Journal of Ethnopharmacology 210 (2018) 23–30

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Anti-inflammatory effect of stem bark of *Paulownia tomentosa* Steud. in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and LPS-induced murine model of acute lung injury

Jae-Won Lee[1], Kyeong-Hwa Seo[1], Hyung Won Ryu, Heung Joo Yuk, Hyun Ah Park, YouRim Lim, Kyung-Seop Ahn, Sei-Ryang Oh*

Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, 30 Yeongudanji-ro, Ochang-eup, Cheongwon-gu, Chungju-si, Chungbuk 363-883, Republic of Korea

A R T I C L E I N F O

Chemical compounds studied in this article:
Verbascoside (PubChem CID: 5281800)
Isoverbascoside (PubChem CID: 6476333)

Keywords:
Paulownia tomentosa stem bark extract
Anti-inflammatory effect
ALI NF-κB
SOD3

A B S T R A C T

Ethnopharmacological relevance: The leaves, bark, and flowers of *Paulownia tomentosa* Steud. have been widely used as a traditional medicine in East Asia to treat inflammatory and infectious diseases.
Aim of the study: We investigated the protective effect of the methanol stem bark extract of *P. tomentosa* using an animal model of lipopolysaccharide (LPS)-induced acute lung injury (ALI).
Materials and methods: The UPLC Q-TOF-MS profiles for the methanol extract of *P. tomentosa* stem bark showed that verbascoside and isoverbascoside were the predominant compounds. Raw 264.7 cells were used for inhibitory effects of cytokine production in vitro. C57BL/6 N mice were administered intranasally with LPS (10 μg/per mouse) to induce ALI. H & E staining was used to evaluate histological changes in the lung.
Results: Treatment with *P. tomentosa* stem bark extract (PTBE) suppressed the production of IL-6 and TNF-α in LPS-stimulated RAW 264.7 macrophages, and the recruitment of neutrophils and macrophages in the BALF of mice with LPS-induced ALI. PTBE also decreased the levels of reactive oxygen species (ROS) and pro-inflammatory cytokines in the BALF. PTBE reduced the levels of nitric oxide (NO) in the serum and of inducible nitric oxide synthase (iNOS) in the lung of ALI mice. PTBE also attenuated the infiltration of inflammatory cells and the expression of monocyte chemoattractant protein-1 (MCP-1) in the lung. In addition, PTBE suppressed the activation of NF-κB and the reduced expression of superoxide dismutase 3 (SOD3) in the lung.
Conclusion: The results suggest that PTBE has a protective effect on LPS-induced ALI.

1. Introduction

Acute lung injury (ALI) is a disease characterized by acute lung inflammation and tissue damage (Proudfoot et al., 2011), its incidence remains high and the overall mortality rate is approximately 40% (Cao et al., 2016; Johnson and Matthay, 2010). Bacterial infection is the most common cause of ALI and leads to systemic inflammation in the lung (Li et al., 2016). Inappropriate recruitment and activation of neutrophils is a key event in ALI (Grommes and Soehnlein, 2011) that leads to an enhanced reactive oxygen species (ROS) at the site of inflammation inducing disruption of endothelial barriers and tissue injury (Mittal et al., 2014). Macrophages contribute to the pathogenesis of ALI, releasing inflammatory cytokines and chemokines (Herold et al., 2011), and high level of nitric oxide (NO) also play an important role in airway inflammatory response by regulating chemokine secretion (Speyer et al., 2003). Monocyte chemoattractant protein-1 (MCP-1) is one of the key chemokines that regulates the recruitment of neutrophils against bacterial infection (Balamayooran et al., 2012). Pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), stimulate and activate neutrophils, leading to the release of lung damaging oxidants and proteases (Lee et al., 2016b; Takashima et al., 2014).

The transcription factor nuclear factor-kappa B (NF-κB) regulates the production of pro-inflammatory molecules, such as ROS, NO, IL-6

Abbreviations: ALI, acute lung injury; LPS, lipopolysaccharide; BALF, bronchoalveolar lavage fluid; ROS, reactive oxygen species; IL-6, Interleukin-6; TNF-α, tumor necrosis factor-α; NO, nitric oxide; MCP-1, monocyte chemoattractant protein-1; iNOS, inducible nitric oxide synthase; NF-κB, Nuclear factor-κB; IκB, inhibitor of NF-κB; SOD3, superoxide dismutase 3; PTBE, *P. tomentosa* stem bark extract; PTBB, *P. tomentosa* stem bark n-BuOH fraction

* Corresponding author.
E-mail addresses: suc369@kribb.re.kr (J.-W. Lee), kyeonghwseo@korea.kr (K.-H. Seo), cupid6@kribb.re.kr (H.W. Ryu), ryuhw@kribb.re.kr (H.J. Yuk), hyunah00@kribb.re.kr (H.A. Park), youlim@kribb.re.kr (Y. Lim), ksahn@kribb.re.kr (K.-S. Ahn), seiryang@kribb.re.kr (S.-R. Oh).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jep.2017.08.028
Received 21 March 2017; Received in revised form 9 August 2017; Accepted 23 August 2017
Available online 24 August 2017
0378-8741/ © 2017 Elsevier B.V. All rights reserved.

and TNF-α (Lin et al., 2015). Accumulating evidences have showed that NF-κB activation is increased in the lung of mice with LPS-induced ALI (Wu et al., 2016) and NF-κB inhibitor suppressed the influx of neutrophils and lung edema, which are the major characteristics in ALI (Everhart et al., 2006). Superoxide dismutase 3 (SOD3), an important antioxidant enzyme, reduces oxidative stress and lung damage by inhibition of inflammatory cells recruitment and by attenuation of NF-κB activation in ALI (Yeh et al., 2014).

Paulownia tomentosa Steud. (princess tree, Scrophulariaceae) is a fast-growing deciduous tree that is considered as an economic potential because of its value for woods as well as its high biomass production (San Jose Mdel et al., 2014). *P. tomentosa* is traditionally used as an herbal remedy to treat or prevent a variety of diseases, such as hemorrhoid, carbuncle, gonorrhea, parotitis, traumatic bleeding, erysipelas, bacteriological diarrhea, swelling, enteritis, conjunctivitis, hypertension, enteritis, dysentery, and tonsillitis (He et al., 2016; Ji et al., 2015; Zima et al., 2010). The bark of *P. tomentosa* have been shown to have anti-asthma, upper respiratory tract infection, bronchopneumonia and inflammatory bronchitis activity in traditional medicine (He et al., 2016), however *P. tomentosa* stem bark has yet to reveal the biological effects based on the main constituents. Two major metabolites, verbascoside and isoverbascoside of *P. tomentosa* stem bark (PTBE) have been reported as constituents of *Castilleja tenuiflora* to possess anti-inflammatory activity in a mouse ear edema model (Sanchez et al., 2013). Recently, verbascoside (syn. acteoside) isolated from *Rehmannia glutinosa* suppressed the inflammatory cells influx and the production of inflammatory mediators in LPS-induced ALI models (Jing et al., 2015), isoverbascoside possessed the antioxidant capacity in phorbol myristate acetate (PMA)-challenged human neutrophils (Muzila et al., 2016). In the present study, we examined the anti-inflammatory effect of PTBE in RAW 264.7 macrophage cells and the protective activity of it in LPS-induced ALI murine model.

2. Materials and methods

2.1. Plant materials

The stem bark of *P. tomentosa* Steud. was collected at Sancheong, Republic of Korea in June 2015 and positively identified by Dr. Joong Ku Lee (Korea Research Institute of Bioscience & Biotechnology). A voucher specimen (KRIB 0059121-0059123) was deposited at the Plant Extract Bank of KRIBB in Daejeon, Korea.

2.2. Extraction and sample preparation

The dried stem bark (800 g) was chopped and extracted with 100% MeOH (7 L × 3) at room temperature for 24 h, filtered and concentrated in vacuo, and yielded total extract (PTBE, 105 g). The constituent profile of PTBE was analyzed by UPLC-PDA-QTof-MS, and two major compounds were found as major constituents of PTBE. The isolation and structure elucidation process and structural data of two compounds were described in supplementary data (Supplementary material, Figs. S1–6).

2.3. Cell culture

RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS in the presence of 100 U/ml penicillin and 100 µg/ml streptomycin and were incubated at 37 °C in a 5% CO_2 incubator. The cells were incubated in the absence or presence of PTBE before the addition of LPS (500 ng/ml).

2.4. Cell viability

The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) assay (Lee et al., 2016c). The RAW 264.7 macrophages were plated into 12-well culture plates and incubated with PTBE at various concentration for 24 h at 37 °C and 5% CO_2. The resulting formazan crystals were dissolved in DMSO. The absorbance was determined at 570 nm. The results were expressed as a percentage of surviving cells over control cells.

2.5. Animal model of lipopolysaccharide (LPS)-induced ALI

Male C57BL/6N (6-week-old) mice were obtained from Koatech Co. (Pyeongtaek, Korea) and were acclimated to a specific pathogen-free condition with food at least 1 week before the experiments. The mice were divided into 4 groups as follows: i) the normal control (NC) group; ii) the lipopolysaccharide (LPS) group; iii) the dexamethasone (DEX; 1 mg/kg) group (used as a positive control); and iv) PTBE group (administered 20 and 40 mg/kg, per oral). DEX and PTBE were dissolved with 1% DMSO and 1% Tween-20 in PBS and were administered orally from 1 to day 3. The mice were exposed to LPS (10 µg per mouse) intranasally 1 h after the final DEX and PTBE treatment, as previously described (Lee et al., 2016b). All the experimental procedures were performed in accordance with the procedures approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology and performed in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and Korean National Laws for Animal Welfare.

2.6. Collection of bronchoalveolar lavage fluid (BALF) and inflammatory cell counts

BALF collection was performed as previously described (Lee et al., 2016a). To obtain the BALF, the lungs were washed two times with ice-cold PBS (700 µl) using a tracheal cannula (a total volume of 1.4 ml). The collected BALF was centrifuged at 1500 rpm for 5 min at 4 °C. The BALF differential cell count was determined by counting the cells in the pellet with Diff-Quik® staining regent according to the manufacturer's instruction.

2.7. Measurement of inflammatory mediators such as reactive oxygen species (ROS), nitric oxide (NO) and pro-inflammatory cytokines in the BALF

To examine the inhibitory effect of PTBE on the production of ROS, BALF cells were washed with PBS and incubated with 20 µM of 2′,7′-dichlorofluorescin diacetate (DCFH-DA; Sigma Aldrich, St. Louis, MO, USA) for 10 min at 37 °C (Lee et al., 2015). The level of intracellular ROS was detected by measuring the fluorescence at 488 nm excitation and 525 nm emission on a fluorescence plate reader (Perkin-Elmer, Waltham, MA, USA). The releases of pro-inflammatory cytokines (IL-6 and TNF-α) in the BALF were detected using ELISA kits according to the manufacturer's instruction (R & D System, Minneapolis, MN, USA). The absorbance was measured at 450 using an ELISA reader (Molecular Devices, Sunnyvale, CA, USA). To evaluate the level of NO, Griess reagent were mixed with an equal volume of serum. Light absorbance was read at 540 nm.

2.8. Western blot analysis

The lung tissues were homogenized (1/10 w/v) and extracted with tissue lysis/extraction reagent, containing a protease inhibitor cocktail (Sigma-Aldrich). Equal amounts of the total cellular protein were loaded per well on 8–12% SDS-polyacrylamide gels and transferred to PVDF membranes (Bio-Rad Laboratories, Hercules, CA, USA). The

Download English Version:

https://daneshyari.com/en/article/5555929

Download Persian Version:

https://daneshyari.com/article/5555929

Daneshyari.com