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A novel *in situ* model of liver cold ischemia–reperfusion in rats

Rui Li, MD,[a] *Yuetang Mi, MD, PhD,*[a] *Gang Tan, MD, PhD,*[b] *Wei Zhang, MD,*[a] *Guixia Li, MD,*[a] *and Xueying Sun, MD, PhD*[b,*]

[a] *Department of General Surgery, Liaocheng People's Hospital, Liaocheng, China*
[b] *Department of General Surgery, The Hepatosplenic Surgery Center, The First Affiliated Hospital of Harbin Medical University, Harbin, China*

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ABSTRACT

Background: Orthotopic liver transplantation (OLT) is being used for studying cold ischemia reperfusion (I/R)-induced injury in experimental animals, but the technique is complicated and it does not accurately reflect the pathophysiology. Here, we report a novel model, termed "*in situ* liver cold ischemia (ISLCI)", in Wistar rats.

Methods: ISLCI was achieved in rats by establishing a portal-jugular shunt and a cannula shunt in inferior vena cava, and the liver was continuously perfused with lactate Ringer's solution at a speed of 150 mL/h through the portal vein for 60 min. Portal venous pressure, serum levels of total bilirubin, alkaline phosphatase, alanine aminotransferase and γ-glutamyl transpeptidase (GGT), and hepatic histopathology were examined, and compared with rats undergoing OLT, in which the donor liver was subjected to a 60 min cold ischemia.

Results: Livers from ISLCI and OLT rats showed histopathologic changes characteristic of I/R-induced injury when examined on days 1 and 7, with complete recovery 14 d after reperfusion. Compared with OLT rats, ISLCI rats had significantly lower levels of portal venous pressure 1 and 10 min after porta hepatis clamping. They suffered a milder degree of I/R-induced hepatic injury, reflected by significantly lower levels of GGT, alanine aminotransferase, and alkaline phosphatase on day 1, and a significant lower level of GGT and a lower histopathologic score on day 7 after reperfusion.

Conclusions: Our preliminary results indicate that the ISLCI model is reliable and technically easier, and is superior to OLT for studying cold I/R injury.

1. Introduction

During liver transplantation, the liver suffers from cold ischemia reperfusion (I/R)-induced injury, which affects patient outcomes. OLT is being used as an *in vivo* animal model for studying the pathophysiology of cold I/R injury [1,2]. However, the defects of this model are obvious as the technique of OLT is complicated and the rate of fatal complications is relatively high. Here, we report a novel rat model, termed "*in situ* liver cold ischemia (ISLCI)", in which an extracorporeal portal-jugular shunt and a cannula shunt in the inferior vena cava (IVC) were established without surgical removal of the liver, and the liver was continuously perfused with lactate Ringer's solution through the portal vein for 60 min.

* *Corresponding author*. Department of General Surgery, The Hepatosplenic Surgery Center, the First Affiliated Hospital of Harbin Medical University, 23 Youzheng Street, Harbin 150001, China. Tel./fax: +86 451 53643628.
E-mail addresses: kevsun88@hotmail.com or k.sun@auckland.ac.nz (X. Sun).
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2. Materials and methods

2.1. Animal experimental design

Male Wistar rats (200–250 g in weight) were supplied by the Animal Research Center at the First Affiliated Hospital of Harbin Medical University, Harbin, China. The animals were housed in cages under pathogen-free conditions at a temperature of 20°C–25°C, and fed commercial pellets and water *ad libitum*. This study had been approved by the Animal Ethics Committee of Harbin Medical University under a permit of animal use (SYXK20020009). All the animals were euthanized with an intraperitoneal injection of a lethal dose of pentobarbital (150 mg/kg).

Ten rats undergoing sham laparotomy were sacrificed at the end of surgery. Forty-two rats were used for ISLCI, and two of them were sacrificed due to hemorrhage during surgery. Ninety-six rats (48 donors and 48 recipients) were used for OLT, and eight of the recipients were killed due to failure of operation (five cases) and hemorrhage (three cases). Therefore, the ISLCI and OLT groups had 40 rats each. Rats were given liquid paracetamol (120 mg/5 mL in a water bottle) to induce preemptive analgesia 24 h before surgery. They were fasted for 12 h before surgery, but allowed free access to water. All surgical procedures were performed under an aseptic condition and ether anesthesia. Buprenorphine was subcutaneously administered to rats at a dose of 1 mg/kg every 12 h for 3 d after surgery. Ten rats were randomly sacrificed from the ISLCI and OLT groups at the end of surgery, and on days 1, 7, and 14 after surgery, respectively, and livers and blood samples were collected. Serum levels of total bilirubin (TIBL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT) were measured. Liver specimens were histologically analyzed with a scoring system as described previously [3].

2.2. Orthotopic liver transplantation

Syngeneic OLT was performed as described previously [4,5]. The infra-hepatic vena cava (IHVC) was reestablished by the cuff method. The total cold ischemic period (4°C) of donor livers was 60 min before being transplanted into recipients without hepatic artery reconstruction.

2.3. In situ liver cold ischemia

One end of a 100-mm-long polyethylene tube (1.0-mm internal diameter) was inserted into the jugular vein, and the tube was temporarily clamped with a vascular clip. Midline laparotomy was made and the liver skeletonized with the ligation of all identifiable collateral vessels. The supra-hepatic vena cava (SHVC) was isolated, and a 30-cm 1/0 silk placed beneath the SHVC (Fig. 1A). The IHVC was isolated for a length of 6–7 mm. The other end of the tube was inserted into the portal vein, and secured with a silk tie. The clip on the tube was released to open the portal-jugular shunt. A 3-mm venotomy was made longitudinally on the IHVC between the liver and the right renal vein, and a 30-mm-long polyethylene tube (2.0-mm internal diameter) was inserted into the IHVC through the incision to establish an IVC shunt. The upper end of the tube extended above the hepatic superior margin for 3 mm. Two ties were made around the IHVC above and below the incision, letting the blood from the IVC return to the heart through the lumina of the tube (Fig. 1A). After the porta hepatis was clamped, the liver was perfused through the proximal end of portal vein clamp with 3 mL of heparinized cold saline (5U/mL) to flush the blood from the liver into the physical blood pool (Fig. 1A). The SHVC was temporarily tied around the upper end of the tube with the 1-0 silk placed. The cava venotomy was reopened by releasing the tie on the upper part of the venotomy. The portal vein and the cava venotomy were used as the afferent and efferent channels in hypothermic irrigation lasting for 60 min. Lactate Ringer's solution (4°C) was perfused into the liver at a speed of 150 mL/h, and outflowed via the outer space of the cannula (Fig. 1B). After cold ischemia, the tube was removed and the incision on the IVC was sutured with an 11-0 silk. The tube for the portal-jugular shunt was then removed, and the veins were repaired. Reperfusion was achieved by releasing the porta hepatis clip. No intravenous fluid was administered to the rat during the 60-min cold ischemic period.

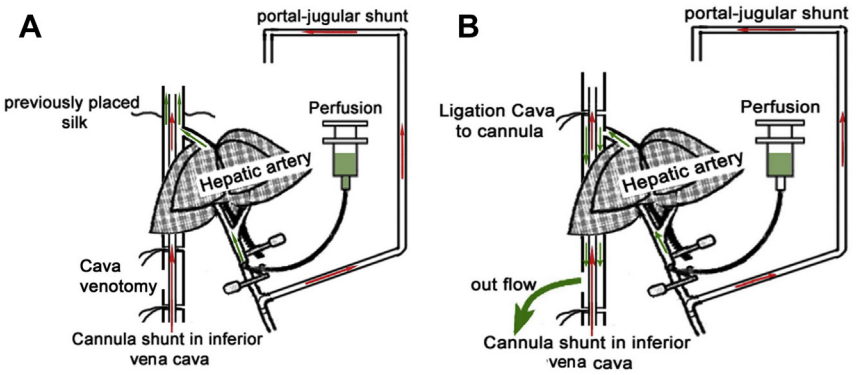

Fig. 1 – Schematic diagram of the ISLCI in rats. (A) After the portal-jugular shunt and a cannula shunt in IVC were established, the liver was perfused to flush all the blood into the physical blood pool. (B) *In situ* hypothermic irrigation of the liver. The SHVC was temporarily tied to the tube with the silk. The portal vein and the cava venotomy were used as the afferent and the efferent channels. The hypothermic irrigation solution outflowed via the outer space of the cannula. (Color version of figure is available online.)