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In-situ perfusion of the liver as a stable model of cold ischemia in orthotopic liver transplantation

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ABSTRACT

Various models of hepatic ischemia/reperfusion (I/R) injury were tested, using both rats (groups 1, 2 and 3, each n=10) and pigs (groups 4 and 5, each n=5). To create cold ischemia for 1 hr, an orthotopic liver transplantation (OLT) was performed in groups 1 and 4 using the cuff technique to reconstruct the portal vein and infra-hepatic vein in group 1, and using the veno-venous bypass during a non-hepatic phase in group 4. In groups 3 and 5, in-situ perfusion of the liver was performed without taking out the liver under a porto-systemic shunt, which was achieved by a splenic transposition (created 4 weeks prior to the surgery) in group 3, and by a pump controlled veno-venous bypass in group 5. In group 2, a simple clamp of the portal triads was applied, without flushing out the blood from the liver. The survival rate at 1 week after surgery was 90% (9/10), 70% (7/10), 100% (10/10), 60% (3/5), 100% (5/5) in groups 1 to 5, respectively. Regarding the macroscopic findings of the liver 1 week after ischemia, lobar necrosis (1 case) and patchy necrosis (3 case) were characteristically found in group 1, but not in either groups 2 or 3. The serum hepatic enzyme levels showed a large deviation from case to case in groups 1, 2, and 4, in response to the same amount of cold ischemia. However, these values were relatively uniform in groups 3 and 5, in which in-situ perfusion was performed. In conclusion, the above described in-situ perfusion model of the liver is thus considered to provide a stable in-vivo model for studying hepatic I/R injury, in both rats and pigs. *Ryukyu Med. J., 18(3)73~77, 1998*

Key words: ischemia reperfusion injury, liver, in-situ perfusion

INTRODUCTION

To investigate ischemia/reperfusion injury (I/R injury) of orthotopic liver transplantation (OLT), a model of either experimental OLT or a simple clamp of the hepatic afferent vessels has been used in previous reports[1, 2]. In the experimental OLT models, the surgical procedures are technically demanding and fatal accidents cannot be avoided within a certain probability. In these models of OLT, it is difficult to obtain even a 100% survival of 24 hr, especially in the pig model[3]. In the simple clamp models, the procedure is not technically demanding, however the occurrence of intestinal congestion during ischemia and remnant blood in the liver may contribute to additional hepatic injury other than I/R injury[2, 4-7]. Since no global standard has been reported in the models of hepatic I/R injury, we have to establish a stable model in this area. We herein tested several models of hepatic I/R injury both in rats and pigs, to prove the usefulness of in-situ perfusion of the liver as the stable model for investigating hepatic I/R injury.

MATERIALS AND METHODS

Surgical procedures in rats:

Cold ischemia for 60 minutes was investigated in 3 different in-vivo models of hepatic I/R injury. In groups 1 to 3, adult male Wistar rats (n=10 in each group), weighing 250 to 350 g were used. In group 1, syngeneic orthotopic liver transplantation (OLT) was performed using the cuff technique described by Kamada and Calne[8]. In brief, the graft liver was perfused with 10 ml of cold lactate ringer (LR) via the abdominal aorta. The harvested liver was transplanted into a syngeneic rat after 1 hour of cold ischemia without reconstructing the hepatic artery (HA). In group 2, the portal triad was simply

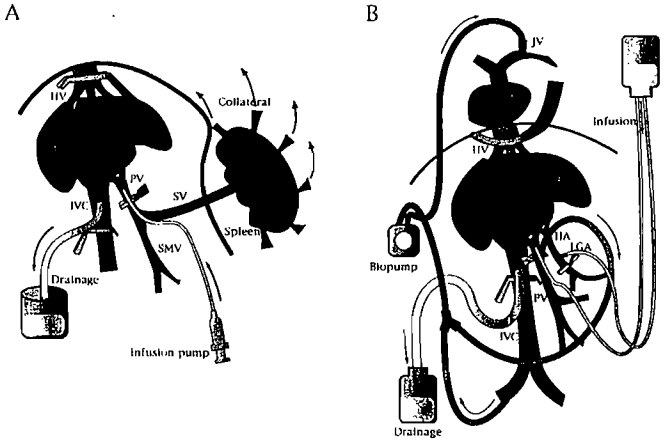

Fig. 1 Schema of in-situ perfusion of the liver in group 3 (A; rat) and in group 5 (B; pig).

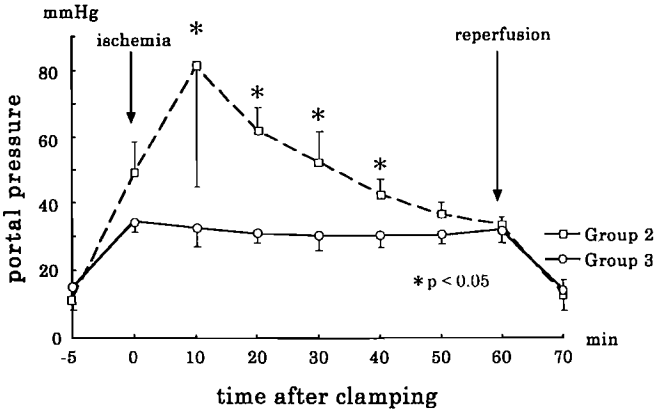

Fig. 2 Portal pressure in groups 2 and 3.

clamped to induce 60 min of hepatic ischemia, after systemic heparinization of 100 units/rat. The liver was also directly cooled by an external cooling with crushed ice, during the non-hepatic phase. In group 3, the rat's spleen was translocated into the subcutaneous space (the left subcostal lesion) to create a porto-systemic shunt[9,10]. Total hepatic ischemia was then induced by clamping the hepatic artery, the portal vein, suprahepatic vena cava (SHVC) and infrahepatic vena cava (IHVC). After clamping, the portal vein (PV) and the IHVC were cannulated with a polyethylene tube and the liver was then perfused through the portal vein with 20ml of heparinized (2.5 IU /ml) cold LR at 4℃, in order to wash out all the blood in the liver. The liver was also directly cooled by surrounding it with crushed ice, during the non-hepatic phase. The tubes were removed after perfusion and the openings of tube insertion were closed. The SHVC and IHVC were then declamped immediately after repairing the vessels to allow for a reflow in the vena cava. (Fig. 1-A). After 60 minutes of cold ischemia, reperfusion of the liver was achieved by removing the portal and hepatic arterial clamps. The portal pressure was measured by the branch of the superior mesenteric vein (SMV) from −5 minutes to +70 minutes after clamping in groups 2 and 3. In group 1, continuous monitoring of the portal pressure was impossible due to the complicated surgical procedures of OLT and the short allowable time of the surgery to obtain a successful outcome. Seven days after the experiment, the animals were sacrificed to collect the liver specimens and serum in groups 1 to 3.

Surgical procedures in pigs:

Cold ischemia for 60 minutes was investigated in 2 different in-vivo models of hepatic I/R injury, using adult female pigs (n=5 in each group) weighing from 20 to 25 kg. In group 4, OLT was performed using the same techniques used in clinical liver transplantation. In brief, a graft liver was perfused using the rapid infusion technique[11], with cold LR and then was harvested. The harvested liver was transplanted orthotopically into the recipient, anastomosing the SHVC, IHVC, PV, hepatic artery, and bile duct in this order, in an end to end fashion. In group 5, the pig livers were surgically isolated from the surrounding tissue, except for the SHVC, IHVC, PV, HA, and bile duct. Two tubes were inserted through the single incised opening of the PV, to enable both a bypass of the portal flow and a hepatic infusion, simultaneously. The blood flow from the PV and IHVC was bypassed, using a pump-controlled veno-venous bypass, to the internal jugular vein. The left gastric artery was canulated with an elastic tube to perfuse the HA. Next, the pancreatico-duodenal artery was ligated and divided, and the common hepatic artery was clamped during liver ischemia. The liver was then flushed with 3000 ml of cold LR solution (4℃) through the PV and HA, and kept in-situ for 1 hr with additional external cooling by crushed ice around the liver. Reperfusion was achieved by declamping all the clamps (Figure. 1-B).

Monitoring and sampling:

During the surgical procedures in groups 2 and 3, the portal pressure was monitored through the polyethylene tube (PE-10, Imamura, Tokyo, Japan), by inserting the end of the tube into the portal branch. Blood samples were collected at 24 hr after reperfusion to assess the serum AST and LDH, and the animals were thereafter sacrificed at 7 days after surgery to obtain liver tissue specimens. In groups 4 and 5, hemodynamic monitoring, including the mean carotid arterial pressure (MAP in mmHg) and the mean pulmonary arterial pressure (MPAP in mmHg) through the Swan-Ganz catheter, were performed. The MPAP and MAP were monitored from −60 to +90 minutes after reperfusion. The animals were then sacrificed by exsanguination under anesthesea, to obtain liver tissue specimens and blood samples at 7 days after surgery.

Table 1 Hepatic enzyme levels in the serum

	AST (U/l)	LDH (U/l)
Group 1	1845±1278/940±599*	8961±5801/4562±2863
Group 2	2546±1518/1255±692	9659±4603/4931±2975
Group 3	1299±515/102±49	5902±2312/1204±591
Group 4	197±165/113±72	4960±3764/2517±1639
Group 5	124±57/92±40	3731±1367/1984±834

*serum level at 24 hr /7 days after reperfusion

Statistical analysis:

All data are expressed as the mean(standard deviation (SD) of the mean value. Group comparisons were performed using the t-test when appropriate. Differences were considered to be statistically significant at $p<0.05$.

RESULTS

Hepatic Ischemia / Reperfusion Models in Rats (Groups 1 to 3):

The survival rate one week after hepatic ischemia was 90% (9/10), 70% (7/10), and 100% (10/10) in groups 1 to 3, respectively. In the macroscopic findings, lobar necrosis was found in 1 case in group 1 (died at 5 days after the OLT), but no such cases were found in groups 2 and 3. Multiple spotty necrotic areas were also characteristically found in 2 cases in group 1. The portal pressure in groups 2 and 3 are shown in Figure 2. In group 2, portal pressure showed a steep increase to 81.0± 35.9 mmHg at 10 minutes after portal clamping, and these high levels were thereafter maintained throughout the non-hepatic phase. This increased portal pressure returned to its pre-ischemic level at 10 min after declamping. In group 3, the portal pressure showed a mild increased of 34.7±13.5 mmHg at 10 min after clamping and then maintained this level throughout the clamping period (0 to 60 min). The portal pressure of group 3 was statistically lower ($p<0.05$) than that of group 2 at 10, 20, 30 and 40 minutes after clamping. The serum aspartate aminotransferase (AST) levels were evaluated at 24 hrs and 7 days after reperfusion in groups 1 to 3. The serum AST and LDH levels in group 3 (1299±515 and 5902±2312 IU/l, respectively) were lower than the same levels in the group 1 (1845±1278 and 8961±5801 IU /l, respectively) and group 2 (2546±1518 and 9659±4203 IU/l, respectively) without a statistical difference, at 24 hrs after reperfusion. The AST levels in group 3 decreased to close to the pre-ischemic levels at 7 days after reperfusion (102±49 IU/l), which were also lower than those of groups 1 (940±599 IU/l) and 2 (1255±692 IU/l) (Table 1).

Hepatic Ischemia/Reperfusion Models in Pigs (Groups 4 to 5):

The survival rates at one week after hepatic ischemia in groups 4 and 5 were 60% (3/5), 100% (5/5), respectively. In the macroscopic findings, subsegmental necrosis was frequently observed in group 4, but was rare in group 5. Two animals with less than 7 days survival in group 4 showed a poor recovery from anesthesia until death. The AST and LDH levels at 24 hr after reperfusion were, 197±165 IU/l and 4960±3764 IU/l respectively in the group 4, and 124±57 IU/l and 3731±136 7 IU/l, respectively, in group 5 (Table 1). The systemic arterial pressure during the operation was stable in both groups until the portal clamp was applied, but was depressed (60 to 80 mmHg) during the non-hepatic phase in both groups without any difference observed between both groups. After reperfusion, the systemic arterial pressure increased immediately (90 to 110 mmHg) in group 5. In group 4, however, the MAP remained low (50 to 80 mmHg) especially in those animals demonstrating early post-operative death within 7 days. The MPAP/MAP ratio showed an apparent increase from −30 to −1 minutes during the ischemic period, in both groups. After reperfusion (+0 min) the MPAP/MAP ratio showed a further increase (0.52±0.19 in group 4 and 0.49±0.11 in group 5, at 1 minute after reperfusion) without any statistical difference. These MPAP/MAP ratios were especially high in the 2 animals that were not able to survive more than 7 days in group 4.

DISCUSSION

To investigate hepatic I/R injury in the rat, either the OLT model or a simple clamping of the portal triad has been extensively used as an in-vivo model of I/R injury[1, 12]. Even though the technique of OLT in rats has already been established by Kamada et al[13], this procedure can not provide uniform results in the early postoperative hepatic function due to the complexity of the procedures. More specifically, the serum enzymes released from the liver immediately after reperfusion vary according to the individuals after 1 hr of cold ischemia in the OLT model, as observed in this report. Moreover, both the lobar necrosis and multiple spotty necrosis observed in this group were thought to be caused by a portal air embolism, which we were unable to completely eliminate even when careful preparations were made to avoid it and it was thus thought to be unavoidable to a certain degree. These unpredictable variations in liver damage were thus affected with subtle differences in the surgical procedures of each case, thus indicating that the OLT model is not an appropriate model for studying experimental I/R injury.

In previous studies of I/R injury, hepatic ischemia was also induced by a simple clamping of both the hepatic artery and portal vein without washing out the

blood from the liver. With this method, it is impossible to exclude the participation of several factors such as neutrophils[2,4], adhesion molecules[5], platelet activating factor[6,7], among others in the remnant blood in the liver. In clinical liver transplantation, such factors are minimized by flushing the blood out of the liver and thus this simple clamp model is again inappropriate as a clinical relevant model of hepatic I/R injury. Since a port-systemic shunt is not usually used in this model, a simple clamp of the portal triad causes other problems such as a severe reduction in the venous return, systemic hypotension, and intestinal congestion, during the hepatic ischemia. These factors are thought to induce even greater hepatic I/R injury due to cytokines or endotoxin in the portal vein and hypotensive organ failure, thus rendering this model even less appropriate as a model to investigate hepatic I/R injury in OLT[14].

To develop a more clinically relevant model of hepatic I/R injury, we tested in-situ perfusion of the rat liver combined with the port-systemic shunt. The port-systemic shunt created by the splenic transposition was able to effectively reduce intestinal congestion, as shown in the prevention of portal hypertension during the non-hepatic phase. With this port-systemic shunt, the rat liver was surgically isolated from the systemic circulation, flushed in-situ, and kept in cold ischemia, which is similar to the cold ischemia of the harvested liver graft in the OLT model. Since the liver was cooled not only by the perfusion of cold LR but also by external cooling with crushed ice, the temperature measured deep inside the liver was always kept under 10℃. These findings thus indicate the main advantage of this model to be the feasibility of providing hepatic cold ischemia with a minimal degree of surgical stress, and thus avoiding such complicated procedures as OLT. In fact the degree of hepatic I/R injury under the simple clamp model or the OLT model was higher than that of the in-situ clamp model as assessed by the serum AST and ALT. Since the aim of this report is to establish a stable experimental model of hepatic I/R injury, amount of injury itself is not the main issue. However, these high values with their large standard deviations directly indicated the presence of additional injuries other than hepatic I/R injury, in the simple clamp model and the OLT model.

Experiments in large animals offer various advantages including a better accessibility of numerous physiological parameters, clinical relevance of the model and the feasibility of repeated sampling. Both porcine or canine models of OLT may thus provide important information on hepatic I/R injury. In these animals, however, the large surgical stress inherent in OLT results in unstable outcomes and subtle differences in the surgical procedures can also cause large differences even in the survival rate as observed in this report. Moreover, a stable hemodynamic status was difficult to maintain after reperfusion, especially in those cases with a short survival after OLT, without any detectable causes such as the massive bleeding, anastomotic failure, or poor reperfusion of the liver. In these cases, the unstable hemodynamic status might thus cause additional hepatic injury, which also contributed to the early post-operative death observed in these cases.

These large animals also have very little endurance regarding portal congestion which results in immediate intestinal congestion and systemic hypotension, and a simple clamp model cannot therefore be performed in these animals. To investigate hepatic I/R injury within these limitations, we also tested the in-situ perfusion of the liver in combination with the porto-systemic shunt, which is similar to the technique used in clinical OLT. Since the hepatic portion of the vena cava in pig does not have any communicating veins behind the vena cava, such as the human vertebral veins, isolated circulation of the liver can easily be achieved by the surgical control of the major vascular vessels around the liver. In this way, the liver can be isolated in-situ with less surgical stress, less hepatic manipulation, and no influence of anastmotic procedures in SHVC and IHVC. The liver was kept cold with a perfusion of cold LR and external cooling, thus mimicking the cold preservation of the harvested liver in clinical OLT. In another line of experiments, we also used University of Wisconsin (UW) solution as the preservation solution to flush out the liver, and no systemic side effects were observed since the hepatic circulation is completely isolated from the systemic circulation in this system. These findings thus indicate in-situ perfusion to be useful in the porcine model of hepatic I/R injury.

In conclusion, in-situ perfusion of the liver in combination with port-systemic shunt was shown to be a stable and reliable model of experimental I/R injury in both the rat and pig.

We followed "Standards Relating to the Care and Management of Experimental Animals" (Notification No. 6, March 27, 1980, Prime Minister's Office, Tokyo, Japan) for care and use of animals. The animals used in our studies were handled humanely in accordance with animal experimental protocols approved by the Animal Care and Use Committee of the University of the Ryukyus.

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