Original articles

Protective effects of d-allose against ischemia reperfusion injury of the rat liver

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Abstract

Background/Purpose. d-Allose, a rare sugar, has been reported to inhibit segmented neutrophil production without causing any significant detrimental clinical effects. Our previous study demonstrated the immunosuppressive effect of d-allose in a rat model of liver transplantation. Neutrophils are closely involved in the process of hepatic ischemia/reperfusion (I/R) injury. One possible mechanism is the adherence of neutrophils to the hepatic sinusoidal endothelium following microcirculatory failure.

Methods. The present study investigated the effects of d-allose on the involvement of neutrophils, with particular emphasis to the microcirculation in a model of hepatic I/R. Ischemia was induced by occluding the hepatoduodenal ligament for 90 min. d-Allose was infused 2 h before ischemia. Normal saline was infused in the control group. Liver tissue blood flow (LTBF) and portal venous flow (PVF) were measured before and after ischemia. Myeloperoxidase (MPO) and ATP were measured at, before inducing ischemia, at the end of ischemia, and at the end of 2-h reperfusion. Liver enzyme analysis and histology were done at the end of reperfusion. Postreperfusion animal survival was followed for 15 days.

Results. d-Allose significantly improved the liver hemodynamics and postreperfusion animal survival, with a significant decrease in liver tissue MPO, liver enzymes, and the number of neutrophils. ATP level was improved significantly in the d-allose group. Histology revealed significant sinusoidal congestion and tissue necrosis after 2-h reperfusion in the control group.

Conclusions. d-Allose exerted its protective effects against liver damage incurred when the liver was injured by warm ischemia and reperfusion mainly by the suppression of activated neutrophils.

Key words Liver ischemia · Reperfusion injury · d-Allose · Portal venous flow · Tissue blood flow

Introduction

d-Allose is a rare aldo-hexose (sugar), produced from d-psicose by using microorganisms and their enzymes. Rare sugars are now attracting attention for their variety of uses; namely, as potential inhibitors of various glycosidases, and as low-calorie carbohydrate sweeteners and bulking agents. Moreover, biologically, d-allose has been reported to inhibit segmented neutrophil production and lower platelet counts in vivo without other significant detrimental clinical effects in rats (Arnold et al. US patent no. 5620960; 1997).

d-Allose is very expensive because of the need to prepare it from d-ribose using the chemical reaction of 1,2-5,6-di-O-isopropylidene-α-d-ribo-hexofuranose-3-ulose hydrate. These chemical methods are elaborate and tedious, and require relatively expensive starting materials. Bhuiyan et al. described a simple method to produce rare sugars on a large scale using microorganisms and their enzymes. Thus depending on the availability, our interest has been focused on the use of d-allose in an in vivo model of ischemia-reperfusion injury. Our previous data (not shown) have also supported the findings of a d-allose-induced decrease in the total number of neutrophils, and demonstrated the immunosuppressive effects of d-allose in a model of allogeneic orthotopic rat liver transplantation, with minimum side effects shown in comparison to those of the renowned immunosuppressive agent, FK506.

Ischemia/reperfusion (I/R) injury of the liver is an important clinical problem. Neutrophils are closely involved in the process of hepatic I/R injury; thus, suppression of neutrophils is mandatory to reduce this injury. Myeloperoxidase (MPO) is an enzyme released from neutrophils. The activity of MPO is directly proportional to the number of activated neutrophils and/or the total number of neutrophils, as well as the number of marginated and/or extravasated neutrophils, seen in histologic
sections. Stimulated neutrophils adhere to vascular endothelium and subsequently produce superoxide anion and other highly reactive oxygen products that increase microvascular permeability and thus induce cellular injury. Therefore, in the present study, we examined the protective efficacy of 

Dose selection of d-allose

In a series of preliminary studies, different doses of d-allose, ranging from 50mg to 1.0g/kg body weight were administered to investigate the effects on liver hemodynamics in normal intact liver and to investigate the effects on survival of the animals. Although d-allose showed no detrimental clinical side effects even at high doses, we observed a slight decrease in body weight in the group treated with high doses compared with the control group. Thus, we decided that the present dose (as above) would have optimal effect.

Materials and methods

Experimental design

Male Lewis rats, weighing 220 to 270g, obtained from Charles River Japan (Yokohama, Japan) were fasted for 12h before surgery, but were allowed to drink water ad libitum. Rats were anesthetized by the inhalation of isoflurane (Abbott Laboratory, Chicago IL, USA) and 99% oxygen at a flow rate of 0.2l/min. The control group received 0.9% normal saline (NS) solution as a vehicle, and the treatment group received d-allose (β-d-allopyranose; Sigma Chemical, St. Louis, MO, USA). D-Allose was dissolved to a concentration of 25mg/ml with normal saline and was administered at a dose of 200mg/kg. The animals were divided into two groups according to treatment; a d-allose group and a control group. In the d-allose group, d-allose was infused continuously with an infusion pump at a dose of 200mg/kg for 60min. In the control group, normal saline was infused as a vehicle at the same infusion rate.

Surgical procedure

After anesthesia, the abdominal cavity was opened through a transverse incision close to the xyphosternum, extending on both sides up to the mid-axillary line. During the experiment, the abdomen was covered with a piece of gauze moistened with warm saline to prevent the visceral organs from drying. The liver was exposed, including the hepatoduodenal ligament. A polyethylene catheter (Intramedic PE-50; Clay Adams, Parsippany, NJ, USA) was inserted into the femoral vein for d-allose or vehicle administration. Two hours prior to the inducing of liver ischemia, d-allose was infused by an infusion pump. Then liver ischemia was induced by occluding the hepatoduodenal ligament for 90min. Splanchnic decompression was accomplished by a portosystemic shunt connecting the ileocecal vein and the left external jugular vein. After 90-min total ischemia, the abdominal wall was closed. The animals were housed in separate cages maintained at room temperature and were allowed free access to food and water for the follow-up of postoperative survival, for a period of 15 days.

Measurements

Hemodynamics

The peripheral tissue blood flow (PTBF) of the liver was measured with a laser-Doppler tissue flow meter (Advance, Tokyo, Japan). The probe was fixed on the liver surface. All the flow values were recorded every minute. A transit time flow meter (Transonic Systems, Ithaca, NY, USA) was used to measure the portal venous flow (PVF). A probe, model 45151, was placed on the portal vein and was fixed with ultrasound transmission gel (Parker Laboratories, Orange, NJ, USA). A 15-min stabilization period was allowed to permit all the vascular parameters to stabilize after surgery. Flows were recorded for 15min before the infusion was started. D-Allose or vehicle was infused and flows were recorded. Then the portal vein probe was withdrawn and the proximal portal vein, including hepatic artery and common bile duct, was occluded for 90min. The portal vein probe was replaced just before reperfusion was started and the flow values were recorded every minute until the end of reperfusion.

Myeloperoxidase

Liver tissues were weighed and homogenized with hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50mM potassium phosphate buffer, pH 6.0) with a Polytron homogenizer (Brinkmann, Westbury, NY, USA) and MPO activity was measured spectrophotometrically by the method of Krawisz et al.7

Sampling

After 120-min reperfusion, venous blood was collected for biochemical measurements and livers were preserved for histopathology. Blood samples were centrifuged at 2000rpm for 5min and the plasma supernatant was stored at −80°C until measurement was done.

ATP

About 100mg of the liver tissue was quickly frozen in liquid nitrogen. Soon after the measurement of tissue weight, the frozen tissue was homogenized in 1ml of