

Original Article

Warm ischemia may damage peribiliary vascular plexus during DCD liver transplantation

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Abstract: Biliary complications cause significant morbidity and mortality in liver transplantation. Warm ischemia can induce biliary duct injury. This study aimed to investigate the effects of warm ischemia on the peribiliary vascular plexus in rat liver transplantation. A total of 102 Sprague-Dawley rats were divided into three groups: sham-operation group, non-ischemic group, and ischemic group. Liver transplantation was performed in both the non-ischemic group and the ischemic group. The animals were sacrificed on day 1, 3, 7, and 14 to collect the blood and liver samples. Serum levels of bile duct obstruction, viz, alkaline phosphatase and gamma-glutamyl transpeptidase, as well as direct and indirect bilirubin were measured. Liver biopsy samples were examined with hematoxylin-eosin staining and transmission electron microscopy. The levels of enzymes and bilirubin were significantly higher in the ischemic group than the non-ischemic group and sham-operated animals ($P < 0.05$), with return to normal levels in the ischemic group after two weeks. Morphological examination showed microthrombi and endothelial damage in the bile ducts and the peribiliary vascular plexus of the ischemic group. Warm ischemia/reperfusion injury can damage the endothelium of the peribiliary vascular plexus, which might compromise the bile duct microcirculation and lead to ischemic cholangiopathy after liver transplantation.

Keywords: Liver transplantation, warm ischemia, reperfusion injury, liver microcirculation, BHD, DCD

Introduction

Liver transplantation is often the only effective treatment for end-stage liver disease and acute liver failure. However, ischemic cholangiopathy is still an important complication after liver transplantation, causing significant morbidity and mortality [1]. Ischemia/reperfusion injury to the bile ducts is a major contributing factor of bile duct complications post-transplantation [2-7].

The bile ducts are supplied by the peribiliary vascular plexus, which arises from the hepatic artery [8]. Several recent studies found that warm ischemia/reperfusion injury can cause bile duct injury in rat liver transplantation [9-12]. However, the specific mechanisms underlying the biliary injury caused by warm ischemia are still unclear. It has been shown that the bile duct epithelium is sensitive to ischemia/reperfusion injury [13, 14]. Therefore, we hypothesized that ischemia/reperfusion injury

during liver transplantation has the potential to induce epithelial damage, leading to compromised microcirculation and bile duct complications.

In the present study, we established a model of warm ischemia/reperfusion injury in rat liver transplantation, similar to donation after cardiac death (DCD) model, and evaluated its effects on the epithelium of bile ducts and peribiliary vascular plexus to understand the mechanisms underlying ischemic cholangiopathy after liver transplantation.

Materials and methods

Animals

A total of 102 male Sprague-Dawley rats (190-250 g) were provided by the Laboratory Animal Center of Fujian Medical University. The study protocol was approved by the Ethics Committee of the PLA 180 Hospital. The rats were kept

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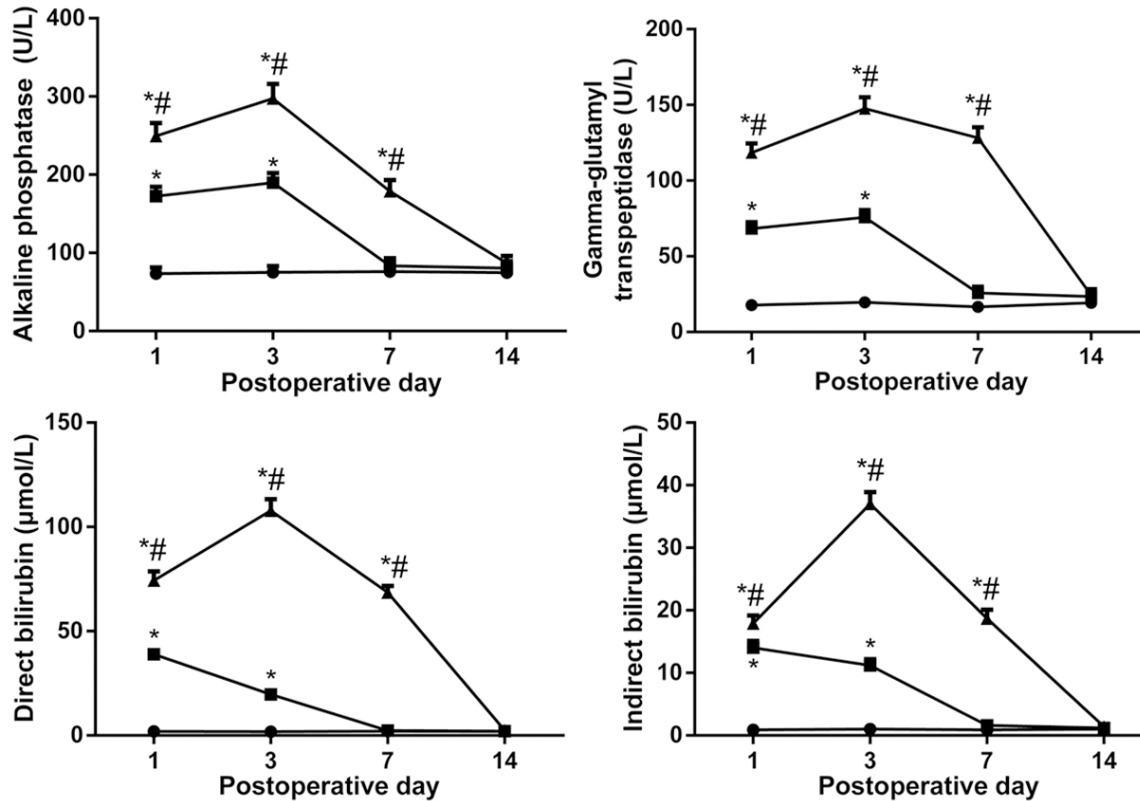


Figure 1. Both the ischemic and non-ischemic groups showed significantly increased levels of ALP, GGT, DBIL, and IBIL compared with the sham-operation group. The ischemic group also had higher levels of enzymes and bilirubin than the non-ischemic group. ▲ Ischemic group; ■ non-ischemic group; ● sham-operation group. *, vs the sham-operation group, $P < 0.05$; #, vs the non-ischemic group, $P < 0.05$.

under a 12 h/12 h light/dark cycle with free access to rat chow and water. Animals were randomly divided into three groups: a sham-operation group ($n=6$), non-ischemic group representing beating heart donor (BHD) model ($n=48$), and ischemic group representing donation after cardiac death (DCD) ($n=48$).

The sham-operation group underwent laparotomy and liver ligament dissection. In both the non-ischemic and ischemic groups, half of the group was used as the donor and the other half as the recipient of liver transplantation.

In the ischemic group, a median incision was placed in the chest to expose the heart. The heart base was clamped to induce cardiac arrest and warm ischemia for 10 min. Then in both the non-ischemic and ischemic groups, a median incision was placed in the abdominal wall. Ringer's solution 30 ml (including heparin 25 U/ml) was slowly infused through the abdominal aorta. The thoracic aorta was clamped and the inferior vena cava was transected.

Liver transplantation was performed according to the procedures described by Noack K [13] and Lahmann TG [15, 16]. No animals died during the study. The rats were sacrificed on postoperative day 1, 3, 7, and 14 to collect the blood and liver samples.

Serum analysis

From each animal, 4 ml blood was collected from the inferior vena cava during harvesting of tissues. The blood was centrifuged to obtain serum. Levels of alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), direct bilirubin, and indirect bilirubin were measured with an automatic biochemistry analyzer (HITACHI 7150, Japan).

Morphological examination

The liver samples were fixed in 10% formalin for 24 h and embedded in paraffin. The tissue blocks were cut into 3-μm sections for hematoxylin-eosin (HE) staining and fibrin staining (modified MSB methods). For transmission

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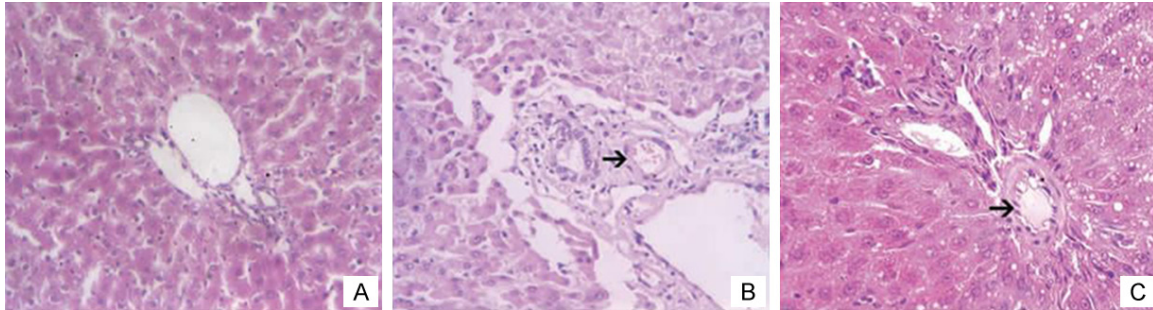


Figure 2. HE staining of the liver on postoperative day 1. A. Normal liver histology in the sham-operated rats (100×). B. The non-ischemic group showed only red blood cell clot (arrow, 400×). C. The ischemic-group showed microthrombi in addition to red blood cell clot (arrow, 400×).

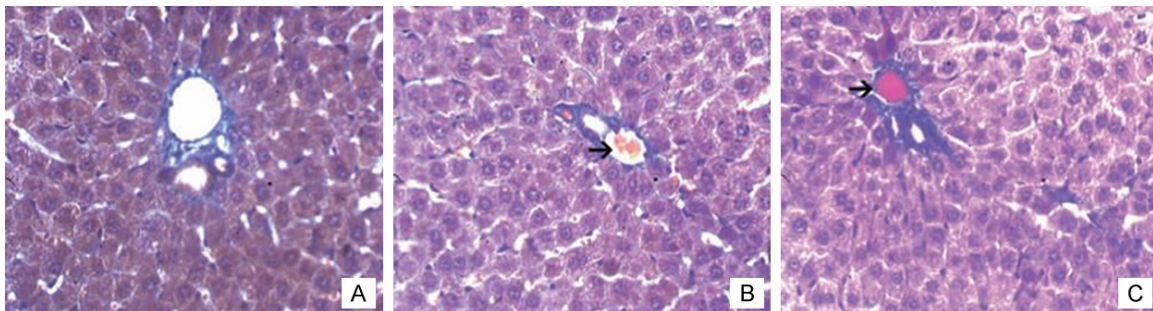


Figure 3. Fibrin staining of the liver on postoperative day 1. A. Normal structure of the peribiliary vascular plexus and bile ducts (400×). The collagen was stained blue and the nuclei was blue-black. B. Intravascular red blood clot was seen in the non-ischemic group (arrow, 400×). Red blood cells were stained yellow. C. Microthrombi were stained red in the ischemic group (arrow, 400×).

electron microscopy, the liver samples were fixed in 2.5% glutaraldehyde at 4°C, embedded, and sectioned, and then observed under an electron microscope (JEOL JEM-1400, Japan).

Statistical analysis

The continuous data were expressed as mean \pm standard deviation (SD). Data was analyzed using SPSS 13.0 software (SPSS, USA). Comparisons among the groups were made using analysis of variance, followed by the Fisher's least significant different test. A *P*-value less than 0.05 was considered statistically significant.

Results

Levels of enzymes and bilirubin

Both the ischemic and non-ischemic groups showed significantly increased levels of ALP, GGT, direct and indirect bilirubin compared with

the sham-operation group ($P < 0.05$) (**Figure 1**). In addition, enzymes and bilirubin in the ischemic and non-ischemic groups had delayed return to normal levels (postoperative day 14) compared with the sham-operated rats (postoperative day 7). This intriguing observation probably resulted from initial defects in the biliary tree, which were ameliorated over the course of two weeks.

Pathological changes

No significant change was observed in the peribiliary vascular plexus of the sham-operation group (**Figure 2A**). However, in the non-ischemic group, swollen epithelium in the bile ducts and the peribiliary vascular plexus and intravascular debris likely representing clots were seen (**Figure 2B**). In the ischemic group, in addition to the swollen epithelium, cell necrosis and apoptosis were also seen, with red blood cell clot and microthrombi (**Figure 2C**). No thrombus was seen in the hepatic artery and its large branches (data not shown). Fibrin staining

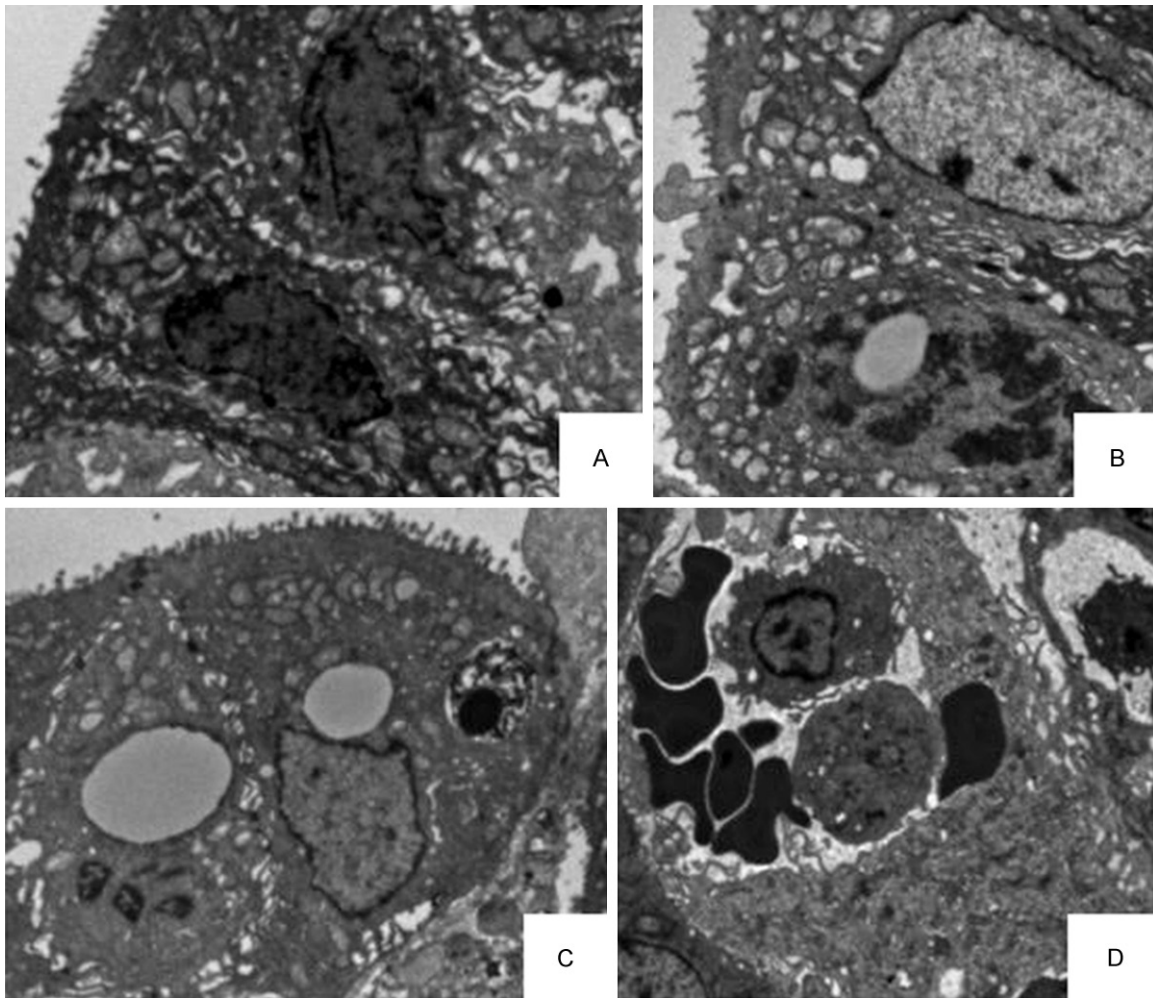


Figure 4. Transmission electron microscopy of the liver on postoperative day 3. A. Normal microstructures of the bile duct epithelium (5000 \times). B. Swollen mitochondria were seen in the non-ischemic group but without red blood cell blot or microthrombi (5000 \times). C. Significant microstructural changes were seen in the epithelium of the ischemic group, such as lipid droplets, karyopyknosis, and swollen mitochondria (5000 \times). D. Microthrombi was seen in the ischemic group together with abundant red blood cells and neutrophils.

showed that the microthrombi in the ischemic group were hyaline thrombi (**Figure 3C**), while the fibrin staining was negative in the sham-operation group and the non-ischemic group (**Figure 3A, 3B**).

The transmission electron microscopy results were consistent with that of histological staining. No significant microstructural change was seen in the sham-operation group (**Figure 4A**). Microstructural changes were seen in both the ischemic and non-ischemic groups (**Figure 4B-D**).

Discussion

Warm ischemia/reperfusion injury has been suggested to cause bile duct complications

after liver transplantation. Bile duct injury after liver transplantation might be associated with compromised microcirculation caused by the warm ischemia [17]. In this study, we investigated the levels of serum enzymes and bilirubin and the peribiliary vascular plexus structure in a DCD model and compared with BHD and sham-operated conditions. It was found that warm ischemia induces epithelial damage in the bile ducts and the peribiliary vascular plexus, resulting in intravascular microthrombi and compromised microcirculation of the bile ducts. This might contribute to the underlying mechanisms for ischemic cholangiopathy after liver transplantation.

The liver enzymes and bile duct enzymes showed differential trends after liver transplan-

tation. The serum levels of aspartate transaminase and alanine aminotransferase peaked within hours to 2 days after liver transplantation, and returned to normal levels around postoperative 1 week. However, the serum bile duct enzymes such as GGT and total bilirubin maintain at high levels until postoperative 2 weeks, suggesting a prolonged reperfusion injury in the bile ducts.

The association between warm ischemia/reperfusion injury and bile duct injury has been suggested [3-10]. However, the specific mechanisms underlying the biliary damage are not clear. Peribiliary vascular plexus is the terminal vascular network nourishing the bile ducts [8]. In the present study, we found that warm ischemia can induce epithelial damage in the peribiliary vascular plexus and microthrombi formation. The microthrombi may lead to compromised microcirculation of the biliary tree, and subsequent epithelial damage in the bile ducts and elevated bile duct enzymes. This might also explain the severe bile duct complications after liver transplantation such as bile duct necrosis and cholestasis, which are important reasons for non-anastomotic biliary strictures.

A surprising finding was the return of the enzymes like GGT to normal after two weeks. This likely resulted from healing of the initial pathophysiology that might be attributable to the biliary ducts. This may have resulted either from resolution of the microthrombi or regenerative capacity of the bile ductules. Further studies are required to resolve these issues.

The biliary tree undergoes the first and the second warm ischemia during liver transplantation. The first ischemia happens when the liver is harvested and therefore is generally inevitable. Then, the portal venous recanalization to hepatic arterial recanalization induces the second warm ischemia to the biliary tree. This is a special phase of biliary warm ischemia in the graft and induces relatively more severe damage to the biliary tree than the first warm ischemia. Due to the inevitability of the first warm ischemia in the harvesting of donor livers, the second warm ischemia and its mechanisms are of clinical importance for developing possible treatment solutions [18].

In conclusion, this study demonstrated that warm ischemia induces epithelial damage in

the peribiliary vascular plexus, compromising the microcirculation of the biliary tree and damaging the bile duct epithelium, finally leading to ischemic cholangiopathy after liver transplantation. These findings may help to find novel therapeutic methods for biliary complications after liver transplantation.

Disclosure of conflict of interest

None.

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