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Protective effect of catalpol on lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Catalpol, an iridiod glucoside isolated from *Rehmannia glutinosa*, has been reported to have anti-inflammatory properties. Although anti-inflammatory activity of catalpol already reported, its involvement in lung protection has not been reported. Thus, we investigated the role of catalpol on lipopolysaccharide (LPS)-induced acute lung injury in this study. Mice acute lung injury model was induced by intranasal instillation of LPS. Catalpol was administrated 1 h prior to or after LPS exposure. The severity of pulmonary injury was evaluated 12 h after LPS administration. The results showed that catalpol inhibited lung W/D ratio, myeloperoxidase activity of lung samples, the amounts of inflammatory cells and TNF-α, IL-6, IL-4 and IL-1β in BALF induced by LPS. The production of IL-10 in BALF was up-regulated by catalpol. In vitro, catalpol inhibited TNF-α, IL-6, IL-4 and IL-1β production and up-regulated IL-10 expression in LPS-stimulated alveolar macrophages. Moreover, western blot analysis showed that the activation of NF-κB and MAPK signaling pathways was inhibited by catalpol. Furthermore, catalpol was found to inhibit TLR4 expression induced by LPS. In conclusion, catalpol potently protected against LPS-induced ALI. The protective effect may attribute to the inhibition of TLR4-mediated NF-κB and MAPK signaling pathways.

1. Introduction

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is the leading cause of death in critical care [1,2]. It was characterized by neutrophil recruitment, interstitial edema, and lung parenchymal injury [3,4]. It can be induced by severe infection, trauma, acidosis or burn [5–7]. LPS, the outer membrane of gram-negative bacteria, has been referred to be an important risk factor of acute lung injury [8]. LPS induces the production of inflammatory cytokines such as TNF-α, IL-6, IL-1β (Deakin et al., 1995; Jang et al., 2006) which play critical roles in the development of ALI (Bhatia and Moochhala, 2004). Despite extensive effects in research and clinical medicine, ARDS still has a high mortality rate of approximately 40% [9]. Therefore, the development of definitive and targeted drug therapies for ALI is urgently needed.

Catalpol, an iridoid glycoside extracted from the roots of *Rehmannia glutinosa*, has been shown to have antioxidant, anti-apoptosis and anti-inflammatory activities [10–12]. Catalpol was found to inhibit LPS and IFN-r-induced inflammatory response in astrocytes [13]. Studies showed that catalpol inhibited inflammatory cytokines in the senescent mice induced by D-galactose [10]. However, the effect of catalpol on LPS-induced acute lung injury remains unclear. In this study, we sought to assess the preventive effects and potential mechanism of catalpol on LPS-induced acute lung injury.

2. Materials

2.1. Materials

Catalpol was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Mouse TNF-α, IL-10, IL-4, IL-6 and IL-1β ELISA kits were purchased from R&D Systems (Minneapolis, MN). LPS was purchased from Sigma (St. Louis, MO, USA). The myeloperoxidase (MPO) determination kit was provided by the Jiancheng Bioengineering Institute of Nanjing (Nanjing, Jiangsu, China). Anti-pNF-κB p65, anti-p-p38, anti-p38, anti-pJNK, anti-JNK, anti-pERK, anti-ERK, anti-TLR4, and anti-β-actin monoclonal antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). All other reagents were of analytical grade.

2.2. Animals

Male BALB/c mice, weighing approximately 18 to 22 g, were purchased from the Center of Experimental Animals of Harbin Medical

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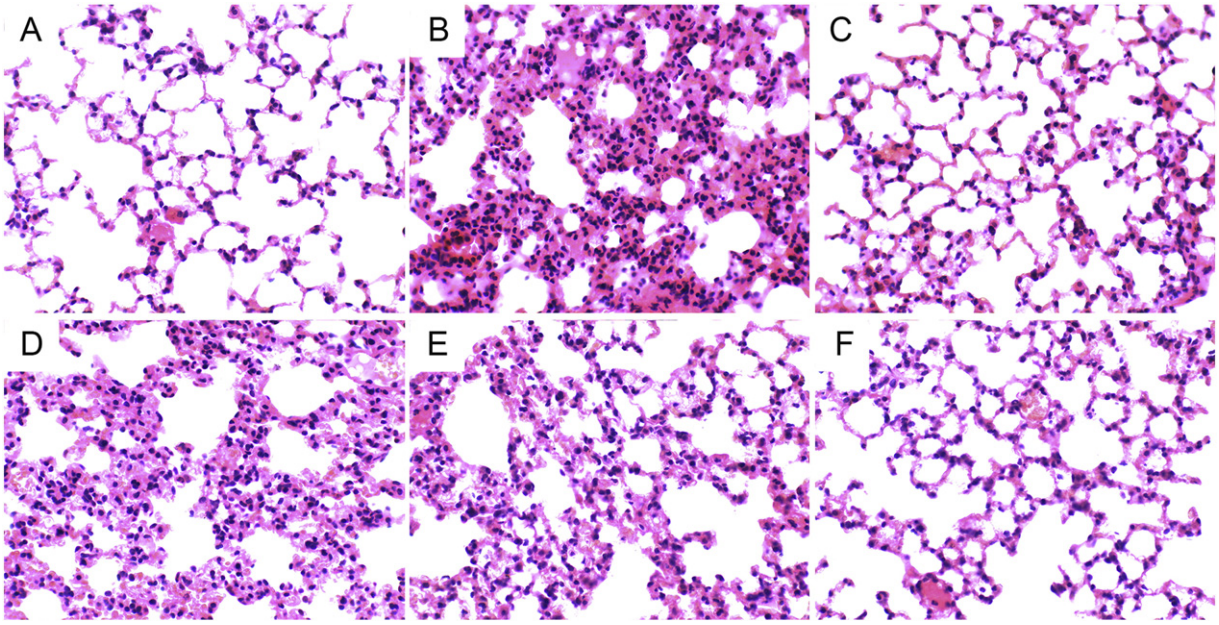

Fig. 1. Effects of catalpol on histopathological changes in lung tissues in LPS-induced ALI mice. Representative histological changes of lung obtained from mice of different groups. A: Control group, B: LPS group, C: LPS + DEX group, D: LPS + catalpol (2.5 mg/kg) group, E: LPS + catalpol (5 mg/kg) group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

University (Hei Longjiang, China). The mice were housed in an air-conditioned room with controlled temperature (24 ± 1 °C) with 40%–80% humidity. They were allowed free access to food and water. All animal experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.3. Experimental design

The mice were randomly divided into seven groups: control group, LPS group, catalpol (2.5, 5 and 10 mg/kg) + LPS group, dexamethasone (DEX) + LPS group and LPS + catalpol (10 mg/kg) group. DEX in this study was used as a positive control. Catalpol (2.5, 5 and 10 mg/kg) and DEX (5 mg/kg) were given through intraperitoneal injection (i.p.) 1 h before LPS administration. 10 μg of LPS in 50 μl PBS was instilled intranasal (i.n.) to induce lung injury. Control mice were given 50 μl PBS without LPS. 12 h after LPS administration, animals were euthanized. The lung tissues and bronchoalveolar lavage fluid (BALF) were received.

2.4. Cytokine assays

Levels of the cytokines, including TNF-α, IL-6, IL-10, IL-4 and IL-1β, in the BALF and cell-free supernatants were measured using ELISA kits (R&D) according to the manufacturer's instructions.

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

Lungs were excised and weighed to obtain the 'wet' weight. Then the lungs were blotted dry, weighed, and then placed in an oven at 80 °C for 48 h to obtain the "dry" weight. The ratio of the wet lung to the dry lung was calculated by dividing the wet weight by the dry weight.

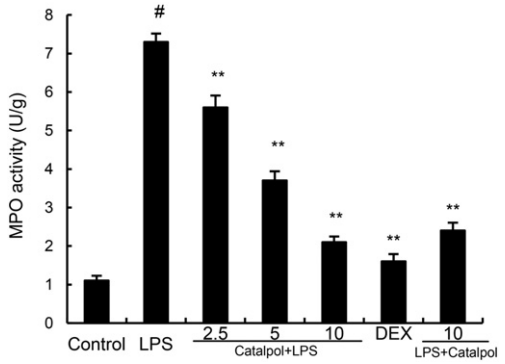

Fig. 2. Effects of catalpol on MPO activity in lung tissues of LPS-induced ALI. The values presented are the mean ± SEM (n = 12 in each group). p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS group.

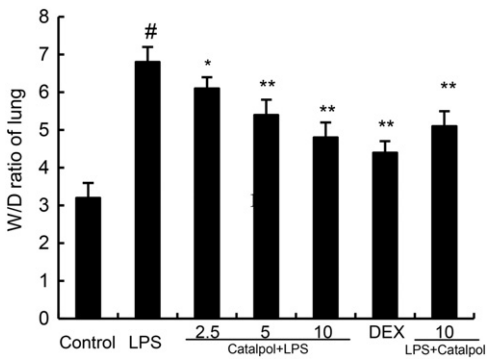

Fig. 3. Effects of catalpol on the lung W/D ratio of LPS-induced ALI mice. The values presented are the means ± SEM (n = 12 in each group). #p < 0.01 vs. control group, *p < 0.05 and **p < 0.01 vs. LPS group.