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Bupleurum chinense DC polysaccharides attenuates lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Bupleurum chinense DC had hepato-protective, anti-inflammatory, antipyretic, analgesic, and immunomodulatory effect in traditional Chinese medicine. This study was to determine whether the crude polysaccharides isolated from the roots of *Bupleurum chinense* DC (BCPs) attenuated lipopolysaccharide (LPS)-induced acute lung injury in mice. Mice were challenged with LPS intratracheally 2 h before BCPs (20, 40 and 80 mg/kg) administration. The bronchoalveolar lavage fluid (BALF) was collected 24 h after LPS challenge. Treatment with BCPs reduced lung wet-to-dry weight ratio. The elevated number of total cells and protein concentration in BALF was reduced. The increased level of myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α) in BALF, and serum nitric oxide (NO) were also inhibited. BCPs significantly attenuated lung injury with improved lung morphology and reduced complement deposition. These results suggested that the effect of BCPs against ALI might be related with its inhibitory effect on excessive activation of complement and on the production of proinflammatory mediators.

Introduction

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a prototype human inflammatory disease. ALI or ARDS is associated with acute lung inflammation involving the local neutrophil recruitment, the release of proinflammatory mediators (Yeh et al. 2006), the increased pulmonary vascular permeability and the development of interstitial edema (Liu et al. 2010). In addition, the pathogenesis of ALI/ARDS involves the immunity damage and the over-activation of complement system (Flierl et al. 2008a). Although intensive investigations into new strategies for treatment and improved therapy methods have shown the great possibility, the mortality rate from ALI/ARDS remains around 40% (Rubenfeld and Herridge 2007). The development of efficient therapeutic approaches that could improve or complement for current strategies is urgently needed (Liu et al. 2010).

Animal models of ALI could be established by tracheal (i.t.) or intranasal (i.n.) instillation of lipopolysaccharide (LPS) (Yeh et al. 2006; Liu et al. 2010), ventilator (Slutsky 2005), or smoking inhalation (Traber et al. 1985) in mice. LPS is an important component of the cell walls of gram-negative bacteria, which is often used to establish the experimental model of ALI (Ulich et al. 1994; Sato et al. 2002). Thus, it was designed to instill LPS i.t. in physiological saline (NS) in this experiment to induce acute lung injury in mice.

Bupleurum chinense DC, called "Bei Chaihu" in China, has hepato-protective, anti-inflammatory, antipyretic, analgesic, and immunomodulatory effects in traditional Chinese medicine (Xie et al. 2007; Sun 2006). Some reports showed that anti-inflammatory and immunomodulatory effects could be closely associated with reduced complement activation (Wang et al. 2009; Sahu and Lambris 2000). Complement activation, which can cause neutrophil recruitment and damage to the alveolar-capillary membrane, plays a pivotal role in the pathogenesis of ALI (Flierl et al. 2008a). C5a increases the recruitment and influx of neutrophils and monocytes into lung, resulting in host tissue destruction and inflammation response in ALI (Flierl et al. 2008b).

In our laboratory, two homogeneous polysaccharides have been isolated and characterized as anti-complementary agents from the crude polysaccharides from *Bupleurum chinense* DC (BCPs) *in vitro* (in press). As it is difficult to get enough homogeneous polysaccharides for the animal experiments in mice, the crude polysaccharides

Abbreviations: BCPs, crude polysaccharides from *Bupleurum chinese* DC; ALI, acute lung injury; LPS, lipopolysaccharide; DXM, dexamethasone; MPO, myeloperoxidase; ELISA, enzyme-linked immunosorbent assay; NS, normal saline; CMC, carboxymethyl cellulose; BALF, bronchoalveolar lavage fluid; NO, nitric oxide; TNF-α, tumor necrosis factor-α; HRP, horseradish peroxides; DAB, 3,3′-diaminobenzidine; VBS^{2+}, isotonic veronal-buffered saline; EAs, sensitized erythrocytes.

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with anti-complementary activity were used in this research. The present work was to study the effect of BCPs on the experimental model of acute lung injury induced by the intratracheal instillation of LPS.

Materials and methods

Animals

Male Kunming mice, which weighed 24–28 g, were purchased from Slaccas-Shanghai Lab Animal Ltd. (Certificate; No. SCXK2007-0005). The mice were kept in a specific pathogen free condition and received food and water ad libitum. Laboratory temperature was 24 ± 1 °C and relative humidity was 40–80%. Guinea pig serum was obtained from animals which were purchased from the Laboratory Animals Research Institute of Fudan University, Shanghai, China. All experimental protocols shown in this study were approved by the Animal Ethical Committee of School of Pharmacy at Fudan University.

Plant materials

The root of *Bupleurum chinense* DC was purchased from Anguo herb market, Hebei Province of China, in October of 2007. The plant material was authenticated by Prof. Shengli Pan at Fudan University, and the voucher specimen (DFC-BC20071015) has been deposited at the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, PR China.

Reagents

LPS (*Escherichia coli* 055:B5) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Dexamethasone (DXM) Sodium Phosphate Injection (No. H37020289) was purchased from Shandong Xinhua Pharmaceutical Co., Ltd. (Xinhua, Shandong, China). Mouse TNF-α ELISA kits (No. B1007233) were purchased from Shanghai Chuanfu Biotechnology Co., Ltd. (Chuanfu, Shanghai, China). Myeloperoxidase (MPO) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The trifluoroacetic acid was purchased from Fluka. β-galactosidase and β-glucosidase were purchased from Sigma–Aldrich Co. Polymyxin B sulfate was purchased from Amresco. All other reagents were of the highest quality available.

Isolation and characterization of the crude polysaccharides from Bupleurum chinese DC

General methods

The dried roots were grounded into fine particles and defatted using 95% C_2H_5OH then the residues were dried in the shade and extracted with hot water for 2 h. The hot water extract was concentrated to the equivalent of 0.12 g crude drug per ml, and then precipitated by adding 4 volumes of 95% C_2H_5OH. The precipitation was dissolved with water and the proteins existed in water solution were precipitated with 7% trichloroacetic acid at 4 °C. The supernatant was neutralized by 10% NaOH and extensively dialyzed against running water for 3 days. After centrifugation, the supernatant was concentrated and then lyophilized to produce the crude polysaccharides (BCPs) for the following *in vitro* and *in vivo* experiments.

BCPs appeared as a light-brown powder and it is soluble in water. The total carbohydrate and uronic acid contents were determined by the phenol-sulfuric acid (Dubois et al. 1956) and *m*-hydroxybiphenyl (Blumenkr and Asboehan 1973) methods respectively, using D-galactose and D-galacturonic acid as respective standards. The concentration of total protein contents in BCPs was estimated by Coomassie brilliant blue test.

Monosaccharide composition and protein contents analysis

BCPs (4.1 mg) were hydrolyzed with 4 ml 2 M trifluoroacetic acid at 110 °C for 4 h. After reduction with 20 mg of $NaBH_4$, the monosaccharide alditol acetates were prepared using the method described by Jones and Albersheim (1972). The alditol acetates were subjected to GC analysis on an HP6890 GC (Hewlett-Packard, Wilmington, USA) fitted with a capillary column DB-225 (0.25 mm × 30 m).

Protein contents of BCPs were measured by Coomassie brilliant blue test (Wang et al. 2009). A standard curve was performed with an albumin (bovine serum) standard. 0.1 ml BCPs solution were mixed with 5 ml Coomassie brilliant blue. The samples were incubated for 5 min. The optical densities were measured at 595 nm.

Acid hydrolysis

BCPs (16.6 mg) were hydrolyzed with 16 ml 2 M trifluoroacetic acid at 110 °C for 4 h. The excessive trifluoroacetic acid was replaced by methyl alcohol. The methyl alcohol solution was evaporated to produce the acid hydrolyzed BCPs for complementary activity detection.

Glycosidase hydrolysis

10 ml BCPs solution (1 mg/ml) was hydrolyzed with glycosidase (100 u β-glucosidase and β-galactosidase) at 50 °C for 2 h, and then in boiling water bath for 10 min. The solution was precipitated at 4 °C for 24 h. After centrifugation, the supernatant was concentrated and then lyophilized to produce the glycosidase hydrolyzed BCPs for complementary activity detection.

Evaluate the action of contaminated endotoxin on complementary activity

The content of LPS in BCPs was determined by limulus amebocyte lysate reagent method (Chang and Sack 2001). A calibration curve was performed with LPS O55:B5 (Sigma) to determine the amount of LPS. The results showed that 1 mg BCPs contained 267 EU (59 ng) LPS.

According to the research of Jacobs and Morrison (1977), 10-fold higher concentrations of polymyxin B could achieve approximately the degree of inhibition obtained with LPS. Polymyxin-treated BCPs was prepared by adding 900 ng polymyxin to 1 ml of a solution of BCPs (1.5 mg/ml). The amount of LPS in BCPs was doubled by adding extra pure LPS, this product and polymyxin-treated BCPs were diluted for complementary activity detection.

Complementary activity through the classical pathway

Based on Mayer's modified method (1961), sensitized erythrocytes (EAs) were prepared by incubation of sheep erythrocytes (4.0×10^8 cells/ml) with rabbit anti-sheep erythrocyte antibody (1:1000) in VBS^{2+} (containing 0.5 mM Mg^{2+} and 0.15 mM Ca^{2+}). Samples were dissolved in VBS^{2+}. Guinea pig serum was used as the complement source. The 1:120 diluted Guinea pig serum was chosen to give submaximal lysis in the absence of complement inhibitors. In brief, various dilutions of tested samples (200 μl) were mixed with 200 μl of Guinea pig serum, and 200 μl of EAs was added, then the mixture was incubated at 37 °C for 30 min. The reaction volume was 600 μl. The different assay controls were incubated in the same conditions: (1) vehicle control: 200 μl EAs in 400 μl VBS^{2+}; (2) 100% lysis: 200 μl EAs in 400 μl ultrapure water; and (3) samples background: 200 μl dilution of each sample in 400 μl VBS^{2+}. The reacted mixture was centrifuged immediately at 4 °C. Optical density of the supernatant was measured