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Anti-inflammatory effect of SQC-β-CD on lipopolysaccharide-induced acute lung injury

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ABSTRACT

Aim of the study: Shuang-Qing-Cao (SQC) is a folk Chinese medicinal formula. The therapeutic effects of inclusion complexation of SQC extract in β-cyclodextrin (SQC-β-CD) against lipopolysaccharide (LPS)-induced acute lung injury (ALI) were studied in mice.
Materials and Methods: Two protocols were designed for administration of SQC-β-CD (10 and 20 mg/kg body weight) or DEX (2 mg/kg). According to Protocol A we intraperitoneally injected diluent (saline with 0.5% Tween 80), SQC-β-CD or DEX respectively into mice 30 min and 3 h after LPS challenge. Alternatively, in Protocol B we administered diluent, SQC-β-CD or DEX 3 h before and 30 min after LPS challenge.
Results: The histological results showed that SQC-β-CD (20 mg/kg) protected mice from LPS-induced ALI such as oedema, haemorrhage, blood vessel and alveolar structural damage. Furthermore, SQC-β-CD inhibited LPS-increased pulmonary MPO activity and migration of polymorphonuclear neutrophils (PMNs) into bronchoalveolar lavage fluid (BALF). Immunohistochemical experiment demonstrated that SQC-β-CD decreased inducible nitric oxide synthase (iNOS) expression in lung 24 h after LPS administration. Consequently, SQC-β-CD prevented LPS-induced nitric oxide (NO) release in BALF.
Conclusions: The results indicated that SQC-β-CD is greatly effective in inhibiting ALI. The present study indicated that SQC-β-CD acted as a potential therapeutic reagent for treating ALI.

1. Introduction

Lipopolysaccharide (LPS) is the major constituent of the outer cell wall of Gram-negative bacteria and the predominant inducer of inflammatory responses to these bacteria (Beutler and Rietschel, 2003). Intratracheal administration LPS to mice triggers an acute inflammation, which is characterized by increased capillary permeability, interstitial and alveolar edema and an influx of circulating inflammatory cells (Ingenito et al., 2001; Kitamura et al., 2001; Karmpaliotis et al., 2002). Previous reports have indicated that lung injury due to LPS instillation is initiated by a rapid influx of neutrophils into the air spaces within 24 h and then resulted in excessive inflammation (Chignard and Balloy, 2000; Rowe et al., 2002). Polymorphonuclear neutrophils (PMNs) play an important role in mediating the acute injury characteristic of acute lung inflammation. In the normal lower respiratory tract, macrophages whereas PMNs are almost absent. However, PMNs can accumulate within the lung structure, just as in the aftermath of injury, trauma or infection (Sibille and Reynolds, 1990). In fact, PMNs have the potential to harm lung tissues in different ways, such as the release of neutral proteinases, myeloperoxidase (MPO) and lysozyme and the generation of reactive oxygen species (Dallegri and Ottonello, 1997) These mediators play a fundamental role in the degradation of the alveolar matrix that is associated with a variety of pulmonary diseases (Stockley, 1994; Venaille et al., 1998).

In LPS-induced acute lung injury, expression of lung iNOS increased mainly due to infiltration of iNOS producing leukocytes, such as neutrophils or alveolar macrophages (Agorreta et al., 2003). Over production of NO catalyzed by iNOS is thought to have deleterious effects and has been implicated in the pathophysiologic mechanisms of ALI (Kristof et al., 1998; Baldus et al., 2001; Sittipunt et al., 2001). There are several candidates including anti-tumor necrosis factor-α (TNF-α) antibodies, iNOS or neutrophil elastase inhibitors were considered to reduce lung injury (Chignard and Balloy, 2000; Rowe et al., 2002; Su et al., 2007). Despite intense research and multiple diverse therapeutic trials, specific therapies effective in preventing or reversing the severe pulmonary inflammation remain elusive (Dellinger, 1999; Anzueto, 2002).

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Shuang-Qing-Cao (SQC) is a folk Chinese medicinal formula composed of three traditional indigenous medicines which include *Lonicera japonica* Thunb (Caprifoliaceae), *Isatis indigotica* Fort (Cruciferae) and *Houttuynia cordata* Thunb (Saururuaceae). *Lonicera japonica* has been used for centuries in traditional Chinese medicine practice for the treatment of sores, carbuncles, furuncles, swelling and affections caused by exopathogenic wind-heat or epidemic febrile diseases at the early stage (Chai et al., 2005). *Isatis tinctoria*, a biennial herbaceous plant distributed in Europe, Asia and Africa, which has an extensive and well documented history in European and Chinese medicines as an anti-inflammatory drug (Danz et al., 2002). In China, *Isatis tinctoria* is used to treat various kinds of virus influenza, epidemic parotitis, virus hepatitis and other infectious diseases clinically and is monographed in Chinese Pharmacopeia. *Houttuynia cordata* was recorded in Bencao Gangmu (Compendium of Materia Medica). It is one of most widely used herbal drug to cure infectious disease, refractory hemoptysis, malignant pleural effusion, nephrotic syndrome, and so on (Hayashi et al., 1995; Chang et al., 2001; Lu et al., 2006a). Furthermore, these three medicinal herbs of SQC formula also act as the key composition in different prescription of Chinese medicines for treating pneumonia and bronchitis in China. Although SQC is used for treating diseases related with inflammation in the folk of China, the anti-inflammatory mechanism of SQC action has never been investigated. Here we studied the effects of SQC on an experimental model of acute lung inflammation induced by the intratracheal instillation of LPS in vivo and tried to clarify the mechanism involved.

β-Cyclodextrin (β-CD) is one of cyclic oligosaccharides that form inclusion complexes with many analytes. Its hydrophobic interior and hydrophilic exterior allow β-CD to bind organic molecules through hydrophobic interactions, hydrogen bonding and electrostatic interactions. The form of β-CD molecules resembles truncated cones with the secondary hydroxyl groups located at the wider edge of the ring and the primary groups on the narrower edge. β-CD is widely used in the pharmaceutical industry for their capability of improving bioavailability, solubility, or stability of drugs via the formation of soluble inclusion complexes (Rajewski and Stella, 1996; Davis and Brewster, 2004; Sun and Stenken, 2007).

Because of volatility, instability and poor dissolubility of some active components in SQC, we prepared SQC-β-CD clathrate (inclusion complexation of SQC in β-CD) for our pharmacological experiments.

2. Materials and methods

2.1. Chemicals, antibodies and drugs

Lipopolysaccharides (from *Escherichia coli* 0111: B4) and β-cyclodextrin (β-CD) were purchased from Sigma–Aldrich (St. Louis, USA). Rabbit anti-iNOS polyclonal antibody was purchased from Chemicon (Temecula, USA). SABC kit for iNOS immunohistochemistry was purchased from Boster Biotechnology Co. (Wuhan, China). Dexamethasone (DEX) Sodium Phosphate Injection was purchased from Lianshui Medicine Co. (Batch No. 0507203, China).

2.2. Preparation of extract of Shuang-Qing-Cao

All medicines formulating Shuang-Qing-Cao, viz. bud of *Lonicera japonica* thub), leaf of *Isatis tinctoria* Fort and overground part of *Houttuynia cordata* Thuhb plant, were purchased from the Jiangsu Medicinal Material Company. The voucher specimens of *Lonicera japonica*, *Isatis tinctoria* and *Houttuynia cordata* identified by Prof. L.X. Zhang were preserved at the Herbarium of Nanjing University, Nanjing, China.

100 g (dry weight) of SQC (*Lonicera japonica* Thunb: *Isatis indigotica* Fort: *Houttuynia cordata* Thunb is 1:1:1) was extracted by the refluxing method with 1000 ml ethanol for 1 h and filtrated, followed by refluxing extraction of precipitation with 500 ml ethanol for another 1 h and filtration. Evaporation of the solvent from reduplicated refluxing extraction under a rotavapor gave 19 g SQC extract (concrete). Inclusion complexes of SQC and β-CD were prepared by the suspension method. Above SQC concrete (19 g) added with 380 g β-CD was suspended in 400 ml distilled water and was magnetically stirred at 40 °C for 14 h. After the contact period (12 h, 5 °C) the suspension was filtered. The resulting precipitation was washed with cold water twice and dried in a vacuum in order to collect the solid products. As a result, we get 381 g inclusion complexes of SQC in β-CD (SQC-β-CD).

2.3. Animals

Male ICR mice (6–7 weeks) weighing 18–22 g were purchased from Shanghai Experimental Animal Center, China Academy of Science. Laboratory animal handling and experimental procedures were performed in accordance with the requirements of Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by Science and Technology Department of Jiangsu Province.

2.4. LPS-induced acute lung injury model

The general procedure was modified in a manner similar to previous reports (Asti et al., 2000; Rowe et al., 2002). Briefly, mice were anesthetized with pentobarbital sodium (30 mg/kg) and fixed on a board at angle of 50° in a supine position. 50 μl sterile saline or sterile saline containing 10 μg LPS was instilled into the mouse trachea with a 3-gauge needle respectively. After intratracheal instillation, the mouse was placed in a vertical position and rotated for 0.5–1 min to distribute the instillate evenly within the lungs.

Two protocols were designed for administration of SQC-β-CD (10 and 20 mg/kg body weight) or DEX (2 mg/kg). According to protocol A we intraperitoneally injected diluent (saline with 0.5% Tween 80) or SQC-β-CD or DEX into mice 30 min and 3 h after challenge with LPS. Alternatively, in protocol B we administered diluent or SQC-β-CD or DEX 3 h before and 30 min after the challenge of LPS. DEX (2 mg/kg) was used as a positive control in this experiment.

2.5. Myeloperoxidase assay

Six hours after LPS treatment, the mice were anaesthetized with pentobarbital sodium (30 mg/kg). Lungs were perfused via the right ventricle with 2 ml of sterile PBS and then whole lungs were homogenized and sonicated in 0.5% hexadecyltrimethylammonium bromide (HTAB) buffer. After centrifugation at 12,000 × *g* for 10 min at 4 °C, the supernatant fluids containing MPO were incubated in a 50 mM KPO_4 buffer containing the substrate H_2O_2 (1.5 M) and *o*-dianisidine dihydrochloride (167 mg/ml; Sigma–Aldrich) for 30 min. The enzymatic activity was determined spectrophotometrically by measuring the change in absorbance at 460 nm using a 96-well plate reader.

2.6. Bronchoalveolar lavage and cell counting

The mice were anesthetized and were inserted with a plastic cannula into the trachea. Bronchoalveolar lavage (BAL) was performed with three aliquots of 0.6 ml PBS (PH 7.2) instilled up to a total volume of 1.8 ml, and withdrawn three times each, the fluid recovery rate was 87 ± 2%. Bronchoalveolar lavage fluid (BALF) samples were centrifuged (700 × *g*, 4 °C) for 10 min. The sediment cells