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Isoalantolactone protects LPS-induced acute lung injury through Nrf2 activation

Cheng-bo Yuan, Lin Tian, Bo Yang, Hai-yan Zhou*

Department of Respirology, The Affiliated Hospital of Changchun University of Chinese Medicine, Changchun, 130000, Jilin, China

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ABSTRACT

Isoalantolactone (ISO), a sesquiterpene lactone isolated from *Inula helenium*, is known to have anti-inflammatory activity. Here, using a mouse model of acute lung injury, we investigated the effects of ISO on lung inflammation in vivo. ISO (2.5, 5, 10 mg/kg) was administered 1 h before LPS treatment. Histopathological changes suggested that ISO attenuated the injury of lung tissues induced by LPS. ISO also inhibited LPS-induced MPO activity, MDA content, lung W/D ratio, and the production of inflammatory cytokines TNF-α and IL-1β. LPS decreased the activities of the antioxidant enzymes SOD, GPX, and CAT and the decreases were inhibited by ISO. Further studies were performed to detect the Nrf2 and NF-κB signaling pathway. The results showed that ISO significantly suppressed LPS-induced NF-κB activation, as well as PI3K and AKT phosphorylation. Additionally, the expression of Nrf2 and HO-1 were dose-dependently up-regulated by the treatment of ISO. Taken together, the results indicate the protective action of ISO against LPS-induced ALI were through activation of the Nrf2 signaling pathway.

1. Introduction

Acute lung injury (ALI) is a clinical syndrome characterized by inflammatory cell infiltration, diffuse damage of microvascular and alveolar epithelium, pulmonary edema, and interstitial fibrosis of the lung [1]. It can be caused by many factors, such as trauma, pneumonia, and sepsis [2]. Previous studies showed that up to 40% of patients with sepsis had associated ALI [3]. LPS, the major component of gram negative bacteria, is known as one of the important pathogenic factors of sepsis [4]. LPS can lead to lung injury by releasing inflammatory cytokine production [5]. Inhibition of inflammatory cytokines could attenuate the injury of lung tissues induced by LPS [6]. ALI is an important cause of death in patients with sepsis [7]. Therefore, seeking effective strategies for the prevention and treatment of ALI has important clinical significance.

Isoalantolactone (ISO), a sesquiterpene lactone isolated from Inula helenium, has been known to have anti-inflammatory activity. ISO has been known to inhibit LPS-induced inflammatory cytokines production in peritoneal macrophages and protect mice against sepsis [8]. Also, ISO has been reported to induce ROS-dependent apoptosis in U2OS cells via a novel mechanism involving inhibition of NF-κB activation [9]. Furthermore, ISO was found to induce apoptosis in SGC-7901 cells via regulating PI3K/AKT signaling pathway [10]. In addition, ISO was found to induce the detoxifying enzymes in HepG2-C8 cells [11]. However, whether ISO had anti-inflammatory effects against lung injury induced by LPS had not been reported. The purpose of this study was to investigate the protective effects of ISO on LPS-induced lung injury in mice.

2. Materials and methods

2.1. Materials

ISO (purity > 98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). ELISA kits for TNF-α and IL-1β were purchased from BioLegend (CA, USA). GPX, SOD, CAT, MPO and MDA assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). LPS and dexamethasone (DEX) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2. Experimental design

BALB/c mice, weighing approximately 20–25 g, were obtained from the Center of Experiment Animals of Jilin University (Changchun, China). All animal experimental procedures followed the guidelines of

* Corresponding author. Department of Respirology, The Affiliated Hospital of Changchun University of Chinese Medicine, Changchun, 130000, Jilin, China.
E-mail address: zhouhaiyan211@126.com (H.-y. Zhou).

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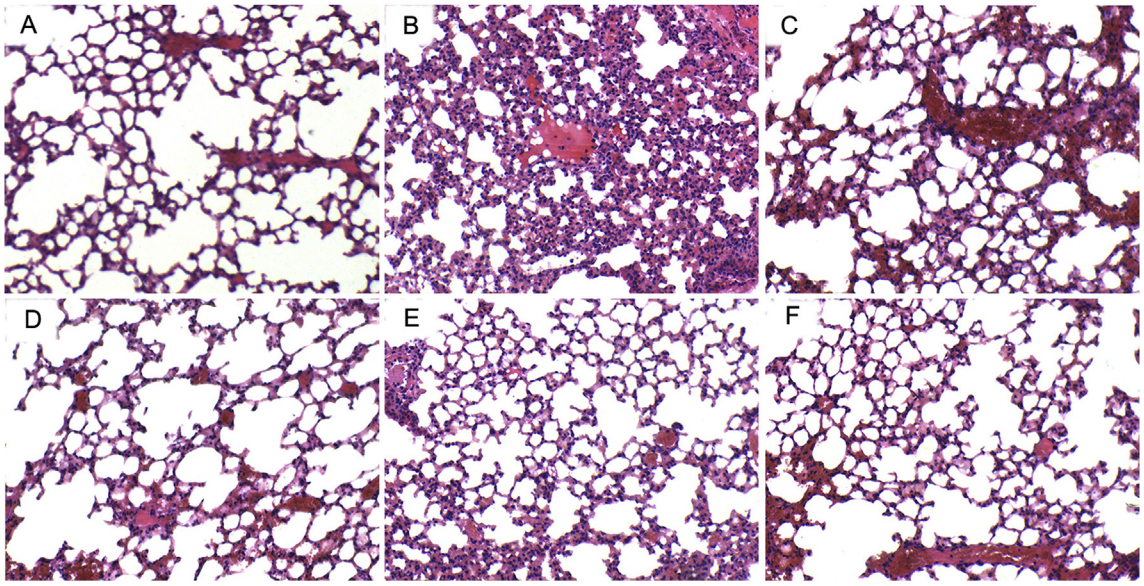

Fig. 1. Effects of ISO on histopathological changes in lung tissues in LPS-induced ALI mice. Representative histological changes of lung obtained from mice of different groups. A: Control group, B: LPS group, C: LPS + ISO (2.5 mg/kg) group, D: LPS + ISO (5 mg/kg) group, E: LPS + ISO (10 mg/kg) group, F: LPS + DEX group (Hematoxylin and eosin staining, magnification 200×).

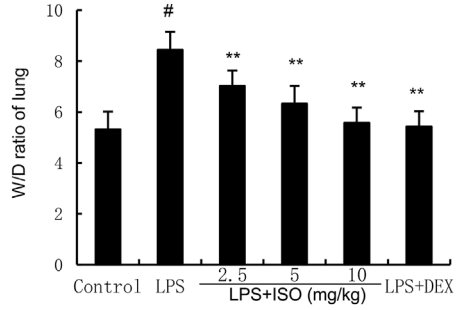

Fig. 2. Effects of ISO on the lung W/D ratio of LPS-induced ALI mice. The values presented are the means ± SEM of three independent experiments. #p < *0.01 vs.* control group, *p < *0.05* and **p < *0.01 vs.* LPS group.

the US National Institute of Health. The mice were divided into six groups: the control group, LPS group, LPS + ISO (2.5, 5, 10 mg/kg) groups, and LPS + DEX (5 mg/kg) group. LPS-induced ALI was induced by giving 10 μg of LPS dissolved in 50 μl of PBS by intratracheal instillation. ISO (2.5, 5, 10 mg/kg) or DEX (5 mg/kg) was given intraperitoneal 1 h before LPS treatment. The doses of ISO used in this study were based on a previous study [8].

2.3. Lung wet-to-dry weight (W/D) ratio

The lung tissues were collected and the wet weight was recorded. Subsequently, the tissues were placed in an incubator at 80 °C for 48 h. Then, the lung tissues were weighted to obtain the 'dry' weight. The ratio of wet lung to dry lung was calculated to assess tissue edema.

2.4. MPO and MDA assay

Lung tissues were weighted and homogenized in cold PBS. The supernatants were collected and the MPO activity and MDA content in lung tissues were detected by the assay kits according to the manufacturer's instructions.

2.5. Histopathological evaluation of the lung tissue

The right lobes of the lung tissues were fixed in 10% formaldehyde for 48 h. Then, the tissues were exposed to a series of graded ethanol for dehydration, and embedded in paraffin. Finally, the tissues were cut into sections (5 μm), and stained with hematoxylin and eosin (H & E). Pathologic examination was assessed using a light microscope (Olympus, Japan).

2.6. ELISA assay

The production of TNF-α and IL-1β in the BALF was measured in this study using the commercially available ELISA kits (BioLegend, CA, USA) according to the instructions of the manufacturer.

2.7. Western blot analysis

Lung tissues were collected and lysed with RIPA buffer to collect proteins. The protein concentration was measured by BCA kit. Subsequently, the proteins were separated on 10% SDS-PAGE and transferred onto PVDF membranes. After blocking, the membranes were incubated with primary antibodies: NF-κB p65, NF-κB p-p65, IκBα, p-IκBα, Nrf2, and HO-1. Then, the membranes were washed three times and incubated with secondary antibodies. Finally, the proteins were visualized using enhanced chemiluminescence reagents (ECL) (Thermo, IL, USA).

2.8. Statistical analysis

All data were expressed as means ± SEM and analyzed using SPSS software (Chicago, IL, USA). The differences among multiple groups were analyzed using one-way ANOVA and the LSD method. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Effects of ISO on LPS-induced lung histopathological changes

The effects of ISO on lung histopathology were tested by H&E staining. The results demonstrated that the lung tissues of the control group exhibited normal alveolar structure. However, the lung tissues of the LPS group exhibited severe pathological damage, such as inflammatory cell infiltration, lung edema, and obvious alveolar wall