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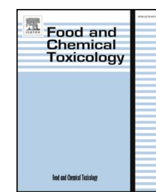

Tyrosol attenuates lipopolysaccharide-induced acute lung injury by inhibiting the inflammatory response and maintaining the alveolar capillary barrier

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ABSTRACT

Acute lung injury (ALI) is a life-threatening disease characterized by increased pulmonary vascular permeability because of alveolar capillary barrier dysfunction and increased immune responses. This study determined the anti-inflammatory effect of tyrosol on lipopolysaccharide (LPS)-induced ALI and its underlying mechanisms of action. BALB/c mice were orally administered with tyrosol (0.1, 1, and 10 mg/kg) 1 h before an intratracheal injection of LPS (25 μg/50 μL). Oral treatment with tyrosol inhibited lung vascular permeability, histopathological changes, wet/dry lung weight ratio, and pulmonary vascular cell infiltration. The LPS-induced imbalance in the activity of enzymes, such as superoxide dismutase and myeloperoxidase, was regulated by tyrosol. Pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-6, were reduced by tyrosol in bronchoalveolar lavage fluid and lung tissue. The activation of inflammatory molecules, including inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and phosphorylated-IκBα, was suppressed by the presence of tyrosol in the lung tissue. In addition, tyrosol attenuated the production of NO, the expression of pro-inflammatory cytokines, the expression of iNOS and COX-2, and the nuclear translocation of nuclear factor-κB in LPS-stimulated RAW 264.7 macrophages. These results suggested that tyrosol is a potential therapeutic agent for treating inflammatory lung diseases.

1. Introduction

Acute lung injury (ALI) is characterized by excessive inflammation and increased production of oxidizing agents in the lungs because of both direct (aspiration pneumonia and inhalation injury) and indirect (sepsis, pancreatitis, and blood transfusion) causes. It involves inflammatory processes such as the influx of polymorphonuclear neutrophils into the alveoli and subsequent damage to the alveolar-capillary membrane that increases vascular, alveolar epithelial, and microvascular endothelial permeability, specialized fibroblast generation, and protein-rich pulmonary edema fluid accumulation in airspaces (Bhatia et al., 2012).

Lipopolysaccharide (LPS), an outer membrane component of gram-negative bacteria, has been recognized as a cause of inflammation and sepsis (Chen, 2011). Damaged and inflamed alveoli predominantly contain activated alveolar macrophages and neutrophils (Idell, 2003; Wang et al., 2008). In particular, macrophages are one of the important players in host defense, bridging innate and adaptive immunity during inflammatory responses through the secretion of various pro-inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), metalloproteinases, and prostaglandin E2

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(PGE2) (Korhonen et al., 2005; Wu et al., 2013). In addition to the acute inflammatory response, these inflammatory mediators trigger pulmonary dysfunction, including the epithelial fluid transport disruption and surfactant production. Moreover, macrophages promote neutrophil infiltration into the lungs and exacerbate alveolar barrier dysfunction and inflammatory response to induce lung tissue damage (Niesler et al., 2014; Vergadi et al., 2014).

Transcription factor nuclear factor-κB (NF-κB) regulates the balance between pro-inflammatory and anti-inflammatory responses and plays an important role in the pathogenesis of lung diseases (Schuliga, 2015). NF-κB is required for the optimal transcription of various inflammatory cytokines, including TNF-α, IL-1β, and IL-6, and is thought to be important in their expression (Caamano and Hunter, 2002). Therefore, it has been suggested that the inhibition of NF-κB function may be a potential therapeutic target for treating ALI (Yang et al., 2012; Lee and Yang, 2013).

ALI treatment is based on ventilatory and nonventilatory therapies involving surfactants and many other drugs. However, both the therapies are nonspecific, induce short-term healing, and are associated with various side effects (Johnson and Matthay, 2010). Therefore, safer, continuous, and more effective drugs are needed for the therapy of ALI. Natural products are considered an abundant source of new drug candidates (Newman and Cragg, 2016). Extract of *Amomum xanthioides* has been extensively used in Asia because its numerous beneficial properties, including anti-allergic, anti-inflammatory, and hepatoprotective properties (Kim et al., 2007; Wang et al., 2013). Tyrosol, (2-(4-hydroxyphenyl)ethanol), a phenolic compound isolated from *A. xanthioides*, has been reported to possess a wide range of biological activities, including anti-oxidative, anti-apoptotic, and anti-inflammatory activities (Bertelli et al., 2002; Tuck and Hayball, 2002). Our previous study showed that tyrosol attenuated mast cell-mediated allergic inflammation by reducing NF-κB activation (Je et al., 2015). In the present study, we investigated the effect of tyrosol in a mouse model of LPS-induced ALI and in RAW 264.7 macrophages.

2. Materials and methods

2.1. Plant material

The fruits of *A. xanthioides* collected between September and November of 2011 were purchased from Yak-Ryung-Si Market (Daegu, Korea) and identified by Dr Seung Ho Lee (College of Pharmacy, Yeungnam University). A voucher specimen was deposited in the Natural Product Laboratory at the College of Pharmacy, Yeungnam University, South Korea (YU00194). The dried fruits of *A. xanthioides* (9.5 kg) were extracted with methanol (MeOH) (15 L × 3) at a temperature of 22 ± 2 °C. The extract was concentrated *in vacuo* to yield a black gum (440.0 g), which was dissolved in H_2O and then continuously partitioned with the solvents *n*-hexane, methylene chloride (MC, 32.7 g), and ethyl acetate (EtOAc, 9.8 g). After partitioning, the solutions were concentrated to obtain separate extracts of water and each organic solvent.

2.2. Purification and structure analysis of tyrosol

The MC-soluble extract of *A. xanthioides* (32.7 g) was separated by silica gel liquid chromatography and eluted with a gradient mixture from 100% MC to 100% acetone to obtain seven fractions (AF1–AF7). Tyrosol was isolated from fraction AF3 (101.2 mg) by preparative HPLC (C_{18}, 250 × 21.2 mm; flow rate, 6 mL/min) with MeOH:H_2O as the eluent (gradient, 10:90 to 55:45). The stationary phases used were Merck silica gel (70–30 mesh) and Merck LiChroprep RP-18 gel (40–63 μm), and the HPLC apparatus consisted of a Gilson system, UV detector, and a Luna C_{18} F_{254} column (Merck, Darmstadt, Germany). The compounds were detected under UV light and visualized by spraying the plates with vanillin-sulfuric acid reagents (1% ethanolic vanillin solution plus 10% ethanolic sulfuric acid) and 10% sulfuric acid, and heating at 110 °C for 1 min.

^{1}H (250 MHz) and ^{13}C (63 MHz) NMR spectra were recorded with a Bruker 250 MHz (DMX 250) using tetramethylsilane as an internal standard. NMR spectra were recorded on JEOL ECA-500 (^{1}H, 500 MHz, ^{13}C, 125 MHz) spectrometers. All NMR experiments were performed at 21 °C. The chemical structure of the isolated compound was elucidated by the comparison of 1D NMR data with reported values. The presence of an aromatic ring was suggested by the ^{1}H NMR spectrum, which showed two *ortho* coupled aromatic signals at δ_H 7.01 (2H, d, J = 8.7 Hz) and 6.66 (2H, d, J = 8.7 Hz), together with an ethyl group moiety at 3.65 (2H, t, J = 3.6 Hz) and 2.69 (2H, t, J = 3.6 Hz). In the ^{13}C NMR spectrum, four benzyl carbon signals at δ_C 155.7, 130.1, 129.8, and 115.1, and two methylene carbons at δ_C 63.5, and 38.7, were observed (Pouchert and Behnke, 1993). Tyrosol was obtained as a white amorphous powder; ^{1}H NMR ($CDCl_3$, 250 MHz) δ_H 7.01 (2H, d, J = 8.7 Hz, H-2,6), 6.66 (2H, d, J = 8.7 Hz, H-3, 5), 3.65 (2H, t, J = 3.6 Hz, H-8), 2.69 (2H, t, J = 3.6 Hz, H-7); ^{13}C NMR ($CDCl_3$, 250 MHz) δ_C 155.7 (C-4), 130.1 (C-3), 129.8 (C-2,6), 115.1 (C-3,5), 63.5 (C-8), 38.7 (C-7).

For experimental use, tyrosol was dissolved in dimethyl sulfoxide (DMSO) and diluted with pathogen-free phosphate-buffered saline (PBS).

2.3. Animals and reagents

Six-week-old male BALB/c mice, weighing approximately 18–20 g, were purchased from the Dae-Han Experimental Animal Center (Daejeon, Korea). All mice had *ad libitum* access to standard rodent chow and filtered water during the study. Five mice were housed per cage in a laminar airflow room; throughout the study, the following conditions were maintained: temperature, 22 ± 2 °C; relative humidity, 55 ± 5%; and a 12-h light:dark cycle. The care and treatment of the animals was in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Kyungpook National University.

LPS (*Escherichia coli* 055:B5), dexamethasone (DEX), Evans blue, sulfanilamide, *N*-(1-naphthyl)-ethylenediamine dihydrochloride, 2.5% phosphoric acid, and a superoxide dismutase (SOD) assay kit were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were dissolved in pathogen-free PBS. All other reagents were purchased from Sigma-Aldrich unless otherwise stated.

2.4. In vivo studies

2.4.1. Experimental design

Thirty mice were randomly divided into six treatment groups with five mice in each group: PBS only; LPS only; LPS and 0.1, 1, and 10 mg/kg tyrosol; and 2 mg/kg DEX. Tyrosol and DEX (positive control) were orally administered as a pretreatment for 1 h. Subsequently, all the mice were anesthetized by intraperitoneally injecting of PBS:ketamine:xylazine (7:2:1) mixture and were intratracheally injected with LPS (25 μg in 50 μL) to induce ALI. Control mice received 50 μL of PBS. After 5 h of LPS treatment, the mice were euthanized by CO_2 inhalation and their bronchoalveolar lavage fluid (BALF) was collected.

2.4.2. Bronchoalveolar lavage

After 5 h of LPS injection, the mice were euthanized and bronchoalveolar lavage was performed three times through the tracheal cannula with 0.5 mL of autoclaved PBS to obtain BALF, which was then centrifuged at 400 *g* for 10 min at 4 °C. The supernatants were stored at -80 °C until further analysis. The pellets were resuspended in saline (100 μL), fixed onto slides, and stained with Diff-Quik staining (Sysmex Co., Kobe, Japan). The number of neutrophils and macrophages were

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