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A new liver graft preparation method for uncontrolled non-heart-beating donors, combining short oxygenated warm perfusion and prostaglandin E1

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ABSTRACT

Background: To resolve the shortage of donors associated with liver transplantation, the potential uncontrolled non-heart-beating donor (UNHBD) pool is expected to increase. However, warm ischemia-reperfusion injury leads to inferior survival in transplantation using the grafts from UNHBD compared with those from heart-beating donors. To overcome this problem, we developed a new method for preparation of liver grafts from UNHBDs consisting of a combination of short oxygenated warm perfusion (SOWP) and prostaglandin E1 (PGE1).

Methods: Using an *ex vivo* perfusion rat model, we examined the effectiveness of this new method.

Results: Using SOWP and PGE1 treatment, the total amount of bile production during reperfusion in UNHBD grafts was increased to the same level as that in the heart-beating donor grafts. The addition of PGE1 to SOWP buffer decreased aspartate aminotransferase/alanine aminotransferase and tumor necrosis factor α levels during 1 h of reperfusion. Necrosis and apoptosis were significantly decreased by SOWP + PGE1 treatment. SOWP + PGE1 ameliorated induction of mitochondrial permeability transition, and the total amount of mitochondrial cytochrome c in the SOWP + PGE1 group after reperfusion was kept significantly higher than that in the no treatment group. Cytosolic c-Jun N-terminal protein kinase activation was significantly suppressed by SOWP + PGE1. Decrease in mitochondrial Bcl-2 was suppressed by SOWP alone and SOWP + PGE1 treatment, and Bax in the mitochondria was significantly suppressed by SOWP + PGE1.

Conclusion: SOWP and PGE1 prior to cold preservation significantly improved the function of liver grafts that underwent warm ischemia-reperfusion injury. Therefore, this method might be useful in liver transplantation using UNHBD grafts.

1. Introduction

Liver transplantation is a well-established treatment for various end-stage liver diseases and acute liver failure. As the number of patients waiting for liver transplantation grows, the shortage of donors and increase in waiting list mortality has become a worldwide issue. The addition of non-heart-beating donors (NHBDs) to the potential donor pool is

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expected to help resolve this problem. NHBDs have increased to approximately 10%–20% of the liver donor pool for liver transplantation in some European countries [1].

NHBDs are largely classified into two categories—controlled and uncontrolled [2]. Uncontrolled NHBDs (UNHBDs) are thought to have the highest potential as donors and also have fewer ethical problems compared to controlled NHBDs. However, cardiac arrest in UNHBDs is a sudden and unexpected event as there is variation in agonal status and warm ischemia time during resuscitation [1]. During the course of agonal status and the resuscitation period, low arterial blood pressure and portal flow, as well as endotoxin and tumor necrosis factor (TNF)-α from ischemic intestines, damage UNHBD grafts more severely than controlled NHBD grafts [3,4].

ATP depletion caused by hypo-oxygenation after cardiac arrest leads to plasma membrane dysfunction, cellular edema, and, finally, cell death [5]. These changes lead to microcirculation disturbance and further cell death after reperfusion. Reperfusion also causes Kupffer cells to activate and release toxic mediators, e.g., reactive oxygen species and inflammatory cytokines, which lead to further cellular injury and cell death [6,7].

Oxygenation of liver grafts that have experienced warm ischemia has been considered promising, and several clinical trials have reported normothermic extracorporeal membrane oxygenation [8], machine perfusion during preservation [9], and hypothermic oxygenated extracorporeal perfusion after cold preservation [10,11]. Experimental and clinical results using these strategies have been reported to be relatively good, but inferior when compared to heart-beating donor (HBD) liver grafts. On the other hand, during liver surgery, liver function is well preserved despite the Pringle maneuver.

We hypothesized that liver graft function could be improved by short oxygenation of the graft after cardiac arrest, which might have ameliorated the energy status of the organ, and thereby preserve plasma membrane function. If the liver grafts were perfused *ex vivo*, it might be possible to avoid several effects caused by various mediators in the portal venous system as encountered with systemic oxygenation of donors. Ethical problems elicited by administration of drugs to donors prior to declaration of death and side effects of drugs in recipients after liver transplantation might also be avoided.

Furthermore, concomitant use of vasodilative agents could contribute to improvement in microcirculation disturbances. Prostaglandin E1 (PGE1) is well known to have not only vasodilative but also various hepatoprotective effects [12–15]. Therefore, in an attempt to improve grafts from UNHBDs, we studied the effects of short oxygenated warm perfusion (SOWP) before cold preservation with PGE1 on livers from UNHBDs.

Mitochondria have recently been reported to play a central role in both necrotic and apoptotic cell death [16,17]. Focal necrosis and broad sinusoidal circulatory failure was histopathologically observed in liver tissues that underwent warm ischemia-reperfusion injury [18,19]. Thus, in this study, we analyzed how SOWP + PGE1 worked, focusing on mitochondrial function and cell death.

2. Materials and methods

2.1. Experimental design and operation procedure

Male Wistar rats, weighing 272–314 g, were used for all experiments. Rats were permitted free access to laboratory food and tap water until the start of experiments. All animals in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The animal studies were approved by the Animal Care and Use Committee of Tohoku University.

Rats were anesthetized with pentobarbital sodium (50 mg/kg) intraperitoneally. After laparotomy, the common bile duct was cannulated. In the heart-beating (HB) group, the portal vein was cannulated and the liver flushed with 20 mL of cold University of Wisconsin (UW) solution at 4°C *via* the portal vein under the heart-beating condition. After flushing, livers were retrieved and preserved in UW solution at 4°C for 6 h. During cold preservation, the infrahepatic inferior vena cava was ligated and the suprahepatic inferior vena cava was cannulated.

In uncontrolled non-heart-beating (UNHB) models, all livers were subjected to 30 min of warm ischemia after approximately 10 min in agonal state induced by thoracotomy followed by cardiac arrest. UNHB models consisted of no treatment (NT), SOWP, and SOWP + PGE1 groups. In the NT group, livers were flushed in the same manner as in the HB group after warm ischemia. In the SOWP and SOWP + PGE1 groups, livers were retrieved in the same manner as in the NT group and perfused for 30 min on a perfusion apparatus. In the SOWP + PGE1 group, 10 ng/mL PGE1 was added to the perfusate in SOWP. After 30 min of perfusion, livers were flushed with cold UW solution and preserved. No anticoagulants were administered to rats in this study.

2.2. Normothermic perfusion and sample collection

Normothermic perfusion was performed using a non-recirculating perfusion machine (Perfusion System, PS-1; Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). Livers were maintained in the moist perfusion chamber at 37°C and connected to the perfusion machine. All livers were perfused with oxygenated (95% O_2/5% CO_2) Krebs–Henseleit solution (PO_2: 450–500 mm Hg) at a pressure of 7 mm Hg at 37°C *via* the portal vein. The portal vein pressure was continuously measured with a pressure transducer (Research Grade Blood Pressure Transducer, 110 VAG/60 Hz; Hugo Sachs Elektronik-Harvard Apparatus) during perfusion. In all the groups, the perfusate from the suprahepatic inferior vena cava and bile were collected, and the volumes of each were measured. Mitochondria and cytosolic fractions were isolated from fresh liver tissues by differential centrifugation, as reported previously [20]. The protein concentration of the mitochondrial suspension and cytosolic fraction was quantified using a BCA protein assay kit (Thermo Scientific Pierce, Rockford, IL).

2.3. Liver enzymes and cytokines

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), TNF-α, and interleukin 1β (IL-1β) in the perfusate were