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Hyperoxygenated Solution Preconditioning Attenuates Lung Injury Induced by Intestinal Ischemia Reperfusion in Rabbits[1]

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Background. **The current study was undertaken to elucidate the possible therapeutic effects of hyperoxygenated solution (HOS) preconditioning on lung injury induced by intestinal ischemia/reperfusion (I/R) in rabbits.**

Materials and methods. **Eighty rabbits were randomly divided into four groups (n = 20 each) as follows: (1) control group in which sham operation was performed (sham group), (2) HOS pretreatment group and sham operation (HOS + sham group), (3) ischemia/reperfusion group (I/R group), (4) HOS pretreatment and ischemia/reperfusion group (HOS + I/R group). Intestinal I/R model was produced by clamping superior mesenteric artery with an atraumatic vascular clamp for 1 h, and followed by reperfusion for 2 h. Animals in HOS + sham group and HOS + I/R group received intravenous HOS infusion (20 mL/kg, 10 mL/kg·h for 2 h) every day for 5 d before operation, and animals in sham group and I/R group received the same amount of normal saline in the same way. At the end of reperfusion, 8 animals from every group were sacrificed and histopathological changes of lung were observed; pulmonary edema, lung myeloperoxidase activity, superoxide dismutase activity, and malondialdehyde levels in lung tissues were also detected. The rest 12 animals in every group underwent 60 min of intestinal ischemia followed by 72 h of reperfusion, and effects of HOS pretreatment on survival in rabbits with lung injury induced by intestinal I/R was observed.**

Results. **When rabbits were subjected to 60 min of intestinal ischemia, a high incidence of mortality was observed within 24 h. In this situation, HOS preconditioning before the start of ischemia/reperfusion significantly reduced the mortality. HOS preconditioning also decreased lung wet/dry ratio, neutrophil infiltration, lipid membrane peroxidation, and increased superoxide dismutase activity in the lungs after intestinal I/R compared with the I/R-treated rabbit lungs without HOS treatment. Histopathological analysis also indicated the effectiveness of HOS pretreatment.**

Conclusions. **HOS preconditioning could preserve superoxide dismutase activity, decrease lipid membrane peroxidation and neutrophil infiltration in the lungs, then ameliorate the deleterious changes in pulmonary injury induced by intestinal I/R.**

Key Words: **hyperoxygenated solution; intestinal ischemia; lung injury; preconditioning; antioxidant defenses; oxidative stress.**

INTRODUCTION

Intestine injury resulting from ischemia/reperfusion (I/R) is an important clinical event in disorders such as trauma, burns, 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) [1]. Intestinal I/R is also a critical and triggering event in the development of distal organ dysfunction, frequently involving the lungs. Respiratory failure is a common cause of death and complications after intestinal I/R [2, 3].

Clinical and experimental studies suggest that oxidative stress induced by reactive oxygen species is one of the most important mediators in the pathogenesis of intestinal I/R [4, 5]. Reactive oxygen species can cause

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direct oxidative damage to DNA, proteins, and lipids [5, 6]. It has been known that neutrophils attached to the injured tissues produce a large amount of reactive oxygen species and cause tissue injury [6]. Ito and his colleagues have reported that pharmacological preconditioning with doxorubicin, an anticancer drug, protects acute lung injury induced by intestinal I/R [6]. In addition, it has been shown that the free radical scavenger methylene blue prevents the development of polymorphonuclear cell-related lung injury after intestinal I/R in rat [7].

Recently, Xu and colleagues developed a kind of medical solution called hyperoxygenated solution (HOS) [8]. 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 hyperoxygenated solutions, and oxygen partial pressure (PO_2) in these solutions can reach 750 to 900 mmHg. At the same time, there is about 10 to 20 μg/mL of O_3 in the fresh-made HOS [8]. Administration of HOS in animal experiments has been shown to preserve organ function and integrity after I/R injury of the myocardium, spinal cord, brain, and intestine [8–10]. Recently, we have found that HOS preconditioning could preserve the endogenous antioxidant systems and decrease lipid peroxidation in rabbit with intestinal I/R injury [11].

The current study was undertaken to elucidate the possible therapeutic effects of HOS preconditioning on lung injury induced by intestinal I/R in rabbits.

MATERIALS

The procedure of preparing HOS has been described in our previous study [10]. Medical oxygen is introduced into the "medical hyperoxygenated solution apparatus" (Patent number: 922412936, Xi'an Medical Equipments Company, Xi'an, China) at an inflow of 3 L per min for 15 min. Treated with ultraviolet light (wavelength = 180 to 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 PO_2 in the fresh-made HOS can reach 750 to 900 mmHg, which is measured with the I-stat hand-held blood gas analyzer (iSTAT Corporation, Princeton, NJ). The indigo method [12] as described, with slight modification, is used to determine O_3 in HOS; the concentration of O_3 in the fresh-made HOS is about 10 to 20 μg/mL.

All reagents used in determinations of superoxide dismutase (SOD), malondialdehyde (MDA), and myeloperoxidase (MPO) were purchased from Nanjing Jiancheng Biotechnology Company, Nanjing, China. Other reagents of analytical grade were obtained from normal commercial sources.

METHODS

Rabbit Intestinal I/R Model

All procedures used in this study were approved by the Ethics Committee for Animal Experimentation and was conducted according to the Guidelines for Animal Experimentation of our institutes [9]. The animals were studied at Tangdu Hospital of the Fourth Military Medical University (Xi'an, China). Male New Zealand white rabbits (weight, 2.5 to 3.2 kg) were used in this study. The animals were housed in wire-bottom cages at 24°C (room temperature) with a 12-h light-dark cycle and were fed standard rabbit chow and water. 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. Ringer's lactated solution (5 mL/kg/h) was given intravenously via auricular vein during the experimental protocol to prevent dehydration of the rabbits. 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

Eighty male New Zealand white rabbits were randomly divided into four groups (n = 20 each) as follows: (1) control group in which sham operation was performed (sham group), (2) HOS pretreatment and sham operation group (HOS + sham group), (3) ischemia/reperfusion group (I/R group), (4) HOS pretreatment and ischemia/reperfusion group (HOS + I/R group). Immobilized in the self-made table, animals in HOS + sham group and HOS + I/R group received intravenous HOS infusion (20 mL/kg, 10 mL/kg·h for 2 h) with an infusion pump via auricular vein every day for 5 d before operation, and animals in sham group and I/R group received the same amount of normal saline in the same way. At the end of the 2 h reperfusion period, 8 animals from every group were sacrificed and the following assessments were performed. The rest 12 animals in every group underwent 60 min of intestinal ischemia followed by 72 h of reperfusion, and effects of HOS pretreatment on survival in rabbits with lung injury induced by intestinal I/R was observed. The animals for survival research came around automatically after I/R, and no analgesic was used for the survival animals during the post-operative period.

Histopathological Evaluations

For histopathological analysis, 60 min of intestinal ischemia followed by 120 min of reperfusion was conducted. At the indicated times rabbits were sacrificed by removing the blood from the heart. Isolated lungs were fixed by inflation with a buffered 10% formalin solution for 24 h and embedded in paraffin. Ten consecutive longitudinal sections of the lungs were stained with hematoxylin and eosin. All tissue sections were examined microscopically for characterization of histopathological changes by an experienced pathologist who was blinded to the treatment conditions. Scoring of the lung injury was done by grading the following criteria from 0 to 3 (0: none, 1: mild, 2: moderate, 3: severe): (a) vascular congestion, (b) interstitial edema, (c) alveolar structural disturbance, (d) leukocyte infiltration, and (e) general edema, giving a maximum score of 15 [13].

Pulmonary Edema

At the end of reperfusion, the lungs were removed from the thoracic cavity, and the inferior third of the left lung was weighed and then placed in a drying oven at 90°C for 24 h. After this drying procedure, the specimen was reweighed, and the ratio of the weight before and after drying was calculated. Lung edema is represented by an increase in this ratio.

Lung MPO Activity

Activity of MPO, an enzyme present in neutrophils, was used as a marker of neutrophil infiltration. Lung MPO activity was determined as described [14]. Lung tissue (100 mg wet wt) was homoge-