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Peucedanum japonicum extract attenuates allergic airway inflammation by inhibiting Th2 cell activation and production of pro-inflammatory mediators

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ABSTRACT

Ethnopharmacological relevance: The root of *Peucedanum japonicum* Thunberg is traditionally used to treat coughs, colds, headache and inflammatory diseases in Korea and Japan. Its effects on allergic lung inflammation have not been investigated.

Aim of the study: To investigate the anti-asthmatic effects of *Peucedanum japonicum* extract (PJE) using a murine model of asthma and a lipopolysaccharide (LPS)-stimulated macrophage cell line.

Materials and methods: Mice underwent two rounds of sensitization with ovalbumin 1 week apart followed by four intranasal ovalbumin challenges on days 13–16. The control group received saline only. Two ovalbumin-sensitized groups were orally administered vehicle or PJE (200 mg/kg) 5 days a week starting 1 week before the first ovalbumin sensitization. The third group was orally administered the asthma medication Montelukast (10 mg/kg) on days 12–16. All animals were sacrificed on day 17. The lungs were assessed for histological features, inflammatory cell infiltration, Th2 cell activation and GATA-binding protein-3 (GATA-3) expression. The bronchoalveolar lavage fluid (BALF) was assessed for type 2 cytokine levels. The effect of PJE on the *in vitro* Th2 polarization of naïve $CD4^+$ splenocytes and the production of pro-inflammatory mediators and cytokines by LPS-stimulated RAW 264.7 cells was evaluated.

Results: PJE treatment inhibited OVA-induced inflammatory cell infiltration, eosinophilia, Th2 activation, and GATA-3 expression in the lung, reduced the interleukin (IL)-5 and IL-13 levels in BALF, down-regulated Th2 activation *in vitro*, and inhibited the macrophage production of inducible nitric oxide, cyclooxygenase-2, tumor necrosis factor-α, and IL-6.

Conclusion: PJE attenuated allergic airway inflammation by inhibiting Th2 cell activation and macrophage production of inflammatory mediators. *Peucedanum japonicum* may be candidate therapy for allergic lung inflammation.

1. Introduction

Allergic asthma is a chronic inflammatory disease that is characterized by airway obstruction, eosinophil infiltration, mucus hypersecretion, and inflammatory cytokine and chemokine overproduction (Murdoch and Lloyd, 2010). Although the pathophysiology and underlying mechanisms of allergic asthma are still not fully understood, recent studies have established that T helper 2 (Th2) cells play a pivotal role, as they orchestrate the initiation and progression of the disease *via* the production of type 2 cytokines (Afshar et al., 2008). In particular, activated Th2 cells secrete interleukin (IL)-5 and IL-13, which induce eosinophil infiltration, goblet cell hyperplasia, and excessive mucus secretion (Murdoch and Lloyd, 2010). Th2 cells also produce IL-4, which induces B cells to secrete of immunoglobulin E. The immunoglobulin E in turn stimulates mast cells, which then release additional allergenic and inflammatory factors (Poon et al., 2012; Lloyd and Saglani, 2013). Therefore, therapeutic targets in Th2-related allergic asthma include the activation of Th2 cells and their production of inflammatory cytokines.

Abbreviations: BALF, bronchoalveolar lavage fluid; COX-2, cyclooxygenase-2; GATA-3, GATA-binding protein-3; LC/MS, liquid chromatography mass spectrometry; IgE, immunoglobulin E; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NO, nitric oxide; OVA, ovalbumin; PJ, *Peucedanum japonicum*; PJE, *Peucedanum japonicum* extract; PGE_2, prostaglandin E_2; Th2, T helper 2; TNF-α, tumor necrosis factor-α

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Many studies have sought to elucidate the inflammatory pathways that underlie asthma pathogenesis (Fang et al., 2008). In particular, the NF-κB/Rel transcription family, which plays a key role in many inflammatory diseases, has been shown to participate in the progression of inflammation in asthma (Tak and Firestein, 2001) by elevating the production of inflammatory mediators by various cells, especially macrophages. These inflammatory mediators include nitric oxide (NO), which is an important immunological mediator that is generated by inducible NO synthase (iNOS). Large amounts of NO are produced in allergic asthma (Tufvesson et al., 2016). This sustained production of NO in allergic asthma promotes the cytotoxic activity of macrophages in resident tissues, particularly the lungs and airways (MacMicking et al., 1997). In asthma, macrophages also secrete the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and IL-6 (Thomas, 2001; Chu et al., 2015). In addition, the macrophages of asthma patients produce high levels of cyclooxygenase (COX)-2 (Profita et al., 2003), which converts arachidonic acid into prostaglandin E_2 (PGE_2). PGE_2 is not only a physiologically active lipid compound; it also mediates several immune diseases, including allergic asthma (Profita et al., 2003). All of these molecules play key roles in the pathophysiological changes that occur in chronic asthma and are increasingly being recognized as important treatment targets in asthma (Barnes et al., 1998; Jeon et al., 2014).

Recent studies suggest that various natural products can ameliorate asthma by inhibiting the release of pro-inflammatory mediators such as those described above. One of these natural products may be the root of *Peucedanum japonicum* Thunberg (PJ). PJ is a medicinal herb that is used in traditional medicine in Korea and Japan to treat coughs, colds, headaches, fever, rheumatoid arthritis, and other inflammatory diseases (Ikeshiro et al., 1992; WHO, 1998). These ethnomedicinal uses of PJ root suggest that it has anti-inflammatory properties, which in turn suggests that it may also be useful for treating inflammatory airway disease. However, while several studies also show that PJ possesses antioxidant (Hisamoto et al., 2003), anti-obesity (Okabe et al., 2011; Nugara et al., 2014a, 2014b), anti-platelet aggregation (Hsiao et al., 1998), antidiabetic (Lee et al., 2004), anti-metastatic, and anti-cancer invasion properties (Kim et al., 2016), it remains unclear whether PJ also has anti-asthmatic effects.

Considerable efforts have been made to identify new drugs that will quickly relieve the symptoms of asthma and suppress allergic inflammation. However, the current conventional therapies have limited efficacy in some patients and can have side effects (Dahl, 2006). In addition, there are no preventive strategies that can reduce the prevalence of asthma. Therefore, in this study, we sought to determine whether the traditional clinical effects of PJE on inflammation can also ameliorate airway inflammation. For this purpose, we employed the ovalbumin (OVA)-induced mouse model of asthma and *in vitro* experiments with naïve splenic T cells and a lipopolysaccharide (LPS)-stimulated macrophage cell line (RAW 264.7). First, mice were treated five times per week with PJ extract (PJE) starting 1 week before OVA sensitization and continuing until the last day of OVA challenge. The effect of PJE treatment on airway inflammation was then assessed. Second, the effect of PJE treatment on Th2 cell activation of naïve $CD4^+$ T cells and the production of inflammatory mediators and cytokines by LPS-stimulated macrophages was determined.

2. Materials and methods

2.1. Peucedanum japonicum Thunberg

The roots of *P. japonicum* were purchased from Kwangmyongdang Co. (Ulsan, Korea) and authenticated on the basis of the macroscopic characteristics described by Dr. Goya Choi of Korea institute of Oriental Medicine. A voucher specimen (no. 2014 PJE-1) was deposited in the Korean Herbarium of Standard Herbal Resources.

2.2. Preparation of ethanolic extract from Peucedanum japonicum root

In this study, crude extracts were prepared using the reflux extraction method, as described previously (Sultana et al., 2009; Wang et al., 2010). Briefly, the dried roots of *P. japonicum* (289.8 g) were extracted twice with 70% ethanol using a 2 h reflux and then concentrated under reduced pressure (yield: 34.0%). Before use in the animal and *in vitro* experiments, this lyophilized powder was dissolved before use in vehicle (0.25% carboxymethylcellulose or 10% dimethyl sulfoxide, respectively).

2.3. Murine studies

2.3.1. Mice

Female C57BL6 mice (8 weeks old) were purchased from Daehan Biolink Co., Ltd. (Chungcheongbuk-do, Korea) and housed in specific pathogen-free conditions with freely available food and water. All animal experiments were performed with the approval of the Animal Care committee of the Korea institute of Oriental Medicine (15-028).

2.3.2. Asthma induction and drug treatment

OVA (chicken egg ovalbumin; Sigma-Aldrich, St. Louis, MO) sensitization and challenge were performed as described previously (Lee et al., 2017). Briefly, to induce allergic lung inflammation, mice were sensitized with OVA twice 7 days apart (*i.e.*, on Days 0 and 7) by intraperitoneal injection of OVA (50 μg) emulsified with 200 μL aluminum sulfate (InvivoGen, San Diego, CA). Thereafter, on Days 13–16, the mice were challenged intra-nasally with 50 μL of OVA (25 μg).

There were four groups of mice (four mice in each group). One was injected with saline only (control group). The remaining three groups were sensitized to OVA. Two of these groups were treated orally with vehicle alone or 200 mg/kg PJE five times weekly starting 7 days before the first OVA sensitization (Day – 7) and ending on the last day of challenge (Day 16). The remaining group was treated orally with Montelukast (10 mg/kg) on Days 12–16 (*i.e.*, on the day before starting OVA challenge and all 4 days of OVA challenge). The Montelukast group served as a positive control (Yuan et al., 2013). The scheme of the experimental procedure is shown in Fig. 1.

2.3.3. Histological analysis

On day 17, all animals were euthanized with an overdose of pentobarbital sodium (a short acting barbiturate) in accordance with IACUC guidelines. The lung tissues were removed, fixed in 10% formalin, embedded in paraffin, and cut into 5 μm thick sections. To estimate inflammatory cell accumulation, goblet cell hyperplasia, and mucus production in the airway epithelium, the tissue sections were placed on glass slides, deparaffinized, and stained with hematoxylin and eosin (H & E; Sigma-Aldrich) or periodic acid-Schiff solution (PAS; Sigma-Aldrich). The tissue slides were viewed under a microscope and photographed (Olympus, Olympus Optical Co. Tokyo, Japan). The extent of peri-bronchial infiltration by inflammatory cells was quantified in lung tissue as previously described (Su et al., 2016). The scoring system was as follows; 0, no inflammatory cells detectable around the

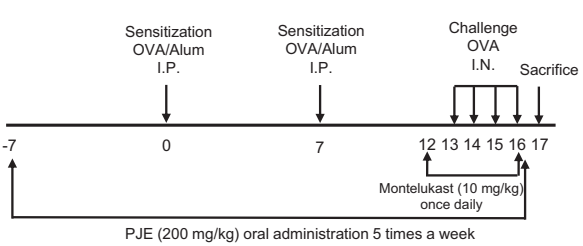

Fig. 1. The experimental protocol used to induce asthma and administer *Peucedanum japonicum* extract. I.N., intranasal; I.P., intraperitoneal; OVA, ovalbumin; PJE, *Peucedanum japonicum* extract.