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Cold Preservation of Pig Liver Grafts with Warm Ischemia and Pentoxifylline-UW Solution

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Background. We undertook this study to investigate the safe time limits of cold preservation in UW solution of liver grafts subjected to warm ischemia (WI) for 20 min and the changes of the limits when pentoxifylline is added to UW solution.

Methods. The safe time limit was studied in a simple porcine orthotopic liver transplantation (LTx) model. In donors, livers were subjected to 20 min of WI and subsequent 12-h (group 1, $n = 5$), 16-h (group 2, $n = 5$), and 20-h (group 3, $n = 3$) cold preservation in UW solution, respectively. After the safe time limits were clear, another group (group 4, $n = 5$) was built to test whether or not the limits can be changed when pentoxifylline is added to UW solution in an unsafe time limit group.

Results. All five animals in group 1 survived up to 7 days of the survey endpoint. In group 2, only one animal survived up to the same survey endpoint and all animals in group 3 died within 12 h. The 1-week survival rate of group 1 was significantly higher than the other two groups. Group 1 had a lower level of alanine aminotransferase (ALT) or aspartase aminotransferase (AST) after LTx, less pathological damage, higher concentration of adenosine triphosphate (ATP) and higher microcirculation blood flux in the grafted liver tissue at 1 h after reperfusion than the other two groups. The results primarily showed that 12-h cold preservation was safe, 16 h was unsafe, and 20 h was highly unsafe. But when pentoxifylline was added to UW solution in cold preservation (16-h group, group 4), in contrast to group 2, the incidence of liver tissue necrosis and primary graft nonfunction was significantly lower in group 4 than in group 2. The 1-week survival rate of the pigs was 100% in the former and 20% in latter group. Levels of ALT and AST in recipients' artery blood, malondialdehyde and TNF-α concentration in grafted liver tissue, resistance of portal vein and hepatic artery after preservation in group 4 were significantly reduced, whereas microcirculation blood flux of the grafted liver, superoxide dismutase concentration and ATP concentration in grafted liver tissue were significantly elevated.

Conclusions. The safe time limit of cold preservation in UW solution of liver grafts subjected to WI for 20 min was about 12 h and the limits can be prolonged to 16 h when pentoxifylline is added to UW solution. Many mechanisms were involved.

Key Words: Liver transplantation, Warm ischemia, Cold preservation, Safe time limit, Pentoxifylline.

Introduction

It is suggested that warm ischemia (WI), cold preservation/reperfusion injuries of the grafts are risk factors for postoperative graft dysfunction after liver transplantation (LTx) (1–2). Prolonged WI or cold storage time will result in

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LTx failure. Although some reports including our research suggested that the safe time limit of tolerance to WI of liver grafts from pigs was about 30 min (3,4), such liver grafts cannot be preserved in UW solution unlimitedly. Then how long is the safe time limit of cold preservation in UW solution of liver grafts with WI under 30 min? In our previous research, we conclude that the safe time limit of cold preservation in UW solution of liver grafts with 30 min of WI from non-heart-beating donors (NHBDs) was about 6 h (5). But it was not yet clear when the liver graft had 20 min of WI. In this study, we investigated the safe time limits of cold preservation in UW solution of liver grafts subjected to WI for 20 min (Part A) and whether or not the limits can be changed when pentoxifylline (PTX) was added to UW solution (Part B).

Part A: Cold Preservation Limits of Pig Liver Grafts with Twenty Minutes Warm Ischemia in UW Solution

Materials and Methods

In accordance with the Chinese legislation regarding protection of animals and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 8623, revised 1985), 26 Chinese Guangxi BAMA miniature swine weighing 8–11 kg were used after 36 h of fasting. Orthotopic LTx was performed as previously described with some modifications (6). Five minutes after administration of 12,500 IU IV heparin in the donor, the liver was subjected to in situ WI for 20 min by clamping all vessels of the liver and at the same time take blood from the animal until the arrest of the heart for recipient using next (mimic NHBDs). At the end of the WI period, the liver was washed for about 15 min with 4°C lactated Ringer's solution (300 mL) and UW solution (300 mL) until the liver changed to a yellow-white color. The liver was harvested and stored for different times (12, 16, 20 h, respectively) at 4°C in UW solution 300 mL. In the recipients, during the anhepatic period, venovenous bypass (VVB) was not used. At the time of the suprahepatic vena cava anastomosis, the liver allograft was cooled with 500 mL of 4°C lactated Ringer's solution for flow-out UW solution, preventing hyperkalemia. After completion of the suprahepatic vena cava anastomosis and portal vein anastomosis, the liver allograft was reperfused. Then the infrahepatic vena cava and hepatic artery and bile duct were reconstructed by end-to-end suture. No immunosuppressant was administered during or after the operation.

Pigs were divided into three groups: group 1 ($n = 5$), the liver grafts were subjected to 20 min of WI and a subsequent 12-h cold preservation; group 2 ($n = 5$), the liver grafts were subjected to 20 min of WI and a subsequent 16-h cold preservation; group 3 ($n = 3$), the liver grafts were with 20 min of WI and a subsequent 20-h cold preservation.

In the recipients, postoperative survival was assessed for 7 days. Serum alanine aminotransferase (ALT) and aspartase aminotransferase (AST) concentrations in arterial blood were measured using an autoanalyzer. For assays of adenine nucleotides, performed at donor laparotomy, after cold preservation and at 1 h at reperfusion (RPF) in the recipient, specimens of graft liver were clamped and frozen in situ with stainless steel tongs precooled with liquid nitrogen. The concentration of ATP was determined enzymatically (7). Liver microcirculation blood flux was assessed by means of Laser Doppler Fluxmetry (Perimed AB, Stockholm, Sweden). Tissue samples of the graft for hematoxylin and eosin histological examination were obtained at the following time points: laparotomy, end of WI and after cold preservation before implantation and 1 h after RPF. Electron microscopic examination was also taken from some of these representative tissue samples.

Values are expressed as mean ± SD. Analysis of variance and Student-Newman-Keuls test (q test) were used to determine significance. Differences in survival were determined using the Kaplan–Meier analysis and log-rank test. A p value <0.05 was considered significant.

Results

Survival

Figure 1 shows the postoperative survival rate of the recipient pigs. All five animals in group 1 survived up to 7 days of the survey endpoint. In group 2, only one animal survived to the survey endpoint, the other four animals died within 12 h after LTx because of noncorrectable metabolic acidosis or not resuming consciousness. All animals in group 3 died within 12 h from primary graft failure. At autopsy, neither intraabdominal bleeding nor anastomotic failure was found. The 1-week survival rate of group 1 was significantly higher than the other two groups.

ALT and AST

Serum ALT concentrations at 1 or 6 h after RPF of the graft in group 2 or group 3 were significantly higher than in group 1 and there was the same tendency for AST among the three groups at 1 or 6 h after RPF. These results showed that injuries of the liver function in group 1 were less than the other groups.

ATP

After preservation, ATP concentration decreased significantly in group 2 or group 3 compared with group 1 ($p < 0.05$). At 1 h after RPF, ATP concentration was restored to 90% of the initial value in group 1. In group 2, although the survivor showed a similar successful pattern of ATP recovery, recovery of the nonsurvivors was suppressed, and the mean values at 1 h after RPF in the group was significantly lower than in group 1 ($p < 0.05$). The