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Soyasaponin Ab inhibits lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Soyasaponin Ab (SA) has been reported to have anti-inflammatory effect. However, the effects of SA on lipopolysaccharide (LPS)-induced acute lung injury (ALI) have not been reported. The aim of this study was to investigate the anti-inflammatory effects of SA on LPS-induced ALI and clarify the possible mechanism. The mice were stimulated with LPS to induce ALI. SA was given 1 h after LPS treatment. 12 h later, lung tissues were collected to assess pathological changes and edema. Bronchoalveolar lavage fluid (BALF) was collected to assess inflammatory cytokines and nitric oxide (NO) production. In vitro, mice alveolar macrophages were used to investigate the anti-inflammatory mechanism of SA. Our results showed that SA attenuated LPS-induced lung pathological changes, edema, the expression of cycloxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in lung tissues, as well as TNF-α, IL-6, IL-1β, and NO production in mice. Meanwhile, SA up-regulated the activities of superoxide dismutase (SOD) and catalase decreased by LPS in mice. SA also inhibited LPS-induced TNF-α, IL-6 and IL-1β production as well as NF-κB activation in alveolar macrophages. Furthermore, SA could activate Liver X Receptor Alpha (LXRα) and knockdown of LXRα by RNAi abrogated the anti-inflammatory effects of SA. In conclusion, the current study demonstrated that SA exhibited protective effects against LPS-induced acute lung injury and the possible mechanism was involved in activating LXRα, thereby inhibiting LPS-induced inflammatory response.

1. Introduction

Acute respiratory distress syndrome (ARDS) is a life-threatening syndrome associated with acute lung inflammation [1]. Uncontrolled and self-amplified pulmonary inflammation leads to the pathology of this disease [2,3]. Growing evidences have shown that oxidative stress had been involved in the pathological process of ARDS [4,5]. LPS has been identified as one of the most important risk factors that caused ARDS [2,6,7]. Stimulating alveolar macrophages by LPS leads to the production of inflammatory cytokines TNF-α, IL-1β, and IL-6 [8–11]. Also, LPS could induce the production of ROS, as well as NO and PGE_2 [12]. These inflammatory mediators initiate and amplify the inflammatory response and oxidative stress, which result in the development of lung injury. Studies showed that inhibition of inflammation and oxidative stress could attenuate the development of lung injury [13].

It is well known that NF-κB plays critical roles in the regulation of inflammatory mediators, such as IL-6 and TNF-α, [14]. In normal conditions, NF-κB is sequestered in the cytoplasm. Once stimulated by LPS, NF-κB p65 translocates into the nucleus to regulate inflammatory cytokine gene transcription [15]. The liver X receptors (LXRα and LXRβ), members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, have been reported to have anti-inflammatory effects [16,17]. Recently, studies showed that activation of liver X receptor could prevent LPS-induced lung injury by inhibiting NF-κB activation [18].

Soy (*Glycine max*, family Leguminosae), which contains isoflavones and saponins as main constituents, has been reported to have protective effects on cardiovascular, chronic renal diseases, and breast cancers [19]. Soyasaponin Ab is isolated from soybean and has been demonstrated to have anti-inflammatory effects [20]. SA has been reported to suppress TNF-α and IL-1β production in LPS-stimulated peritoneal macrophages [21]. Furthermore, SA was found to attenuate 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice [21]. However, the anti-inflammatory effects of SA on LPS-induced ALI have not been reported. Thus, in this study, we investigated the protective effects and the possible mechanism of SA on LPS-induced ALI.

2. Materials and methods

2.1. Reagents

3-(4, 5-Dimethylthiazol-2-y1)-2, 5-diphenyltetrazolium bromide (MTT) and LPS (Escherichia coli 055:B5) were purchased from Sigma

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Chemical Co. (St. Louis, MO, USA). Soyasaponin Ab (Purity > 98%) was purchased from Shanghai Tianyuan Biotechnology Co., Ltd. (Shanghai, China). Dexamethasone (DEX) was purchased from Changle Pharmaceutical Co. (Xinxiang, Henan, China). RPMI 1640 and fatal bovine serum (FBS) were purchased from Invitrogen-Gibco (Grand Island, NY). The myeloperoxidase (MPO), SOD, and Catalase determination kits were provided by the Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China). DharmaFECT transfection reagent was purchased from Thermo Fisher Scientific (USA). Mouse TNF-α, IL-6 and IL-1β ELISA kits were purchased from Biolegend (San Diego, CA). Antibodies specific for LXRα, NF-κB, IκBα, iNOS, COX-2, and β-actin were purchased from Cell Signaling Technology Inc. (Beverly, MA). All other chemicals were of reagent grade.

2.2. Animals

Male BALB/c mice (18–22 g) were purchased from the Center of Experimental Animals of Zhengzhou University (Henan, China). All mice were kept on a 12 h light/dark cycle with free access to food and water. All animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.3. Experimental and administration

Eighty-four mice were randomly divided into seven groups and each group contained twelve mice: Control, SA alone group, LPS, LPS + SA (12.5, 25 and 50 mg/kg) and LPS + DEX group. 10 μg of LPS in 50 μl Phosphate Buffered Saline (PBS) was instilled intranasal (i.n.) to induce lung injury. 1 h later, Soyasaponin Ab (12.5, 25 and 50 mg/kg) and DEX (5 mg/kg) were given intraperitoneally. The dose of DEX used in this study was based on previous studies [22,23]. The mice of normal control group were received equal amounts of PBS. 12 h later, bronchoalveolar lavage fluid (BALF) was collected. BALF was collected three times through a tracheal cannula with autoclaved PBS and instilled up to a total volume of 1.3 ml.

2.4. Lung wet-to-dry weight ratio

After the mice were euthanized, the right lungs were excised and weighed to obtain the 'wet' weight. Then the lungs were dried at 80 °C for 48 h in an oven to obtain the 'dry' weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

2.5. MPO, SOD, and catalase activities in lung tissues

MPO, SOD, and catalase activities in lung tissues were determined using relevant test kits purchased from Jiancheng Bioengineering Institute of Nanjing (Jiangsu, China) according to the instructions.

2.6. Histological analysis

The lung tissues were harvested and fixed in 10% formalin. Then the lung tissues were dehydrated, embedded in paraffin and rehydrated with graded alcohol for staining. The sections were stained with hematoxylin and eosin (H&E) stain and pathological changes of lung tissues were visualized with a microscope (Olympus, Japan). The histological changes in the lungs were scored as previously described [23–25]. The score was categorized according to the sum of the score for damage level such as thickening of alveolar walls and epithelium, the numbers of infiltration cells, as well as increases in peribronchial and perivascular cuff area. Each histological characteristic was scored 0 to 5.

2.7. Cell culture and treatment

Murine alveolar macrophages line MH-S was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured at 37 °C in 5% CO_2 in RPMI 1640 supplemented with 10% FBS. In all experiments, MH-S cells were incubated with the indicated concentrations of SA 1 h before LPS (1 μg/ml) treatment.

2.8. MTT assay

MTT assay was used to evaluate the cell viability. Briefly, MH-S cells (4×10^5 cells/ml) were seeded in 96-well plates. The cells were treated with Soyasaponin Ab (0–10 μM) 1 h before LPS treatment for 24 h. Then 20 μl MTT (5 mg/ml) was added to each well for an additional 4 h. The supernatant was removed and the formation of formazan was measured at 540 nm using a microplate reader (TECAN, Austria).

2.9. Cytokines and NO assay

The levels of TNF-α, IL-6, and IL-1β in the BALF and cell-free supernatants were measured using ELISA kits according to the manufacturer's instructions (Biolegend, Inc., San Diego, CA, USA). The level of NO in BALF was detected by measuring the intermediate and end products, NOx, by a Sievers Nitric Oxide Analyzer (Sievers 280 NOA, Sievers, Boulder, CO, USA) as previously described [26].

2.10. Western blot analysis

The cells were lysed with RIPA lysis buffer in the presence of protease inhibitors. Total proteins from lung tissues were extracted using detecting kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Protein concentration was determined through BCA method. Equal amounts of protein were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to a nitrocellulose membrane. Then the membranes were blocked by 5% nonfat dry milk for 2 h. After blocking, the membranes were incubated with the specific primary antibodies LXRα (1:1000, 50 kD), NF-κB p65 (1:1000, 65 kD), NF-κB p-p65 (1:1000, 65 kD), p-IκBα (1:1000, 41 kD), IκBα (1:1000, 39 kD), iNOS (1:1000, 130 kD), and COX-2 (1:1000, 74 kD) at 4 °C for 12 h. Subsequently, the membranes were incubated with peroxidase-conjugated secondary antibody at room temperature for 2 h. Protein bands were detected with the Super Signal West Pico Chemiluminescent Substrate (Thermo, MA, USA). Densitometric analysis was performed using a MicroChemi 4.2 system (DNR Bio Imaging Systems, Jerusalem, Israel).

2.11. Transient transfection of siRNA against LXRα

Si-LXRα and si-control stock solutions (20 μM) were diluted with diethyl pyrocarbonate (DEPC) water to form 5-μM solutions. The DharmaFECT transfection reagent was mixed with 5 μM si-LXRα or si-control, incubated for 20 min and then added to the culture medium at a final concentration of 25 nM. The cells were incubated with si-LXRα and si-control for 48 h.

2.12. Statistical analysis

Data are expressed as a mean ± standard deviation (SD). Statistically significant differences between groups were determined by one-way ANOVA with the Tukey's post hoc test for multigroup comparisons. Statistical significance was accepted $P < 0.05$ or $P < 0.01$.

3. Results

3.1. SA attenuated LPS-induced lung histopathologic changes

Lung histological changes were measured 12 h after LPS administration. As shown in Fig. 1, lung histological sections of normal control group and SA alone group showed normal structure of lungs (Fig. 1A, G). Lung histological sections of LPS-challenged mice showed interstitial