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Oral administration of cilostazol improves survival rate after rat liver ischemia/reperfusion injury

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ABSTRACT

Background: Cilostazol is a type III phosphodiesterase inhibitor used to treat the symptoms of intermittent claudication. Recent studies have shown that cilostazol decreases ischemia/reperfusion (I/R) injury in several organs.

Materials and methods: We evaluated the effects of cilostazol in a rat model of liver I/R injury. Thirty male Wistar rats with liver I/R injury were divided into a cilostazol or saline (control) group ($n = 15$ each). Each rat was orally administered cilostazol or saline for 3 d before I/R injury. Liver I/R injury was induced via 1 h of warm ischemia of the median and left lateral liver lobes, followed by 3 h of reperfusion. The rats were then euthanized. Serum aspartate aminotransferase, alanine aminotransferase, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α levels were measured. The Mann–Whitney *U* test was used to compare the differences between the treatment groups. Histologic examination was performed on the liver tissues. We also conducted a survival study to confirm the effect of cilostazol on the mortality rate in rats. For the survival study, a liver I/R injury model with an ischemia time of 1.5 h was used, and the rats were observed for 1 wk.

Results: Serum aspartate aminotransferase, alanine aminotransferase, IL-1β, and IL-6 levels were significantly lower in the cilostazol group than in the saline group. Treatment with cilostazol significantly improved pathological findings associated with liver I/R injury and increased survival rate compared to that in controls.

Conclusions: Cilostazol reduced mortality and alleviated the effects of liver I/R injury in Wistar rats.

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Introduction

Ischemia/reperfusion (I/R) injury is a serious complication of liver resection and liver transplantation and a major contributor to the associated morbidities.[1] Alleviating liver I/R injury can improve survival rate by minimizing postoperative liver injury. Data from studies in laboratory animals have suggested that I/R injury can be alleviated by ischemic preconditioning and postconditioning, some pharmacologic agents, gene therapy, and machine perfusion; however, none of these interventions have been recommended for use in clinical practice.[2] I/R injury is typified by an inflammatory response; liver I/R injury involves a complex web of interactions between Kupffer cells, CD4+ lymphocytes, neutrophils, and hepatocytes, as well as various cytokines, chemokines, and complement proteins.[3]

Over the last 20 y, cilostazol (6-[4-(1-cyclohexyl-1h-tetrazol-5-yl) butyloxy]-3, 4-dihydroquinolin-2(1H)-one) has been used worldwide to treat the symptoms of intermittent claudication caused by peripheral arterial disease. The Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II) has suggested cilostazol as the first-line drug for the relief of claudication symptoms because it improves treadmill exercise performance and quality of life.[4] Cilostazol is a phosphodiesterase III inhibitor that suppresses degradation of cyclic adenosine monophosphate (cAMP), resulting in an increased concentration of cAMP in platelets and blood vessels, which leads to inhibition of platelet aggregation and vasodilation, respectively.[5,6] In addition, cilostazol inhibits leukocyte adhesion to the endothelium[7] and appears to tighten the endothelial barrier *in situ* by partly inhibiting cAMP-degrading enzymes in the endothelium.[8] A study reported that cilostazol suppresses neointimal formation by suppressing proliferation of vascular smooth muscle cells.[9] Recent studies have shown that cilostazol alleviates I/R injury in the brain, spinal cord, and small bowel.[10–12]

Previously, we investigated the mechanism of liver I/R injury.[13–15] Based on our findings, in the present study, we hypothesized that cilostazol is beneficial for the treatment of liver I/R injury. We also examined the effect of cilostazol on the survival rate in rats with liver I/R injury.

Materials and methods

Animals

Male Wistar rats aged 8 wk (weighing 250-300 g) were purchased from Japan SLC, Inc (Hamamatsu, Shizuoka, Japan) and housed in a temperature- and humidity-controlled room under a 12-h light/dark cycle. The animals were given free access to water and food. All experiments were performed in accordance with the guidelines for the use of experimental animals by the National Institutes of Health and were approved by the Laboratory Animal Care and Use Committee of Keio University School of Medicine.

Surgical procedure

Liver I/R injury was induced in the rats according to a previously reported procedure.[16] Briefly, a midline incision was made after shaving the abdomen, and the abdominal cavity was then exposed with the aid of retractors. Next, the left hepatoduodenal ligament containing the hepatic artery, portal vein, and bile duct of the left lateral and median liver lobes was clamped for 60 or 90 min with a microvascular clamp to induce partial (70%) warm ischemia. The 90-min ischemia model was used to determine survival and the 60-min ischemia model was for all other experiments; 60 min of partial ischemia is nonlethal.[17] Thereafter, the clip was removed to initiate hepatic reperfusion, and the abdominal cavity was closed. All the procedures were performed under general anesthesia induced with isoflurane. The rats were subjected to warm ischemia for 60 min followed by 3 h of reperfusion. The animals were then euthanized by total blood collection from the aorta by exsanguination. Liver tissues and blood samples were taken for analysis.

Experimental protocol

The rats were randomly assigned to either a control group (I/R + saline, $n = 15$) or cilostazol group (I/R + cilostazol, $n = 15$). Cilostazol (Otsuka Pharmaceutical, Tokushima, Japan) was dissolved in a 0.5% carboxymethylcellulose sodium salt (Wako Pure Chemical Industries Ltd, Osaka, Japan) solution at a concentration of 10 mg/mL. Each rat was administered cilostazol (20 mg/kg/d) or saline (0.5 mL/body/d) through a 3-Fr feeding tube (NIPRO, Osaka, Japan) into the stomach. These administrations were started 3 d before the surgery. On the day of the surgery, cilostazol or saline was administered 30 min before the induction of ischemia.[18] Cilostazol or saline was administered 4 times before the surgery was performed (Fig. 1).

Laboratory measurements

Blood samples were obtained from the aorta at the end of the experimental protocol and centrifuged at 3000 rpm for 10 min to obtain serum, which was immediately stored at −80°C. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using ultraviolet spectrophotometric and enzymatic assays. We also measured serum interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) levels using an R&D Rat Magnetic Luminex Screening Assay Kit (R&D Systems Inc, Minneapolis, MN).

Histologic examination

Liver tissues for histologic examination were sampled from the left and median liver lobes at the end of the experimental protocol. The specimens were fixed in a 10% buffered formaldehyde solution, embedded in paraffin, and stained with hematoxylin and eosin. The tissue samples were sectioned into 5-μm-thick pieces for analysis. Histologic examination was performed based on randomly selected tissue sections ($n = 5$ from each group) using the scoring system proposed by