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Ischemic Postconditioning Attenuates Lung Reperfusion Injury and Reduces Systemic Proinflammatory Cytokine Release *Via* Heme Oxygenase 1

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Objective. **Systemic inflammatory response following ischemia-reperfusion injury (IRI) to a specific organ may cause injuries in multiple remote organs. The emergence of ischemic postconditioning (IPO) provides a potential method for experimentally and clinically attenuating various types of organ postischemic injuries. We have shown that IPO can attenuate lung IRI by up-regulating the protein expression of heme oxygenase-1(HO-1). This study tested the hypothesis that IPO attenuates systemic inflammatory responses following lung IRI by activating HO-1.**

Methods. **Anaesthetized and mechanically ventilated adult Sprague–Dawley rats were randomly assigned to one of the following groups ($n = 8$ each): the sham-operated control group, the ischemia-reperfusion (IR) group (40 min of left-lung ischemia and 120 min of reperfusion), the IPO group (three successive cycles of 30-s reperfusion per 30-s occlusion before restoring full perfusion), and the zinc protoporphyrin IX (ZnP) plus IPO group (ZnP, an inhibitor of HO-1, was injected intraperitoneally at 20 mg/kg 24 h prior to the experiment, and the rest of the procedures were similar to that of the IPO group). Lung injury was assessed by arterial blood gas analysis, wet-to-dry lung weight ratio and tissue histologic and biochemical changes. The lung tissue and plasma levels of lipid peroxidation were determined by measuring the contents of malondialdehyde (MDA) production. Protein expression of HO-1 was determined by Western blotting. Pulmonary neutrophil was counted. Lung tissue myeloperoxidase (MPO) activity as well as plasma levels of proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukines 6 and 8 (IL-6, IL-8) were determined by spectrophotography.**

Results. **Lung ischemia-reperfusion led to severe lung pathologic morphologic changes and increased pulmonary MDA production, neutrophil count, and MPO activity and reduced arterial oxygen partial pressure (all $P < 0.05$ IR *versus* sham), accompanied with a compensatory increase in HO-1 protein and activity. Plasma levels of TNF-α, IL-6, and IL-8 were increased in the IR group (all $P < 0.05$ *versus* sham). IPO attenuated or prevented all the above changes, except that it further increased lung HO-1 activity. Treatment with ZnP abolished all the protective effects of postconditioning.**

Conclusion. **Postconditioning attenuated pulmonary neutrophil accumulation and activation and lung IRI and reduced systemic inflammatory responses by activating HO-1.**

Key Words: **ischemic postconditioning; heme oxygenase 1; lung ischemia reperfusion; proinflammatory cytokines.**

INTRODUCTION

Pulmonary ischemia-reperfusion injury (IRI) occurs after various clinical procedures, including lung transplantation, cardiopulmonary bypass, pulmonary thrombo-endarterectomy, and trauma [1, 2]. During lung transplantation, the recipient may suffer detrimental effects, from operative trauma and IRI to the graft. These effects cause severe systemic inflammatory

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responses that result in a hypermetabolic status, which interferes with recovery. Lung IRI causes significant morbidity and mortality and is characterized by neutrophil extravasation, interstitial edema, disruption of epithelial integrity, and leakage of protein into the alveolar space that are associated with severe alterations in gas exchange [3]. Therefore, effective modulation of the postoperative systemic inflammatory response may be beneficial to lung transplant recipients.

Recent studies have demonstrated that brief intermittent cycles of ischemia alternating with reperfusion applied after the prolonged ischemic event, a novel approach termed "ischemic postconditioning (IPO), attenuated IRI in a wide range of organs, including the heart, brain, spinal cord, liver, and kidney [4]. Our most recent study shows that IPO can also attenuate lung IRI, and IPO does so by up-regulating the protein expression of heme oxygenase-1(HO-1) [5], a molecular that has recently been shown to play a critical role in ameliorating myocardial, pulmonary, and vascular endothelial injuries [6, 7]. It is unknown, however, whether or not IPO can attenuate systemic inflammatory response after acute lung IRI.

A most recent study shows that postconditioning caused a significant reduction in systemic inflammatory response evidenced as reduced production of tumor necrosis factor-α (TNF-α) and reactive oxygen species [8]. On the other hand, deficiency of heme oxygenase-1 impairs renal hemodynamics and exaggerates systemic inflammatory responses to renal ischemia [9]. In this study, we hypothesized that IPO may attenuate systemic inflammatory responses following lung IRI by up-regulating HO-1 protein expression.

METHODS

Animals

The experimental procedures and protocols used in this investigation were approved by institutional Animal Use Committee. Specific pathogen-free Sprague–Dawley (SD) rats of either sex, weighing between 200 and 230 g, were housed under constant temperature (23 ± 1°C) with 12 h light/dark cycles. All rats were fed with water and rodent chows ad libitum.

Surgical Procedure and Experimental Protocol

The animals were anaesthetized with 7% chloral hydrate (5 mL/kg, i.p.). A 14-gauge angiocatheter was inserted into the trachea through a midline neck incision. The animals were then connected to a volume-controlled ventilator (DW-2000; Jiapeng Keji, Shanghai, China) with room air at a breath rate of 40 times per min, a tidal volume of 12 mL/kg and a positive end expiratory pressure of 2 cm H_2O. The left femoral vein was catheterized, and 3:1 crystalloid to colloidal fluid mixture was infused intravenously. The right femoral artery was catheterized for continuous monitoring of mean arterial pressure (MAP) and for blood sampling. A heating pad was applied during anesthesia to keep the body temperature between 36.5°C and 37.5°C.

The animals were randomly assigned to one of the four groups (n = 8 per group). Under aseptic conditions, an *in situ* unilateral lung warm ischemia model was used. In brief, a left anterolateral thoracotomy in the fifth intercostal space was made. The left lung was mobilized, the pulmonary hilum was dissected, and perivascular and peribronchial tissues were removed. Then, all animals received 500 U/kg of heparin intravenously in saline (total volume 500 mL). In group 1 (sham control, group SC), animals underwent a sham thoracotomy and hilar dissection, but the lungs were not rendered ischemic. In group 2 (group IR), 5 min after heparin administration, the left pulmonary artery, bronchus, and pulmonary vein were occluded with a noncrushing microvascular clamp, maintaining the lung in a partially inflated state. Lungs were kept moist with periodic applications of warm, sterile saline, and the incision was covered to minimize evaporative losses. The period of ischemia was held constant at 40 min, after which the clamp was removed and the lung reperfused for up to 120 min. In group 3 (IPO), postconditioning was performed by three successive cycles of 30-s reperfusion per 30-s occlusion, starting immediately after release of the index ischemia. In group 4 (zinc protoporphyrin IX + IPO group, zinc protoporphyrin IX [ZnP] + IPO), rats were intraperitoneally injected with zinc protoporphyrin IX (Sigma, St. Louis, MO) a specific HO-1 inhibitor, at a dose of 20 mg/kg 24 h prior to the experiment, and the rest of the procedures were similar to that of the IPO group. The rats, which were not administered with any preoperative treatment of ZnPP, were injected with an isovolume of normal saline.

Arterial Blood Gas Analysis

Arterial blood sample for blood gas analysis were taken at 20 min of mechanical ventilation (baseline) and 120 min after reperfusion (postoperative). Arterial blood specimens were analyzed for PaO_2 and $PaCO_2$ using blood gas analyzer.

Lung Wet-to-Dry Weight Ratio

At the end of the experiments, the left lower lobe of the lung was dissected and dried at a constant temperature of 80°C for 24 h to obtain a dehydrated consistency. The ratio of wet weight to dry weight (W/D) was calculated to assess tissue edema, as described in references [5, 10].

Lung Histopathologic Analysis

At the end of the experiments, the left upper lobe of lung was fixed in 10% buffered formalin and 4-mm sections were prepared from paraffin-embedded tissues. The level of histologic tissue injury was assessed by hematoxylin-eosin (H&E) staining. Slides were examined in 400-fold magnification by light microscopy (Leica DMLB; Leica Microsystems) and photo-documented with a digital camera (Leica DC 300 F; Leica Microsystems, Wetzlar, Germany). For each animal, five random tissue sections (three fields per section) were examined. The severity of lung injury was graded by an investigator who was initially blinded to research groups, using a four-point scale according to combined assessments of amount of alveolar congestion, hemorrhage, infiltration, or aggregation of neutrophils in the airspace or vessel wall, and thickness of alveolar wall/hyaline membrane formation [11]. The following criteria were considered: 0 = no damage, 1 = mild damage, 2 = moderate damage, and 3 = severe damage. Further, the number of neutrophils was determined in five randomly selected sections per lung by an investigator blinded to the origin of the sample.

Myeloperoxidase Determination in the Lung Tissue

In the lung tissues, myeloperoxidase (MPO) activities were determined using the technique described by Krawisz *et al.* [12]. Tissue