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Patchouli alcohol protects against lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Background: Patchouli alcohol (PA), a natural compound isolated from *Pogostemon cablin*, has been reported to possess anti-inflammatory activity. However, the effects of PA on lipopolysaccharide (LPS)-induced acute lung injury (ALI) have not yet been studied. In the present study, we investigated *in vivo* the effect of PA on ALI induced by LPS.

Methods: Mice were administrated intranasally with LPS to induce lung injury. PA was administrated intraperitoneally 1 h before or after the LPS challenge.

Results: The results showed that PA significantly decreased the wet-to-dry weight ratio of lungs and the number of total cells, neutrophils, and macrophages in bronchoalveolar lavage fluid at 7 h after the LPS challenge. In addition, PA also suppressed the production of inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1β, and interleukin-6 in bronchoalveolar lavage fluid. Furthermore, Western blot analysis showed that PA inhibited the phosphorylation of IκB-α and p65 nuclear factor κB (NF-κB) induced by LPS.

Conclusions: Our results suggest that the anti-inflammatory effects of PA against LPS-induced ALI may be due to its ability to inhibit NF-κB signaling pathways.

1. Introduction

The acute respiratory distress syndrome, a clinically important complication of severe acute lung injury (ALI) in humans, is a significant cause of morbidity and mortality in critically ill patients [1]. It is characterized by intense pulmonary inflammatory responses, involving neutrophil recruitment, interstitial edema, disruption of epithelial integrity, and lung parenchymal injury [2,3]. Lipopolysaccharide (LPS), a main component of the outer membrane of gram-negative bacteria, is one of the major factors that induce ALI [4]. Stimulating lung epithelial cells and macrophages by LPS induces the production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β. These proinflammatory mediators lead to lung inflammation and lung tissue injury [5]. The mouse model of LPS-induced ALI has been used for preliminary pharmacologic studies of potential therapeutic drugs and agents [6,7].

Patchouli alcohol (PA; Fig. 1), a tricyclic sesquiterpene isolated from *Pogostemonis Herba*, is known to possess a number of pharmacologic activities, such as antioxidant, antitumor, and anti-inflammatory effects [8,9]. PA was found to inhibit TNF-α, IL-1β, and IL-6 production in LPS-stimulated RAW264.7 cells and IL-6 production in TNF-α stimulated HT-29 cells [8,10]. In addition, PA had been reported to have anti-inflammatory effects in xylene-induced ear edema in mice and carrageenan-induced paw edema in rats [11]. However, whether PA has the ability to attenuate LPS-induced ALI and

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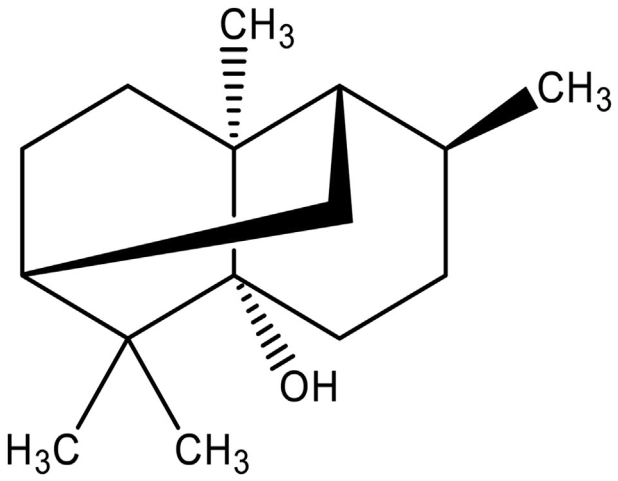

Fig. 1 – Chemical structure of PA.

its underlying molecular mechanisms remains unclear. In this study, we sought to assess the effects of PA on LPS-induced mouse ALI model and elucidate the potential anti-inflammatory mechanism.

2. Materials and methods

2.1. Materials and chemicals

PA was purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Enzyme-linked immunosorbent assay (ELISA) kits of TNF-α, IL-6, and IL-1β were purchased from R&D Corporation (R&D Systems Inc, Minneapolis, MN). Anti-pNF-κB p65, anti-NF-κB p65, anti-IκBα, anti-pIκBα, anti-Lamin B, and anti-β-actin monoclonal antibodies were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA). The myeloperoxidase (MPO) determination kit was provided by the Jiancheng Bioengineering Institute of Nanjing (Nanjing, Jiangsu, China). All other chemicals were of reagent grade.

2.2. Animals

Male BALB/c mice, 6–8 wk, weighing approximately 18–20 g were purchased from the Experimental Animal Center of Shandong University (Shandong, China). The mice were housed in microisolator cages and received food and water. The laboratory temperature was 24 ± 1°C, and relative humidity was 40%–80%. Mice were housed for 4–6 d to adapt the environment before experimentation. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. LPS-induced ALI in mice

After adjustment to the environment, 108 male BALB/c mice were randomly divided into eight groups, and each group contained 12 mice as follows: control group, LPS group, PA (10, 20, and 40 mg/kg; 1 h before LPS) + LPS groups, dexamethasone (DEX; 1 h before LPS) + LPS group, PA (40 mg/kg; 1 h after LPS) + LPS group, pyrrolidine dithiocarbamate (PDTC) (nuclear factor κB [NF-κB] antagonist) (100 mg/kg) + LPS group, and PDTC (100 mg/kg) + PA (40 mg/kg) + LPS group. DEX was used in this study as a positive control, and the dose of DEX used in this study was based on previous studies [12]. PA (10, 20, and 40 mg/kg), PDTC (100 mg/kg), and DEX (5 mg/kg) were given intraperitoneally. DEX has been reported to have a well protective effect on LPS-induced ALI, and many studies used DEX as a positive control. PA was suspended in 0.1% Tween 80 in phosphate-buffered saline (PBS) [11]. Mice from the control and LPS groups received an equal volume of 0.1% Tween 80 instead of PA or DEX. One hour later, mice were slightly anesthetized with an inhalation of diethyl ether, 10 μg of LPS in 50-μL PBS was instilled intranasally to induce lung injury. Control mice were given 50-μL PBS without LPS. All the mice were alive after 7 h of LPS treatment. Collection of bronchoalveolar lavage fluid (BALF) was performed three times through a tracheal cannula with autoclaved PBS, instilled up to a total volume of 1.3 mL.

2.4. Lung wet-to-dry weight ratio measurement

After the mice were euthanized, the lungs were excised, blotted dry, weighed to obtain the "wet" weight, and then placed in an oven at 60°C for 24 h to obtain the "dry" weight. The ratio of wet lung to dry lung was calculated to assess tissue edema.

2.5. Inflammatory cell counts of BALF

The fluid recovered from each sample was 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. Cytospins were prepared for differential cell counts by staining with the Wright–Giemsa staining method.

2.6. Cytokine ELISA

Levels of TNF-α, IL-6, and IL-1β in the supernatants of the BALF were determined using commercially available ELISA kits according to the manufacturer's instructions.

2.7. Pulmonary MPO activity in ALI mice

The accumulation of neutrophils in the lung tissue was assessed by MPO activity. Briefly, the lung tissue samples were frozen and homogenized in cool normal saline (lung tissue to normal saline 1:10). Then, the homogenate was done according to the manufacturer's instructions. MPO activity was measured with a spectrophotometer at 460 nm.

2.8. Histologic study

Histopathologic examination was performed on mice that were not subjected to BALF collection. The lung tissues were fixed with 4% paraformaldehyde for 24 h and embedded in paraffin. After deparaffinization and dehydration, the lungs were cut into 5-μm sections and stained with hematoxylin and eosin.