died of pneumothorax-caused respiratory failure, the mortality was 4% (1/25), accounting for 14% of total early deaths. The early death in liver transplantation models in rhesus monkey established using improved method was significantly less than that of classical method ($P < 0.05$; Table 1).

Table 1 Comparison of the cause for early death in liver transplantation models in rhesus monkey (*n*)

Group	Models	Abdominal bleeding	Primary liver graft nonfunction	Pneumothorax-caused respiratory failure
Pre-improved	9	4	1	1
Improved	16	1	0	0
P		0.040	0.286	0.286

DISCUSSION

Liver transplantation model in rhesus monkeys is chiefly established with classical approach, because the post-hepatic inferior vena cava is entirely embedded and sneaked in liver parenchyma of rhesus monkeys, thus it is difficult to implement piggyback liver transplantation. This outcome was consistent with the reported surgical approach in monkey model of liver transplantation[1-2]. Rhesus monkeys is relatively weak and susceptible to early death, or can not live for the required age. The present experiment adopted double-cuff method which is widely used[17-18], thus significantly shortening the anhepatic period and reducing the incidence of portal vein bleeding and stenosis, the early death rate was significantly lower compared with the classical approach.

Abdominal hemorrhage after liver transplantation in rhesus monkey

This study showed that, abdominal hemorrhage was the main

cause for early death after liver transplantation in rhesus monkeys. It also dominates the death after reduced size liver transplantation in small animals, such as rats[19-22]. Many factors trigger abdominal hemorrhage, such as anastomotic bleeding of suprahepatic and infrahepatic inferior vena cava, followed by bleeding of reduced volume ligation, liver capsule staxis, right adrenal and lumbar veins bleeding, liver damage bleeding, portal vein and infrahepatic inferior vena cava canula bleeding[23]. In this study, abdominal hemorrhage in rhesus monkey after liver transplantation was a sequence of portal vein and infrahepatic inferior vena cava junction bleeding, especially bleeding in portal vein; other parts of abdominal hemorrhage were due to anastomotic bleeding of suprahepatic and infrahepatic inferior vena cava, liver bed bleeding, liver damage bleeding and liver capsule staxis, as well as right adrenal and lumbar veins bleeding. This was similar with the abdominal hemorrhage after liver transplantation in rats. Once the portal vein bleeding occurred, the majority of receptors in rhesus monkeys died within 6 hours after the opening of veins, and rarely able to live through 6 hours. Moreover, abdominal hemorrhage is characterized by multiple bleeding sites in the same receptor. This study found that, rhesus monkeys was weakly tolerant to the blood loss in liver transplantation, peripheral circulation disturbance may occur at 100 mL blood loss, coagulation function transferred from preoperative hypercoagulable state to postoperative low coagulation state[1-2]. This further affects the stability of rhesus monkey model of liver transplantation, so the careful hemostasis and the intraoperative and postoperative fluid supplement are very important for liver transplantation in rhesus monkeys. Abdominal hemorrhage after liver transplantation in rhesus monkeys is induced by many factors, so the successful establishment of liver transplantation in rhesus monkey depends on intraoperative and postoperative abdominal hemorrhage, calm management in each surgical step, excellent surgical techniques and experienced surgical technique (including microsurgical techniques), as well as tacit agreement between surgeons[23-24]. The classical approach adopted direct anastomosis, the surgical techniques were complicated, the operation cost long time, anhepatic period was significantly longer than that of improved method, thus resulting in systemic circulation and internal environment disorder in rhesus monkey after liver transplantation and the opening of portal vein, accordingly leading to dysfunction of blood coagulation and obvious bleeding of surgical wound. The present experimental results showed that, 44.4% of receptors dieing of abdominal hemorrhage within 6 hours after liver transplantation established with modified method in rhesus monkey, while only one case (6%) died after modeling with improved approach.

Other causes of early death after liver transplantation in rhesus monkey

Early death after liver transplantation in rhesus monkeys was also induced by primary liver nonfunction in 1 case and pneumothorax-caused respiratory failure in 1 case, and both occurred in the pre-modified model. Primary liver nonfunction may due to the edge of the donor liver (fatty liver, 50% fatty degeneration), great variation of the liver weight between the donor and the receptor, long anhepatic period (nearly 1 hour), and intraoperative blood loss. The transplanted liver does not work or work poorly after the opening of portal vein, no bile flew out when the abdomen was closed, liver function was extremely poor after surgery, bilirubin levels were high, indicating liver failure. Sigh-like breathing was obvious upon death, accompanied with dilated pupils, cardiac arrest followed the cessation of breathing. Liver autopsy found that the liver was poorly color, a large number of abdominal ascites (no bleeding) were observed, gastrointestinal tract showed apparent flatulence. One case suffered from the intraoperative pneumothorax caused by the diaphragm shear due to incorrect surgical operations, and died of respiratory failure although the remedial measures were given timely. There were a variety of factors contributing to the death, among them the intraoperative pneumothorax is very important predisposing factor. Autopsy found that the liver was normal, no abdominal hemorrhage or significantly abdominal ascites were observed, diaphragm bulged to abdominal cavity and gas flew when the chest opened, the bilateral lung showed poor performance of expansion after significant compression. Compared with rats[25], monkeys and other large animals are more difficult to establish stable model of orthotopic liver transplantation[10, 26-28]. The following issues should be noted to improve the early survival rate of rhesus monkey model of liver transplantation: (1) the liver is ensured in good quality, liver edge is not used. (2) The weight of donors and receptors is not greatly varied, otherwise donor liver can not enough work to meet the receptor requirements. (3) The surgery should be carefully operated, intraoperative bleeding is reduced as much as possible, the blood is removed thoroughly; while reducing damage to other organs and tissues. (4) The modified vascular cuff method tries to shorten the anhepatic period and surgical anesthesia time, thereby minimizing the influence on the body circulation and interval environment. (5) Perioperative management is also very important. Analyzing the cause for the early death after liver transplantation in rhesus monkeys can guide us to further improve the rhesus monkey model of liver transplantation, thus promoting surgical success rate and early survival rate of models; also it provides the necessary preconditions for experimental study based on rhesus monkey models of liver transplantation.

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恒河猴肝移植后早期死亡的原因分析*☆

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摘要

背景：影响恒河猴肝移植模型建立的因素较多，模型的成功率和长期生存率较低。

目的：分析恒河猴肝移植后早期死亡的原因。

方法：实验采用改良前和改良后两种方法建立恒河猴肝移植模型。改良后供体采用腹部“十”字形大切口进行快速切取供肝，在修肝时将肝腔静脉、门静脉套管和胆道支撑管留置好，受体采用经典式原位肝移植+二袖套法+胆道支撑管建立稳定的恒河猴肝移植模型。

结果与结论：成功实施的 25 对恒河猴肝移植模型中，早期死亡 7 只，其中应用改良前方法移植的 9 只中死亡 6 只，用改良后方法移植的 16 只中死亡 1 只。死亡 7 只中因腹腔出血而死亡 5 只，原发性肝脏无功能死亡 1 只，气胸导致呼吸衰竭死亡 1 只。结果表明，恒河猴肝移植后早期死亡的主要原因是腹腔出血；改良后的恒河猴肝移植方法对减少肝移植后出血有明显效果，提高了肝移植后早期生存率。

关键词：恒河猴；大动物模型；肝移植；死亡；腹腔出血；器官移植
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利益冲突：课题设计为第一作者，动物模型制作由第一至七作者完成，第八作者为指导者；数据的收集和统计由第二，七作者完成；实验的评估和论文写作由第一、二作者完成。采用盲法评估。

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本文创新性：

提供证据：检索 CNKI 数据库和 Kjmed.com.cn(西文生物医学期刊文献数据库)，检索时间范围 1987-01/2010-12；检索关键词为：肝移植，恒河猴；liver transplantation, Rhesus Monkey，或者为 Monkey。检索到 2 篇国内文献与模型建立有关的报道，其中 1 篇为本课题文章，另外 1 篇为其他作者文章。检索结果没有对于恒河猴肝移植模型的早期死亡原因分析的报道。提示本课题文章在国内外处于领先地位。

创新性说明：文章分析了恒河猴肝移植后早期死亡的原因主要是腹腔出血，结果表明改良后的恒河猴肝移植的方法对于减少肝移植术后出血有较明显的效果。查阅国内外的有关文献，均未见到有相关的报道。