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Diazoxide reduces local and remote organ damage in a rat model of intestinal ischemia reperfusion

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ABSTRACT

Background: Intestinal ischemia reperfusion is a common clinical condition that causes functional impairment. Once tight junctions are damaged, barrier function is compromised, and the intestines become a source for entry of bacterial and inflammatory mediators into the circulation, leading to systemic inflammatory response syndrome, multiple organ failure, and death. It is possible that diazoxide could protect the intestines against ischemia reperfusion. The aim of this study is to determine whether diazoxide can provide protection in a rat model of intestinal ischemia reperfusion.

Methods: A total of 32 adult male specific pathogen-free Wistar rats were randomized into three groups: a control group, $n = 6$; a saline group, $n = 13$; and a diazoxide group, $n = 13$. The saline and diazoxide groups underwent clamping of the superior mesenteric artery for 1 h, with samples in all the groups being collected 12 h later.

Results: Intestinal histology showed greater damage in the intestinal ischemia reperfusion groups. mRNA expression of zonula occludens-1 and occludin (tight junction proteins) and interleukin-6 and cyclooxygenase-2 was the highest in the Saline group. The Diazoxide group showed a reduction in aspartate aminotransferase serum levels compared with the other groups.

Conclusions: Increased expression of zonula occludens-1, occludin, and cyclooxygenase-2 suggested a greater regenerative effort because of more severe lesions in the saline group. In addition, increased expression of interleukin-6 in the saline group was suggestive of inflammation, indicating that diazoxide had protective effects in the diazoxide group. Reduced aspartate aminotransferase in the diazoxide group suggested liver protection. Diazoxide protects the intestines and liver from intestinal ischemia reperfusion lesions in rats.

Introduction

Intestinal ischemia reperfusion (IR) is a clinical condition caused by blood flow obstruction or low perfusion states[1] and is common in major vascular and abdominal procedures, hemorrhagic shock, major trauma, sepsis, and intestinal transplantation.[2] Because of difficulties in diagnosis and clinical care, it has high morbidity and mortality worldwide.[3,4]

Once enterocytes are damaged after IR, the ability of the intestines to act as an immunological and physical barrier to

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the environment is impaired, and the intestines become a major source of bacteria and inflammatory mediators and can result in bacterial translocation, systemic inflammatory response syndrome, multiple organ failure, and death.[5-8] Multiple organ failure is the most important cause of death in surgical intensive care units.[9]

A layer of enterocytes (one-cell thick) forms the main intestinal physical barrier, with paracellular spaces sealed by tight junction (TJ) complexes. In TJs, cytoskeletons between adjacent cells are linked by intramembranous proteins such as occludin and claudins and extracellular proteins such as zonula occludens-1 (ZO-1).[10] During IR, TJ proteins undergo changes in distribution and expression, resulting in increased permeability and bacterial translocation and cytokine release into the circulation.[11,12] Once the intestines become a cytokine-generating organ, it can cause systemic inflammatory response syndrome. A study has reported that after 1 h of ischemia and 1 h of reperfusion, elevated level of lactate, interleukin (IL)-6, IL-10, IL-1β, and tumor necrosis factor-alpha (TNF-α) was observed in systemic and mesenteric blood.[9] Some of these factors are suggested to be responsible for damage to TJs.[12] Finally, the inflammation spreads to remote organs, causing additional damage and leading to multiple organ failure.[13,14]

Studies have sought to identify agents that are effective at ameliorating IR lesions. One of those suggested is diazoxide, a selective mitochondrial adenosine triphosphate-sensitive potassium channel opener with antihypertensive and hyperglycemic effects. The drug maintains transport of potassium into mitochondria during IR, with optimization of volume and damage reduction.[15,16] Diazoxide has been reported to protect various organs in IR, such as the liver,[15] heart,[17] spinal cord,[18] brain,[19] and others,[20] but no studies have examined its effects during intestinal IR. The aim of the present study was to examine the protective effects of diazoxide on the intestines and remote organs (heart and liver) in a rat model of intestinal IR.

Materials and methods

Ethical statement

The study protocol was approved by the Animal Use Ethical Committee of the Faculty of Medicine, University of São Paulo (CEUA–FMUSP; authorization no. 083/15), and performed in accordance with the principles of the National Council of Animal Experiment Control (Concea).

Study animals

Adult male specific pathogen-free Wistar rats weighing between 250 and 350 g were used. Rats were housed under climate-controlled conditions (22°C ± 1°C) on a 12-h light/dark cycle and had access to food and water *ad libitum*.

Experimental procedure

The procedures took place at the Discipline of Clinical Emergencies Laboratory, University of São Paulo. A total of 32 rats were randomized into three groups: control group, $n = 6$; saline group, $n = 13$; and diazoxide group, $n = 13$. Randomization was performed using sealed envelopes. Anesthesia was induced by intraperitoneal injection of 80-mg/kg ketamine (Ketalar; Parke-Davis, São Paulo, Brazil) and 10-mg/kg xylazine (Rompum; Bayer, São Paulo, Brazil) and maintained by intraperitoneal injection of 15 mg/kg and 5 mg/kg of the same, respectively, every 30 min under spontaneous ventilation. Rats were placed in the supine position under halogen lamps to maintain a body temperature of between 35°C and 37°C (rectal temperature probe). After a 3-cm midline abdominal incision, the superior mesenteric artery was isolated at its origin and occluded with an atraumatic clamp for 1 h.[21] Ischemia was confirmed by intestinal color change and a complete stop in pulsation of the mesenteric arcade, as previously described.[9] Reperfusion was confirmed by the reappearance of pulsation and hyperemia color.

Diazoxide administration

Rats were administered either 3.5 mg/kg of diazoxide or saline at the equivalent volume, as appropriate, 15 min before superior mesenteric artery occlusion. A higher dose of diazoxide, according to previous experiments performed in our laboratory, induces severe hypotension and is detrimental, even though others, such as Zeng *et al.*,[22] have successfully administered higher doses for a similar purpose.

Blood and tissue sampling

Anesthetized rats were killed by exsanguination. Control rats had samples collected without prior surgery. For the saline and diazoxide groups, parameters were determined 12 h after superior mesenteric artery clamp removal. Systemic blood (5 mL) was collected from the abdominal vena cava, and fragments of the last ileal loop were obtained.

Quantitative real-time polymerase chain reaction

Fragments of the distal ileum were assessed to evaluate gene expression of cyclooxygenase-2 (COX-2), TJ proteins (junctional adhesion molecule-A [JAM-A], ZO-1, and occludin), and cytokines (TNF-α, IL-10, and IL-6). Total RNA was extracted using TRIzol reagent following the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Carlsbad, CA) and treated with DNAse I (Invitrogen). Real-time polymerase chain reaction (RT-PCR) reaction mixtures were prepared using Superscript Platinum III One-Step kits incorporating SYBR Green (#11736-051; Invitrogen). Production and amplification of cDNA was performed using a StepOne thermocycler (Applied Biosystems; Thermo Fisher Scientific, Carlsbad, CA), and products were confirmed by size on a 1.5% agarose gel (100 ng of total RNA per sample).

Relative expression was performed using the 2-ΔΔCT method, with the housekeeping gene β-2 microglobulin (β2M) used for normalization.[23] Predeveloped primers and probe assays included IL-10, IL-6, TNF-α, COX-2, occludin, JAM-A, ZO-1, and β2M. Fluorescence levels were normalized to β2M and are expressed as arbitrary units.