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Effects of taraxasterol on ovalbumin-induced allergic asthma in mice

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ABSTRACT

Ethnopharmacological relevance: Taraxasterol was isolated from the Chinese medicinal herb *Taraxacum officinale* which has been frequently used as a remedy for inflammatory diseases. In the present study, we determined the *in vivo* protective effect of taraxasterol on allergic asthma induced by ovalbumin (OVA) in mice.
Materials and methods: Mice were sensitized and challenged with OVA, and were orally treated daily with taraxasterol at 2.5, 5 and 10 mg/kg from day 23 to 27 after sensitization. The number of inflammatory cells in bronchoalveolar lavage fluid (BALF) was determined. Th2 cytokine interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-13 (IL-13) production in BALF and OVA-specific immunoglobulin E (IgE) production in sera were measured using ELISA. Histological changes in lung tissues were examined using hematoxylin and eosin (H&E) and periodic acid-Schiff staining (PAS). Airway hyperresponsiveness (AHR) to inhaled methacholine was assessed.
Results: Taraxasterol dramatically decreased the total inflammatory cell and main inflammatory cell counts, reduced the production of Th2 cytokine IL-4, IL-5, IL-13 in BALF and OVA-specific IgE in sera, and suppressed AHR in a dose-dependent manner. Histological studies demonstrated that taraxasterol substantially suppressed OVA-induced inflammatory cells infiltration into lung tissues and goblet cell hyperplasia in airways.
Conclusions: This finding suggests that taraxasterol protects against OVA-induced allergic asthma in mice.

1. Introduction

Inflammation is a hallmark of many diseases, including pulmonary inflammatory diseases (e.g., acute lung injury, chronic obstructive pulmonary disease, and asthma), infectious diseases and cancer. Allergic asthma is a chronic inflammatory lung disease of the airway, characterized by airway eosinophil accumulation and goblet cell hyperplasia with mucus hypersecretion to inhaled allergens and nonspecific stimuli (Kay, 1991; Bousquet et al., 2000). In particular, eosinophilic inflammation is considered the hallmark of airway inflammation in asthma (Umetsu et al., 2002). The inflammatory process in allergic asthma is dominated by Th2 cells that produce cytokines interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) (Wegmann, 2009; Bosnjak et al., 2011), which activate eosinophils and induce immunoglobulin E (IgE) production by B cells (Nakajima and Hirose, 2010). Although recent evidence-based advances in clinical management and extensive investigations into new strategies for treatment, have shown promise, the morbidity and mortality of asthma remain increasing (Umetsu et al., 2002), and it has been suggested that medications used to treat asthma contribute to this trend. Corticosteroids have long been used as the main therapeutical drugs for asthma (Tritar-Cherif et al., 2002). However, these treatments are not curative, and symptoms return soon after treatment termination, and prolonged use of corticosteroids, especially at higher doses, has been accompanied by concerns about both systemic and local side effects (Kleiman and Tuckermann, 2007; Manson et al., 2009). Thus, the development of efficient therapeutic drugs for allergic and inflammatory lung disease is urgently needed.

Taraxacum officinale has long been used in traditional oriental medicine for its lactating, choleretic, diuretic, antirheumatic and anti-inflammatory properties (Ahmad et al., 2000; Kisiel and Barszcz, 2000). It is widely used for treating various inflammatory or infectious diseases clinically such as hepatitis, upper respiratory tract infections, bronchitis, pneumonia, and as a compress for its anti-mastopathy activity (Leu et al., 2005; Sweeney et al., 2005). Pharmacological activities of *Taraxacum officinale* including its anti-angiogenic, anti-inflammatory, and anti-nociceptive activities have been in part evaluated so far (Schütz et al., 2006; Jeon et al., 2008). Recently, *Taraxacum officinale* extracts have been shown to inhibit lipopolysaccharide (LPS)-induced inflammatory responses by reducing nitric

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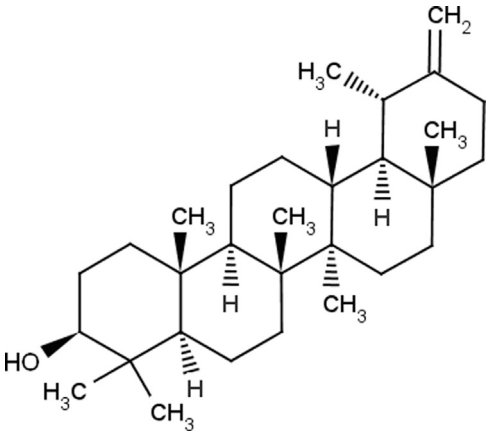

Fig. 1. Chemical structure of taraxasterol.

oxide (NO), prostaglandin E_2 (PGE_2) and pro-inflammatory cytokines production via inactivation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signal pathway in RAW 264.7 cells (Koh et al., 2010; Park et al., 2011). Moreover, the aqueous extract of *Taraxacum officinale* was assessed to contain acute anti-inflammatory activity by showing its protective effects against cholecystokinin-induced acute pancreatitis in rats (Seo et al., 2005), and we also reported its protective effects against LPS-induced acute lung injury in mice (Liu et al., 2010). Taraxasterol (Fig. 1) isolated from *Taraxacum officinale* is one of its main active constituents. Recently, we have reported that taraxasterol has the *in vitro* anti-inflammatory activity by supressing the production of various cytokines and inflammatory mediators in lipopolysaccharide (LPS)-induced murine RAW 264.7 macrophages (Zhang et al., 2012). However, no study thus far has addressed whether taraxasterol has protective effect on chronic inflammatory lung disease asthma and what the underlying mechanisms. Therefore, as a part of our on-going screening program to evaluate the anti-inflammatory potentials of natural compounds, we studied the effects of taraxasterol on allergic airway inflammation in the experimental murine model of asthma induced by ovalbumin (OVA) *in vivo* and tried to clarify the mechanism involved. This study might provide new insights for understanding immune modulation by taraxasterol as well as the potential application of taraxasterol as a modality for asthma treatment.

2. Materials and methods

2.1. Animals

Female BALB/c mice, 8–12 weeks old, were purchased from the Center of Experimental Animals of Yanbian Medical College of Yanbian University (Yanji, Jilin, China). The mice were kept in microisolator cages and received food and water *ad libitum*. Before experimentation, the mice were allowed to adapt to the experimental environment for a minimum of 1 week. All animal experimental procedures were performed in accordance with the guidelines of the Ethical Committee for the Experimental Use of Animals at Yanbian University (Yanji, Jilin, China).

2.2. Reagents

Taraxasterol was obtained from Chengdu Fenruisi Biotechnology Co. (Chengdu, Sichuan, China), and its purity was 99.5% based on HPLC analysis. OVA (Grade V) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dexamethasone (DXM) Sodium Phosphate Injection (No.H41020055) was purchased from Changle Pharmaceutical Co. (Xinxiang, Henan, China). Mouse IL-4, IL-5, IL-13 and IgE ELISA kits were purchased from Biolegend, Inc. (San Diego, CA, USA).

2.3. Establishment of a murine model of asthma and treatment regimen

Mice were sensitized and challenged with OVA, as previously described (Ci et al., 2012). The mice were randomly divided into six groups ($n=10$): control group, OVA group, taraxasterol (at doses of 2.5, 5 and 10 mg/kg, respectively)+OVA groups, and DXM+OVA group. The mice were sensitized with 20 μg OVA adsorbed with 100 μg/ml of Imject Alum by intraperitoneal injection on days 0, 7, 14. On day 14, mice were anesthetized with an inhalation of diethyl ether and challenged by intranasal instillations of 100 μg OVA in 50 μl phosphate-buffered saline (PBS) or PBS alone for negative control (unsensitized mice). On days 25, 26 and 27, mice were again anesthetized and challenged by intranasal instillations of 50 μg OVA in 50 μl PBS or PBS alone for negative control (unsensitized mice). On days 23–27, taraxasterol (2.5, 5 and 10 mg/kg) in 0.5% Sodium Tvlose was administered orally once per day, and DXM at 2 mg/kg was given with an intraperitoneal injection once 1 h prior to OVA administration as a positive control. Animals recovered quickly from the procedure with only mild discomfort.

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

Twenty four hours after the last OVA challenge and mice were anesthetized with an inhalation of diethyl ether. Blood was drawn from the brachial plexus, sera were collected by centrifugation (4 °C, 420*g*, 15 min) and stored at −80 °C for serum determination of OVA-specific IgE. After blood collection, mice were sacrificed by exsanguination. BALF was obtained by intratracheal instillation, and the lungs were lavaged three times with 0.8 ml of sterile PBS. The BALF from each sample was centrifuged (4 °C, 420*g*, 15 min), and supernatants were stored at −80 °C for subsequent analysis of cytokine levels. Cell pellets were re-suspended in PBS for total cell counts using a hemacytometer, and cytospins were prepared for differential cell counts by staining with a modified Giemsa method and cells with red cytoplasmic granules were counted as eosinophils. At least 200 cells were counted per slide.

2.5. Cytokine analysis

The levels of cytokine IL-4, IL-5 and IL-13 in the supernatants of the BALF were measured by a sandwich ELISA kit using commercially available reagents, according to the manufacturer's instructions. Briefly, microwell plates were coated overnight at 4 °C with mouse IL-4, IL-5 or IL-13 capture antibody, and blocked at room temperature for 1 h with 1% BSA in PBS with shaking. Samples from the BALF of mice and internal standard were incubated at room temperature for 2 h with shaking, followed by mouse IL-4, IL-5 or IL-13 biotinylated detecion antibody for 1 h and an avidin horseradish peroxidase (Av-HRP) conjugate for 30 min. TMB substrate solution was added and incubated in the dark for 15 min. The reaction was stopped by addition of 1 M H_2SO_4 and absorbance was measured at 450 nm on a microplate reader (TECAN-GENious, Austria). The levels of IL-4, IL-5 and IL-13 were expressed based on the appropriate standard curve. The detection limits were 4 pg/ml for each cytokine.

2.6. Measurement of serum IgE level

The serum level of OVA-specific IgE was measured by a sandwich ELISA kit using commercially available reagents,