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Protective effects of syringin against lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Background: Syringin, a major active substance isolated from *Eleutherococcus senticosus*, has been found to have anti-inflammatory effect. The aim of this study was to investigate the effects and underlying mechanisms of syringin on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice.

Methods: We established an LPS-induced ALI model in mice. We also detected the lung wet-to-dry ratio, myeloperoxidase activity, and inflammatory cytokines tumor necrosis factor alpha, interleukin (IL)-1β, and IL-6 to estimate the index of lung injury in mice. Furthermore, the expression of nuclear factor-erythroid 2-related factor-2 (Nrf2), heme oxygenase-1, and nuclear factor κB (NF-κB) was detected by Western blot analysis.

Results: The results showed that the increases in lung wet-to-dry ratio, myeloperoxidase activity, malondialdehyde content, and levels of tumor necrosis factor alpha, IL-1β, and IL-6 induced by LPS were significantly inhibited by treatment of syringin. The phosphorylation of IκB-α and p65 NF-κB caused by LPS was inhibited by syringin. Furthermore, syringin was found to upregulate the expression of Nrf2 and heme oxygenase 1.

Conclusions: In conclusion, the results suggest that syringin protects against LPS-induced ALI by activating Nrf2 and inhibiting NF-κB signaling pathway.

Introduction

Acute lung injury (ALI) is a critical illness syndrome with high morbidity and mortality in critically ill patients.[1] ALI is characterized by severe lung inflammation and increased capillary permeability.[2] Lipopolysaccharide (LPS) has been identified as the major factor that leads to ALI.[3] In mice with ALI, LPS significantly upregulates the expression of inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.[4] Studies showed that these cytokines cause the aggregation of neutrophilic leukocytes and lead to lung injury.[5] Therefore, inhibition of these cytokines has drawn a great attention in the treatment of LPS-induced ALI. Nuclear factor κB (NF-κB) is a nuclear transcription factor, which plays an important role in the regulation of inflammatory cytokines.[6,7] Inhibition of NF-κB activation has the ability to attenuate LPS-induced ALI.[8,9]

Syringin is a major active substance isolated from *Eleutherococcus senticosus*. It has been found that syringin has broad pharmacologic effects, such as anti-inflammatory and

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antinociceptive effects.[10] Syringin has been reported to protect against LPS and D-galactosamine–induced hepatic failure.[11] Syringin also inhibits LPS-induced TNF-α production in RAW264.7 cells.[12] Furthermore, syringin has been reported to exert sleep-potentiating effects through the nitric oxide synthases–nitric oxide pathway.[13] However, the effects of syringin on LPS-induced ALI have not been reported. Therefore, in this study, we investigated the effects of syringin on LPS-induced ALI in mice.

Materials and methods

Reagents

Mouse TNF-α, IL-6, and IL-1β enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioLegend (San Diego, CA). The myeloperoxidase (MPO) determination kit was purchased by the Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China). Mouse nuclear factor-erythroid 2-related factor-2 (Nrf2), HO-1, NF-κB p65, NF-κB p-p65, IκB, p-IκB, and β-actin were provided from Cell Signaling Technology Inc (Beverly, MA). Syringin was purchased from Chengdu Must Bio-technology Co, Ltd (Chengdu, China). LPS (Escherichia coli 055:B5) was purchased from Sigma Chemical Co (St. Louis, MO). All other chemicals were of reagent grade.

Animal

Male BALB/c mice of 18-22 g weight (Animals Experiment Center, Baiqiuen Medical College of Jilin University, Jilin, China) were housed in microisolator cages with controlled environment at a temperature of 22°C-25°C. All studies involving animals were performed according to the National Institutes of Health guide for the Care and Use of Laboratory Animal.

Study design and grouping

Sixty mice were randomly divided into three groups: control group, LPS group, and LPS + syringin (25, 50, and 100 mg/kg) groups. The mice of LPS group were received 50-μL LPS given intranasally to induce ALI. The mice of LPS + syringin (25, 50, and 100 mg/kg) groups were given intraperitoneally to the mice 1 h after LPS treatment. The mice of control group were given equal amount of phosphate-buffered saline.

Lung wet-to-dry weight ratio

Twelve hour after LPS treatment, the mice were condemned to death, and the lungs were collected to obtain the wet weight. Next, we obtained dry weight after keeping the lungs at 80°C for 48 h. The lung wet-to-dry (W/D) ratio was calculated by dividing the wet weight by the dry weight.

MPO and malondialdehyde assay

Twelve hour after LPS treatment, the lung tissues were homogenized and the MPO and malondialdehyde (MDA) contents were measured by MPO determination kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

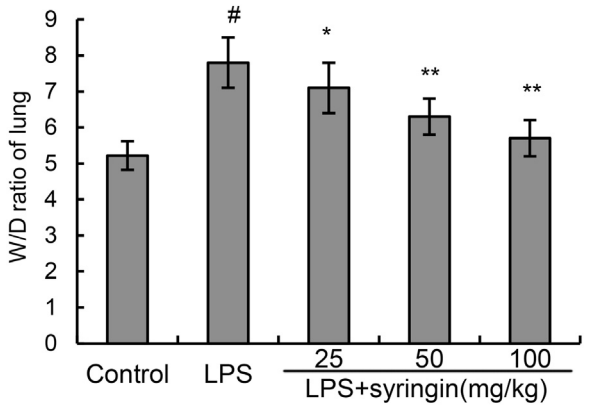

Fig. 1 – Effects of syringin on the lung W/D ratio of LPS-induced ALI mice. The values presented are the means ± standard error of the mean of three independent experiments. #$P < 0.01$ *versus* control group, *$P < 0.05$ and **$P < 0.01$ *versus* LPS group.

Hematoxylin and eosin staining

Twelve hour after LPS treatment, the lung tissues were collected and fixed in 4% formaldehyde. The samples were

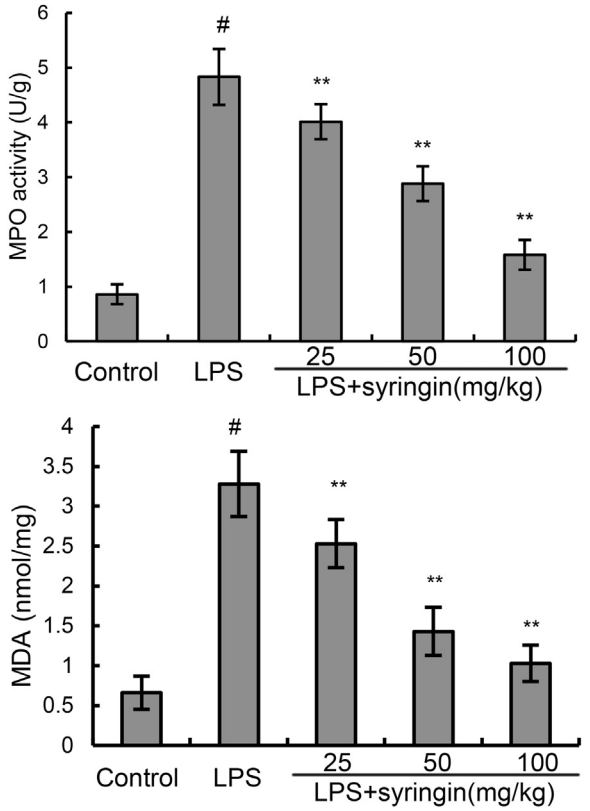

Fig. 2 – Effects of syringin on MPO and MDA contents. The values presented are the mean ± standard error of the mean of three independent experiments. #$P < 0.01$ *versus* control group, **$P < 0.01$ *versus* LPS group.