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# Causes of Death of Rhesus Monkeys Undergoing Liver Transplantation

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## 1. Introduction

As non-human primates have similar pathophysiological reactions to humans, experimental data evaluating acute rejection reactions following liver transplantation in rhesus monkeys are clinically significant. However, the success rate and long-term survival rate are low, and post-transplant death is one of the major factors influencing survival in rhesus monkeys undergoing experimental liver transplantation. Non-human primates provide the ideal model for clinical studies of liver transplantation. A large number of factors can influence the establishment of a stable and reproducible non-human primate model. Post-transplant death is a major problem in experimental liver transplantation in rhesus monkeys. This study investigates causes of rhesus monkey death following liver transplantation.

## 2. Materials and methods

### 2.1 Materials

Healthy rhesus monkeys provided by the Laboratory Animal Center of Kunming Medical University were used as donor and recipient. Recipients were male rhesus monkeys weighing 7.2–11.5 kg, and donors were of either gender weighing 5.3–8.1 kg. The animals were housed in the Laboratory Animal Center of Kunming Medical University, and were allowed free access to food and water. Food access for donors was restricted, and recipients were starved of food for 12 hours and of water for 6 hours preoperatively. Recipients were given cefazolin sodium (0.1 g/kg) before transplantation. Experimental procedures were performed in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of the People's Republic of China.

### 2.2 Surgery for donor and recipient animals

We operated on 9 rhesus monkeys using our original surgical model, and then modified our model for the remaining 16 monkeys. In the original model, the hepatic vein, portal vein and hepatic artery were directly anastomosed, and a supporting tube was placed in the biliary tract. The modified model is described below.

### 2.3 Donor surgery and liver perfusion

Donor animals were anesthetized by intravenous injection of 3% pentobarbital sodium dissolved in normal saline (0.5 mL/kg). Under sterile conditions, a large, crucial incision was made in the abdominal wall and the liver was harvested. One cannula was placed in the portal vein and another was placed in the inferior vena cava below the liver. The splenic and renal veins were ligated. The liver was perfused with HTK solution at 4 °C, and bleeding tissues were ligated. A 2 mm diameter supporting tube was placed in the bile duct.

### 2.4 Recipient surgery

Recipient animals were anesthetized by intravenous injection of 3% pentobarbital sodium dissolved in normal saline (0.5 mL/kg), followed by subcutaneous injection of atropine (0.03–0.04 mg/kg). Recipient surgery was undertaken while the donor liver was undergoing Histidine- Tryptophan- Ketoglutarate (HTK) perfusion. Briefly, a large, cross-shaped incision was made in the abdominal wall. The perihepatic ligaments and inferior vena cava above and below the liver were separated from adjacent structures. The hepatic artery, portal vein and biliary tract were separated at the porta hepatis. Tissues surrounding the inferior vena cava below the liver between the right renal and right adrenal veins were separated over 0.5–1.0 cm. The right suprarenal and lumbar veins were ligated adjacent to the inferior vena cava using a 4-0 suture. Blood was collected from the liver according to the autotransfusion method described for rat liver transplantation. The inferior vena cava below the liver and the portal vein were clamped, and 60–100 mL of sterile balanced salt solution was slowly injected into the portal vein until the liver became khaki in color. The inferior vena cava above the liver was then immediately clamped. The inferior vena cava above the liver was cut adjacent to the liver, and was trimmed into a bellmouth shape at the bifurcation of the portal vein. The inferior vena cava was cut below the liver with some liver tissue included. The donor liver was transplanted using standard orthotopic liver transplantation techniques (double-cuff and one support tube). The inferior vena cava above the liver was anastomosed using 5-0 prolene, the cuff of the portal vein was anastomosed and the portal vein was declamped. When blood was observed flowing from the inferior vena cava below the liver, the cuff was anastomosed. The inferior vena cava above and below the liver were declamped to terminate the anhepatic phase. The liver and gastrointestinal tract were perfused with 0.9% sodium chloride injection at 40–50 °C for rewarming until the color of the liver was restored. The common hepatic artery was anastomosed and a supporting tube was placed in the common bile duct. The abdominal cavity was washed with warm saline. If no hemorrhage or bile leakage was detected, the abdominal wall was closed.

### 2.5 Postoperative observation and treatment

Animal activities, facial expressions, food and water intake and reactions to stimulation were observed. Animals who died were immediately dissected to obtain samples and to analyze the cause of death. Each monkey was caged separately at 22–25 °C and was allowed access to water after 24 hours and food after 48 hours. Intramuscular cefazolin sodium (0.1 g/kg) was administered twice a day for 2 days. Colloid and sugar water (500–1 000 mL per day) was administered postoperatively to maintain electrolyte and acid-base balance.

### 3. Conclusion

We successfully performed liver transplantation in 25 pairs of rhesus monkeys. In the early postoperative period (within 6 hours after portal vein opening), seven animals (25%) died; five (20%) due to abdominal hemorrhage, one (4%) due to primary nonfunction and 1 (4%) due to pneumothorax-induced respiratory failure. In the short-term postoperative period (12–72 hours after portal vein opening), seven animals (28%) died; one due to hyperacute rejection within 12 hours, one due to hyperacute rejection and arterial thrombosis within 12 hours, one due to pulmonary infection and one due to accidental death at 72 hours. In the long-term postoperative period (> 72 hours after portal vein opening), eleven animals (44%) died; six due to acute rejection, three due to arterial thrombosis and two due to pulmonary infection. Abdominal hemorrhage occurred mainly in the early and short-term postoperative periods, and acute rejection occurred mainly in the long-term postoperative period.

### 4. Discussion

Liver transplantation in the rhesus monkey frequently uses the classical model because the inferior vena cava is embedded in the parenchyma of the posterior segment of the liver, making the anatomy unsuitable for piggyback liver transplantation. The rhesus monkey is fragile and often dies early following experimental transplantation. In this study, seven animals (66.7%) died within 6 hours after portal vein opening, seven died at 12–72 hours and eleven died at > 72 hours. The double-cuff method has been used extensively in established liver transplantation models. This model significantly shortens the duration of the anhepatic phase, decreases the incidence of portal vein bleeding and stenosis, and decreases the incidence of early death.

#### 4.1 Abdominal hemorrhage in rhesus monkeys following liver transplantation

In this study, the main cause of death was abdominal hemorrhage in the early postoperative period (within 6 hours after portal vein opening). In small animals such as rats, abdominal hemorrhage is also the major cause of death after reduced-size liver transplantation. This bleeding is often from the inferior vena cava anastomosis above the liver but has also been observed from the ligation points, liver capsule, right adrenal vein, lumbar veins, portal vein and inferior vena cava below the liver. In this study, abdominal hemorrhage was most often from the anastomoses of the portal vein and the inferior vena cava below the liver, and was also observed from the anastomosis of the inferior vena cava above the liver, liver bed, liver capsule, right adrenal vein and lumbar veins, similar to the bleeding points observed in rats. Abdominal hemorrhage sometimes involved multiple sites in one animal. In this study, the animals did not tolerate bleeding well and showed signs of decreased peripheral circulation after a blood loss of 100 mL. Animals with abdominal hemorrhage commonly died within 6 hours after portal vein opening. Rhesus monkeys may also have a preoperative hypercoagulable state and a postoperative hypocoagulable state, greatly influencing the stability of this model. Hemostasis and fluid balance are therefore very important for successful liver transplantation in the rhesus monkey. As a variety of factors contribute to the development of abdominal hemorrhage, surgeons should be familiar with the surgical procedures used including microsurgical techniques. Our original model prior to

modification used direct anastomosis, which is a complex and time-consuming process, increasing the duration of the anhepatic phase and resulting in circulatory and other systemic problems following portal vein opening. Liver transplantation may also lead to coagulation disorders, resulting in wound hematoma. In this study, seven animals (28%) died of abdominal hemorrhage following transplantation; five (71.4%) in the early postoperative period and two (28.6%) in the short-term postoperative period.

#### 4.2 Rejection

Hyperacute rejection occurs within 24 hours after anastomosis of the major vessels. It is a complement-mediated response caused by the recipient having pre-existing IgM antibodies to donor antigens (such as ABO, platelet and HLA antigens). Hyperacute rejection of unmatched blood type is mainly mediated by IgG antibodies. In this study, hyperacute rejection caused two deaths. Acute rejection is the most common rejection reaction after transplantation. It is clinically manifested by fever, general malaise, pain, transplant swelling and functional impairment. CD4 Th1 cell-mediated delayed hypersensitivity is the main cause of transplant injury. Transplant HLA stimulates T lymphocyte differentiation and proliferation in the recipient, producing large numbers of sensitized lymphocytes which can damage or kill target cells by releasing lymphokines. In this study, acute rejection caused six deaths in the long-term postoperative period. CD4+T and CD8+T cells may change significantly in acute rejection, followed by worsening of liver function and sometimes death. The pathological characteristics of acute rejection are: (1) inflammatory cell infiltration of the portal area, including activated lymphocytes, neutrophils and eosinophilic granulocytes, (2) endothelial cell inflammation of the portal vein or central vein and (3) bile duct inflammation and injury. Animals with at least two of these characteristics in association with liver dysfunction can be diagnosed with acute rejection. Inflammatory cell infiltration involving > 50% of the bile duct or involving the portal area or central vein are evidence of acute rejection.

#### 4.3 Hepatic artery thrombosis

Studies have reported that hepatic artery anastomosis in a rat model of orthotopic liver transplantation does not influence survival rate or survival time. However, hepatic artery anastomosis is critical in clinical liver transplantation, because hepatic artery thrombosis can cause transplant loss in a short period of time, requiring emergency surgery or even repeat transplantation. Dissection of rhesus monkeys shows that the outer diameter of the proper hepatic artery is 3–4 mm and the inner diameter is 1–2 mm, supplying a large amount of blood to the liver. Liver parenchyma and bile duct necrosis may therefore occur if the hepatic artery is not reconstructed, affecting post-transplant acute rejection and decreasing the survival rate. The cut end of the common hepatic artery can be trimmed into a bellmouth shape to allow full anastomosis. This simplifies the arterial anastomosis for clinical liver transplantation. In this study, the hepatic artery was reconstructed using microsurgical techniques. There were no deaths due to hepatic artery thrombosis or stenosis in the early postoperative period, but hepatic artery thrombosis caused one death in the short-term postoperative period and three deaths in the long-term postoperative period. Hepatic artery thrombosis is mainly caused by the following factors: (1) the artery is very thin and the intima is fragile and easily injured during surgery, which significantly increases the

incidence of thrombosis, (2) the rhesus monkey has a relatively low body mass, (3) a hypercoagulable state is common in the rhesus monkey and (4) rejection causes damage to the tunica intima, resulting in degeneration or necrosis and subsequent arterial thrombosis.

#### **4.4 Pulmonary infection**

Pulmonary infection is one of the causes of early death following reduced-size liver transplantation in rats, and may be due to an infection focus prior to surgery or to aspiration during surgery. Kamada et al proposed that an anhepatic phase of 26 minutes was safe. Shortening of the anhepatic phase to restore organ perfusion and maintain hemodynamic function is one method to reduce the rate of pulmonary infection following reduced-size liver transplantation, as pulmonary infection is associated with prolonged blood vessels clamping. Prolonged clamping of the portal vein causes prolonged intestinal tract congestion, increasing the likelihood of enteric bacteria entering the circulation and of inflammation-induced lung injury. Preoperative intramuscular atropine to reduce respiratory secretions, small tidal volume anesthesia to reduce aspiration and comfortable living environment and surgical conditions can help to prevent pulmonary infection in rats. In this study, pulmonary infection caused one death in the short-term postoperative period and two deaths in the long-term postoperative period.

#### **4.5 Other causes of death following liver transplantation**

One animal in this study died due to primary nonfunction and one due to pneumothorax-induced respiratory failure. Both these animals underwent transplantation using our original surgical model. The primary nonfunction may have been due to the significant fatty degeneration (> 50%) of the donor liver and the differences in weight between donor and recipient causing microhepatia. This animal underwent an anhepatic phase of approximately 1 hour with significant blood loss after portal vein opening, and deteriorated postoperatively with high bilirubin levels, hypoventilation, mydriasis, respiratory arrest and cardiac arrest. Autopsy showed no bleeding, a large amount of ascites in the abdominal cavity and gaseous distension of the gastrointestinal tract. In one animal, the diaphragm was damaged during surgery, resulting in pneumothorax. This animal died due to respiratory failure despite attempted treatment. Autopsy showed a normal liver, no abdominal bleeding or ascites, a bulging diaphragm, gas in the abdominal cavity and collapse of both lungs.

Compared with the rat model, establishment of an orthotopic liver transplantation model in large animal such as the monkey is more difficult. There are some important issues to consider to improve animal survival rate following liver transplantation. The quality of the donor liver is a key factor, and donation of an unhealthy liver is not appropriate. The weight of recipient and donor livers should be similar for the donor liver to function well. Intraoperative blood loss and injury to tissues and organs should be minimized. The modified cuff technique can minimize the duration of the anhepatic phase and of anesthesia, reducing circulatory and other systemic problems. Care should also be taken in perioperative management. This study analyzes the causes of death of rhesus monkeys at different stages following liver transplantation, which can help to modify models of liver transplantation to improve survival rate and to increase the quality of future experimental studies.

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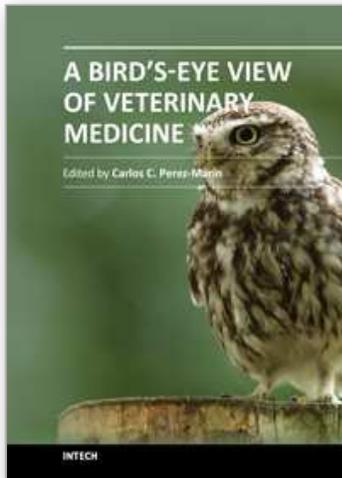
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