International Immunopharmacology 41 (2016) 90–97

Contents lists available at ScienceDirect

International Immunopharmacology

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

Therapeutic effects of rosmarinic acid on airway responses in a murine model of asthma

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ARTICLE INFO

Article history:
Received 27 March 2016
Received in revised form 6 October 2016
Accepted 13 October 2016
Available online 5 November 2016

Keywords:
Rosmarinic acid
Therapeutic
Allergic airway inflammation
AHR
NF-κB
MAPK

ABSTRACT

Rosmarinic acid (RA) is an active component of a traditional Chinese herbal medicine. Previously, we reported that RA exerted a strong anti-inflammatory effect in a mouse acute lung injury model. Therefore, we hypothesized that RA might also have potential therapeutic effects in a murine model of asthma. In this study, we aimed to evaluate the anti-asthmatic activity of RA and explored its possible molecular mechanisms of action. Female BALB/c mice that had been sensitized to and challenged with ovalbumin (Ova) were treated with RA (20 mg/kg) 1 h after challenge. The results showed that RA greatly diminished the number of inflammatory cells and the production of Th2 cytokines in the bronchoalveolar lavage fluid (BALF); significantly reduced the secretion of total IgE, Ova-specific IgE, and eotaxin; and markedly ameliorated airway hyperresponsiveness (AHR) compared with Ova-induced mice. Histological studies further revealed that RA substantially decreased inflammatory cells infiltration and mucus hypersecretion compared with Ova-induced mice. Moreover, our results suggested that the protective effects of RA were mediated by the inhibition of JNK and p38 MAPK phosphorylation and nuclear factor-κB (NF-κB) activation. Furthermore, RA treatment resulted in a significant reduction in the mRNA expression of AMCase, CCL11, CCR3, Ym2 and E-selectin in lung tissue. These findings suggest that RA may effectively delay the development of airway inflammation and could thus be used as a therapy for allergic asthma.

1. Introduction

Allergic asthma is increasing in prevalence in both developed and developing countries [1]. Asthma is a serious health issue and affects people of all ages [2,3], and although great progress has been made in the diagnosis and treatment of asthma, asthma remains a severe public health problem. Moreover, there has been a substantial increase in the global prevalence, morbidity and mortality of asthma over the past two decades due to the off-target and side effects of conventionally available anti-asthma drugs, particularly in children [4].

Asthma is a chronic airway disorder characterized by a variety of symptoms, including airway inflammation, mucus hypersecretion and airway hyperresponsiveness (AHR) [5]. Dozens of studies have shown that these symptoms are part of an inflammatory reaction that is caused by Th2 cells and other inflammatory cells, such as eosinophils, macrophages and neutrophils, as well as inflammatory cytokines and chemokines [6–8]. Interleukin (IL)-4, IL-5 and IL-13 have been shown to play an important role in allergic airway inflammation [9]. IL-4 is pivotal for the initiation of Th2 inflammatory responses [10]; IL-5 is known to play a vital role in eosinophil maturation, differentiation, recruitment and survival [11]; and IL-13 plays a dominant role in the effector phase of Th2 responses, such as AHR, eosinophilic inflammation and mucus secretion [12]. IL-4 and IL-13 are also required for IgE class switching in B cells. In particular, IL-13 is thought to play the most important and central role in allergic asthma [13]. In addition, CCL11 is a chemokine that binds to the receptor CCR3 and is involved in eosinophil, neutrophil, and macrophage recruitment and Th2 cell infiltration [14].

Currently, inhaled glucocorticoids play a main role in combating asthma. Although inhaled glucocorticoids are usually effective, their use at high doses or for a prolonged time can lead to certain side effects, such as impaired growth, adrenal inhibition, and an increased risk of fracture [15]. Recent studies have supported the development of novel asthma treatments based on anti-inflammatory strategies [15,16]. For instance, a number of previous studies have shown that sustained activation of nuclear factor κB (NF-κB) and suppression of the MAPK signalling pathway in the lung may have potential therapeutic value in the treatment of allergic asthma [17–19]. NF-κB plays a vital role in the production of Th2 cytokines and the recruitment of inflammatory cells in the airways in murine models of asthma [20,21]. In addition, the MAPK signalling cascade is known to be important for the activation of various immune cells [22]. p38 MAPK and ERK have been shown to

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http://dx.doi.org/10.1016/j.intimp.2016.10.010
1567-5769/

participate in the regulation of IL-5 and other cytokines [23]. Meanwhile, JNK has been demonstrated to be associated with IgE class switching [24]. Therefore, NF-κB and MAPK have emerged as promising molecular targets for asthma treatment. Likewise, more and more studies have shown that many components of Chinese medicinal herbs exert notable anti-inflammatory effects via the negative regulation of the NF-κB and MAPK signalling pathways [25–27].

Rosmarinic acid (RA) is a polyphenolic phytochemical that is found in numerous herbal plants, including rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*) and mint (commonly *Mentha spicata*). RA shows a broad spectrum of biological effects, such as anti-microbial, anti-inflammatory and immunomodulatory actions [28]. Our previous study revealed the anti-inflammatory effects in a mouse model of acute lung injury induced by lipopolysaccharide (LPS) via the administration of RA at the dose of 20 mg/kg [29]. These results suggested that RA may be used for the treatment of asthma. Moreover, no previous study has evaluated the effectiveness of RA in ameliorating asthmatic symptoms. Here, we evaluated the effects of RA on an ovalbumin (Ova)-induced mouse asthma model and its potential mechanism of action, attempting to provide a new underlying mechanism for the treatment of allergic asthma using traditional Chinese herbal medicine.

2. Materials and methods

2.1. Animals

Female BALB/c mice weighing approximately 18–20 g were purchased from the Centre of Experimental Animals of Guangdong (Certificate: SCXK2013-0002; Foshan, China). The animals were housed in micro-isolator cages and received food and water ad libitum. The laboratory temperature was maintained at 24 ± 1 °C, with a relative humidity of 40–80%. The mice were acclimated for 7 days before the experiments. The experiments were approved by the Ethical Committee on Animal Research of Guangxi University. All animal experiments were conducted according to The Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. Reagents

IL-4, IL-5 and IL-13 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Neobioscience (Beijing, China). The total IgE and anti-Ova IgE ELISA kits were purchased from Cayman Chemical (Ann Arbor, Michigan, USA). The Mouse Eotaxin Platinum ELISA kit was purchased from Affymetrix (eBioscience, Vienna, Austria), and Ova (Grade V) was purchased from Sigma-Aldrich (St. Louis, MO, USA). RA (purity >98%) and dexamethasone (Dex, purity: N 99.7%) were purchased from the National Institute for Food and Drug Control (Beijing, China). The MAPK Family Antibody Sampler Kit, Phospho-MAPK Family Antibody Sampler Kit and NF-κB Pathway Sampler Kit were obtained from Cell Signalling Technologies Inc. (Beverly, MA, USA). The purity of all chemical reagents was at least analytical grade.

2.3. Sensitization, challenge and treatment of mice

The mice were randomly divided into five groups (n = 12 in each group): (1) Cont group; (2) RA 20 group; (3) Ova group; (4) Ova + RA 20 group; and (5) Ova + Dex group. The mice were sensitized via an intraperitoneal injection with 0.2 mL of sensitization fluid containing Ova (20 μg) adsorbed to Imject Alum (100 μg/mL, Pierce, Rockford, USA) on days 0, 7 and 14. On days 22–27, the mice were anesthetized and intranasally challenged with Ova (100 μg) in PBS (50 μL) [27]. The mice in Cont group and RA 20 group were sham-sensitized and challenged with PBS using the same protocol. RA (20 mg/kg, based on the results of our previous study [29]) and Dex (2 mg/kg) were dissolved in normal saline and administered intraperitoneally 1 h after each corresponding Ova challenge on days 25–27. The control mice were administered equivalent volumes of normal saline. The schematic diagram of the treatment schedule is presented in Fig. 1.

2.4. Collection of blood and bronchoalveolar lavage fluid (BALF)

The mice were euthanized on day 28. Blood was withdrawn and centrifuged at 3000 rpm for 10 min at 4 °C, and the serum was stored at −80 °C prior to the IgE and eotaxin measurements. The BALF was processed to separate the cell pellets and supernatants. Briefly, the BALF was lavaged three times using a tracheal cannula and 0.5 mL of ice-cold PBS [27]. The fluid recovered from each sample was centrifuged (4 °C, 3000 rpm, 10 min) to pellet the cells, and the supernatant was immediately stored at −80 °C prior to the cytokine, Ova-IgE and eotaxin assessments. The cell pellets were resuspended in PBS and stained with Wright-Giemsa, and the numbers of different inflammatory cells were counted. At least 200 cells were counted per slide.

2.5. Measurements of Th2 cytokines, eotaxin and IgE production

The production of IL-4, IL-5, IL-13 and eotaxin was measured with sandwich ELISA kits according to the manufacturer's instructions. In addition, the total IgE and Ova-specific IgE concentrations were determined with an ELISA according to the manufacturer's instructions.

2.6. Lung histology

Twenty-four hours after the last inhalation exposure, the lungs were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 3-μm sections, and stained with haematoxylin and eosin (H&E) to observe the general morphology or alcian blue-periodic acid-Schiff (AB-PAS) to observe mucus production and identify the goblet cells in lung tissues. Semi-quantitative analyses of inflammatory cell accumulation and

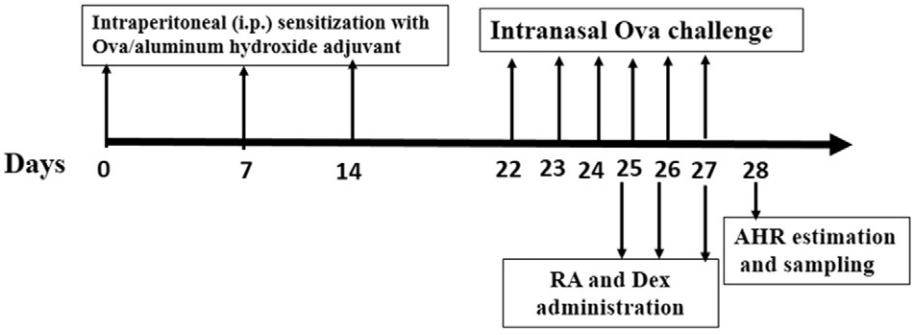

Fig. 1. Experimental protocol for the development of allergic asthma and treatment with RA or Dex. The mice were divided into five groups (n = 12 in each group) and sensitized to Ova on days 0, 7 and 14. Subsequently, the mice were given an intranasal instillation of 100 μg Ova in 50 μL of PBS on days 22–27. The mice were intraperitoneally injected with RA (20 mg/kg) or Dex (2 mg/kg) 1 h after each corresponding Ova or PBS challenge from days 25 to 27.