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Artemisia argyi attenuates airway inflammation in ovalbumin-induced asthmatic animals

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ABSTRACT

Ethnopharmacological relevance: *Artemisia argyi* is a traditional herbal medicine in Korea and commonly called as mugwort. It is traditionally used as food source and tea to control abdominal pain, dysmenorrhea, uterine hemorrhage, and inflammation.
Aim of the study: We investigated the effects of *A. argyi* (TOTAL) and dehydromatricarin A (DA), its active component on ovalbumin (OVA)-induced allergic asthma.
Materials and methods: The animals were sensitized on day 0 and 14 by intraperitoneal injection of OVA with aluminum hydroxide. On day 21, 22 and 23 after the initial sensitization, the animals received an airway challenge with OVA for 1 h using an ultrasonic nebulizer. TOTAL (50 and 100 mg/kg) or DA (10 and 20 mg/kg) were administered to mice by oral gavage once daily from day 18–23. Airway hyperresponsiveness (AHR) was measured 24 h after final OVA challenge.
Result: TOTAL and DA treated animals reduced inflammatory cell counts, cytokines and AHR in asthmatic animals, which was accompanied with inflammatory cell accumulation and mucus hypersecretion. Furthermore, TOTAL and DA significantly declined Erk phosphorylation and the expression of MMP-9 in asthmatic animals.
Conclusion: In conclusion, we indicate that Total and DA suppress allergic inflammatory responses caused by OVA challenge. It was considered that *A. argyi* has a potential for treating allergic asthma.

1. Introduction

Inflammation is an important defense system against harmful stimuli, however; excess responses causes various diseases such as allergic asthma, arthritis and dermatitis via destruction of normal physiological condition (Kim et al., 2012). Of inflammatory diseases, allergic asthma is induced by exposure to specific allergens including pollens, chemicals and air pollutants and featured as airway inflammation, mucus hypersecretion and airway hyperresponsiveness (AHR), resulting in aggravating normal lung functions (Xiong et al., 2012; Lee et al., 2012a, 2012b; Zhao et al., 2017). Allergic inflammation is mainly characterized by eosinophil infiltration and production of allergen-specific immunoglobulin (Ig) E (Lee et al., 2012a, 2012b). Eosinophil infiltration is controlled by Th2 cytokines and IgE and plays a crucial role in the manifestation of asthmatic clinical signs, such as mucus hypersecretion and airway hyperresponsiveness. Excess eosinophil infiltration is also associated with the activation of inflammatory signaling pathways with the release of cytotoxic granule proteins and enzymes, including matrix metalloproteinases (MMPs) (Choi et al., 2012; Kim et al., 2016; Moore et al., 2011).

MMPs play a key role in maintaining normal physiological functions due to their capacity to degrade structural materials, such as elastin and collagens (Mehra et al., 2010; Cui et al., 2017). These functions of MMPs are closely associated with airway remodeling in the development of allergic asthma and are involved in the production of cytokines, chemokines, and growth factors (Grzela et al., 2016). MMP-9 is associated with inflammatory cell accumulation, remodeling processes, and healing of injured tissue (Lee et al., 2012a, 2012b; Bruschi et al., 2014). MMP-9 production can be induced by extracellular signal-regulated kinase (Erk) signaling (Deng et al., 2013; Li et al., 2010). Erks also play an important role in eosinophil differentiation and activation, and are closely involved in development and

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aggravation of allergic asthma via modulation of interleukin (IL)-5 production (Alam and Gorska, 2011; Liang et al., 2016).

The genus *Artemisia*, commonly called mugwort, has been widely used as a tea, spice, and food ingredient in East Asia (Kim et al., 2015a, 2015b). The mugwort is a Korean native, *A. argyi,* which is frequently used as a traditional medicine for patients with abdominal pain, dysmenorrhea, uterine hemorrhage, and inflammation (Kim et al., 2015a, 2015b; Yun et al., 2016). According to previous reports (Kim et al., 2015a, 2015b; Lee et al., 2015; Yao et al., 2016), mugwort possesses antioxidant and anti-inflammatory properties and contains high amounts of vitamin C and total phenolic compounds. However, there have been no studies on the effect of *A. argyi* against allergic asthma.

Thus, we have explored the therapeutic effects of *A. argyi* (TOTAL) constituent, dehydromatricarin A (DA), against airway inflammation, using a murine asthma model induced by ovalbumin (OVA). We have also investigated the expression and production of allergic, asthma-related inflammatory mediators to elucidate a possible mechanism of *A. argyi*.

2. Materials and methods

2.1. Plant material

Artemisia argyi was collected from a farm in Eumseong-gun, South Korea, in May of 2016 (National Institute of Horticultural and Herbal Science, NIHHS). After harvesting, the aerial parts were immediately freeze-dried and stored at −40 °C until analysis. A voucher specimen (KRIBB 0076330) was deposited at the Plant Extract Bank of Korea Research Institute of Bioscience and Biotechnology (KRIBB) in Daejeon, south Korea.

2.2. Extraction and isolation

First, *A. argyi* was washed with clean, sterile water. The raw material was cut into small pieces, and the chopped leaves were mixed with three volumes of methanol. The mixture was sonicated three times for 1 h. Evaporation of solvent under reduced pressure yielded the crude extracts (94.0 g, 10.5% yield), which were used for analysis of biological activities and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTof-MS).

2.3. UPLC-QTof-MS analysis

Profiling of TOTAL was conducted on an ACQUITY UPLC™ system (Waters Corporation, Milford, MA, USA) equipped with a binary solvent delivery manager and a sample manager. This was coupled to a Micromass Q-TOF Premier™ mass spectrometer (Waters Corporation) with an electrospray ionization (ESI) interface and MassLynex V4.1 software. Chromatographic separation was conducted on an ACQUITY BEH C_{18} chromatography column (2.1 × 100 mm, 1.7 μm). The column was maintained at 35 °C, and the mobile phases A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. The gradient elution program was as follows: 0.0–1.0 min, 15% B; 1.0–6.0 min, 15–20% B; 6.0–14.0 min, 20–55% B; 14.0–16.3 min, 55–100% B; wash for 1.5 min with 100% B; and then a 2.0 min recycle time. The injection volume and flow rate were 5.0 μL and 0.4 mL/min, respectively. The mass spectrometer was operated in negative ion mode. Leucine-enkephalin and N_2 were used as reference compound (*m/z* 556.2771 in the positive mode) and desolvation gas, respectively. The flow rate was 500 L/h, desolvation temperature was 350 °C, source temperature was 100 °C, capillary voltage was 2700 V, and cone voltage was 27 V. The data were collected for each test sample from 200 Da to 1500 Da with a 0.25-s scan time and 0.01-s interscan delay over a 25-min analysis time.

2.4. Experimental procedure

Female BALB/c mice (20–25 g, 6 weeks old, Samtako Co., Osan, Korea) were maintained in groups (n = 8 per group) in a controlled environment (temperature 22 ± 2 °C and humidity 55 ± 5%). The experimental design was approved by the Institutional Animal Care and Use Committee of Chonnam National University. The animals were randomly divided into 7 groups: normal control group (NC; no treatment and phosphate-buffered saline (PBS) challenge), ovalbumin (OVA) challenge group (OVA; no treatment, sensitization, and OVA challenge), dexamethasone group (DEX; 3 mg/kg dexamethasone, sensitization, and OVA challenge), *A. argyi* groups (TOTAL50 and TOTAL100; 50 and 100 mg/kg *A. argyi*, respectively, sensitization, and OVA challenge), and dehydromatricarin A groups (DA10 and DA20; 10 and 20 mg/kg dehydromatricarin A, respectively, sensitization, and OVA challenge). The dose of A. argyi was determined according to preliminary study. In preliminary study, A.argyi was administered to mice at doses of 100 and 200 mg/kg body weights. The experimental procedure was the same with present study. BALF cell counts were observed the significant decreases in both 100 and 200 mg/kg treated mice. However, 30 mg/kg treated mice was more effective than 60 mg/kg treated mice. Based on these results, we determined 100 mg/kg, an as experimental dose. OVA induced allergic asthma model were designed as previously described (Lee et al., 2012a, 2012b; Shin et al., 2015). To induce allergic inflammation, sensitization was performed via intraperitoneal injection of OVA (20 μg) mixed with aluminum hydroxide (2 mg) on days 0 and 14. The animals received nebulized 1% (w/v) OVA solution for 1 h from day 21–23, using a nebulizer (Omron, Tokyo, Japan). Drug administration was performed 1 h before the OVA challenge from day 18–23. Airway hyperresponsiveness (AHR) was measured using whole-body plethysmography (Allmedicus, Seoul, Korea) on day 24. AHR was measured following methacholine challenge (doses of 0, 5, 10, and 15 mg/mL in PBS) for 3 min. Results were recorded as the dimensionless parameter, enhanced pause (Penh).

2.5. BALF analysis

Twenty-four hours after AHR, bronchoalveolar lavage fluids (BALF) were collected as previously described (Shin et al., 2015). Briefly, a tracheostomy was performed under slight anesthesia and inserted endotracheal tube into trachea. Ice-cold PBS (0.7 mL) was infused into the lung and withdrawn through tracheal cannulation two times (total volume 1.4 mL). To count inflammatory cells, 100 μL of BALF was centrifuged onto slides using a Cytospin (Hanil, Seoul, Korea) and the slide were dried and stained with Diff-Quik® reagent (IMEB Inc., Deerfield, IL). Serum was collected by centrifugation of blood obtained from the caudal vena cava. Quantitative analyses of IgE (Biolegends, San Diego, CA) and Th2 cytokines (R & D System, Minneapolis, MN) were performed using ELISA kits with serum and BALF, respectively. Optical absorbance was measured using a spectrophotometer (Bio-Rad, Hercules, CA, USA).

2.6. Immunoblotting

To perform immunoblotting, lung tissues were homogenized with a tissue lysis buffer (Sigma-Aldrich) and its protein concentration was assayed with Bradford solution (Bio-Rad). Immunoblotting was performed as previously described (Shin et al., 2015). Equal amounts of protein (30 μg) were resolved by 10% SDS-polyacrylamide gel deletrophoresis and transferred to nitrocellulose membrane. The membrane was incubated with 5% skim milk, followed by an overnight incubation with the primary antibodies. Anti-β-actin, anti-Erk, anti-phospho-Erk (all from Cell Signaling Technology, Danver, MA), and anti-MMP-9 (Abcam, Cambridge, MA) were used as primary antibodies. The membranes were washed for three times with Tris-buffered