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Effects of *Astragalus* and *Codonopsis pilosula* polysaccharides on alveolar macrophage phagocytosis and inflammation in chronic obstructive pulmonary disease mice exposed to PM2.5

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ABSTRACT

Astragalus and *Codonopsis pilosula* are used for their immunomodulatory and anti-inflammatory effects. Here, we investigated the effects of *Astragalus* polysaccharides (APS) and *Codonopsis pilosula* polysaccharides (CPP) on alveolar macrophage (AM) phagocytosis and inflammation in chronic obstructive pulmonary disease (COPD) associated with exposure to particulate matter with a mean aerodynamic diameter ≤2.5 μm (PM2.5). A mouse model of COPD was established by cigarette smoke exposure. PM2.5 exposure was performed by inhalation of a PM2.5 solution aerosol. APS and CPP were administered intragastrically. COPD showed defective AM phagocytosis and increased levels of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α in bronchoalveolar lavage fluid and serum. PM2.5 exposure aggravated the damage, and this effect was reversed by APS and CPP gavage. The results indicate that APS and CPP may promote defective AM phagocytosis and ameliorate the inflammatory response in COPD with or without PM2.5 exposure.

1. Introduction

Alveolar macrophages (AM) play a central role in chronic obstructive pulmonary disease (COPD) development by orchestrating the inflammatory response (Barnes, 2014). Phagocytosis by AM is critical for the uptake and degradation of infectious agents and senescent cells, and it participates in development, tissue remodeling, immune response, and inflammation (Barnes, 2014; Donnelly and Barnes, 2012). The defective phagocytotic ability of AM, which indicates a suppressive adaptive immune response and results in vital for infection, has been reported in COPD patients. (Donnelly and Barnes, 2012). Because of the abundant bacterial colonization within the lower respiratory tract of COPD patients, persistent infection by bacteria is the predominant cause of chronic airway inflammation and it increases the risk of acute exacerbations (Finney et al., 2014). COPD progression is associated with the upregulation of inflammatory mediators such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 both in the sputum and the circulation, while the failure of resolution of inflammation causes damage to the organism (Barnes, 2014; Khurana et al., 2014; Rubinsztajn et al., 2015). Inflammation is not restricted to the lungs of COPD patients and may spill over to become a systemic inflammation (Barnes, 2014). Fine particulate matter with a mean aerodynamic diameter ≤2.5 μm (PM2.5) is closely related to respiratory disease. Short-term exposure to a 10 $\mu g/m^3$ increment of ambient PM2.5 is associated with increased COPD hospitalizations and mortality (Li et al., 2016). Long-term exposure to air pollution and PM2.5 is related to markers of inflammation such as IL-6 and the susceptibility of lung infection (Hajat et al., 2015; Zhao et al., 2014). Lanzhou city in the northwest China is seriously polluted, which resulted in an increase in COPD-related hospitalizations of 2.8% per inter-quartile range increase in PM10 (Tao et al., 2013).

Astragalus and *Codonopsis pilosula* are two traditional Chinese herbal medicines that are widely used for the treatment of chronic information diseases (Shergis et al., 2014; Yang et al., 2013b). However, the exact mechanism has not been fully elucidated. Among the many constituents of the two herbs, polysaccharides show prominent medical effects that have been recognized by modern pharmacognosy studies (Wang and Huang, 2014; Xin et al., 2012). The bioactive fractions of *Astragalus, such as Astragalus* polysaccharide (APS), possess anti-inflammatory properties and decrease the release of inflammatory mediators secreted from macrophages. Xu at al. (2007) reported that macrophage phagocytic activity is significantly increased after treatment with APS. Purified fractions of

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Codonopsis pilosula such as *Codonopsis pilosula* polysaccharide (CPP) can increase the thymus and spleen index, as well as the phagocytic activity of peritoneal macrophages (He et al., 2015). CPP inhibits the production of inflammatory mediators by macrophages (He et al., 2015; Kim et al., 2014). However, there are few studies on the effects of APS and CPP on COPD and PM2.5-associated damage.

In the present study, a cigarette smoke exposed COPD mouse model was established. We studied the effects of PM2.5 on AM phagocytosis, and pulmonary and systemic inflammation in COPD mice. Otherwise, we also investigated the effects of APS or/and CPP on AM phagocytosis and inflammation response in COPD mice with or without PM2.5 exposure.

2. Materials and Methods

2.1. Chemicals

APS (purity: 70.2%) was purchased from Meilun biotechnology Company (Dalian, China). CPP (purity: 61.3%) was obtained from Zelang Medical Technologic Co. Ltd. (Nanjing, China). Cigarettes were acquired from Gansu tobacco industry Co. Ltd. (Lanzhou, China). Fluorescein isothiocyanate-labeled *Escherichia coli* (FITC-*E.coli*) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). Phycoerytherin (PE)-labeled anti-CD 68 mouse monoclonal antibodies were obtained from BioLegend Inc. (San Diego, CA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for IL-6 and TNF-α were obtained from Dakewe Bioengineering Co. Ltd. (Shenzhen, China). ELISA kits for IL-8 were purchased from NeoBioscience Co. Ltd. (Shenzhen, China). All other reagents used were acquired locally and were standard laboratory reagents of analytical grade.

2.2. PM2.5 preparation

PM2.5 was prepared as described by Shang et al. (2013) with modifications. PM2.5 was collected from Lanzhou atmosphere by an integrated intelligent air total suspended particulates sampler (Laoying 2050, Laoshan Applicable Technology Institute, Qingdao, China) with a PM2.5 cutter in it on the 18th floor platform near a traffic road. PM2.5 was collected on glass fiber filters placed inside the sampler beforehand. Then, the filters were collected and washed in distilled water by an ultrasonic cleaning machine. The residue of the filters was filtered and the particles were freeze-dried in a vacuum. The PM2.5 obtained was stored at −20 °C in the dark. Suspensions were prepared before inhalation.

2.3. Animals and experimental groups

Balb/c mice were purchased from the medical animal center of Lanzhou University (Lanzhou, China). Mice were acclimated for 1 week before experiments and randomly divided into nine groups (12 mice in each group) as follows: Control, COPD, COPD + PM2.5, COPD + APS, COPD + CPP, COPD + APS + CPP, COPD + PM2.5 + APS, COPD + PM2.5 + CPP, and COPD + PM2.5 + APS + CPP. All mice were housed at 20–22 °C with 50 ± 10% humidity and a 12/12-h light/dark cycle. Mice and housing conditions were pathogen free. Mice were given free access to food and water and were allowed activity in the cages. Experiments were performed in compliance with the People's Republic of China legislation on the use and care of laboratory animals.

2.4. COPD mouse model

A mouse model was established as described by Li et al. (2012a) with modifications. A 50 cm × 30 cm × 35 cm plexiglass container was made for cigarette smoke exposure. Four cigarette bars were placed in the container and burned entirely. Then, 12 mice were placed on a clapboard hanging in the middle of the container and allowed to move freely. Each exposure time was 45 min followed by a rest period of 1 h. Mice were exposed to cigarette smoke four times every day and the exposure period lasted for 90 continuous days. After the model was established, peak inspiratory flow (PIF) and peak expiratory flow (PEF) were measured using a noninvasive body plethysmograph (GYD-003 noninvasive mice pulmonary function instrument, Emka technologies, SAS, Paris, France).

After mice were sacrificed, the thorax was opened and lungs were removed. Then left lobes of lungs were fixed in 10% paraformaldehyde and embedded in paraffin. They were cut into 3-μm sections and stained with hematoxylin and eosin for histological analysis.

2.5. APS or/and CPP treatment on COPD mice with PM2.5 or without PM2.5

APS was intragastrically administered to mice at 200 mg/kg and CPP at 300 mg/kg. The control group received an equal volume of distilled water using the same method. The gavage lasted throughout the experimental period from the first day when mice began to receive cigarette smoke exposure to the last day. As described by Laskin et al. (2010), a 20 cm × 15 cm × 10 cm plexiglass container was made for PM2.5 inhalation. The concentration of PM2.5 was 770 μg/m^3 and it was generated by an ultrasonic pump atomizer (REF90543 Medel family silver aerosol, Medel group S.p.A, Parma, Italy) connected to the container. Mice were placed in the container and allowed to freely move around. Exposure lasted for 30 min and was performed four times per day, and the exposure period was the same as that of the cigarette smoke exposure. Filtered air was inhaled by mice of other groups as a control.

2.6. Isolation of alveolar macrophages

AMs were isolated from mice lung tissue according to the protocol described by Chana et al. (2014). After mice were sacrificed, blood was collected and serum was separated from the blood. 0.9%NaCl Solution was injected slowly into the lung using an 18-G needle syringe to lavage. Bronchoalveolar lavage fluid (BALF) was collected several times. The lung lobes were then dissected from the thorax and immersed into RPMI 1640 medium (RPMI 1640 with 2 mmol/L [1% vol/vol] L-glutamine, 100 U/mL [1% vol/vol] penicillin, 100 μg/mL [1% vol/vol] streptomycin, 5% vol/vol fetal calf serum, and 50 mmol/L Ethylene Diamine Tetraacetie Acid) on a 200 mesh strainer. Lobes were gently ground by a sterile Pasteur pipette. The cell suspension was then centrifuged and separated. The cell suspension was layered gently on the top of a discontinuous Ficoll-Hypaque density gradient (100%/50%) and was centrifuged (2000 rpm) for 20 min. The fraction between 100% and 50% Ficoll-Hypaque was collected and washed twice using PBS. Then, cells were suspended in complete medium (RPMI 1640 with 2 mmo/L [1% vol/vol] L-glutamine, 100 U/mL [1% vol/vol] penicillin, 100 μg/mL [1% vol/vol] streptomycin, and 15% vol/vol fetal calf serum) at 1 × 10^6/mL. Cells were seeded into 24-well cell culture plates. After 4 h in the incubator, nonadherent cells were removed and the adherent AMs were incubated overnight. The purity of AMs was verified by flow cytometry (BD LSRFortessa flow cytometry, Becton, Dickinson and Company, NY, USA) with CD68 labeling.

2.7. AM phagocytosis was detected by flow cytometry

FITC-*E. coli* was diluted to 0.04 mg/mL and then it was dropped into the cell suspension. After incubation for 4 h, non-phagocytosed FITC-*E. coli* were removed. Trypan blue (0.25/ml) was added for 1 min to quench the extracellular FITC-labelled *E.coli*. Then AM was detached by vigorous pipetting. Flow cytometry was used to detect