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Protective Effects of Hyperoxygenated Solution Preconditioning on Intestinal Ischemia–Reperfusion Injury in Rabbits

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Background. **The aim of this study was to investigate the protective effects of hyperoxygenated solution (HOS) preconditioning on intestinal ischemia–reperfusion (IR) injury in rabbits.**

Materials and methods. **Thirty-two rabbits were randomly divided into four groups as follows: (1) control group in which sham operation was performed (Sham group); (2) sham operation and HOS treatment group (sham+H group); (3) ischemia–reperfusion group (IR group); (4) ischemia–reperfusion and HOS treatment group (H group). Intestinal IR model was produced by clamping superior mesenteric artery with an atraumatic vascular clamp for 1 h, followed by reperfusion for 2 h. Animals in H group received intravenous HOS infusion (20 mL/kg) every day for 5 days before ischemia–reperfusion; animals in the sham+H group received the same amount of HOS before sham operation, and animals in IR group received the same amount of normal saline in the same way. At the end of reperfusion, histopathological changes of intestine were observed, and malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities in intestinal tissues were also detected. Intestinal barrier function was assessed by blood D-lactate levels and bacterial translocation (BT).**

Results. **The H group showed significantly lower MDA levels and higher activities of SOD, CAT, and GSH-Px in the intestinal tissue compared with the IR group. Furthermore, the mean D-lactate levels and incidence of BT in the H group were significantly lower than those in the IR group. Histopathological analysis also indicated that there were significant histological improvements in the H group compared with the IR group.**

Conclusions. **HOS preconditioning at an appropriate dose ameliorates the deleterious changes in intestinal mucosal injury and barrier function associated with IR by effectively preventing a decrease in the intestinal antioxidant defense system, which is another simple and effective measure to protect intestine from IR injury.**

Key Words: **hyperoxygenated solution; intestines; reperfusion injury; preconditioning; antioxidant defenses.**

INTRODUCTION

Among the internal organs, the intestine is probably the most sensitive to ischemia–reperfusion (IR) injury. The intestine is composed of labile cells that are easily injured by episodes of ischemia. Subsequent reperfusion of the intestine results in further damage to the mucosa [1]. Intestine injury resulting from IR is an important clinical event in disorders such as trauma, burn, septic or hypovolemic shock, strangulated hernias, neonatal necrotizing enterocolitis, mesenteric insufficiency, abdominal aortic aneurysm surgery, cardiopulmonary bypass, and intestinal transplantation and plays an important role in the pathogenesis of systemic inflammation and multiple organ failure (MOF) [2]. The mechanisms of IR injury of the intestine and protective strategies against injury have been under extensive research for almost decades. It has been thought that a large number of events are involved in the intestinal IR injury, such as oxygen-free radicals formation, release of iron storage, inflammatory cytokines, complement activation, neutrophil infiltration, and enteric bacteria translocation [3–5]. Several potential therapeutic modalities have been used to amelio-

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rate IR injury of the intestine in animal models. However, these ameliorative strategies, including ischemic preconditioning (IPC), antioxidants, free-radical scavengers, nitric oxide (NO) supplementation, anticomplement therapy, antileukocyte therapy, glutamine supplementation, and glycine supplementation are still inadequate [6–9].

In 1928, Hancock made a miraculous therapeutic effect in the treatment of septicemia with ultraviolet blood irradiation and oxygenation (UBIO) for the first time [10]. Subsequent research has proven that UBIO could also improve oxygen-carrying capacity, regulate immunological function, and ameliorate microcirculation [11]. Chinese scientists derived inspiration from the achievements of UBIO and developed a kind of medical solution called hyperoxygenated solution (HOS) [12]. With photochemistry techniques, some oxygen transforms into ozone (O_3) and oxygen can be dissolved largely in commonly used medical solutions (such as 5% glucose solution, normal saline, lactated Ringer solution, etc.); then these solutions are turned into HOS, and oxygen partial pressure (PO_2) in these solutions can reach 750–900 mmHg. At the same time, there is about 10–20 μg/mL of O_3 in the fresh-made HOS [12]. Administration of HOS in animal experiments has been proven to preserve organ function and integrity after IR injury of the myocardium, spinal cord, and brain [12, 13]. We have found the beneficial effects of intraluminal HOS in intestinal IR injury in rabbits [14]. The current study was undertaken to determine if HOS pretreatment could ameliorate the deleterious changes in intestinal mucosal injury and barrier function caused by IR in rabbits and to develop a new protective strategy against IR injury of the intestine.

MATERIALS AND METHODS

Materials

Fig. 1 displays the schema of preparing HOS. Medical oxygen is introduced into the "Medical hyperoxygenated solution apparatus" (Patent number 922412936; Xi'an Medical Equipment Co., China) at an inflow of 3 L/min for 15 min. Treated with ultraviolet light (wavelength = 180–240 μm), some oxygen transforms into O_3. The O_2/O_3 mixture flows into the airtight base solution (normal saline solution), and this base solution turns into HOS. The amount of oxygen dissolved in the water is a linear function of the partial pressure of oxygen and contact time. In addition, the solubility coefficient (Cs) of O_3 in the water is 13 times larger than that of O_2, and the greater part of the dissolved O_3 will be decomposed into O_2 in a certain period. Then the oxygen displaces the nitrogen (N_2) dissolved in the water. According to the above theories, the amount of oxygen dissolved in the water increases rapidly in a short time, and oxygen pressure (PO_2) in the fresh-made HOS can reach 750–900 mmHg. The PO_2 in this HOS is measured with the I-stat handheld blood gas analyzer (Princeton, NJ). The indigo method [15] as described with slight modification is used to determine O_3 in HOS and the concentration of O_3 in the fresh-made HOS is about 10–20 μg/mL.

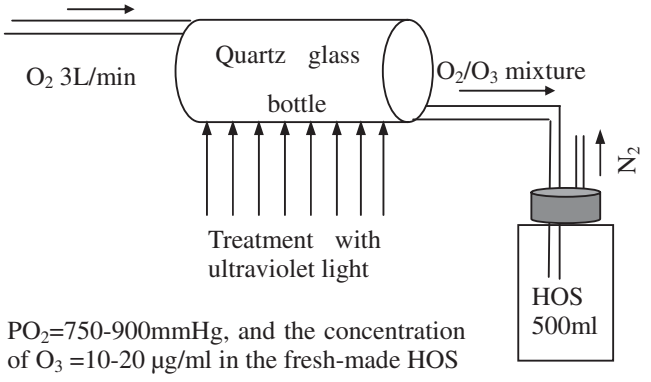

FIG. 1. Schema of preparing HOS.

All reagents used in determinations of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), D-lactate, and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Biotechnology Co. (Nanjing, Jiangsu Province, China). Other reagents of analytical grade were obtained from normal commercial sources.

Methods

Rabbit Intestinal IR Model

All procedures used in this study have been approved by the Ethics Committee for Animal Experimentation and were conducted according to the Guidelines for Animal Experimentation of our institutes. The animals were studied at Tangdu Hospital of the Fourth Military Medical University (Xi'an, China). Thirty-two male New Zealand white rabbits (2.5–3.2 kg) were used in this study. After an overnight fast with unrestricted access to water, the animals were anesthetized with intravenous 3% pentobarbital sodium (30 mg/kg). After a midline laparotomy, the superior mesenteric artery was occluded for 1 h with an atraumatic vascular clamp and followed by reperfusion for 2 h. Between surgical interventions, the midline incision was sutured and covered with plastic wrap to minimize fluid losses. To maintain an adequate anesthetic plane, pentobarbital sodium was administered as necessary. The rabbits were placed on heating pads at 37°C throughout the experiment.

Treatment Groups

Thirty-two male New Zealand white rabbits were randomly divided into four groups (n = 8 each) as follows: (1) control group in which sham operation was performed (Sham group); (2) sham operation and HOS treatment group (sham+H group); (3) ischemia-reperfusion group (IR group); and (4) ischemia-reperfusion and HOS treatment group (H group). Animals in the H group received intravenous HOS infusion (20 mL/kg) every day for 5 days before IR; animals in the sham+H group received the same amount of HOS before sham operation, and animals in the IR group received the same amount of normal saline in the same way. At the end of the 2-h reperfusion period, animals were sacrificed and the following assessments were performed.

Histopathological Evaluations

Intestinal segments from the experimental groups were initially placed in 10% paraformaldehyde and were subsequently paraffin-fixed, sectioned, and stained with hematoxylin and eosin (H&E). One section from each rabbit was graded blindly and semiquantitative histological evaluation was graded from 0 to 5 by a single observer according to the index of Chui *et al.* [16]: sections of intestine with normal morphology were given a score of 0; sections with subepithelial edema and partial separation of apical cells were given a score of 1; sections with moderate lifting of enterocytes from tips of the villi