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Original article

Tetrahydroberberrubine attenuates lipopolysaccharide-induced acute lung injury by down-regulating MAPK, AKT, and NF-κB signaling pathways

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ABSTRACT

Acute lung injury (ALI) is a life-threatening syndrome that is characterized by overwhelming lung inflammation and increased microvascular permeability, which causes a high mortality worldwide. Here, we studied the protective effect of tetrahydroberberrubine (THBru), a berberine derivative, on a mouse model of lipopolysaccharide (LPS)-induced acute lung injury that was established in our previous studies. The results showed that a single oral administration of THBru significantly decreased the lung wet to dry weight (W/D) ratio at doses of 2, 10 and 50 mg/kg administered 1 h prior to LPS challenge (30 mg/kg, intravenous injection). Histopathological changes, such as pulmonary edema, infiltration of inflammatory cells and coagulation, were also attenuated by THBru. In addition, THBru markedly decreased the total cell counts, total protein and nitrate/nitrite content in bronchoalveolar lavage fluid (BALF), significantly decreased tumor necrosis factor-α (TNF-α) and nitrate/nitrite content in the plasma, and reduced the myeloperoxidase (MPO) activity in the lung tissues. Additionally, THBru (10 μM) significantly decreased the content of TNF-α and nitric oxide (NO) in LPS-induced THP-1 cells *in vitro*. Moreover, THBru significantly suppressed the activation of the MAPKs JNK and p38, AKT, and the NF-κB subunit p65 in LPS-induced THP-1 cells. These findings confirm that THBru attenuates LPS-induced acute lung injury by inhibiting the release of inflammatory cytokines and suppressing the activation of MAPKs, AKT, and NF-κB signaling pathways, which implicates it as a potential therapeutic agent for ALI or sepsis.

1. Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are characterized by disruption of the alveolar-capillary barrier, which results in lung edema, neutrophil accumulation, alveolar fibrin deposition and consequent impairment of arterial oxygenation [1–3]. Although there is an approved therapy with mechanical ventilation to treat ALI/ARDS patients, the mortality has remained high over the last decade [4,5]. Therefore, the development of efficient therapeutic approaches is urgently required. Sepsis is a major cause underlying the development of ALI, wherein gram-negative bacteria are the dominating factor. An injection of lipopolysaccharide (LPS), a major component in gram-negative bacteria, mimics human gram-negative ALI by inducing excessive inflammatory response [6]. It has been widely accepted that the inappropriate activation of inflammatory cells, including polymorphonuclear neutrophils (PMNs), circulating monocytes and tissue resident macrophages, and the increased release of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and nitric oxide (NO), play a key role in the pathogenesis of sepsis-induced ALI [7–9]. Furthermore, toll like receptor 4 (TLR4), a well-characterized PRR, recognizes the LPS and leads to the activation of the MYD88-mediated nuclear factor-κB (NF-κB), Mitogen-activated protein kinase (MAPK) and AKT/phosphoinositide3-kinase (PI3K) pathways in sepsis-induced ALI [10,11].

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; BBR, berberine; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; NO, nitric oxide; PI3K, phosphoinositide3-kinase; PMNs, polymorphonuclear neutrophils; TGF-β, transforming growth factor beta; THP-1, the human monoblastic leukemia cell line; TLR4, toll like receptor 4; TNF-α, tumor necrosis factor alpha; THBru, tetrahydroberberrubine.

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Due to remarkable synergic therapeutic effects and relatively low toxicity of herbal medicines, considerably more attention has been given to natural anti-oxidant and anti-inflammatory products and their derivatives [12–14]. It has been reported that berberine attenuates bleomycin-induced pulmonary toxicity and fibrosis by suppressing NF-κB-dependent transforming growth factor beta (TGF-β) activation and that it inhibits cigarette smoke- or LPS-induced acute lung inflammation [15–17]. Furthermore, berberine inhibited the LPS-induced over-expression and procoagulant activity of tissue factors by regulating the p38 MAPK and NF-κB/p65 pathways in THP-1 cells [18]. However, accumulating evidence has demonstrated that berberine is characterized by a very low oral bioavailability, which limits the clinical application of this compound [19,20]. Therefore, recent studies have focused on the research and development of berberine derivatives [21–23]. Tetrahydroberberrubine (Fig. 1A), a berberine derivative (Fig. 1B), was synthesized by semi-chemical synthesis, in which berberine chloride undergoes pyrolysis monodemethylation to produce a red compound and then reduction using potassium borohydride [24–26]. Our previous research showed that tetrahydroberberrubine inhibited tissue factor procoagulant activity in LPS-induced THP-1 cells [27] and that its derivative has anxiolytic effects [28]. However, the pharmacological actions of tetrahydroberberrubine on LPS-induced acute lung injury and its possible mechanism have not been clarified. Therefore, our study was designed to assess the protective effects of tetrahydroberberrubine compared with berberine in an LPS-induced acute lung injury mouse model and to elucidate the potential anti-inflammatory mechanism in THP-1 cells *in vitro*.

2. Materials and methods

2.1. Drugs and reagents

Tetrahydroberberrubine sulfate (THBru) was provided by Dr. Haixia Ge, and its purity, as analyzed by high performance liquid chromatography, was 98%. Berberine chloride (BBR) was obtained from Nanjing Qingze Medical Technology Company (Nanjing, China). Dexamethasone (DEX) was obtained from Zhejiang Xianju Pharmaceutical Co. Ltd (Zhejiang, China). LPS (from Escherichia coli O55:B5) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The MPO kit, nitrate/nitrite colorimetric assay kit and TNF-α ELISA kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The BCA assay and BSA were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Lysis buffer and the ECL kit were purchased from Vazyme Biotech Co. Ltd (Nanjing, China). PVDF membrane was acquired from Millipore Company (Shanghai, China). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies were acquired from Shanghai KangChen Bio-tech Inc. (Shanghai, China). Anti-p65, anti-phospho-NF-κB/p65, anti-JNK, anti-phospho-JNK, anti-p38MAPK, anti-phospho-p38MAPK, anti-AKT, and anti-phospho-AKT antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). Goat anti-mouse IgG antibody and goat anti-rabbit IgG antibody were purchased from Bioworld Technology (St. Louis Park, MN, USA). Alexa Fluor 488-labeled secondary antibody was purchased from Life Technologies (Carlsbad, CA, USA). All other reagents were of analytical grade.

2.2. Cell culture and pharmacological treatments

The human monoblastic leukemia cell line (THP-1) was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. THP-1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 °C. THP-1 cells were grown in serum-free medium for 2 h, pretreated with tetrahydroberberrubine (1, 5, 10 μM) for 1 h and stimulated with 500 ng/mL LPS for 5 h or 30 min.

2.3. Animals

Male ICR mice that were 6–8 weeks old were obtained from Shanghai Slac Laboratory Animal Co. Ltd (Shanghai, China). They were kept in plastic cages at 22 ± 2 °C, with free access to pellet food and water on a 12 h light/dark cycle. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and related regulations of China Pharmaceutical University.

2.4. LPS-induced acute lung injury in mice

Mice were randomly divided into seven groups (n = 8 in each group). LPS (30 mg/kg) was administered intravenously to induce lung injury, and sterile saline was used as the control. The solvent (sterile saline with 5% ethanol), THBru (2, 10 and 50 mg/kg), BBR (50 mg/kg) and DEX (3.0 mg/kg) were administered orally 1 h prior to LPS or saline administration. The chosen doses of these drugs were based on our previous studies and preliminary experiments [16,29,30].

2.5. Lung wet to dry weight ratio measurement and histopathological analysis

The left lung was removed and the wet weight was determined at 6 h after LPS injection. Then, the lung tissue was placed in an oven at 60 °C for 24 h to obtain the dry weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema. Samples of the upper lobe of right lung were harvested at 6 h after LPS injection and fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 24 h at 4 °C. For examination by light microscopy, lung tissues was dehydrated with graded alcohol and then embedded in

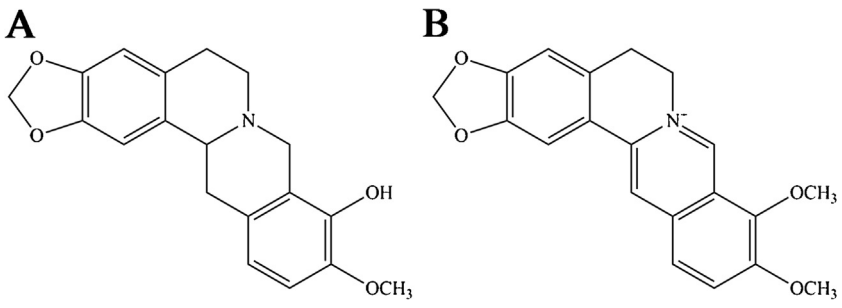

Fig. 1. The chemical structure of tetrahydroberberrubine (A) and berberine (B).