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# Ischemic postconditioning downregulates Egr-1 expression and attenuates postischemic pulmonary inflammatory cytokine release and tissue injury in rats

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ABSTRACT

*Background:* The early growth response-1 (Egr-1) gene is upregulated after an ischemia–reperfusion (IR) challenge and upregulates target genes, such as proinflammatory cytokines. Ischemic postconditioning (IPostC) attenuates lung IR injury and reduces the systemic inflammatory response by activating heme oxygenase-1 (HO-1). However, the role of Egr-1 in IPostC protection against lung IR injury and inflammation and its interplay with HO-1 in IPostC protection is unknown.

*Materials and methods:* Sprague-Dawley rats or cultured A549 cells were subjected to IR or hypoxia/reoxygenation with or without IPostC or hypoxic postconditioning in the presence or absence of Egr-1 inhibition using Egr-1 antisense oligodeoxyrinonucleotide or Egr-1 small interfering RNA transfection. Lung injury was assessed by measuring the lung wet/dry weight ratio, histologic change, and malondialdehyde content. The amount of lactate dehydrogenase release in culture medium was detected to evaluate cell injury. The protein expression of Egr-1, interleukin (IL)-1β, and HO-1 was assessed by Western blot.

*Results:* Inhibition of Egr-1 significantly attenuated lung IR injury and the inflammation response caused by IR or hypoxia/reoxygenation, as shown by the alleviated lung pathologic changes, decreased pulmonary malondialdehyde content, wet/dry ratio, reduced release of the cytokines tumor necrosis factor-α, IL-6, and IL-8 in the bronchoalveolar lavage, and reduced Egr-1, IL-1β, and HO-1 protein expression and HO-1 activity. IPostC or hypoxic postconditioning reduced the postischemic Egr-1 expression and conferred similar protection against lung IR injury as Egr-1 inhibition.

*Conclusions:* Egr-1 plays an important role in regulating the HO-1 production induced by IR or hypoxia/reoxygenation. Thus, downregulation of Egr-1 expression might represent one of the major mechanisms whereby IPostC confers protection against pulmonary IR insult.



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## 1. Introduction

Acute pulmonary injury resulting from ischemia–reperfusion (IR) can occur with various clinical events, including lung transplantation, cardiopulmonary bypass, trauma, resuscitation for circulatory arrest, atherosclerosis, and pulmonary embolism [1–4] and causes high morbidity and mortality in these patients [5]. Although lung IR injury has been well characterized as increased vascular permeability, microvascular damage, neutrophil infiltration, production of various inflammatory mediators, and tissue injury [1], the pathogenetic mechanisms of lung IR injury are more complicated than other organs because of the dual blood supply system and the availability of oxygen from alveolar ventilation [6]. Therefore, it remains a major area of research to elucidate the underlying mechanisms and develop novel therapies to reduce lung IR injury.

Extensive studies have demonstrated that ischemic and pharmacologic preconditioning can reduce the extent of lung IR injury [7,8]. However, its clinical application has been limited because of the unpredictable occurrence of ischemia in patients. However, a novel approach that applies transient brief interruptions of reperfusion to ischemic episodes, termed "ischemic postconditioning (IPostC)," has been shown to be beneficial to the heart, brain, spinal cord, liver, kidneys, and skeletal muscle [9,10]. Our recent studies have shown that IPostC can also attenuate lung IR injury and reduce the systemic inflammatory response in rats by upregulating heme oxygenase-1 (HO-1) [11,12], an induced molecular in response to various pathophysiologic stress to protect cells and tissues against injury in many disease settings [13]. However, the underlying molecular mechanisms by which IPostC regulates HO-1 expression or activity remain incompletely understood.

The early growth response-1 (Egr-1) gene, a transcription factor containing three zinc finger DNA-binding domains [14], presents in promoter regions and activates expression of multiple target downstream genes, especially those with associations with the inflammatory response, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, intercellular adhesion molecule-1, macrophage inflammatory protein-2, monocyte chemotactic protein-1, and tissue factor [15–17]. Studies have shown that Egr-1 is upregulated in many organs after an IR challenge [15,18,19], and Egr-1 knockout or antisense oligodeoxyribonucleotide to block the effects of Egr-1 can prevent or attenuate pathogenesis during IR injury [15,17]. These findings indicate that upregulation of Egr-1 could be the common denominator in IR injury and that Egr-1 might be a good therapeutic target for IR injury. The purpose of the present study was to investigate the role of Egr-1 in IPostC-mediated protection against pulmonary IR injury and to explore the potential interplay between Egr-1 and HO-1 in IPostC-mediated protection.

## 2. Materials and methods

### 2.1. Rats

A total of 50 male Sprague-Dawley rats (aged 8–12 wk) that were free of specific pathogens and weighing 280–320 g were housed in individual cages in a temperature-controlled room with alternating 12-h light/dark cycles. All the rats were acclimated for 1 wk before the study. All experimental procedures and protocols used in the present study were performed in accordance with the institutional animal care guidelines and approved by the institutional animal care and use committee.

### 2.2. Preparation of Egr-1 antisense oligodeoxyribonucletides

The Egr-1 antisense oligodeoxyribonucletides (ODNs) were designed with the following sequence [17]: 3′-TAC CGT CGC CGG TTC-5′, which were commercially synthesized (TaKaRa Biotechnology, Co, Ltd, Dalian, Liaoning, China). Antisense Egr-1 ODNs were administrated at a dose of 4 mg/kg through the sublingual vein 24 h before ischemia. That dosage has previously shown efficacy without evidence of toxicity [20].

### 2.3. Lung IR model and experimental protocol

An *in vivo* model of lung IR injury was established by clamping the hilus, which resulted in complete ischemia and anoxia, as previously described [21]. The rats were randomly assigned to one of five groups according to the intervention ($n = 10$ in each group; Fig. 1). The rats undergoing IR were subjected to 1 h of left lung ischemia (by left hilar occlusion)

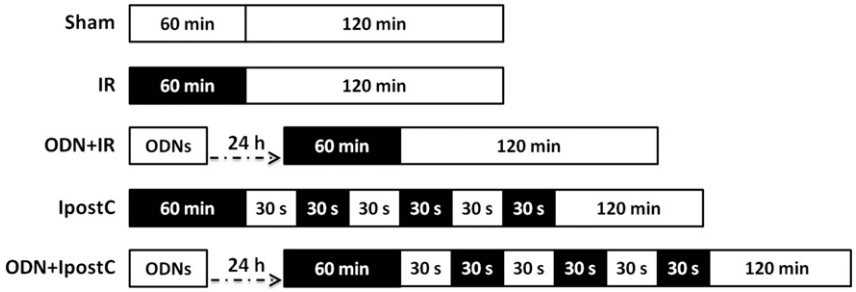


**Fig. 1 – Experimental protocols used to determine effects of IPostC on acute lung injury after IR. White boxes represent ischemia; dark boxes, reperfusion. Sham group underwent surgery without hilar occlusion. Rats in IR group underwent 1 h of left lung ischemia (by left hilar occlusion) followed by 2 h of reperfusion. Rats in IPostC group received three cycles of 30 s reperfusion and 30 s occlusion immediately at onset of reperfusion. Egr-1 antisense ODNs administered though sublingual vein 24 h before surgery and rats subjected to IR with or without IPostC.**