Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

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Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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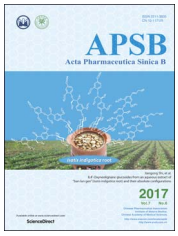

ORIGINAL ARTICLE

Beneficial effects of *Houttuynia cordata* polysaccharides on "two-hit" acute lung injury and endotoxic fever in rats associated with anti-complementary activities

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KEY WORDS

Houttuynia cordata;
Polysaccharides;
Fever;
Acute lung injury;
Anti-complementary

Abstract *Houttuynia cordata* Thunb. is a traditional herb used for clearing heat and eliminating toxins, and has also been used for the treatment of severe acute respiratory syndrome (SARS). *In vitro,* the crude *H. cordata* polysaccharides (CHCP) exhibited potent anti-complementary activity through both the classical and alternative pathways by acting on components C3 and C4 of the complement system without interfering with the coagulation system. This study was to investigate the preventive effects of CHCP on acute lung injury (ALI) induced by hemorrhagic shock plus lipopolysaccharide (LPS) instillation (two-hit) and LPS-induced fever in rats. CHCP significantly attenuated pulmonary injury in the "two-hit" ALI model by reducing pulmonary edema and protein exudation in bronchoalveolar lavage fluid (BALF). In addition, it reduced the deposit of complement activation products in the lung and improved oxidant-antioxidant imbalance. Moreover, CHCP administration inhibited fever in rats, reduced the number of leukocytes and restored serum complement levels. The inhibition on the inappropriate activation of complement system by CHCP may play an important role in its beneficial effects on inflammatory diseases. The anti-complementary polysaccharides are likely to be among the key substances for the heat-clearing function of *H. cordata*.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2017.11.003

Please cite this article as: Lu Yan, et al. Beneficial effects of *Houttuynia cordata* polysaccharides on "two-hit" acute lung injury and endotoxic fever in rats associated with.... *Acta Pharmaceutica Sinica B* (2017), https://doi.org/10.1016/j.apsb.2017.11.003

1. Introduction

The complement system is composed of more than 30 plasma and membrane-bound proteins, and is considered to be a nonspecific host immune response. It is activated immediately after injury and to a greater degree during resuscitation[1]. However, the inappropriate activation of the complement system has been demonstrated to play a role in the pathogenesis of a wide range of auto-immune disorders, acute inflammatory diseases and tissue injuries[2,3]. C3a, C4a and C5a, the anaphylatoxins cleaved respectively from the complement components C3, C4 and C5 during complement activation, may trigger degranulation of endothelial cells, mast cells or phagocytes, and induce inflammatory responses as well as the fatal shock-like syndrome[4]. Modulation of the complement activity could be beneficial in treating various complement-associated diseases[5,6].

Acute lung injury (ALI) is characterized with respiratory dysfunction, hypoxemia with presence of diffuse pulmonary infiltrates, widespread lung inflammation[7], increased pulmonary vascular permeability and accumulation of activated neutrophils in the lung[8]. Acute respiratory distress syndrome (ARDS) is the most severe form of ALI[9]. Complement activation is an early step in ALI[3] through both the classical pathway and the alternative pathway[10]. The loss of endothelial integrity in the process of ALI/ARDS also causes injured pulmonary cells to release some tissue factors or enzymes, which would lead to the initiation of complement cascade[10,11]. A cycle of complement activation and lung injury is consequently formed. Hence, the application of complement inhibitors should be a novel treatment strategy for infectious fever and ALI.

Fever is another characteristic symptom of infectious diseases. It is one of complex and nonspecific host defense responses to infections[12]. It is reported that hyper-activated complement system also involves in fever[13].

As a traditional Chinese medicine with actions of clearing heat and eliminating toxins, the whole plant of *Houttuynia cordata* Thunb. (Saururaceae) is commonly used for the treatment of pulmonary symptoms (including lung abscess, dyspnea, phlegm and cough), as well as infectious diseases, anaphylaxis, cancer and viral infection[14–17]. A Chinese multiherb remedy with *H. cordata* as the principal ingredient had been used for the treatment and prevention of severe acute respiratory syndrome (SARS) in 2003[18] and was found to have significant anti-complementary activity[19]. Our previous study indicated that the crude *H. cordata* polysaccharides (CHCP)[20] and flavonoids[21] were the major anti-complementary principles of this plant. Some anti-complementary polysaccharides showed potent effects on complement-associated diseases *in vivo*[22,23]. The presence of lipopolysaccharide (LPS), a prototypical endotoxin, in blood can cause the immediate activation of the complement cascade. We had reported that CHCP could ameliorate LPS-induced ALI in mice and reduce the complement activation products deposited in the lung tissue[20]. However, it is still unclear whether this effect was a direct result of CHCP's anti-complementary activity *in vivo*. Herein, the beneficial effect of CHCP on ALI was further evaluated using a "two-hit" ALI rat model induced by hemorrhagic shock plus LPS instillation, which is more susceptive to lung injury[24]. Specifically, the *in vivo* anti-complementary activity of CHCP was also assessed. Besides pulmonary infections, fever is another typical indication for heat-clearing traditional Chinese medicine (TCM). Therefore this paper investigated *in vivo* the anti-pyretic effects and anti-complementary activity of CHCP on a febrile rat model induced by LPS.

2. Materials and methods

2.1. Agents and animals

The dried whole plant of *H. cordata* was purchased from Shanghai Hua-Yu Chinese Materia Medica Co., Ltd. (Shanghai, China) and was identified by one of the authors (Dr. Yan Lu). A voucher specimen (DFC-YXC-2006072601) has been deposited at the Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, China.

CHCP was prepared through the process of water extraction, alcohol precipitation and deproteinization as previously reported[20]. The dried whole plant of *H. cordata* was grounded and defatted with 95% ethanol. The residue was extracted with hot water. The water extract was concentrated and precipitated with trichloroacetic acid to remove proteins. The supernatant was dialyzed in running water for 3 days, and then precipitated by adding 4 volumes of 95% ethanol. The precipitate was lyophilized to yield the polysaccharides (CHCP). The total carbohydrate content was determined with the phenol-sulfuric acid using D-galactose as the standard. The *m*-hydroxybiphenyl method was used to test the uronic acid content with D-galacturonic acid as the standard. The concentration of total protein was evaluated using the Coomassie brilliant blue method, with bovine serum albumin as the standard. High performance gel permeation chromatography (HPGPC) was carried out to analyse the molecular weight of CHCP on a TSKgel GMPWxl column (300 mm × 7.6 mm, TOSOH, Japan). Gas chromatography (GC) was used to analyze the monosaccharide composition of the completely hydrolyzed CHCP on a HP6890 (Hewlett-Packard, Wilmington, USA) fitted with a capillary column DB-225 (30 m × 0.25 mm).

Male Sprague–Dawley (SD) rats (300 to 350 g, SPF II Certificate; No. SCXK 2008-0016), were provided by the Sino British SIPPR/BK Lab Animal Ltd. (Shanghai, China). Male Wistar rats (210 ± 20 g, SPF II Certificate; No. SCXK 2007-0005) were purchased from Slaccas-Shanghai Lab Animal Ltd. (Shanghai, China). All the experimental procedures described in this study were previously approved by the Animal Ethics Committee of School of Pharmacy, Fudan University.

Sheep blood cells were collected in Alsevers' solution. Normal human serum (NHS) was obtained from healthy male donors (at the age of 21–25 years old). Heparin (sodium salt, 160 IU/mg) was from Shanghai Aizite Biotech Co., Ltd. (Shanghai, China). Antisera of C1q, C2 and C9 were from Merck Biosciences (Darmstadt, Germany). Antiserum of C5 was from Shanghai Shengsuo Reagent Co., Ltd. (Shanghai, China). Antisera of C3 and C4 were from Shanghai Taiyang Biotech Co., Ltd. (Shanghai, China). LPS (*Escherichia coli* O111:B4 endotoxin) was from Sigma–Aldrich (St. Louis, USA). Malondialdehyde (MDA) and superoxide dismutase (SOD) kits were from Nanjing Jiancheng Biotech Co., Ltd. (Nanjing, China). Complement components C3 and C4 kits were from Taiyang Biotech Co., Ltd. (Shanghai, China).

Buffers: barbitol buffer solution (BBS), containing 0.5 mmol/L Mg^{2+} and 0.15 mmol/L Ca^{2+}. GVB-Mg-EGTA, veronal buffer saline, containing 5 mmol/L Mg^{2+} and 8 mmol/L Ca^{2+}.

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