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Anti-asthmatic effects of *Angelica dahurica* against ovalbumin-induced airway inflammation via upregulation of heme oxygenase-1

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ABSTRACT

Asthma is a chronic immune inflammatory disease characterized by variable airflow obstruction. The present study was undertaken to assess the effects of an *Angelica dahurica* Bentham et Hooker ethanolic extract (AD) on airway inflammation in an ovalbumin (OVA)-induced airway inflammation model. Mice that received AD displayed significantly lower airway eosinophilia, cytokine levels, including interleukin (IL)-4, IL-5, and tumor necrosis factor (TNF)-alpha levels, mucus production and immunoglobulin (Ig)E, compared with OVA-induced mice. In our experiments, AD treatment reduced airway inflammation and suppressed oxidative stress in the OVA-induced asthma model, partly via induction of heme oxygenase (HO)-1. The effects of AD on OVA-induced HO-1 induction were partially reversed by the HO-1 inhibitor, tin protoporphyrin (SnPP). Our results clearly indicate that AD is a suppressor of airway allergic inflammation, and may thus be effectively used as an anti-inflammatory drug in the treatment of asthma.

1. Introduction

Asthma is a chronic inflammatory disease of the airways involving T-lymphocytes and eosinophil infiltration, mucus overproduction and airway hyper-responsiveness. Inflammatory mediators (including lipid mediators) play a critical role in the pathogenesis of chronic airway disease and facilitate the recruitment, activation, and trafficking of inflammatory cells in the airways. Spina and co-workers showed that the pathophysiology of asthma is related to T helper (Th)1/Th2 cell imbalance in the airways (Spina et al., 1998). Cytokines play an important role in airway inflammation in asthma by promoting the development, differentiation, recruitment, activation, and survival of inflammatory cells. Th2 cells participate in asthma pathogenesis by stimulating B cells that produce the allergen-specific immunoglobulin (Ig)E and inducing the infiltration of eosinophils and other inflammatory cells into the airways (Hamelmann et al., 1999; Zhang et al., 1999). While Th2 cytokines, including interleukins (IL)-4, IL-5 and IL-13, are important in asthma (Foster et al., 1996), tumor necrosis factor (TNF)-α, a cytokine usually associated with Th1 responses, has also been implicated in the inflammatory response in asthma (Meiler et al., 2006). Inflammatory cells contribute to the generation of Th2 cytokines (IL-4, IL-5, and IL-13), chemokines (eotaxin and RANTES) and TNF-α, levels of which are increased in asthmatic lungs (Williams and Galli, 2000). TNF-α, a potent proinflammatory cytokine with immunoregulatory activities, is produced by several cell types, including monocytes, macrophages, lymphocytes, neutrophils, eosinophils and mast cells (Bazzoni and Beutler, 1996; Costa et al., 1993; Gordon and Galli, 1991).

Increased reactive oxygen species (ROS) generation, resulting in an imbalance between oxidative forces and the antioxidant defense systems, has been implicated in the pathogenesis of asthma (Dworski, 2000; Rahman and MacNee, 2000). Therefore, regulation of intracellular ROS during inflammation may have a potential advantage with regard to treatment of inflammatory diseases. Asthma is a chronic inflammatory disease of the airways associated with increased reactive oxygen species, nitric oxide (NO) production and increased levels of several inflammatory mediators in the airways, which may induce heme oxygenase (HO)-1 expression (Barnes, 1990; Kharitonov et al., 1996). HO-1 is emerging as an important phase II anti-inflammatory enzyme that is significantly upregulated under conditions of oxidative stress (Choi and Alam, 1996) HO is the rate-limiting enzyme in

Abbreviations: BALF, bronchoalveolar lavage fluid; AD, *Angelica dahurica* Bentham et Hooker ethanolic extract; OVA, ovalbumin; ROS, reactive oxygen species; Ig, immunoglobulin; IL, interleukin; HO, heme oxygenase; NO, nitric oxide; SnPP, tin protoporphyrin; ELISA, Enzyme-linked immunosorbent assays; TNF, tumor necrosis factor; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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the heme-degradation reaction, converting heme to biliverdin IX, carbon monoxide, and iron. Three isoforms of HO have been fully characterized. Specifically, HO-2 and HO-3 are constitutive isozymes whereas HO-1 is induced by a variety of stimuli, including hemin and oxidative stress, in different cell types (Pae et al., 2003). Oxidative stress in ovalbumin-challenged guinea pigs is reduced when HO-1 expression and subsequent bilirubin production are induced by hemin (Almolki et al., 2004).

Several drugs for asthma therapy are currently available. However, most of these agents are relatively inefficient and induce adverse side effects. Therefore, novel drugs with fewer side effects are necessary for the effective treatment of asthma. We investigated the effects of the therapeutic agent *Angelica dahurica*, with a view to identifying safe lead compounds from natural sources with minimal side effects. The medicinal plant, *A. dahurica*, is a perennial herb abundant in Korea, China and Japan. The root of this species is used in Korea as a traditional folk medicine to treat headache, bleeding, menstrual disorders and neuralgia (Kimura et al., 1996). Previous studies focusing on the chemical constituents of *A. dahurica* led to the isolation of more than 20 coumarins (Baek et al., 2000; Kwon et al., 2002; Qiao et al., 1996; Kim et al., 1992). The major active constituents of this herb are simple coumarins and furocoumarins, including byakangelicoloxypeucedanin, imperatorin, phellopterin and isoimperatorin. These compounds have been reported to exhibit pharmacological effects such as inhibition of IL-1β-induced cyclooxygenase-2 (COX-2) (Lin et al., 2002), inhibition of lipopolysaccharide-induced prostaglandin E2 (Ban et al., 2003), inhibition of acetylcholinesterase (Kim et al., 2002) and inhibitory effects on the GABA degradative enzyme (Choi et al., 2005). However, the effects of the *A. dahurica* ethanolic extract (AD) in patients with allergic diseases are yet to be established. Here, we investigated whether AD exerts anti-asthmatic effects in terms of inhibition of oxidative stress, inflammatory infiltration into the airways, mucus production, and expression of Th1 and Th2 cytokines and IgE, in a murine model of ovalbumin (OVA)-induced asthma, and whether HO-1 is involved in its mechanism of action.

2. Materials and methods

2.1. Preparation of the A. dahurica ethanolic extract (AD)

The *A. dahurica* root was purchased in October 2008 from HMAX (Jecheon, Korea). These materials were confirmed taxonomically by Professor Je-Hyun Lee of Dongguk University, Gyeongju, Republic of Korea. A voucher specimen (ST8) has been deposited at the Korea Institute of Oriental Medicine (KIOM), Daejeon, Republic of Korea. Fresh *A. dahurica* Bentham et Hooker was washed three times with tap water to remove salts, epiphytes and sand, and stored at −20 °C. Frozen samples were lyophilized and homogenized in a grinder, prior to extraction. Extracts were prepared from dried roots of *A. dahurica* (200 g) with 70% EtOH (2 L × 3 times), followed by sonication for 1 h. The extract solution was filtered through filter paper and evaporated to dryness (62.19 g). The dried extract yield from starting crude material was 32.1%. Concentrated AD was freshly dissolved in phosphate-buffered saline (PBS), prior to use.

2.2. Animals

Specific 7 week-old pathogen-free inbred female BALB/c mice routinely screened serologically for relevant respiratory pathogens were purchased from Daehan Biolink Co. Ltd., (Seoul, Korea). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to the conduction of experiments, and provided water and standard chow *ad libitum*. All experimental procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of Korea.

2.3. Sensitization and airway challenge

Mice were divided into groups receiving the following treatments: (1) sham sensitization plus challenge with phosphate-buffered saline (PBS; ipNeb) and PBS p.o., (2) sensitization plus challenge with OVA (Sigma A5503; Sigma, St. Louis, MO) (ipNeb), (3) sensitization with OVA (i.p.) plus challenge with OVA (Neb) and AD (25, 50 or 100 mg/kg) or Montelukast (30 mg/kg) p.o. Briefly, mice were sensitized with an intraperitoneal injection of 20 μg OVA emulsified in 2 mg aluminum hydroxide in 200 μL PBS buffer (pH 7.4) on days 0 and 14. Mice were challenged through the airway with OVA (1%, w/v, in PBS) for 30 min using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on days 21, 22, and 23 after initial sensitization. AD (25, 50 or 100 mg/kg) was administered orally once daily on days 19–23. Negative and positive control mice were orally treated with PBS and Montelukast (Mon; 30 mg/kg), respectively, once daily on days 19–23. Tin protoporphyrin (SnPP), an inhibitor of HO activity, was obtained from Porphyrin products (Logan, UT). SnPP treatment (10 mg/kg, i.p) was performed 12 h before AD administrated. Montelukast, a positive control, was developed as a cysteinyl leukotriene-1 receptor antagonist, in which the clinical responses in patients with aspirin-sensitive asthma, noctural exacerbations of asthma and allergic asthma led to montelukast being successfully introduced into the market. Animals were sacrificed 48 h after the final challenge (day 25) to determine the suppressive effects of AD. A schematic diagram of the treatment schedule is shown in Fig. 1.

2.4. Inflammatory cell counts in bronchoalveolar lavage fluid (BALF)

Mice were sacrificed with an overdose (50 mg/kg) of pentobarbital 48 h after the final challenge, and tracheostomy performed. Following instillation of ice-cold PBS (0.6 mL) into the lungs, BALF was obtained with three aspirations (total volume of 1.8 mL) via tracheal cannulation. BALF was centrifuged and the supernatant fractions collected and stored at −70 °C. The total inflammatory cell numbers were assessed by counting cells in at least five squares of a hemocytometer after exclusion of dead cells via trypan blue staining. BALF (100 μL) was pipetted on a slide and centrifuged (200*g*, 4 °C, 10 min) to fix cells using a cytospin machine (Hanil Science Industrial, Seoul, Korea). Cell pellets was suspended in 0.5 mL PBS, and 100 μL of each solution spun onto a slide. After slides were dried, cells were fixed and stained using Diff-Quik® Staining reagent (B4132-1A; Dade Behring Inc., Deerfield, IL), according to the manufacturer's instructions.

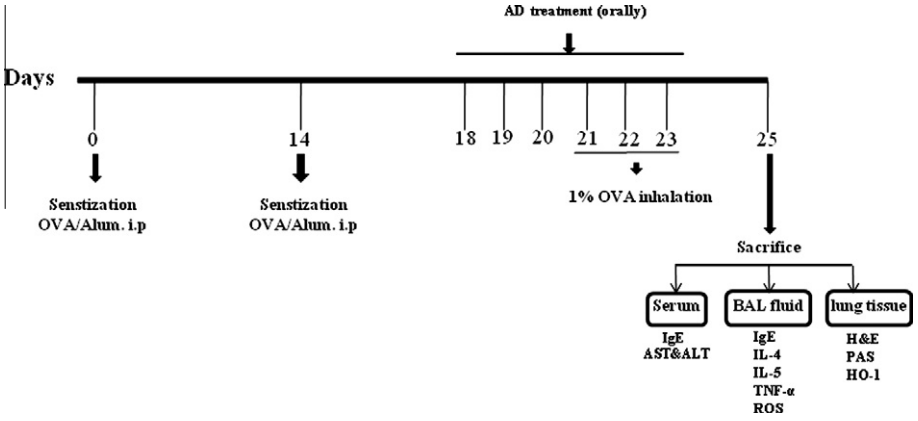

Fig. 1. Mouse model of airway inflammation and effects of treatment with the *A. dahurica* Bentham et Hooker ethanolic extract (AD).