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ORIGINAL ARTICLE

Effects of intravenous administration of pentoxifylline in pancreatic ischaemia–reperfusion injury

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Abstract

Background: Therapeutic strategies to reduce the occurrence of pancreatic ischaemia–reperfusion (I–R) injury might improve outcomes in human pancreas and kidney transplantation. In addition to its haemorrheologic effects, pentoxifylline has an anti-inflammatory effect by inhibiting NF-κB activation. This group has previously demonstrated that pentoxifylline induces an anti-inflammatory response in acute pancreatitis and liver I–R models. This led to the hypothesis that pentoxifylline might reduce pancreatic and renal lesions and the systemic inflammatory response in pancreatic I–R injury. The aim of this experimental study was to evaluate the effect of pentoxifylline administration in a rat model of pancreatic I–R injury.

Methods: Pancreatic I–R was performed in Wistar rats over 1 h by clamping the splenic vessels. The animals submitted to I–R were divided into two groups: Group 1 (n = 20, control) rats received saline solution administered i.v. at 45 min after ischaemia, and Group 2 (n = 20) rats received pentoxifylline (25 mg/kg) administered i.v. at 45 min after ischaemia. Blood samples were collected to enable the determination of amylase, creatinine, tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-10. Pancreatic malondialdehyde (MDA) content, pancreas histology and pulmonary myeloperoxidase (MPO) were also assessed.

Results: Significant reductions in serum TNF-α, IL-6 and IL-10 were observed in Group 2 compared with Group 1 ($P < 0.05$). No differences in pancreatic MDA content or serum amylase levels were observed between the two groups. The histologic score was significantly lower in pentoxifylline-treated animals, denoting less severe pancreatic histologic damage.

Conclusions: Pentoxifylline administration reduced the systemic inflammatory response, the pancreatic histological lesion and renal dysfunction in pancreatic I–R injury and may be a useful tool in pancreas and kidney transplantation.

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Introduction

Ischaemic–reperfusion (I–R) injury occurs relatively frequently in numerous clinical interventions and physiopathological processes, especially in organ transplants.[1] It is caused by an interruption of blood flow or ischaemia and consequent reperfusion, an event that can cause disorders, both local and systemic, that affect the recovery of patients after surgery.[2]

Pancreas transplantation has become the first treatment option for patients with type 1 diabetes. Despite new protocols for immunosuppression, improvements in organ preservation solution and developments in surgical techniques, I–R syndrome remains an important cause of tissue injury in organ transplant recipients.[3] Different experimental studies have tried to identify mediators involved in the systemic inflammatory response, which

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is associated with alterations in microcirculation, such as increased vascular permeability, arterial constriction, stasis of capillary vessels and high levels of circulating pancreatic enzymes.[4] These alterations can lead to several complications such as acute pancreatitis, thrombosis, infection and graft loss.[5]

Graft pancreatitis as a result of I–R injury occurs in 17–35% of patients undergoing pancreas transplantation and may contribute to graft loss and kidney injury, thereby increasing morbidity in these patients.[6,7] It is believed that part of its pathogenesis is related to microcirculatory disorders seen in pancreatic I–R injury. The inhibition of inflammatory mediators involved in this pathogenesis has been studied in some experimental models.[8–11]

Pentoxifylline is a methylxanthine derivative which acts as a phosphodiesterase and NF-κB signal inhibitor with haemorrheologic properties. It is able to increase blood cell deformability, decrease platelet aggregation and lower blood viscosity, reducing thrombus formation. It also exhibits marked anti-inflammatory properties, mediated by inhibition of tumour necrosis factor-α (TNF-α) production.[12,13] A previous study conducted by the present group demonstrated that pentoxifylline achieves an anti-inflammatory response in acute pancreatitis.[14]

Therapeutic strategies to reduce pancreatic I–R injury might improve outcomes in human pancreas and kidney transplantation, and thus pentoxifylline may be useful in reducing the inflammatory response and decreasing complications such as graft loss in patients undergoing pancreas transplant. The aim of this experimental study was to evaluate the effect of pentoxifylline administration in a rat model of pancreatic I–R injury.

Materials and methods

Animals and surgical procedure

Sixty male Wistar rats, weighing 230–250 g, were housed in individual cages under a controlled 12 : 12 h light–dark cycle, with free access to standard chow and water *ad libitum*.

This study was designed in accordance with the Guidelines for the Care and Use of Laboratory Animals (Medical Research Laboratory – 37, Division of Digestive Tract Transplantation, Department of Gastroenterology, University of Sao Paulo). The experiment protocol was approved by the Ethics Committee of the University of São Paulo, São Paulo, Brazil.

The animals were anaesthetized with intraperitoneal ketamine (30 mg/kg) and xylazine (10 mg/kg), submitted to orotracheal intubation, and ventilated with a tidal volume of 0.08 ml/g body weight, at a respiratory rate of 60 breaths/min, and FiO_2 of 0.21 (Small Animal Ventilator 683; Harvard Apparatus, Inc., Holliston, MA, USA). During the surgical procedure, body temperature was monitored using a rectal digital thermometer (Precision™ 4000A; YSI, Inc., Yellow Springs, OH, USA) and was maintained at 37 °C.

Pancreatic I–R model

A pancreatic I–R model was established as described elsewhere.[15] The upper abdomen was opened by a longitudinal laparotomy. The stomach was turned up cranially using two stay sutures (Nylon® 5–0). To minimize pancreatic injury, a no-touch technique was utilized, using the spleen as a grip. The pancreas was carefully separated from the stomach and the short gastric vessels were ligated. Some arterial arcades from the inferior pancreaticoduodenal artery to the splenic artery were ligated. Thus, complete vascular isolation of the pancreatic tail pedunculated on the splenic vessels was achieved. For the induction of ischaemia in the isolated pancreatic tail, one vascular microclamp was applied to the splenic vessels. Ischaemia was induced for 1 h. After achieving ischaemia, the microclamp was removed and pancreatic revascularization was achieved followed by 4 h or 24 h of reperfusion. The abdominal wall remained closed during the I–R period. Blood and tissue samples (pancreas, kidney and lungs) were collected after 4 h h or 24 h of reperfusion.

Pentoxifylline and saline administration

Intravenous administration of pentoxifylline, at 25 mg/kg of animal weight (1.25 ml/kg) (Trental™; Sanofi Aventis Pharma, São Paulo, SP, Brazil), injected via the dorsal penial vein, was performed 15 min before reperfusion and the same dose was repeated after 12 h of reperfusion.

Intravenous administration of saline solution, at 1.25 ml/kg of animal weight, injected via the dorsal penial vein, was performed 15 min before reperfusion and repeated after 12 h of reperfusion.

Experiment design

Animals were divided into three experiment groups: rats in the sham group (n = 20) rats were subjected to laparotomy only without manipulation of the pancreas; rats in Group 1 (n = 20) were subjected to the pancreatic I–R procedure plus i.v. administration of saline solution, and rats in Group 2 (n = 20) were subjected to the pancreatic I–R procedure plus i.v. administration of pentoxifylline.

Rats in Groups 1 and 2 were subjected to laparotomy and 1 h of ischaemia, followed by either 4 h of reperfusion (n = 10 from each group) or 24 h of reperfusion (n = 10 from each group).

Sample preparation

At 4 h and 24 h after reperfusion of the pancreas, animals were re-anaesthetized for blood sampling through cardiac puncture and killed by exsanguination. Serum samples were assayed for amylase activity,[16] inflammatory mediators [TNF-α, interleukin-6 (IL-6) and IL-10], urea and creatinine. The pancreas tissue was obtained from pancreatic tail portions previously submitted to I–R injury and collected for evaluation of malondialdehyde (MDA) and histological examination. Lung myeloperoxidase (MPO) activity was analysed in lung homogenates 24 h after reperfusion.

Cytokine levels

Serum levels of TNF-α, IL-6 and IL-10 were determined by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using commercial kits (Invitrogen Corp., Camarillo, CA, USA).