International Immunopharmacology 24 (2015) 88–94

Contents lists available at ScienceDirect

International Immunopharmacology

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

Paeoniflorin attenuates allergic inflammation in asthmatic mice

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ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 27 October 2014
Accepted 14 November 2014
Available online 26 November 2014

Keywords:
Paeoniflorin
Asthma
IL-5
IL-13
IL-17
MAPK

ABSTRACT

Paeoniflorin (PF), one of the major active ingredients of *Chinese peony*, has demonstrated anti-inflammatory and immunoregulatory effects. However, it has remained unclear whether PF treatment can inhibit allergic inflammation in asthma. In this study, we evaluated the effects of PF on pulmonary function and airway inflammation in asthmatic mice. The allergic asthma models were established in BALB/c mice. The mice were sensitized and challenged with ovalbumin. Airway hyperresponsiveness was detected by direct airway resistance analysis. Lung tissues were examined for inflammatory cell infiltration. IL-5, IL-13, IL-17, and eotaxin in bronchoalveolar lavage fluid (BALF) and their mRNA expression in lung tissue were examined by ELISA and realtime PCR, respectively. The total IgE level in serum was measured by ELISA. The protein expression of p-ERK and p-JNK was detected by western blot. Our data showed that PF oral administration significantly reduced airway hyperresponsiveness to aerosolized methacholine and decreased IL-5, IL-13, IL-17 and eotaxin levels in the BALF, and decreased IgE level in the serum. Histological studies showed that PF administration markedly decreased inflammatory infiltration. Similarly, treatment with PF significantly inhibited IL-5, IL-13, IL-17 and eotaxin mRNA expression in lung tissues. The protein expression levels of p-ERK and p-JNK were substantially decreased after oral administration of PF. In summary, PF displayed anti-inflammatory effects in the OVA-induced asthmatic model by decreasing the expression of IL-5, IL-13, IL-17 and eotaxin. These effects were mediated at least partially by inhibiting the activation of MAPK pathway.

1. Introduction

Bronchial asthma is one of the most common chronic inflammation diseases, characterized by airway hyperresponsiveness (AHR), chronic eosinophilic inflammation, episodic airflow obstruction that is at least partially reversible and airway remodeling that includes subepithelial fibrosis, increased airway smooth muscle, increased vascularization of the airway wall, and goblet cell and submucosal gland hyperplasia [1–3].

Many inflammatory cells and mediators are well-known as critical participants in allergic diseases [4]. In asthma, increased numbers of eosinophils are observed in the circulation and sputum [5]. More and more evidence suggests that eosinophils participate in a wide variety of functions in lung allergic inflammation, including airway epithelial cell damage and loss, airway dysfunction of cholinergic nerve receptors, airway hyperresponsiveness, mucus hypersecretion, and airway remodeling, characterized by fibrosis and collagen deposition [6–8]. In addition, eosinophils express surface membrane receptors with high affinity and specificity for IgE. The interaction of antigen-bound IgE in surface membrane receptors releases histamine, prostaglandins, leukotrienes and cytokines. These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally, cytokine-induced reactions cause tissue inflammation [9]. The number of eosinophils in the lung is associated with disease severity and has been used to guide therapy in severe asthma. Eosinophils are considered as a potential target for the treatment of asthma [6].

A biased immune response towards Type 2 T helper (Th_2) cells is observed in asthma, which is characterized by their production and secretion of cytokines including IL-4, IL-5, IL-9, and IL-13 [10]. These cytokines play an important role in driving eosinophilic inflammation and tissue damage, leading to AHR and the release of additional mediators [11].

Chinese herbal medicines have been used for treating allergic diseases for thousands of years. The effectiveness of herbal medicine has received increasing attention [12,13]. Paeoniflorin (PF) is one of the main active ingredients of Chinese peony which is also known as *Paeonia lactiflora* Pall. PF has demonstrated anti-inflammatory and immunoregulatory effects [14]. A recent study showed that PF could improve IgE-induced anaphylaxis and scratching behaviors [9]. Little is known about the effectiveness of PF treatment in allergic asthma. The aim of this study was to obtain in vivo evidences to show that PF can

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http://dx.doi.org/10.1016/j.intimp.2014.11.016
1567-5769/

improve pulmonary function and reduce inflammation in allergic asthma mice.

2. Materials and methods

2.1. Reagents and animals

PF (purity > 99.8%, Fig. 1) was purchased from Mansite Biotechnology Co. (Chengdu, China); molecular formula: $C_{23}H_{28}O_{11}$; molecular weight: 480.45.

Four-to-six-weeks-old female BALB/c mice weighing 18–22 g were obtained from Department of Laboratory Animal Science, Fudan University. The animals were housed under specific pathogen-free conditions in a temperature and humidity controlled environment and given ad libitum access to water and food. Mice were housed for 7 days for acclimation before experiments.

2.2. Allergic asthma model

The BALB/c mice were sensitized and challenged with OVA (grade V, Sigma, Taufkirchen, Germany) according to the protocol described previously [15]. Briefly, on day 0, mice were intraperitoneally injected with 20 μg of OVA precipitated with 2 mg of aluminum hydroxide gel in 0.2 mL saline. This was repeated on day 7, day 14 and day 21 for sensitization. On day 25, each animal was placed into an individual chamber and inhaled 3% OVA to challenge them for 6 consecutive days, every day for 30 min to replicate allergic asthma (Fig. 2).

2.3. Animal treatment

The whole protocol was approved by the Institutional Animal Care and Use Committee of Fudan University. Mice were randomly assigned to 6 experimental groups (n = 10 per group). Control Group (CON): Did not receive any treatment. Model Group (MOD): Mice were sensitized and challenged with OVA as described above. Low-dose PF Group (LDP), Medium-dose PF Group (MDP) and High-dose PF Group (HDP): Mice in these groups were sensitized and challenged with OVA as described in the model group. Simultaneously from days 24 to 30, they were treated with 10, 25, and 50 mg/kg PF solutions in 0.3 mL saline by gavages 1 h before OVA challenge, respectively.

2.4. Measurement of AHR

Before the methacholine challenge and measurement of airway hyperresponsiveness (AHR), the mice were anesthetized with 1% sodium pentobarbitone (wt/vol) at a dose of 50 mg/kg by intraperitoneal injection.

AHR was evaluated by a Buxco's modular and invasive system (Buxco Electronics Inc., NY). Changes in airway resistance (R_L) and lung dynamic compliance (C_{dyn}) were measured directly as described by Amdur and Mead [16]. Briefly, each anesthetized mouse was tracheostomized and intubated with an appropriate cannula, and then laid supine inside the body plethysmograph chamber connected to the ventilator. After a stable baseline airway pressure (<5% variation over 2.5 min) is reached, saline and increasing concentrations of methacholine (3.125, 6.12, and 12.5 mg/mL) in succession were administered via a jet nebulizer into the head chamber. Minimum values for R_L were determined and expressed as percent change from the baseline value [17].

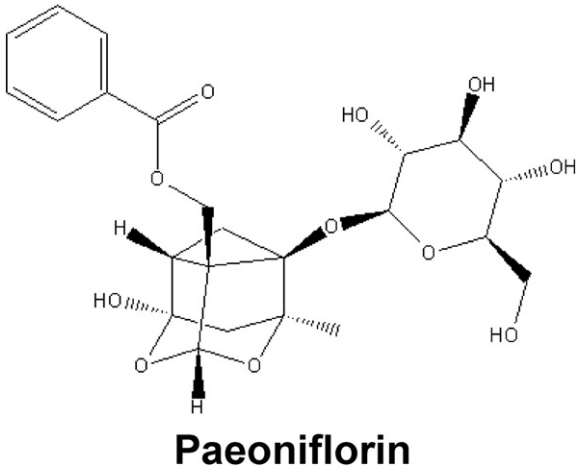

Fig. 1. Chemical structure of paeoniflorin (PF).

2.5. Histopathological evaluation

The right upper lobe of the lung was removed and fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 4 μm sections and stained with hematoxylin and eosin (HE), another was stored at −80 °C. The histological slides with H&E stain were read by a light microscope at high power (200×).

Histopathological assessment (light microscopy) was performed blind on randomized sections. The severity of inflammatory cell infiltration in the lung was evaluated by a 5 point scoring system: 0, no cells; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2–4 cells deep; and 4, a ring of cells >4 cells deep [18].

2.6. Measurement of total IgE in serum

After the methacholine challenge and assessment of AHR, the serum was separated by centrifuging at 1200 ×*g* for 15 min at 4 °C. Aliquot serum was stored at −80 °C. The concentrations of total IgE in serum were determined by using an ELISA kit (R&D, Minneapolis, MN), according to the manufacturer's instructions.

2.7. Preparation and analysis of bronchoalveolar lavage fluids (BALF)

Mouse was anesthetized and a tracheal cannula was inserted via a midcervical incision, the right lung was ligated and the airway of each mouse was lavaged 3 times with 1 mL of PBS. The collected lavage fluid was centrifuged at 1000 ×*g* at 4 °C for 10 min. The supernatants were harvested and stored at −80 °C for measurements of cytokine production. The levels of interleukin (IL)-5, IL-13, IL-17, and eotaxin were analyzed in BALF by using the ELISA kits (R&D, Minneapolis, MN), following the manufacturer's instructions.

2.8. RNA extraction and quantitative real-time PCR

Total RNA was extracted from the lung tissues with Trizol (TaKaRa Biotechnology Co., Ltd.), and the quality of RNA was subsequently evaluated by measuring the ratio of the absorbance at 260/280 nm. For reverse transcription, the First Strand cDNA Synthesis Kit was used. For PCR amplification, the following mouse-specific sense and antisense primers were used: IL-5, 5′ AAG GCT GAG GTT ACA GA 3′ (forward) and 5′ ATG AGG GGG AGG GAG TAT 3′ (reverse); IL-13, 5′ CCA CAC AGG GCA ACT GAG 3′ (forward) and 5′ G GCA TAG GCA GCA AAC CAT 3′ (reverse); IL-17, 5′ ATT CAG AGG CAG ATT CAG 3′ (forward) and 5′ AAA AAC AAA CAC GAA GCA G 3′ (reverse); eotaxin, 5′ CAC CCT GAA AGC CAT AGT 3′ (forward) and 5′ GT CAA GAG AGG AGG TTG TT 3′ (reverse); and GAPDH, 5′ TGG TGA AGG TCG GTG TG 3′ (forward) and 5′ GG TCA ATG AAG GGG TCG TT 3′ (reverse). The amplification was carried out in the ABI-7500 instrument (Applied Biosystems, USA) under the following conditions: initial denaturation at 95 °C for 10 min, 40 cycles of amplification at 95 °C for 15 s, annealing at 60 °C for 25 s, and then extension at 72 °C for 30 s.