Journal of Ethnopharmacology 189 (2016) 22–30

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Pheretima aspergillum decoction suppresses inflammation and relieves asthma in a mouse model of bronchial asthma by NF-κB inhibition

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ARTICLE INFO

Article history:
Received 7 August 2015
Received in revised form
19 February 2016
Accepted 11 May 2016
Available online 13 May 2016

Jel classification:
Respiratory system

Keywords:
OVA
Asthma
Traditional Chinese medicine
Airway
Earthworm

Chemical compounds studied in this article:
Hypoxanthine (PubChem CID: 790)
Choline (PubChem CID: 305)
l-alanine (PubChem CID: 5950)
l-lysine (PubChem CID: 5962)
l-leucine (PubChem CID: 6106)
l-valine (PubChem CID: 6287)

ABSTRACT

Ethnopharmacological relevance and aim of the study: Guang-Pheretima, the live form of the earthworm *Pheretima aspergillum*, is a traditional Chinese medicine commonly used for the treatment of asthma, cough, stroke, epilepsy and other diseases due to its anti-inflammatory, anti-asthmatic, anti-seizure, thrombolytic and diuretic properties. Although Guang-Pheretima is effective in the relief of asthma, its pharmacological activity and the underlying molecular mechanisms are not fully understood. Hence, we investigated the effects of a *Pheretima aspergillum* decoction (PAD) against inflammation in a model of ovalbumin (OVA)-induced asthma in BALB/c mice, as well as the nuclear factor-κB (NF-κB) pathway involved in this process.

Materials and methods: OVA was used to sensitize and challenge the airway of the mice, and PAD was administrated by gavage. We measured airway hyperresponsiveness (AHR) in the mice 24 h following a final methacholine challenge with whole-body plethysmography. The bronchoalveolar lavage fluid (BALF), serum and pulmonary tissues were collected 48 h after the last challenge. The levels of inflammatory factors and the related mRNAs were determined by enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR), respectively. The number of differential inflammatory cells in the BALF was counted. Serum total and OVA-specific IgE levels were measured with ELISA. The activation of NF-κB signaling in the lung was detected by western blotting. In addition, the lung tissues were stained with hematoxylin and eosin or periodic acid Schiff stain for histopathological examination.

Results: PAD treatment significantly alleviated AHR in the asthmatic mice, decreased the mRNA and protein levels of IL-4, IL-5 and IL-13 and downregulated IgE. In addition, PAD treatment attenuated mucus secretion and infiltration of inflammatory cells in the lung while inhibiting the activation of NF-κB signaling.

Conclusions: PAD effectively inhibited the activation of NF-κB signaling in the lungs of mice with OVA-induced asthma, and mitigated AHR and Th2 type inflammatory reactions. Therefore, PAD may serve as a drug candidate for asthma treatment.

1. Introduction

Asthma is a Th2 lymphocyte-driven disease that is characterized by chronic airway inflammation, airway hyperresponsiveness (AHR), excessive mucus secretion, and remodeling of airway walls. Besides, accumulation of eosinophils in the lungs, enhanced mucus secretion by the goblet cells, and elevation of IgE and a number of cytokines including interleukin (IL)-4, IL-5 and IL-13 are commonly found in asthmatics (Lambrecht and Hammad, 2013; Lloyd and Saglani, 2013). Specifically, IL-4 and IL-13 synergistically regulate B cells and stimulate the secretion of IgE (Kraft et al., 2001), and IL-13 promotes the development of AHR and increases mucus secretion (Wills-Karp et al., 1998). In addition, IL-5 recruits eosinophils that migrate to the airway under the guidance of IL-4 (Woodfolk, 2006). Asthma is considered to be an inflammatory disease, and the progression of inflammation in asthma is associated with multiple transcription factors. Recent studies have shown that excessive activation of nuclear factor-κB (NF-κB) plays a key role in the expression of multiple inflammatory genes and in the progression of airway inflammation (Christman et al., 2000;

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http://dx.doi.org/10.1016/j.jep.2016.05.028
0378-8741/

Oh et al., 2011). On the other hand, steroidal anti-inflammatory drugs are not universally effective in all patients although they are the recommended medication for asthma in clinical practice (Woodruff et al., 2007). Long-term use of steroidal anti-inflammatory drugs can cause complications such as pneumonia, fracture, hyperglycemia and cataract (Mattishent et al., 2014). Therefore, the identification of new therapeutic agents with high potency to combat inflammation and relieve asthma with fewer adverse effects is presently the focus of many scientists and physicians.

Guang-Pheretima, the live form of the earthworm *Pheretima aspergillum* (E. Perrier), is mainly found in the Guangdong and Guangxi provinces of China. *P. aspergillum* is one of the source species of the traditional Chinese medicine "Pheretima" as stated in the Chinese pharmacopoeia, together with *P. vulgaris* (Chen), *P. guillelmi* (Michaelsen) and *P. pectinifera* (Michaelsen). *P. aspergillum* is considered to be the best source of Pheretima as recommended by numerous experts in the history of traditional Chinese medicine for the treatment of a variety of diseases including asthma, cough, stroke and seizure (National-Pharmacopoeia-Committee, 2010). The anti-asthmatic effects of some types of Pheretima have been studied (Chu et al., 2007); however, the anti-asthmatic properties of Guang-Pheretima and the underlying molecular signaling pathways are not fully understood and remain to be elucidated.

The bioactive chemical constituents of *P. aspergillum* have not been comprehensively investigated due to its complex components. Therefore, only purines, various enzymes, proteins, peptides, amino acids, fatty acids, mineral substances and some other compounds are known to be present in this medical material (Wang et al., 1998). As decoction is the classical therapeutic form of *P. aspergillum*, in this study, the effect of a *P. aspergillum* decoction (PAD) on a mouse model with ovalbumin (OVA)-induced asthma was investigated and compared with dexamethasone (DEX) for efficacy in alleviating AHR, reducing inflammatory cytokines, preventing inflammatory cell infiltration and reducing mucus secretion. We further investigated the association of the NF-κB signaling pathway with the anti-asthmatic effects of PAD.

2. Materials and methods

2.1. Preparation of PAD and the fingerprint

P. aspergillum was collected from March to May 2014 in the Guangzhou of Guangdong province of China. After authentication by Professor Wei Li from the Department of Traditional Chinese Medicine Identification in Guangzhou University of Chinese Medicine, individual *P. aspergillum* specimens with obvious clitella and weighing over 10 g were selected. All viscera of the worm and mud were removed and the worms were washed with water 3 times. Guang-Pheretima was obtained by flattening the worms on a porcelain plate and blow-drying them for 5 h at 50 °C. The yield of Guang-Pheretima from live *P. aspergillum* was 7.40% by weight. The Guang-Pheretima was then minced, soaked in a 20-times volume of double distilled water for 30 min, and boiled for 1 h. Boiling was performed twice, and the decoction was pooled together and then filtered with 8 layers of medical gauze. The concentrated decoction was diluted as necessary according to the daily dosage given to the mice.

High performance liquid chromatography (HPLC) fingerprinting (Fig. 1) was performed to ensure the stability and similarity of 10 batches of PAD, which were subjected ethanol precipitation to remove proteins and peptides. HPLC was performed using an Xcalibur Qual Browser system (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a SunFire C18 column (4.6 × 150 mm, 3.5 μm) (Waters, Milford, MA, USA). The mobile phase included pure water (A) and acetonitrile (B) at the flow rate of 0.6 mL/min and the column temperature was 27 °C. The gradient elution program was as follows: 0% B (0–10 min); 0–20% B (10–20 min); 20–50% B (20–35 min); 50–100% B (35–50 min); 100–0% B (50–60 min).

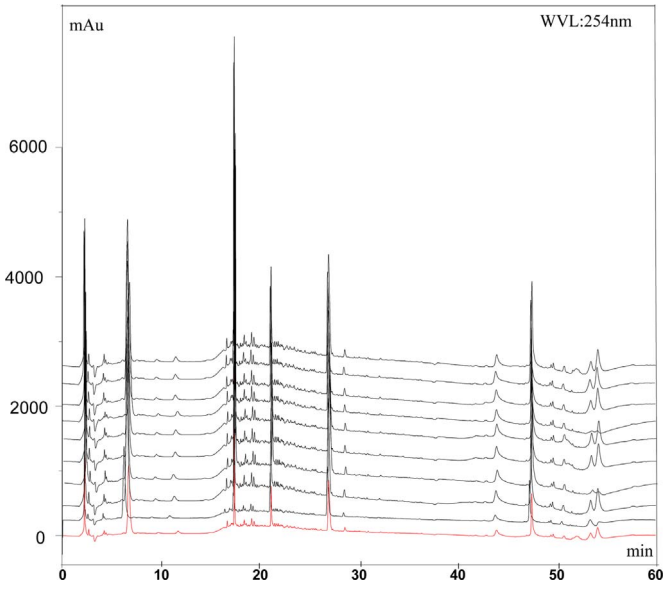

Fig. 1. HPLC fingerprint of *Pheretima aspergillum* decoction (PAD).

2.2. Mice

Six-to-eight-week old female BALB/c mice of specific pathogen free grade were purchased from the Experimental Animal Center in Guangzhou University of Chinese Medicine. The mice were hosted in a barrier environment in the animal center for adaption for 1 week with free access to food and water under an environmental temperature of 22 ± 1 °C and relative humidity of 55 ± 10%. The experimental procedure was conducted in strict accordance with the protocol approved by the Animal Ethics Committee in Guangzhou University of Chinese Medicine.

2.3. Establishment of asthma model and drug administration

The mouse model of asthma with OVA (Grade V; Sigma-Aldrich, St. Louis, MO, USA) sensitization and challenge was constructed based on previously reported methods (Inoue et al., 2009; Oh et al., 2002) with minor modifications. Briefly, 500 μg/mL OVA (in PBS) was mixed with an equal volume of 10% (w/v) aluminum potassium sulfate (Sigma-Aldrich), and the pH was adjusted to 6.5 by titrating with 10 N sodium hydroxide (Sinopharm, Shanghai, China) solution. The solution was then incubated at room temperature for 60 min and centrifuged at 750*g* for 5 min The supernatant was discarded and the samples were re-solubilized with double-distilled water to the original volume in order to obtain the sensitizing solution. On 0 and 14 d, each mouse was sensitized by an intraperitoneal injection of 0.2 mL sensitizing solution at multiple sites. On days 21–27, all mice, except those in the normal control (NC) group, were placed into a 55 cm × 30 cm × 30 cm plexiglass box and challenged by an atomized (403 T; Yuwell Medical Equipment & Supply Co. Ltd., Nanjing, China) 5% (w/v) OVA-PBS solution. The medication was given to the mice on days 14–27. Mice in the NC and OVA-challenged groups received saline orally at a dose of 0.2 mL/10 g body weight. The mice in the positive control group (receiving DEX,