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Skullcapflavone II attenuates ovalbumin-induced allergic rhinitis through the blocking of Th2 cytokine production and mast cell histamine release

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ABSTRACT

Allergic rhinitis is a common heterogeneous chronic upper airway disorder and is an IgE-mediated inflammation characterized by one or more nasal symptoms such as sneezing, itching, nasal discharge, rhinorrhea, post nasal drainage and nasal blockage. In the present study, the effects of skullcapflavone II (SCFII) on upper airway inflammation, Th2 cytokines, and NF-κB signaling in an ovalbumin (OVA)-induced allergic rhinitis (AR) murine model *in vivo* were investigated. OVA-induced AR mice increased nasal symptoms, eosinophils and mast cells infiltration into nasal cavity, OVA-specific IgE/IgG1 and histamine in serum, Th2 cytokines including IL-13 and GATA3, and NF-κB signaling in NALF and lung homogenate. Interestingly, treatment of SCFII reduced the levels of OVA-specific IgE/IgG1 and histamine in serum, of Th2 cytokines and of NF-κB signaling in the NALF and the lung homogenate, and histopathological changes in the nasal tissue and the lung. Also, dexamethasone suppressed such increases. The results of this study suggested that SCFII may ameliorate allergic inflammation of upper airway in AR mice model by blocking the Th2 cytokine production, the NF-κB signal pathway and the mast cell histamine release. Taken together, we suggest that SCFII may be used as a therapeutic agent for patients with Th2-mediated or mast cell-mediated allergic diseases.

1. Introduction

Allergic rhinitis (AR) is a common heterogeneous chronic upper airway disorder and is an IgE-mediated inflammation characterized by one or more nasal symptoms such as sneezing, itching, nasal discharge, rhinorrhea, post nasal drainage and nasal blockage [1,2]. AR is triggered after allergen-specific IgE and Th2 cells recognize inhalant allergen in the environment, and its inflammatory process involves many different inflammatory cells including eosinophils and mast cells, cytokines, and other regulatory molecules [3]. Among immune cells, mast cells and B cells play pivot roles in the pathogenesis of allergic reaction in AR. In allergic reactions, mast cells activate when an allergen interacts with IgE [4]. After antigen-IgE stimulation, mast cells release allergic mediators including histamine, cytokines, chemokines and arachidonic acid derivatives, mediating acute and chronic inflammation [5]. These steps are associated to induce the recruitment of inflammatory cells and eosinophils into affected site.

Even though AR is currently regulated with steroidal and non-steroidal anti-inflammatory agent, the rates of side effects and recurrence have been increasing [6]. Since these agents are still not perfect, development of new better drugs is need. Herbal medicines have been used for the traditional treatment of various allergic diseases with their potential safety and efficacy. There has been growing interest in natural product-based anti-inflammatory agents, which have high safety and less side-effect.

Scutellaria baicalensis Georgi is one of the most popular and multi-purpose herbal medicines or medicinal plants used in oriental countries including China, Japan, and Korea to treat inflammation, allergy, and bacterial and viral infections [7–10]. Recently, investigations have shown that *S. baicalensis* has beneficial properties such as anti-oxidative effect [11], and inhibits anti-dinitrophenyl IgE-medicated anaphylactic reactions and compound 48/80-induced histamine release mast cells [12]. Among flavonoids isolated from the dry roots of *S. baicalensis*, Skullcapflavone II (SCFII; 5,2′-dihydroxy-6,7,8,6′-tetramethoxy-flavone) has been known a wide range of biological activities such as anti-inflammatory, antibacterial, and antioxidant effects [12–16]. However, there is no report regarding the effects of SCFII on AR in a mouse model. Here, we investigated the effects of SCFII on upper airway inflammation, Th2 cytokine production, histamine secretion and NF-κB signal pathway in an ovalbumin (OVA)-induced AR murine model.

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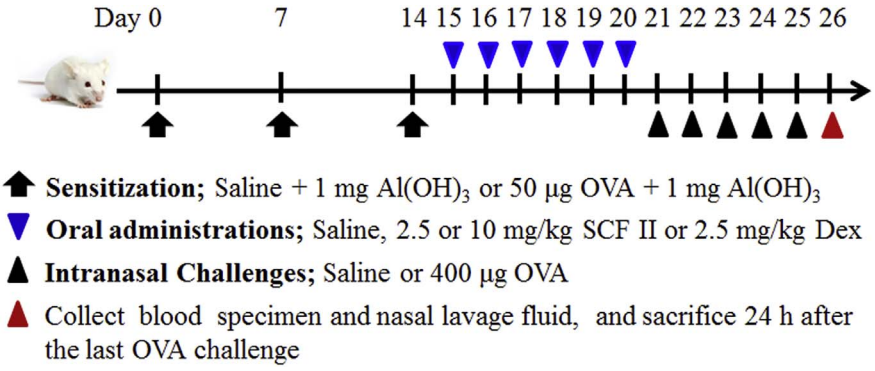

Fig. 1. Sensitization, challenge, and oral administration protocol for allergic rhinitis mouse model. Mice were sensitized on days 0, 7 and 14 and challenged on days 21–25 by ovalbumin (OVA). Mice in control group was sensitized and challenged by saline. Mice in the Skullcapflavone II (SCFII) or Dexamethasone (Dex) treatment groups were sensitized and challenged by OVA and administered orally once a day at 2.5, 10 mg/kg SCFII or 2.5 mg/kg Dex for 6 days.

2. Materials and methods

2.1. Animals

BALB/c male mice, aged 5–6 weeks, were purchased from Damool Science (Daejeon, Korea). These animals were housed in an air-conditioned room with a 12 h light/dark cycle and had unrestricted access to OVA-free food and water. All animal experiments were performed in accordance with the Guidelines on Animal Care and Use and were approved by the Institutional Animal Care and Use Committee of Chonbuk National University Laboratory Animal Center (CBN 2016-37).

2.2. Induction of AR murine model

Mice were divided into 5 groups according to treatment: (1) control group, (2) OVA group, (3) 2.5 mg/kg/day SCFII, (4) 10 mg/kg/day SCFII, and (5) 2.5 mg/kg/day dexamethasone (Dex) as reference drug for positive control in OVA-induced AR mice. The experimental procedure for the mouse model of AR is summarized in Fig. 1. Independent experiments of each group were repeated twice. Mice of groups (2), (3), (4) and (5) were immunized by intraperitoneal injection of 50 μg OVA (Sigma, St. Louis, MO, USA) with 1 mg Imject Alum (Pierce, Rockford, IL, USA) in a total volume of 200 μl on days 0, 7, and 14 while control group was injected with alum in saline on the same schedule. Next, on 5 consecutive days from 21 to 25 after the beginning of the sensitization period, these mice were challenged with 400 μg OVA (10 mg/ml, 20 μl/nostril) through intranasal instillation of both nostrils. On days 15 to 20, the treatment groups were also orally treated once daily with SCFII (Sigma, St. Louis, MO, USA) or Dex (Innovative Research of America, Toledo, Ohio, USA). OVA group was orally received saline instead of SCFII or Dex. Animals were sacrificed 24 h after the last challenge on day 26 to investigate the inhibitory effect of SCFII on OVA-induced AR.

2.3. Clinical symptoms

Nasal symptoms were evaluated by counting the frequencies of nasal rubbing and sneezing. After 400 μg of OVA intranasal challenge on days 21 to 25, the numbers of sneezing and nasal rubbing behaviors were recorded during a 20-minute period and were counted by blinded observers. And then, mice were sacrificed 24 h after the last OVA challenge, and head, lung, and nasal lavage fluid (NALF) were obtained for analysis.

2.4. Collection of blood sample

Twenty-four hours after the final OVA challenge, blood specimens were collected from retro orbital plexus and serum was obtained by centrifugation 1000 *g* for 10 min at 4 °C (Centrifuge 5403; Eppendorf, Hamburg, Germany). Samples were stored at − 20 °C until use.

2.5. Collection and analysis of NALF

Twenty-four hours after the last OVA challenge, NALF was collected by cannulating the upper part of the trachea into the nasal cavity direction and lavaging. The total number of viable cells in NALF was determined by trypan blue exclusion using a hemocytometer. Differential cell counts were determined with cytospin (Centrifuge 5403; Eppendorf, Hamburg, Germany) preparation, followed by Diff Quik staining (Sysmex Co., Kobe, Japan).

2.6. Histopathologic analysis

Heads of mice were excised and fixed in 10% neutral formalin for 2 days at room temperature. After fixation, the heads were decalcified in histological decalcifying agent containing hydrochloric acid and EDTA (National diagnostics, Atlanta, GA) for 6 days and then embedded in paraffin. Histopathologic analysis of lung was performed according routine procedures. Samples were cut into 4 μm thickness sections and sections were stained with either hematoxylin-eosin for overall inflammation, periodic acid-Schiff (PAS) for goblet cells and mucus, and Giemsa for eosinophils and mast cells. After Giemsa staining, the mast cells under high magnification (× 1000) were classified as follows: (1) severely degranulated (> 50% of the cytoplasmic granules exhibiting fusion, staining alteration, and extrusion from the cells), (2) slight to moderately degranulated (10–50% of the granules exhibiting fusion or discharge), or (3) normal.

2.7. Measurements of Th1and Th2 cytokines, OVA-specific IgE, IgG1, and IgG2a and NF-κB, IκB

The levels of Th1 cytokines such as IFN-gamma, Th2-related cytokines such as TNF-α, IL-4, IL-6, IL-13 and GATA3 in the NALF and lung homogenates from each mouse using the appropriate ELISA (BioSource International, Camarillo, CA, USA) were measured. OVA-specific IgE, IgG1, IgG2a in serum were quantified by using ELISA kits (R & D Systems Inc., USA) according to the manufacturer's instructions. Also, NF-κB and IκB in NALF and lung homogenate were examined by using ELISA kits (eBioscience Inc. USA) according to the protocols from the manufacturer.

2.8. Measurement of histamine in serum and NALF

The concentrations of histamine in serum and NALF from each mouse using the appropriate ELISA (MyBioSource, USA) were measured according to the manufacturer's instructions.

2.9. Statistical analysis

Results were expressed as mean ± standard deviation for the number of experiments. Student's *t*-test and ANOVA with Dunnett's test were used for statistical comparison among the groups. Results with