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Pulvis Fellis Suis extract attenuates ovalbumin-induced airway inflammation in murine model of asthma

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ABSTRACT

Ethnopharmacological relevance: Pulvis Fellis Suis (PFS), named with "Zhu Danfen" in China, has been extensively used for the therapy of enteritis, acute pharyngitis, whooping cough and asthma in folk medicine. Although PFS shows anti-inflammatory activities, its effect on airway inflammation in asthma has not been studied.

Aim of the study: To explore the protective effect of PFS ethanol extract against airway inflammation in asthmatic mice.

Materials and methods: Allergic asthma in mice was sensitized and challenged by OVA. Mice were administered in oral with PFS daily at doses of 100, 200 and 400 mg/kg on days 21–27. Inflammatory cell counts and classification in bronchoalveolar lavage fluid (BALF) were analyzed. Histopathological evaluation of the lung tissue was performed by hematoxylin and eosin (H & E) and periodic acid-schiff (PAS) staining. The IgE level in serum was measured by using enzyme-linked immunoassay (ELISA). ELISA was also used to detect the levels of Th1/Th2 cytokine and eotaxin in BALF.

Results: Histological results revealed that PFS could ameliorate OVA-induced histological changes by attenuating inflammatory cell infiltration, mucus hypersecretion and goblet cell hyperplasia in the lung. Treatment with different doses of PFS significantly decreased the elevated inflammatory cell numbers in BALF and IgE production in serum. PFS treatment reduced the production of Th2 cytokine IL-4, IL-5, IL-13, and promoted Th1 cytokine IFN-γ production in BALF. In addition, PFS also decreased the levels of eotaxin and TNF-α in BALF.

Conclusions: These findings suggest that PFS has a markedly anti-inflammatory effect on OVA-induced allergic asthma in mice, and could be a promising protective agent recommended for allergic asthma patients.

1. Introduction

Asthma is a common chronic inflammatory respiratory disease, which characterized by airway hyperresponsiveness (AHR) and airway inflammation (Busse and Lemanske, 2001; Tiwari et al., 2014). Asthma usually has varied reversible airflow limitation, which causes recurrent episodes of wheezing, shortness of breath, chest tightness and cough, often attack at night and in the early morning (Holgate et al., 2008; Shin et al., 2014). Due to the climate, environmental and some other changes, the incidence of asthma in the world is on the rise resulting in increased mortality rates in recent years. The prevalence of allergic asthma has been particularly increased among children and has become an important problem (Mirabelli et al., 2016).

Asthma is caused by a combination of immunological, genetic and environmental interactions. In asthma, the inflammatory responses are linked to the activation of T-helper 2 (Th2) cells, which can induce AHR and airway inflammation by releasing cytokines (include interleukin (IL)-4, IL-5 and IL-13). Furthermore, Th2 cells can help B cells to activate mast cells releasing more allergic and inflammatory mediators, and produce IgE (Bosnjak et al., 2011; Larché et al.,

Abbreviations: AHR, airway hyperresponsiveness; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BALF, bronchoalveolar lavage fluid; DEX, dexamethasone; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; IL, interleukin; OVA, ovalbumin; PBS, phosphate buffered saline; PAS, periodic acid-schiff; PFS, Pulvis Fellis Suis; SEM, standard error of mean; Th2, T-helper 2; TNF-α, tumor necrosis factor-α

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2003; Liu et al., 2013). In the last decades, the medications for asthma therapy mainly include corticosteroids, antileukotrienes, anti-IgE monoclonal antibody, beta2 agonists, and anticholinergic (Chini et al., 2014). Corticosteroids are the most common therapeutical drugs in the clinics (Tritar-Cherif et al., 2002). However, the symptoms could not be prevented by these drugs in a proportion of patients, such as smokers and obese asthma patients. On the other hand, unacceptable side effects limited the use of these drugs (Manson et al., 2009). Therefore, the development of new drugs which can directly reduce or alleviate the symptoms of asthma is urgently needed.

Traditional Chinese medicine is widely used for the treatment of allergic diseases, especially in asthma (Cheng et al., 2014; Wei et al., 2015). Pulvis Fellis Suis (PFS), named with “Zhu Danfen” in China, is the dried bile of *Sus scrofa domestica* Brisson. It has been used extensively in folk medicine in the therapy of respiratory diseases, mainly used for treating acute pharyngitis, bronchitis, cough, asthma and other diseases (Liu and Geng, 1992; Pharmacopoeia of the People's Republic of China, 2015). Bile acids, especially conjugated cholic acids, are the main chemical components in PFS. The recent pharmacological research revealed that bile acids have anti-inflammatory, analgesic, and antibacterial effects. Bile acids from PFS could inhibit cough central excitatory and relieve bronchial spasm (He et al., 2011; Li et al., 2008; Song, 2001; Wu, 1993). Although PFS has been used in the therapy of respiratory disease, its inhibition on airway inflammation in asthma has not been studied yet. Therefore, the present study was designed to investigate the protective effects of ethanol extracts of PFS against allergic asthma induced by ovalbumin (OVA) in mice.

2. Materials and methods

2.1. Chemicals and drugs

Ovalbumin (OVA) was purchased from Sigma-Aldrich Trading Co., Ltd., (Shanghai, China). IL-4, IL-5, IL-13, tumor necrosis factor-α (TNF-α), IFN-γ, eotaxin and IgE enzyme-linked immunosorbent assay (ELISA) kits were purchased from R & D Systems (Minneapolis, MN, USA). Dexamethasone (DEX, No. 140806) was purchased from Xianju Pharmaceutical Co. Ltd. (Zhejiang, China). Other chemical reagents used in the experiments were analytical grade.

2.2. Preparation of the extract

Pulvis Fellis Suis was purchased from Wanshou Road Chinese Herbal Medicine Market (Xi’an, China), and was authenticated by Prof. W.J. Sun of Northwest University, Xi’an, China. The voucher specimen (ZDF-150301) was deposited at Life Science College of Northwest University, Xi’an, China. PFS (50 g) was added into a chromatographic column containing 1500 g aluminum oxide active neutral (8 cm×50 cm), and sequentially eluted with 95% (*v/v*) ethanol. The effluents were then mixed and concentrated in vacuum at 50–60 °C by using a rotary evaporator. The dried residue obtained with a yield of 4.75 g was suspended in saline and subjected to in vivo experiments. The mice received the drugs with a range of doses which are equivalent to 100, 200, and 400 mg of the dried extract per kg body weight respectively.

2.3. Animals

Female ICR mice (18–22 g) were purchased from Experimental Animal Center in Xi’an Jiaotong University (Shaanxi, China). Mice were maintained in the Specific Pathogen Free (SPF) Animal Lab, fed with standard laboratory chow and water ad libitum. The temperature of SPF Lab was controlled at 23 ± 2 °C, and the relative humidity was set at 40–60% with a 12 h light/dark cycle. All animal experimental procedures were conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH Publications, 85-23, revised 1985), and approved by ethical committee for Animal Care and Use of the Northwest University (No. 20150519).

2.4. Establishment of allergic asthma model and treatment

Mice were sensitized and challenged with OVA. Briefly, on day 0, mice were sensitized with 0.2 ml phosphate buffered saline (PBS, pH 7.4) containing 20 μg OVA and 2 mg aluminum hydroxide gel by intraperitoneal injection. The sensitization was repeated on day 7, 14 and 21. On day 24–27, 1 h after the drug treatment, each group mice were placed into an individual chamber, challenged through inhalation atomization with 1% OVA (w/v) in PBS for 30 min by using an ultrasonic nebulizer (JLN -2304AS, Bang Lijian corp., Shenzhen, China). Normal control mice were sensitized and challenged with PBS buffer alone using the same technique.

Mice were divided into six groups (n = 10). Group 1 was normal mice without any treatment (normal control group). Group 2–6 were sensitized and challenged with OVA as described above. Group 2 (OVA control group) was not received any treatment. Group 3 was treated with DEX (2 mg/kg). Group 4, 5 and 6 were given with PFS at 100, 200, 400 mg/kg, respectively. All drugs were given in oral from day 21–27 consecutively. A schematic diagram of the treatment is shown in Fig. 1.

2.5. Preparation of bronchoalveolar lavage fluid (BALF) and inflammatory cell counting

Mice were euthanized under anesthesia 24 h after the last OVA challenge. Following by three times instillation of ice-cold PBS (0.5 ml) into the lung, BALF was aspirated via tracheal catheter (total volume 1.5 ml). BALF from each mouse was recovered and centrifuged, and the supernatants were collected and stored at −80 °C for cytokine assays. Cell pellet in each tube was re-suspended in PBS. The hemacytometer was used for total cell counts, and the differential cell counts were performed by using Wright's and Giemsa stain.

2.6. Histological assessment of lung

Neutral formalin (10%, *v/v*) was used to fix lung tissue, then the tissue was dehydrated with increasing concentrations of ethanol, embedded in paraffin, cut into 5 μm thickness sections and stained with hematoxylin and eosin (H & E) solution. Using a treatment-blind observer, the score of lung inflammation was assessed as the following scale: 0, no inflammation; 1, mild inflammation; 2, moderate inflammation; 3, marked inflammation; and 4, severe inflammation. When

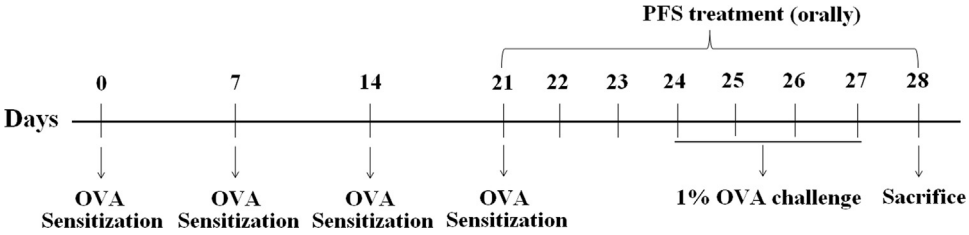

Fig. 1. Mouse model of airway inflammation and treatment with PFS ethanol extract.