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The interaction between oxidative stress and mast cell activation plays a role in acute lung injuries induced by intestinal ischemia–reperfusion

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ABSTRACT

Background: Both oxidative stress and mast cells are involved in acute lung injuries (ALIs) that are induced by intestinal ischemia–reperfusion (IIR). The aim of this study was to further investigate the interaction between oxidative stress and mast cells during the process of IIR-induced ALI.

Materials and methods: Thirty adult Sprague–Dawley rats were randomly divided into five groups: sham, IIR, IIR + compound 48/80 (CP), N-acetylcysteine (NAC) + IIR, and NAC + IIR + CP. All rats except those in the sham group were subjected to 75 min of superior mesenteric artery occlusion, followed by 2 h of reperfusion. The rats in the NAC + IIR and NAC + IIR + CP groups were injected intraperitoneally with NAC (0.5 g/kg) for three successive days before undergoing IIR. The rats in the IIR + CP and NAC + IIR + CP groups were treated with CP (0.75 mg/kg), which was administered intravenously 5 min before the reperfusion. At the end of the experiment, lung tissue was obtained for pathologic and biochemical assays.

Results: IIR resulted in ALI, which was detected by elevated pathology scores, a higher lung wet-to-dry ratio, and decreased expression of prosurfactant protein C ($P < 0.05$). Concomitant elevations were observed in the expression levels of the nicotinamide adenine dinucleotide phosphate oxidase subunits $p47^{phox}$ and $gp91^{phox}$ and the levels of hydrogen peroxide and malondialdehyde. However, superoxide dismutase activity in the lung was reduced ($P < 0.05$). The level of interleukin 6, the activity of myeloperoxidase, and the expression of intercellular adhesion molecule 1 were also increased in the lung. IIR led to pulmonary mast cell degranulation and increases in the plasma and pulmonary β-hexosaminidase levels, mast cell counts, and tryptase expression in lung tissue. CP aggravated these conditions, altering the measurements further, whereas NAC attenuated the IIR-induced ALI and all biochemical changes ($P < 0.05$). However, CP abolished some of the protective effects of NAC.

Conclusions: Oxidative stress and mast cells interact with each other and promote IIR-induced ALI.

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1. Introduction

It has been demonstrated that intestinal ischemia–reperfusion (IIR) causes local injuries and leads to the severe destruction of remote organs [1]. Lung damage, such as that incurred via acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), is particularly likely. ALI and ARDS increase mortality in critically ill patients. Thus, it is imperative to elucidate the molecular mechanisms of IIR-induced ALI to develop effective therapeutic strategies. Pulmonary inflammation has been suggested as the main mechanism of lung injury in such cases [2–4]. However, anti-inflammatory treatments have not significantly decreased the mortality of IIR-induced ALI [5]. The mechanisms underlying IIR are very complicated and poorly understood, and specific therapeutic strategies are currently undefined.

Mast cells are a type of immune-regulated cell. They are ubiquitous in the lung, and their primary role is immune surveillance [6–11]. However, in situations of ongoing tissue damage, the activation of mast cells by the sustained release of numerous proinflammatory mediators, proteases, and cytokines can contribute to the pathophysiology of lung allergic and inflammatory diseases [12,13]. Our previous studies have demonstrated that mast cells are primarily involved in IIR-induced ALI via mast cell degranulation, which increases pulmonary inflammation, whereas mast cell stabilizers ameliorate these symptoms [14,15].

Previous studies have yielded a significant amount of evidence, indicating that oxidative stress is the primary participant in the pathogenesis of lung injuries induced by IIR [16–21]. Intestinal reperfusion allows reactive oxygen species (ROS) to "escape" the microvasculature of the gut into the systemic circulation and to generate a cascade of various biological events, including ALI. However, the relationship between mast cells and oxidative stress in the process of IIR-dependent induction of ALI remains largely unknown. *In vitro*, ROS can stimulate mast cells to produce proinflammatory mediators [22,23]. In this study, we postulate that the ROS generated during IIR might trigger pulmonary mast cell degranulation and aggravate ALI. Additionally, we show that the interaction between oxidative stress and activated mast cells participates in IIR-induced ALI using *N*-acetylcysteine (NAC) to reduce ROS and compound 48/80 (CP) to activate mast cells.

2. Materials and methods

2.1. Animals

Healthy adult Sprague–Dawley rats (provided by the Animal Center of Sun Yat-sen University), weighing 180–220 g at the beginning of the experiment, were used. The animals were housed under standard laboratory conditions. Water and regular rat chow were available *ad libitum*, and the animals were kept on a 12-h dark/light cycle throughout the experiment. Efforts were made to minimize animal suffering and reduce the number of animals used. All experimental procedures received prior approval from the Institutional Animal Care and Use Committee of Sun Yat-sen University (GuangZhou, PR China), in accordance with the ethical guidelines for the investigation of experimental pain in conscious animals.

2.2. Experimental design and the IIR experimental model

The rats were divided into five groups: sham, IIR, IIR + CP, NAC + IIR, and NAC + IIR + CP. Each group consisted of six rats. All rats were anesthetized via an intraperitoneal injection of 10% chloral hydrate (3.5 mL/kg) after they were fasted overnight with free access to water. After ensuring an adequate depth of anesthesia, the rats were fixed in the supine position. In the IIR-inclusive groups, the abdomen was opened, and the superior mesenteric artery (SMA) was identified and clamped for 75 min; subsequently, the clamp was released, and the splanchnic region was reperfused for 2 h. In the sham-operated group (sham group), the abdomen was opened; however, the SMA was not clamped. In the NAC + IIR and NAC + IIR + CP groups, the rats were intraperitoneally injected with NAC (0.5 g/kg) for three successive days as a pretreatment before SMA clamping, with the aim of determining the lone effect of antioxidants on IIR and the combined effect of antioxidants and a mast cell degranulator. All the other groups underwent intraperitoneal treatment with an equal dose of normal saline. In the IIR + CP and NAC + IIR + CP groups, the rats subjected to IIR were also treated with CP (0.75 mg/kg; Sigma, MO), which promotes mast cell degranulation and was administered intravenously 5 min before the start of reperfusion, with the aim of examining whether mast cell degranulation can alter the state of oxidative stress and inflammation. The rats in the other groups received the same volume of normal saline. The doses of NAC and CP were selected in accordance with previous publications [9–11,13–15,21,25,26].

2.3. Preparation of specimens and specimen measurements

Two hours after reperfusion, the rats were killed with an overdose of chloral hydrate. Then, blood was obtained from the abdominal artery to measure β-hexosaminidase levels, and a thoracotomy was rapidly performed. The right upper lungs were removed, fixed in 10% formaldehyde, and embedded in paraffin for sectioning. The right middle lungs were used to determine the lung wet-to-dry ratio. The remainder of the lungs was washed with cold saline, dried with suction paper, and stored at −80°C for further analysis.

2.4. Histopathologic examinations

After immersion in a 10% formaldehyde fixative for 24 h, the lung tissue was rinsed with tap water to remove the fixative. For light microscopy examination, the lung tissue was dehydrated with graded alcohol and embedded in paraffin at 60°C. One series of microsections (4 μm) was stained with hematoxylin and eosin, whereas another series of microsections (4 μm) was stained with toluidine blue to detect pulmonary mast cells. Blue–purple granules in the cytoplasm were recognized as positive staining for mast cells, which were counted in five randomly selected areas per slide at ×400