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Protective effects of scoparone against lipopolysaccharide-induced acute lung injury

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ABSTRACT

The purpose of this study was to investigate the protective effects and molecular mechanisms of scoparone on lipopolysaccharide (LPS)-induced acute lung injury in mice. Mice model of acute lung injury (ALI), induced by intranasal instillation of LPS, was used to investigate the protective effects of scoparone in vivo. The alveolar macrophages were used to investigate the molecular mechanisms of scoparone in vitro. The results showed that scoparone treatment remarkably attenuated LPS-induced pulmonary edema, histological severities, myeloperoxidase activity, and TNF-α, IL-6 and IL-1β production in vivo. We also found that scoparone inhibited LPS-induced TLR4 expression, NF-κB activation, TNF-α, IL-6 and IL-1β production in alveolar macrophages in vitro. In conclusion, our results suggest that scoparone has a protective effect on LPS-induced ALI via suppression of TLR4-mediated NF-κB signaling pathways.

1. Introduction

Acute respiratory distress syndrome (ARDS) is characterized by overwhelming lung inflammation and increased microvascular permeability [1]. ARDS often results in multi-organ failure with high mortality in critically ill patients [2]. Most causes such as sepsis, trauma and burn could induce ARDS and the most common cause of ARDS is sepsis resulting from bacterial infection [3]. Current treatments such as surfactants, glucocorticoids, and stem cells do not significantly reduce lung injury and mortality [4]. Therefore, the developments of new and effective strategies to treat ARDS are urgently needed.

LPS, the outer membrane of gram-negative bacteria, has been reported to be an important risk factor of ALI [5–7]. LPS stimulates alveolar macrophages to induce TLR4 activation, which finally induces the production of inflammatory cytokines, such as TNF-α, IL-6 and IL-1β [8–10]. These cytokines amplify the inflammatory responses and lung injury [11]. The pathophysiological mechanism of ARDS is believed to be associated with the uncontrolled inflammatory response in lungs [12]. Nowadays, studies showed that treatments aimed at modulating TLR4 signaling pathway to alleviate inflammatory response may have potential therapeutic advantages for ARDS [13].

Scoparone, a major component of the shoot of *Artemisia capillaris*, has been reported to have anti-inflammatory and anti-tumor effects [14,15]. Several studies showed that scoparone inhibited IL-8 and MCP-1 production in U937 cells and TNF-α, IL-6 and IL-1β production in LPS-stimulated RAW264.7 cells [16,17]. Furthermore, scoparone was found to have a protective effect on D-galactosamine/lipopolysaccharide-induced hepatic failure in mice [18]. However, the anti-inflammatory effect of scoparone on LPS-induced ALI has not been reported. The aim of this study was to investigate the protective effects and molecular mechanisms of scoparone on lipopolysaccharide (LPS)-induced acute lung injury.

2. Materials and methods

2.1. Reagents

Scoparone and LPS (*Escherichia coli* 055:B5) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse TNF-α, IL-6 and IL-1β assay kits were purchased from R&D Systems (Minneapolis, MN). Anti-pNF-κB p65, anti-NF-κB p65, anti-TLR4, and anti-β-actin monoclonal antibodies were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA). All other chemicals were of reagent grade.

2.2. Animals

Male BALB/c mice weighing 18–22 g were obtained from the Center of Experimental Animals of Peking University (Beijing, China). The mice were maintained in a pathogen-free and light-controlled room (12 h light and 12 h dark) with free access to food and water. All animal experiments were performed in accordance with the Health's Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

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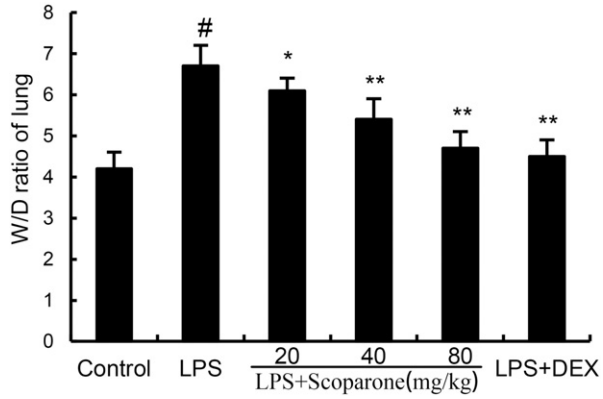

Fig. 1. Effects of scoparone on LPS-induced lung edema. The lung edema was assessed by the lung W/D ratio. Data were presented as means ± SEM (n = 12 in each group). #p < *0.01* compared to control group, *p < *0.05* and **p < *0.01* compared to LPS group.

2.3. Experimental design and grouping

All mice were randomly divided into six groups: Control, LPS, LPS + scoparone (20, 40 and 80 mg/kg) and LPS + DEX group. Scoparone (20, 40 and 80 mg/kg) and DEX (5 mg/kg) were given intraperitoneally. 1 h later, mice were slightly anesthetized with an inhalation of diethyl ether, 10 μg of LPS in 50 μl PBS was instilled intranasal (i.n.) to induce lung injury.

2.4. MPO activity assay

Lung tissues were homogenized in 50 mM HEPES and subjected to three-thaw cycles. Then the homogenate was centrifuged at 13,000 *g* for 30 min and used for MPO assay. MPO activity was detected using test kits purchased from Nanjing Jiancheng Bioengineering Institute (China) according to the instructions.

2.5. Histopathologic evaluation of the lung tissue

The lungs were excised and fixed in 4% paraformaldehyde in 0.1 M PBS (Ph 7.4) for 24 h. Then lung tissue was dehydrated with alcohol, imbedded in paraffin and sliced. The sections stained with hematoxylin and eosin (H&E) stain. Then pathological changes of lung tissues were observed under a light microscope.

2.6. Inflammatory cell counts of BALF

The BALF samples were centrifuged (4 °C, 3000 rpm, 10 min) to pellet the cells. The cell pellets were resuspended in PBS for total cell counts using a hemacytometer. We prepared cytospins for differential cell counts by staining using the Wright–Giemsa staining method.

2.7. Lung wet to dry lung weight ratio (W/D ratio) measurement

The left lungs were obtained immediately and the wet weight was determined. The lungs were placed in an oven at 80 °C for 24 h to obtain the dry weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

2.8. qRT-PCR assay for CXCL1, CXCL2 and CCL2 expressions

Total RNA from mammary gland tissues was extracted using TRIzol reagent. Then the RNA was reversed transcribed following the manufacturer's instructions of first strand cDNA synthesis kit from Fermentas (Burlington, Canada). The primers were: CXCL1, sense 5′-GCCTATCGCCAATGAGCT-3′and antisense 5′-TGACTTCGGTTTGGGTGC-3′; CXCL2 sense 5′-ACCAACCACCAGGCTACA-3′and antisense 5′-CTTC AGGGTCAAGGCAAA-3′; CCL2, sense 5′-TGGGTCCAGACATACATT-3′ and antisense 5′- ACGGGTCAACTTCACATT-3′; β-actin, sense 5′-TAAA ACGCAGCTCAGTAACAGTCG-3′and antisense 5′-TGCAATCCTGTGGCAT CCATGAAAC-3′. The mRNA expression levels were evaluated by qRT-PCR using the SYBR Green QuantiTect RT-PCR kit (TaKaRa Biotechnology Co., Ltd) and the 7500 Fast Real-Time PCR System (applied Biosystems). The relative expression of each gene was normalized to β-actin.

2.9. Cell culture and treatment

Murine alveolar macrophages were isolated as described by Liu et al. with some modifications [19]. The lungs were lavaged with fluids and then centrifuged at 1000 *g* for 10 min. The cells were

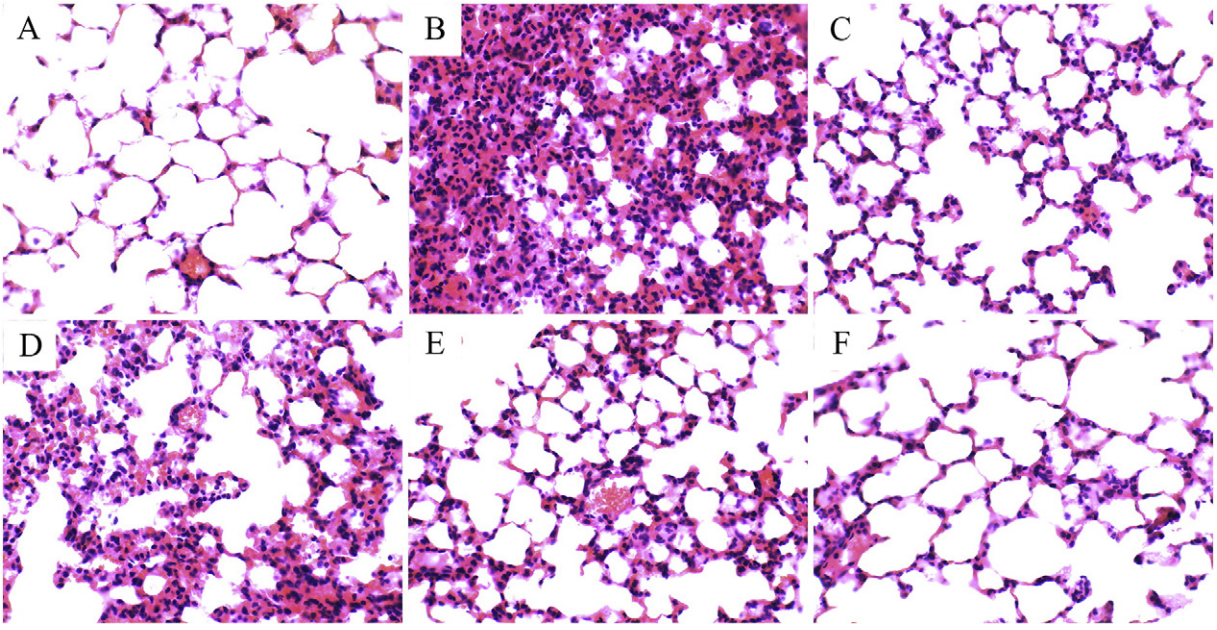

Fig. 2. Effects of scoparone on histopathological changes in lung tissues. Mice were administrated with scoparone 1 h before intratracheal instillation of LPS. The histopathological assays (200× magnification) in lungs were performed at 7 h after LPS instillation. A: Control group, B: LPS group, C: LPS + DEX group, D: LPS + scoparone (20 mg/kg) group, E: LPS + scoparone (40 mg/kg) group F: LPS + scoparone (80 mg/kg) group.