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Fucosterol attenuates lipopolysaccharide-induced acute lung injury in mice

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ARTICLE INFO

Article history:
Received 19 October 2014
Received in revised form
13 December 2014
Accepted 31 December 2014
Available online 10 January 2015

Keywords:
Fucosterol
LPS
Acute lung injury
NF-κB

ABSTRACT

Background: Fucosterol has been reported to have antioxidant, antidiabetic, and anti-inflammatory effects. In this study, we investigated the protective effect and the possible mechanism of fucosterol on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice.

Methods: Lung injury was assessed by a histologic study, pulmonary edema, and inflammatory cytokines production in bronchoalveolar lavage fluid. Alveolar macrophages were stimulated with LPS in the presence or absence of fucosterol. The expressions of inflammatory cytokines were determined by enzyme-linked immunosorbant assay. Nuclear factor-kappa B (NF-κB) expression was detected by Western blotting.

Results: The results showed that fucosterol attenuated lung histopathologic changes, wet-to-dry ratio, and tumor necrosis factor-α, interleukin (IL)-6 and IL-1β production in LPS-induced ALI in mice. Meanwhile, fucosterol inhibited NF-κB activation and tumor necrosis factor-α, IL-6, and IL-1β production in LPS-stimulated alveolar macrophages.

Conclusions: In conclusion, the present study demonstrated that fucosterol exhibited a protective effect on LPS-induced acute lung injury, and the possible mechanism is involved in inhibiting NF-κB activation, thereby inhibiting LPS-induced inflammatory response.

1. Introduction

Acute respiratory distress syndrome is associated with acute lung inflammation involving the neutrophil recruitment, interstitial edema, and lung parenchymal injury [1–3]. Lipopolysaccharide (LPS) has been reported to be an important risk factor of acute respiratory distress syndrome [4–6]. LPS leads to release of proinflammatory mediators and cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 [7–10]. These inflammatory cytokines result in asthmatic pathologic alterations and lung injury. Recently, studies showed that inhibiting of nuclear factor-kappa B (NF-κB) activation and inflammatory cytokines production could prevent LPS-induced lung injury [11].

Fucosterol, isolated from *Undaria pinnatifida,* has been reported to have antioxidant, antidiabetic, and anti-inflammatory effects [12,13]. Fucosterol was found to inhibit LPS-induced nitric oxide and inflammatory cytokines

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http://dx.doi.org/10.1016/j.jss.2014.12.054

production in RAW264.7 cells [14]. Furthermore, fucosterol was found to have a protective effect on 2,4-dinitrochlorobenzene-induced atopic dermatitis in NC/Nga mice [15]. However, the anti-inflammatory effect of fucosterol on LPS-induced acute lung injury (ALI) remains unclear. Thus, we investigated the protective effect and the possible mechanism of fucosterol on LPS-induced ALI in this study.

2. Materials and methods

2.1. Reagents

Fucosterol and LPS (*Escherichia coli* 055:B5) was purchased from Sigma Chemical Co (St. Louis, MO). Mouse TNF-α, IL-6, and IL-1β enzyme-linked immunosorbant assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN). Antibodies against NF-κB, IκBα, β-actin, and horseradish peroxidase–conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Autogen Bioclear, Santa Cruz, United Kingdom). All other chemicals were of reagent grade.

2.2. Animals

Male BALB/c mice, weighing approximately 18–22 g, were purchased from the Center of Experimental Animals of Zhengzhou University (Henan, China). All animals were housed in a room maintained at 23 ± 2°C with 50 ± 10% humidity and a 12-h light–12-h dark cycle. 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.

2.3. Experimental design and grouping

Eighty-four BALB/c mice were randomly divided into seven groups, and each group contained twelve mice as follows: control, LPS, LPS + fucosterol (15, 30, and 60 mg/kg) + LPS group, dexamethasone (DEX) + LPS group, and LPS + fucosterol (60 mg/kg) group. Mice were slightly anesthetized with an inhalation of diethyl ether, 10 μg of LPS in 50 μL phosphate-buffered saline was instilled intranasally to induce lung injury. Fucosterol (15, 30, and 60 mg/kg) and DEX (5 mg/kg) were given intraperitoneally 1 h prior or after LPS treatment. The experiments were repeated three times.

2.4. Histopathologic studies of lung

After the mice were sacrificed, the right lungs were immediately harvested and fixed with 10% formaldehyde. Then lung tissue was dehydrated, embedded in paraffin, and sliced. The sections stained with hematoxylin and eosin stain according to the regular staining method. Then pathologic changes of lung tissues were observed under a light microscope.

2.5. Wet-to-dry lung weight ratio

The right lungs were excised immediately and weighed to obtain the "wet" weight. Then the lungs were placed at 80°C for 48 h to obtain the "dry" weight. The ratio of the W/D (wet lung to the dry lung) was calculated by dividing the wet weight by the dry weight.

2.6. Myeloperoxidase activity assay

Myeloperoxidase (MPO) activity was measured by MPO kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

2.7. Cell culture and treatment

Murine alveolar macrophages line MH-S was purchased from American Type Culture Collection (Manassas, VA) and maintained in Roswell Park Memorial Institute 1640 with 10% fetal calf serum. Media were changed once every 48 h. In all experiments, alveolar macrophages were incubated in the presence or absence of various concentrations of fucosterol that was always added 1 h before LPS (1 μg/mL) treatment.

2.8. Cell viability assay

Cell viability was measured using a standard MTT assay. Alveolar macrophages were plated and incubated for 3 d in a 96-well plate. Alveolar macrophages were plated at a density of 5 × 103 cells per wells into 96-well plates. Then various concentrations of fucosterol (0–200 μg/mL) were added to the wells, followed by stimulation with 1 μg/mL of LPS for 18 h. Then 20 μL MTT (5 mg/mL) was added to each well, and the cells were further incubated for an additional 4 h. The supernatants were removed, and the formation of formazan was resolved with 150 μL/well of dimethyl sulfoxide. The optical density was measured at 570 nm on a microplate reader (TECAN, San Jose, Austria).

2.9. ELISA assay

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

2.10. Western blot analysis

Total proteins from cells were extracted by Total Protein Extraction Kit (BestBio, Shanghai, China). Protein concentration was determined through bicinchoninic acid method. Aliquots of protein (20 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. Then the membrane was blocked by 5% nonfat dry milk for 2 h. The membrane was incubated with specific primary antibody at 4°C for 12 h. Subsequently, the membrane was washed three times with Tris buffered saline-T for 10 min, and incubation with the secondary antibody conjugated with horseradish peroxidase at room temperature for 1 h. Blots were then developed with the ECL Plus Western Blotting Detection System (Amersham Life Science, Cleveland, United Kingdom).