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Effect of olprinone on liver microstructure in rat partial liver transplantation

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ABSTRACT

Background: Donor safety is a major concern in living-donor liver transplantation. However, partial grafts do not meet the functional demands of recipients and lead to small-for-size syndrome (SFSS). In a previous study, we showed that olprinone (OLP), a selective phosphodiesterase III inhibitor, up-regulates endothelial nitric oxide synthase level in the liver and attenuates shear stress, sinusoidal endothelial cell injury, and hepatocyte apoptosis after excessive liver resection in a rat model. We aimed to examine whether OLP treatment has beneficial effects on SFSS in a rat model of partial liver transplantation (PLT).

Methods: We performed experiments in a rat model of 30% PLT. In the OLP group, we inserted an osmotic pump with OLP into the peritoneal cavity 48 h before liver graft sampling. Recipient rats were not treated with OLP. We examined the liver microstructure by electron microscopy and biochemical examination, and determined the 7-d survival of recipients.

Results: In the OLP group 1 h after PLT, the sinusoidal endothelial cells of the liver were well preserved and we observed few vacuolar structures in hepatocytes. The total serum bilirubin level 1 wk after PLT tended to be lower in the OLP group than in the controls, and the liver microstructures were also well preserved in the OLP group. The probability of survival in the OLP group (100%; 14 of 14 rats) was significantly higher than that in the control group (75%; 15 of 20 rats).

Conclusions: Olprinone treatment was demonstrated to have therapeutic potential to overcome SFSS.

1. Introduction

Adult-to-adult living-donor liver transplantation has become an alternative to deceased-donor liver transplantation [1]. One of the advantages of adult-to-adult living-donor liver transplantation is the reduction in waiting time and dropouts. However, donor safety remains a major concern. With regard to donor operations, the overall complication rate was reported to be significantly higher in the right-lobe graft group than in the left-lobe graft group [2]. The use of minimal graft volume to reduce perioperative risk for living donors has been discussed [3].

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However, partial grafts do not meet the functional demands of the recipient and lead to liver failure, which is referred to as small-for-size syndrome (SFSS) [3]. Liver blood flow per unit immediately increases after partial liver transplantation (PLT), and increased blood flow in the portal vein exerts shear stress, thereby affecting the liver [3,4]. Excessive shear stress causes liver injuries such as hepatocyte apoptosis and necrosis and sinusoidal endothelial cell injury [4]. Small-for-size syndrome is characterized by postoperative liver failure from shear stress as a result of excessive portal flow to the liver [3,5]. Various animal experiments have been performed to establish treatments for SFSS, including splenectomy [6], portosystemic shunt placement [7], ischemic preconditioning [8], and pharmacological modifications [9,10]. Splenectomy is performed to reduce portal pressure if the portal pressure after reflow is greater than 15 mm Hg, to achieve intentional portal pressure control [11].

Olprinone (OLP) has been clinically administered to patients with heart failure and to those undergoing cardiac surgery. It is a selective phosphodiesterase III inhibitor with combined positive inotropic and vasodilator properties that are mediated by the elevation of intracellular cyclic adenosine monophosphate (cAMP) levels in vascular smooth muscle cells and cardiomyocytes by the prevention of cAMP degradation [12]. Nitric oxide produced by endothelial nitric oxide synthase (eNOS) has an important role in reducing sinusoidal constriction during the early phase [13]. The importance of eNOS in hepatic injury after cold/warm reperfusion in transplanted liver grafts has been investigated [14]. In a previous study, we showed that OLP treatment up-regulates eNOS levels in the liver and attenuates shear stress after excessive liver resection in rats to protect against sinusoidal endothelial cell injury and hepatocyte apoptosis [15]. Furthermore, OLP has been reported to protect the liver against ischemia-reperfusion injury (IRI) through an increase in cAMP levels and cytokine production [16]. Ischemia-reperfusion injury is a major cause of primary nonfunction of liver graft after liver transplantation. Moreover, IRI reduces liver regeneration after hepatectomy [17]. Therefore, OLP may be effective in overcoming SFSS and extending the minimum limits of the graft/recipient weight ratio.

The purpose of this study was to examine whether OLP preconditioning has a beneficial effect on SFSS in a rat model of PLT, with a particular focus on the potential hepatoprotective effect of OLP on the microstructure of the graft liver.

2. Materials and methods

2.1. Animals

We used inbred male Lewis rats (age, 9–10 wk; body weight, 240–280 g; SLC, Sizuoka, Japan) in this study. Rats were housed in a standard animal laboratory with free access to water and chow. We kept the animals under constant environmental conditions, with a 12-h light-dark cycle. The Animal Research Committee of Kyoto University approved all animal experiments. All animals were cared for in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*.

2.2. Experimental design and surgical procedure

We conducted the experiment using two groups of rats: an OLP-treated group and a control group. In the OLP group, we gave rats 0.6 μg/kg/min OLP (a gift from Eizai Pharmaceutical Co, Ltd, Tokyo, Japan) using an Alzet osmotic pump (Model 2001; Durect Corp, Cupertino, CA) that we inserted into the peritoneal cavity under sevoflurane anesthesia (Abbott, Osaka, Japan) 48 h before we harvested the donor liver. We used the Alzet peritoneal pump placement to ensure the steady administration of continuous OLP. We did not administer OLP to recipient rats in this study. In the control group, preconditioned was not performed.

In this study, we used a rat model of 30% PLT without arterial reconstruction using the cuff method as an SFSS model [18,19]. After ligation of the middle and right Glisson's sheath, we perfused the liver with 10 mL lactated Ringer's solution through the portal vein. We selected the right and caudate lobes of the liver to be grafted on the back table. The graft was stored in cold Ringer's solution, and the cold ischemia time was 50 min. During liver transplantation, the portal vein was clamped for 20 min. There were no differences in the ischemia reperfusion time between groups. After surgery, we gave the rats food and water *ad libitum*. The donor operation was performed after intraperitoneal administration of 25 mg/kg pentobarbital, and the recipient operation was performed under inhalation anesthesia with sevoflurane and ether. We administered no immunosuppressive agent to the recipient rats.

2.3. Electron microscopy

We examined the morphology of endothelial cells and hepatocytes using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). We collected liver samples from both groups 1 h after portal venous reperfusion. The samples were prepared for electron microscopy as described previously [15]. Furthermore, we compared these samples with three samples collected from each liver at 7 d after 30% PLT with and without OLP preconditioning and after whole liver transplantation.

2.4. Biochemical examination

We collected blood samples from survivors 1 wk after PLT. We stored serum samples at −80°C to measure serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), and hyaluronic acid (HA) levels. Japan Clinical Laboratories, Inc, Kyoto carried out the measurements.

2.5. Survival study

We used a total of 14 rats in the OLP group and 20 rats in the control group for the survival study. The OLP group operations were performed after those of the control group. We performed all PLTs consecutively. Rats that lived for 7 d after PLT were considered survivors.

2.6. Statistical analysis

We used Student's t-test to detect differences in the means of continuous measurements. A P value of <0.05 indicated