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Propofol prevents lung injury after intestinal ischemia–reperfusion by inhibiting the interaction between mast cell activation and oxidative stress

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ABSTRACT

Aims: Both mast cells and oxidative stress are involved in acute lung injury (ALI) induced by intestinal ischemia–reperfusion (IIR). The aim of this study was to investigate whether propofol could improve IIR-induced ALI through inhibiting their interaction.
Main methods: Repetitive, brief IIR or IIR + compound 48/80 was performed in adult Sprague–Dawley rats pretreated with saline, apocynin or propofol. And their lungs were excised for histology, ELISA and protein-expression measurements 2 h after reperfusion.
Key findings: Rats pretreated with saline developed critical ALI 2 h after IIR. We found significant elevations in lung injury scores, lung wet/dry ratio and gp91phox, p47phox, intercellular cell adhesion molecule-1 protein expressions and higher level of malondialdehyde, interleukin-6 contents, and myeloperoxidase activities, as well as significant reductions in superoxide dismutase activities, accompanied with increases in mast cell degranulation evidenced by significant increases in mast cell counts, β-hexosaminidase concentrations, and tryptase expression. And the lung injury was aggravated in the presence of compound 48/80. However, pretreated with propofol and apocynin not only ameliorated the IIR-mediated pulmonary changes beyond the biochemical changes but also reversed the changes that were aggravated by compound 48/80.
Significance: Propofol protects against IIR-mediated ALI, most likely by inhibiting the interaction between oxidative stress and mast cell degranulation.

Introduction

Intestinal ischemia–reperfusion (IIR) usually occurs during different surgical events, such as traumatic shock, cardiac bypass, liver transplantation and intestinal transplantation. Clinical trail demonstrated that the mortality induced by intestinal ischemic reperfusion was 32.1%–77.4 (Schoots et al., 2004). It has been well demonstrated that IIR alone can injure the intestines and destroy the remote organs (de Vries et al., 2010). This organ destruction includes acute lung injury (ALI) or acute respiratory distress syndrome, which increases mortality in critically ill patients. It is imperative to elucidate the molecular mechanisms of IIR-mediated ALI to develop effective therapeutic strategies.

The mechanisms of IIR-induced ALI are rather complex. Mast cell is a type of immune-regulated cell, and it is ubiquitous in the lung and plays a primary role in immune surveillance (Impellizzeri et al., 2011; Irani et al., 1989, 1991). Our previous studies have demonstrated that mast cell degranulation participates in IIR-induced ALI (Gan et al., 2012; Huang et al., 2012; Zhao et al., 2014). Oxidative stress has been strongly implicated in the pathogenesis of ALI induced by different causes (Reyes et al., 2006). Research has demonstrated that reactive oxygen species (ROS) can stimulate in vitro mast cell degranulation (Abdeen et al., 2011). According to these results, we assumed that IIR-induced pulmonary oxidative stress and mast cells interact with one another and promoted the ALI process.

Propofol is a commonly used intravenous anesthetic, mainly used for induction and maintenance of anesthesia, ICU sedation, and in recent years try for treatment of insomnia (Vasileiou et al., 2012). Our previous study showed another antioxidant, N-acetylcysteine inhibited the interaction between mast cells and oxidative stress (Zhao et al., 2014). Recent studies have shown propofol reduced organ ischemia–reperfusion injury,

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including lung injury, through its antioxidant action (Vasileiou et al., 2012; Liu et al., 2008, 2007), but it is still unclear whether propofol is related to the mast cell action in IIR-induced ALI. In the current study, we applied mast cell degranulator (compound 48/80) as the tool drug and NADPH oxidase inhibitor (apocynin) as the control drug. We aimed to demonstrate the interaction between oxidative stress and mast cell activation in IIR-induced ALI and to observe the effect of propofol on both mechanisms.

Materials and methods

Animal groups and the IIR experimental model

Healthy adult Sprague–Dawley rats (purchased from the Animal Center of Sun Yat-sen University) weighing 180–220 g, were 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 investigating experimental pain in conscious animals.

The rat mode of IIR was performed in accordance with our previous studies (Gan et al., 2012). Mast cell degranulator compound 48/80 was used to trigger the mast cell degranulation, while apocynin, an NADPH oxidase inhibitor, was used to inhibit the NADPH oxidase and to decrease the ROS production. Meanwhile, apocynin was also used as the positive control of propofol. The rats were divided into seven groups: SH, IIR, IIR + CP, AP + IIR, AP + IIR + CP, PRO + IIR and PRO + IIR + CP. Each group comprised 18 rats, and all subjects were observed for survival. Six rats were randomly chosen from the surviving animals to detect the relative indices. All rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (3.5 mL/kg). All rats, except for those in the SH group, were subjected to 75 min of superior mesenteric artery occlusion, followed by 2 h of reperfusion. In the AP + IIR, PRO + IIR, PRO + IIR + CP groups, the rats were intraperitoneally injected with apocynin (2.5 mg/kg, AP group, Sigma, America) (Paterniti et al., 2010), and a sedative dose of propofol (50 mg/kg, PRO group, AstraZeneca, Holland) (Liu et al., 2008, 2007) for three successive days as a pretreatment prior to the superior mesenteric artery clamping according to our previous study (Yao et al., 2014). In the IIR + CP, AP + IIR + CP, and PRO + IIR + CP groups, the rats subjected to IIR were treated intravenously for 5 min before being reperfused with compound 48/80 (Sigma, USA; 0.75 mg/kg) (Gan et al., 2012).

Preparation of specimens and specimen measurements

Two hours after the reperfusion, the rats were sacrificed, and blood was taken from the abdominal artery to measure the levels of β-hexosaminidase. The right upper lungs were removed, fixed in 10% formaldehyde and embedded in paraffin for sectioning. The right middle lungs were used to detect the lung wet/dry ratio. The remaining lungs were stored at −80 °C for further analysis.

Survival rates

The survival rates of each group were observed during the 2 h reperfusion after intestinal ischemia.

Histopathological examinations

Harvested lung tissues were handled as routing paraffin embedding and sectioning. Microsection series (4 μm) was stained with hematoxylin and eosin. The lung injury quantification method was performed according to the Hofbauer method (Gan et al., 2012).

Lung wet/dry ratio

The right middle lobes of the lungs were weighed by a precision balance after harvesting and dried for 24 h in an oven at 95 °C and reweighed subsequently. The right middle lung lobes were used according to the following equation: wet/dry = $\text{weight}_{\text{wet}}$ / $\text{weight}_{\text{dry}}$ (Murakami et al., 2000).

Assessment of mast cell counts in lung

Microsection series (4 μm) were prepared from paraffin-embedded lung. And the sections were stained with toluidine blue to detect the pulmonary mast cells. Blue–purple granules in the cytoplasm were recognized as positive staining for the mast cells, which were counted in five randomly selected areas per slide at 400× magnification.

Detecting the pulmonary and plasma β-hexosaminidase levels

The pulmonary homogenates and blood samples were centrifuged and used to measure the β-hexosaminidase levels according to the method described by Smith et al. (Ortega et al., 1989). The resulting samples were assayed to detect their absorbance rates at 405 nm.

Detecting the IL-6 levels in lung tissue

Harvested lung tissues were made into homogenates and detected with enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (KeyGEN Biotech Company, Nanjing, China). The IL-6 levels in the lungs were calculated as ng per g protein.

Detecting the MPO activity in lung tissue

Lung tissues were made into homogenates. The supernatants were used to evaluate the pulmonary (myeloperoxidase) MPO activity with an MPO detection kit (KeyGEN Biotech Company, Nanjing, China) according to the manufacturer's instructions. The MPO activity in the lung tissue was calculated as U per g of protein.

Western blot analysis of proSP-C, tryptase, $p47^{phox}$, $gp91^{phox}$, ICAM-1

Western blot analysis was used in our previous studies (Zhao et al., 2014). Intercellular adhesion molecule-1 (ICAM-1) mouse monoclonal antibody was 1:500 dilution; tryptase rabbit polyclonal antibody was 1:500 dilution; anti-prosurfactant protein C (proSP-C) polyclonal rabbit antibody was 1:500 dilution; p47phox polyclonal rabbit antibody was 1:1000 dilution; gp91phox polyclonal rabbit antibody was 1:1000 dilution. All antibodies above were purchased from Santa Cruz, USA. The secondary antibody, goat anti-mouse or anti-rabbit IgG antibodies, was purchased from Thermo Fisher Scientific, Fremont, CA, USA and the dilution is 1:2000. Anti-β-actin was 1:1500, purchased from Merck Millipore, Germany. Protein–antibody complexes were detected with an enhanced chemiluminescence system (KGP1125, purchased from Nanjing KeyGEN Biotech. Co., Ltd.). Protein band sizes were estimated using AlphaView software (Cell Biosciences, Santa Clara, CA). The density measurement was correlated to the protein levels and normalized to those of β-actin.

Detection of the MDA content and SOD activity in lung tissue

Lung tissues were homogenized and centrifuged. The supernatant was used to evaluate the tissue methane dicarboxylic aldehyde (MDA) levels and the superoxide dismutase (SOD) activities using an MDA detection kit and an SOD detection kit, respectively (KeyGEN Biotech Company, Nanjing, China), according to the manufacturer's instructions.