



Comparison of the efficacy of KOB03, ketotifen, and montelukast in an experimental mouse model of allergic rhinitis



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ABSTRACT

KOB03 is a polyherbal medicine derived from an oriental prescription traditionally used to treat allergic diseases. In the present study, we compared the efficacy of KOB03 with modern drugs such as ketotifen and montelukast in an experimental mouse model of allergic rhinitis (AR). Ketotifen is a H1 receptor antagonist and montelukast is a leukotriene receptor antagonist. Mice were treated with KOB03, ketotifen or montelukast in an established AR mouse model using ovalbumin (OVA)-sensitized/challenged BALB/c mice. The treatment of KOB03 had inhibitory effects on symptom scores, serum levels of OVA-specific IgE, histamine, leukotriene C₄, IL-4, TNF- α , and IL-1 β in AR mice, and the histopathological changes of nasal mucosa with mucin release and inflammation. AR mice treated with KOB03 had significantly lower serum levels of OVA-specific IgE, LTC₄, IL-4, and IL-1 β than mice treated with ketotifen, whereas they only had significantly lower serum levels of OVA-specific IgE and IL-4 than those treated with montelukast. In addition, the histopathological changes of nasal mucosa with eosinophil infiltration were significantly lower in the KOB03-treated mice than those in the ketotifen and montelukast-treated group. These results suggest that KOB03 has therapeutic potential for treating AR like other modern medicines.

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1. Introduction

Allergic rhinitis (AR) is characterized as a chronic inflammatory disease characterized by local infiltration of eosinophils and major symptoms like nasal rubbing, sneezing, rhinorrhea, lacrimation, nasal congestion and obstruction [1,2]. These symptoms arise from the interactions between allergic inflammatory mediators and neural, vascular, and glandular structures within the nasal cavity [3–5]. Many important pharmacological agents have been used to treat AR including H1 receptor antagonists, leukotriene modifiers, corticosteroids, mast cell stabilizers, decongestants and intranasal anticholinergic agents. Ketotifen is a second-generation, noncompetitive H1-antihistamine that is used for long-term prevention of all forms of allergic disorders such as AR, bronchial asthma, allergic bronchitis, and asthma [6–8]. Montelukast is a leukotriene receptor antagonist (LTRA) used to reduce symptoms of seasonal allergies and to maintain treatment of rhinitis, asthma, and urticaria [9–11]. Although several novel drugs are under development for pharmacotherapy of allergic diseases, problems such as adverse side effects and low clinical efficacy still remain. Moreover, many of the current drugs used to manage rhinitis are impractical,

given that they are administered over a period of a few weeks to months.

An alternative to drugs are herbal medicines, which have long been used to treat various allergic diseases and to maintain immune balance. The search for appropriate substitutes of anti-allergic drugs has been focused on plants used in traditional medicines because natural products may have fewer side effects and be more effective than currently used drugs. KOB03 is a polyherbal medicine based on an oriental prescription that has been traditionally used to treat allergic diseases. In our previous study, we showed that KOB03 has an antiallergic property on mast cell-mediated allergic response in ovalbumin (OVA) sensitization/challenge-induced AR mice [12]. In this study, we compared the efficacy of KOB03 and modern medicines such as ketotifen, the H1 receptor antagonist, and montelukast, a LTRA, on OVA- sensitization/challenge-induced AR mice.

2. Materials and methods

2.1. Animals

6-week-old male BALB/c mice (20 $\pm$ 2 g) were purchased from Koatrch Co. Ltd. (Gyeonggi-do, Republic of Korea). The animals were housed under controlled environmental conditions at a temperature of 22 $\pm$ 3 °C with a relative humidity of 55 $\pm$ 5% and a 12-h light/dark cycle throughout the study. The care and treatment of animals were in accordance with the guidelines for the care and use of laboratory animals

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established by the Korean National Institute of Health at the Korean Academy of Medical Sciences and were approved by the Institutional Animal Care and Use Committee of Dongguk University.

2.2. Drugs

KOB03 consists of 5 different herbs as follows: *Atractylodes japonica* Koidz (*Atractylodis Rhizoma* White; 15.4 kg), *Astragalus membranaceus* Bunge (*Astragalus Root*; 11.5 kg), *Saposhnikovia divaricata* Schischkin (*Saposhnikovia Root*; 7.7 kg), *Ostericum koreanum* Maximowicz (*Ostericum Root*; 7.7 kg) and *Scutellaria baicalensis* Georgi (*Scutellaria Root*; 7.7 kg). All herbs were purchased from the Medicinal Materials Company (Kwangmyungdang Medicinal Herbs, Ulsan, South Korea) with test reports, and were authenticated by Professor Y.K. Park, a medical botanist in the Department of Herbology, College of Oriental Medicine, Dongguk University (DUCOM), Republic of Korea. Voucher specimens were deposited in the Herbarium of the DUCOM under registration number 2011-GMP-KOB03-11001. The dried extract of KOB03 was received from Hanpoong Pharm & Food Co., Ltd. (Jeonju, South Korea). Briefly, herbs of KOB03 were mixed according to the ratio of combination (2:1.5:1:1:1), minced with a grinder, extracted with 2000 mL of boiling water (95–100 °C) for 4 h, and filtered with 50 µm and 1 µm cartridge paper. The filtered extract was concentrated at 50 °C for 11 h, and concentrated extract was vacuum-dried (yield: 20.0 kg, 40%).

Ketotifen fumarate and montelukast sodium were purchased from Sigma (St. Louis, MO, USA) and dissolved in sterilized normal saline; mice were treated orally once daily from day 21 to day 28.

2.3. Preparation of AR model in mice

The mice were given free access to a standard laboratory diet and water during the experimental period. OVA solution (1 mg/mL in saline) and $\text{Al}(\text{OH})_3$ (20 mg/mL in saline) were mixed in a 1:1 ratio and then the mice were sensitized with an intraperitoneal injection on days 0, 7, and 14. This was followed by daily intranasal challenge with OVA in conscious mice from day 21 to day 28 (Fig. 1).

KOB03 (500 mg/kg/body weight (bw)), ketotifen (10 mg/kg/bw) or montelukast (10 mg/kg/bw) was orally administered once a day in OVA-sensitized mice during OVA challenge. Normal and OVA-induced AR mice were given saline alone on the same schedule. There were 6 mice in each group.

2.4. Evaluation of nasal symptoms in mice

On day 29 of treatment, mice were placed in an observation cage (31 × 25 × 18 cm) for about 10 min for acclimatization before the experiments. After administration of 20 µl of OVA (4 mg/ml in saline) into bilateral nasal cavities using a micropipette, the mice were placed back into the observation cages (one animal/cage), and the numbers of sneezes and nasal rubbing movements were counted for 15 min.

2.5. Serological analysis

Blood samples were taken from each mouse by cardiac puncture under isoflurane anesthesia on day 29. Serum was obtained by centrifugation at $8350 \times g$ for 10 min at 4 °C and was kept at –70 °C until analysis. The concentrations of OVA-specific IgE (BD sciences, San Diego, CA, USA), histamine, leukotriene C_4 (Cayman Chemical Co., Ann Arbor, MG, USA) and cytokines such as IFN- γ , IL-4, IL-1 β (R&D Systems Inc., MN, USA) and TNF- α (BD Sciences, San Diego, CA, USA) in the sera of mice were measured using commercially available enzyme-linked immunosorbent assay or enzyme immunoassay kits, according to the manufacturer's recommendations. The concentration of each substance was calculated from the equations obtained from standard curve plots for the standard solutions in the kits.

2.6. Histological assay

The mice in each group were killed by deep anesthesia using pentobarbital at 24 h postintranasal provocation on day 29. Nasal mucosa tissues were collected and processed immediately by removing the head and discarding the lower jaw. The nasal cavity was separated from the skin, muscle and soft tissue and immersed in freshly prepared 10% formalin for 48 h. The tissue was then rinsed in running tap water and decalcified in 10% ethylenediaminetetraacetic acid solution for 5 days. After rinsing in tap water, the tissue was dehydrated using graded alcohol then embedded in paraffin. Serial sections (5-µm) were cut at the level of the incisive papillae and the first and second palatal ridges and then stained with hematoxylin and eosin (H&E) and Periodic acid-Schiff (PAS) staining. Sections were examined under a light microscope. The thickness of the olfactory mucosa and olfactory bulb glomerulus was measured by microscopic analysis in H&E staining, and the numbers of goblet cells were also counted in nasal mucosa with PAS staining.

2.7. Immunohistochemistry

The nasal tissues were deparaffinized and rehydrated. After a microwave treatment, the sections were treated with 0.3% hydrogen peroxide in methanol for 15 min to inhibit endogenous peroxidase activity of blood cells. Following hydrogen peroxide treatment, the sections were incubated with 1% bovine serum albumin (BSA) in 0.05 M phosphate/0.1 M NaCl, pH 7.4, that contained a blocking reagent for another 10 min at room temperature. The sections were incubated with primary antibody against TNF- α or IL-1 β at the appropriate concentration (1:100) in PBS with 1% BSA for 12 h or overnight at 4 °C. After extensively washing with PBS containing Triton X-100, each sample was incubated with the secondary antibody (1:100) in PBS with 1% BSA for 3 h at room temperature. After washing again, the sections were stained with the ABC kit (Dako, Seoul, Republic of Korea) that includes DAB for 10 min at room temperature. After washing, these sections were stained with hematoxylin for 1 min (1 drop) at room temperature. After another



Fig. 1. Study plan for development of the experimental in vivo model.

wash, the sections were dehydrated and then mounted with cover glass.

2.8. Statistical analysis

Data are presented as the means $\pm$ standard error (means $\pm$ S.E.) of three samples and are representative of three independent experiments. Statistical analysis was carried out by using one-way ANOVA, pair-wise and multiple-comparison testing between groups. In addition, the Post-Hoc test using the GraphPad Prism 5.0's statistical analysis software (GraphPad Software, Inc., San Diego, CA). Values of $P < 0.05$ were considered significant.

3. Results

3.1. Effect of KOB03, ketotifen and montelukast on nasal symptoms

To compare the efficacy of KOB03, ketotifen and montelukast on relieving nasal symptoms, the symptom scores were measured by counting the numbers of sneezes and nasal rubs in OVA-induced AR mice. Sensitization/challenge of OVA induced significant increases in AR symptoms ($P < 0.01$, Fig. 2). The symptom score was significantly decreased by the administration of KOB03 at 500 mg/kg ($P < 0.05$). The symptom scores revealed no significant differences between the KOB03 and ketotifen or montelukast treatment groups.

3.2. Effect of KOB03, ketotifen and montelukast on the serum levels of allergic mediators

To compare other effects of KOB03, ketotifen and montelukast on allergic responses in vivo, the levels of allergic mediators such as OVA-specific IgE, histamine and LTC₄ were measured in the sera of OVA-sensitized/challenged mice. Sensitization/challenge of OVA caused a significant increase in the serum levels of OVA-specific IgE ($P < 0.001$, Fig. 3A), histamine ($P < 0.001$, Fig. 3B), and LTC₄ ($P < 0.001$, Fig. 3C). The administration of KOB03 significantly decreased the serum levels of OVA-specific IgE ($P < 0.001$), histamine ($P < 0.001$), and leukotriene C₄ ($P < 0.001$) in OVA-sensitized/challenged mice. A direct statistical comparison between KOB03 and ketotifen treatment groups revealed that KOB03 was statistically superior to ketotifen for inhibiting OVA-specific IgE ($P < 0.001$), and LTC₄ ($P < 0.01$). Comparison of the KOB03 and the montelukast treatment groups found that KOB03 was statistically superior to montelukast for inhibiting OVA-specific IgE ($P < 0.05$). There was no significant difference in the serum levels of histamine among KOB03, ketotifen and montelukast treatment groups.

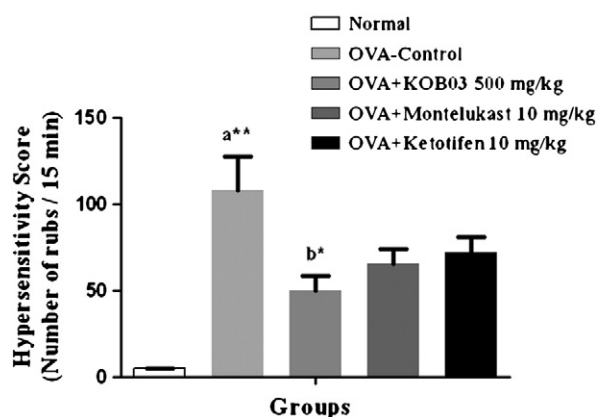


Fig. 2. Effects of KOB03 on the symptoms in OVA-sensitized/challenged mice. Independent experiments were performed ($n = 3$ per group), and the data indicate the means $\pm$ S.E. A difference was considered statistically significant when $*P < 0.05$ and $**P < 0.01$ vs. normal (a) or OVA-control group (b).

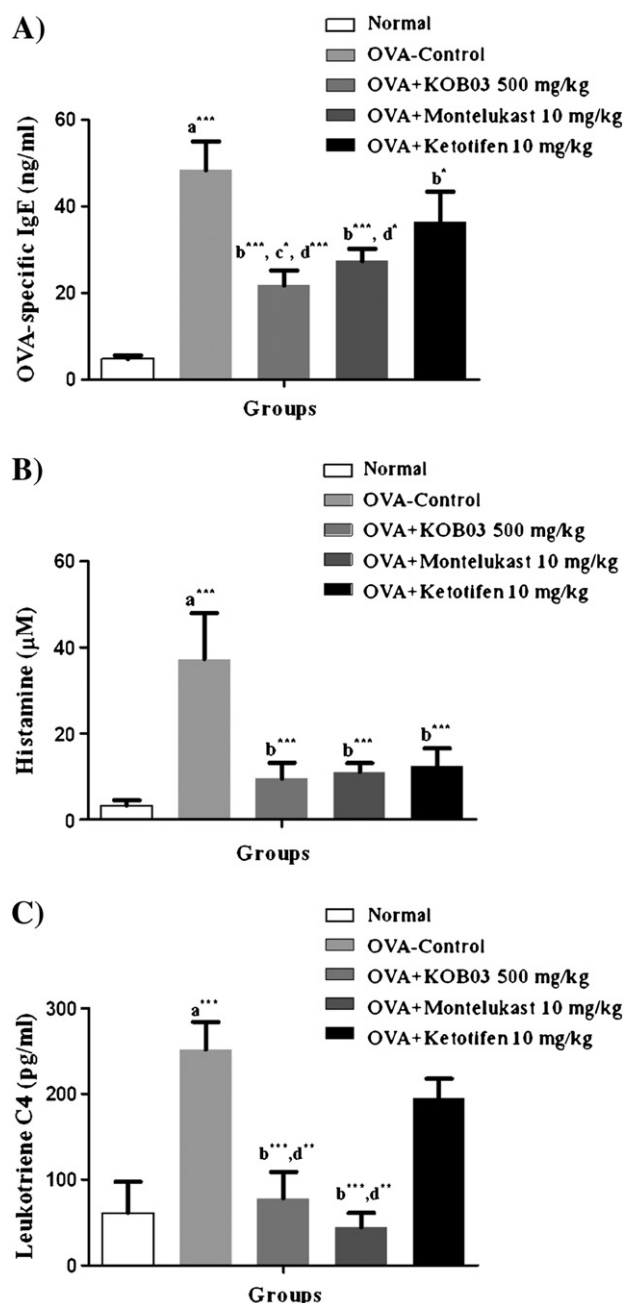


Fig. 3. Effects of KOB03 on the serum levels of allergic mediators in OVA-sensitized/challenged mice. The concentrations of OVA-specific IgE (A), histamine (B), and leukotriene C₄ (C) were measured in the sera by ELISA. Independent experiments were performed ($n = 6$ per group), and the data indicate the means $\pm$ S.E. A difference was considered statistically significant when $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ vs. normal (a), OVA-control group (b), montelukast-treated group (c) and ketotifen-treated group (d).

3.3. Effect of KOB03 on the serum levels of Th1/Th2 cytokines

To compare the efficacy of KOB03, ketotifen and montelukast on allergic responses in vivo, the levels of IFN- γ , a Th1 cytokine, and IL-4, a Th2 cytokine, were measured in the sera of OVA-sensitized/challenged mice. Sensitization/challenge of OVA induced a significant decrease in the serum levels of IFN- γ ($P < 0.001$, Fig. 4A) and a significant increase in the serum level of IL-4 ($P < 0.001$, Fig. 4B). KOB03 treatment significantly increased the serum level of IFN- γ ($P < 0.001$) and significantly decreased the serum level of IL-4 ($P < 0.001$) in OVA-sensitized/challenged mice. Ketotifen and montelukast treatment also induced a significant increase in the serum level of IFN- γ .

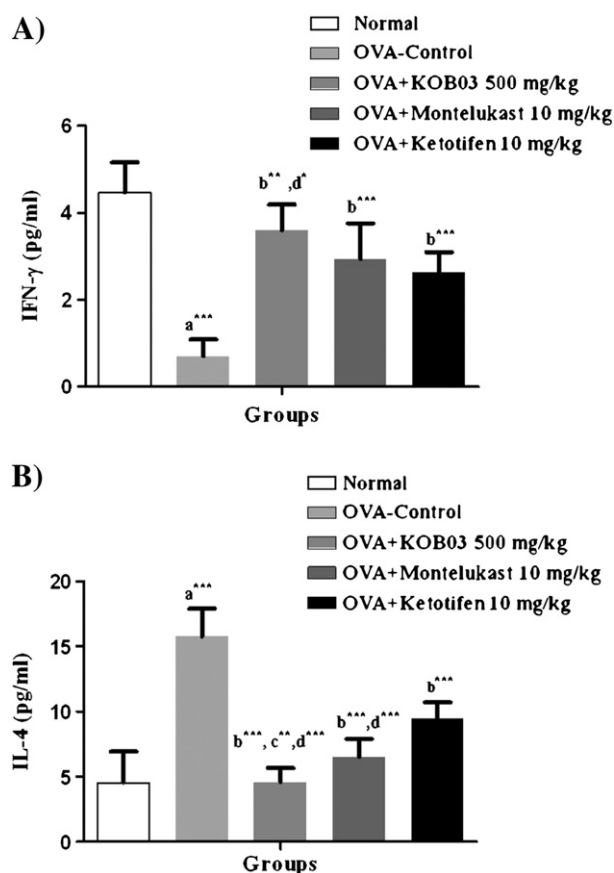


Fig. 4. Effects of KOB03 on the serum levels of cytokine in OVA-sensitized/challenged mice. The concentrations of IFN- γ (A) and IL-4 (B) were measured in the sera by ELISA. Independent experiments were performed ($n = 6$ per group), and the data indicate the means $\pm$ S.E. A difference was considered statistically significant when * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. normal (a), OVA-control group (b), montelukast-treated group (c) and ketotifen-treated group (d).

($P < 0.001$ and $P < 0.001$, respectively) and a significant decrease in the serum level of IL-4 ($P < 0.001$ and $P < 0.001$, respectively). A direct statistical comparison between KOB03 and ketotifen or montelukast treatment groups showed that KOB03 was statistically superior to ketotifen for increment of IFN- γ release ($P < 0.05$), and for inhibition of IL-4 release ($P < 0.001$). In comparison between KOB03 and montelukast treatment groups, KOB03 was statistically superior to montelukast for inhibition of IL-4 ($P < 0.01$). There was no significant difference in the serum levels of IFN- γ between KOB03 and montelukast treatment group.

3.4. Effect of KOB03 on histopathological changes of the nasal cavity

Next, we compared the changes in pathological conditions that KOB03, ketotifen and montelukast induced in the nasal mucosa by using H&E and PAS staining. Sensitization/challenge of OVA resulted in histopathological changes such as epithelial disruption, edema of the olfactory mucosa (Fig. 5A), hypertrophy of the olfactory bulb glomerulus (Fig. 5B) and hyperplasia of goblet cells with mucin release (Fig. 5C). KOB03 treatment significantly decreased the volume of olfactory mucosa edema ($P < 0.05$) and also significantly decreased the thickness of the olfactory bulb glomerulus ($P < 0.001$) and the goblet cell hyperplasia ($P < 0.001$) in OVA-sensitized/challenged mice. In addition, mice treated with KOB03 exhibited greater decreased goblet cell hyperplasia in nasal squamous epithelium ($P < 0.001$) than those treated with ketotifen. Treatment with ketotifen and montelukast also significantly decreased the olfactory

mucosa edema ($P < 0.05$), the olfactory bulb glomerulus thickness ($P < 0.001$), and the goblet cell hyperplasia ($P < 0.001$).

3.5. Effect of KOB03 on the expression of TNF- α and IL-1 β in nasal septum

To compare the efficacy of KOB03, ketotifen and montelukast in the inflammatory response in vivo, the expression of the proinflammatory cytokines, the serum levels of TNF- α and IL-1 β , and their expression in the nasal septum were measured in OVA-sensitized/challenged mice. Sensitization/challenge of OVA induced a significant increase in the serum levels of TNF- α ($P < 0.001$, Fig. 6A) and IL-1 β ($P < 0.001$, Fig. 6A). KOB03 treatment significantly decreased the serum levels of TNF- α ($P < 0.001$) and IL-1 β ($P < 0.001$) in OVA-sensitized/challenged mice. Ketotifen and montelukast treatment also significantly decreased the serum levels of TNF- α ($P < 0.001$ and $P < 0.001$, respectively) and IL-1 β ($P < 0.001$ and $P < 0.05$, respectively). A direct statistical comparison between KOB03 and ketotifen as well as between KOB03 and montelukast treatment groups showed that KOB03 was statistically superior to ketotifen for inhibition of IL-1 β release ($P < 0.05$). In addition, KOB03 treatment inhibited the expression of TNF- α and IL-1 β in the nasal septum of OVA-sensitized/challenged mice (Fig. 6B).

4. Discussion

AR is broadly defined as inflammation of the nasal mucosa. Most of the clinical symptoms of AR, such as rhinorrhea, nasal itching, sneezing and nasal congestion, cause a lot of discomfort to patients [13,14]. The general steps for managing AR include avoiding allergens or various trigger factors, administering symptomatic treatment with various drugs such as antihistamines, antileukotrienes, corticosteroids, decongestants, mast cell stabilizers and anticholinergic agents with different molecular mechanisms, and using therapies based on immunomodulation such as allergen-specific immunotherapy. Although pharmacotherapy is required, the present therapeutic options for AR are not ideal since none of the current drugs is sufficiently effective for nasal obstruction when given as a monotherapy. Moreover, the treatment of AR with modern medicine mostly involves drugs that inhibit histamine release and suppress leukotriene and allergic inflammatory responses. These drugs transiently improve clinical symptoms, but cannot cure the cause of disease. For example, antihistamines such as oral cetirizine, levocetirizine, loratadine, desloratadine and fexofenadine, and the LTRA montelukast have become increasingly popular because of their comparable efficacy and lower incidence of adverse effects relative to their first-generation counterparts. Not to mention, their safety and efficacy have already been established in patients [15]. Ketotifen is a second-generation noncompetitive H₁ type antihistamine and mast cell stabilizer that helps sufferers of asthma and AR. The efficacy and potency of ketotifen have been evaluated by several pharmacologic methods in the treatment of allergic diseases such as asthma and AR [16]. Montelukast is a potent and selective cysteinyl leukotriene (CysLT) 1 receptor antagonist that effectively inhibits the allergic and inflammatory response in asthmatics [17]. The CysLTs are synthesized *de novo* from arachidonic acid and have many pulmonary functions such as induction of smooth muscle contractions in the human airway, proliferation of smooth muscle, chemotaxis, mucous secretion and increase in vascular permeability [18]. In clinical trials, leukotriene modifiers, including montelukast, in mild and moderate persistent asthma have been shown to improve pulmonary function and symptoms when used for rescue medications and a reduction in asthmatic exacerbations [19]. Although LTRAs are widely used as oral medications in the management of chronic asthma for their anti-inflammatory effects, they are well tolerated and cause few class-related adverse effects [20]. However, most drugs for treatment of allergic disorders including asthma and rhinitis are not effective as monotherapies, which are preferable for patient adherence. Rather, they

are only used as an alternative treatment in mild or moderate phase or as a combination of multiple drugs [21].

Herbal medicines have been long used to treat various human diseases and to maintain good health. The search for appropriate anti-allergic agents has been focused on medicinal plants used in traditional medicines because natural products that may provide better treatment options for allergic diseases than currently used drugs. KOB03 is a polyherbal medicine consisting of five different herbs: Astragalus Root, Atractylodis Rhizoma White, Saposhnikovia Root, Ostericum Root and Scutellaria Root. Astragalus Root is known to enhance the immune response, to have an anti-tumor function [22], and to regulate Th1/Th2 cytokine secretion [23]. Atractylodis Rhizoma White has an anti-allergic inflammation function [24]. Peucedani Japonici Radix has anti-oxidizing and anti-inflammatory roles [25]. Ostericum Root has an anti-allergic property in allergen and mast cell-mediated allergic diseases [26]. Scutellariae Radix has anti-inflammatory activity [27]. Therefore, KOB03, through a synergy between these various constituents, might result in a strong anti-allergic drug that is more effective than current therapies. In this study, we compared the efficacy of three drugs, KOB03, ketotifen and montelukast, in terms of their ability to inhibit the clinical symptoms, to release histamine, to produce IgE, and to produce inflammatory cytokines. The results suggest that KOB03 may have anti-allergic effects. The OVA sensitization/challenge in mouse leads to increases in the serum levels of histamine and antigen-specific IgE and the infiltration of inflammatory cells, including eosinophils, in the epithelium and subepithelium of the nasal mucosa [28]. Nasal symptoms of AR are mediated not only by histamine but also by inflammatory mediators including prostaglandins, leukotrienes, trypase and proinflammatory cytokines such as TNF- α and IL-1 β from activated immune cells such as mast cells, eosinophils and basophils. In the present study, KOB03 treatment provided somewhat stronger inhibition of AR symptoms such as sneezing, rhinorrhea, congestion, postnasal drip and itching than ketotifen or montelukast treatment alone. In addition, KOB03 significantly reduced the mean release of allergic mediators such as histamine, and antigen-specific

IgE compared with ketotifen or montelukast. KOB03 also significantly reduced LTC₄, but did not more effective than that of montelukast. Therefore, the effect of KOB03 against AR with clinical symptoms may be mediated, at least in part, through suppression of allergic mediator-induced allergic responses. Previous reports showed KOB03 treatment could reduce airway inflammation via inhibition of allergic and inflammatory mediators such as histamine and IgE as well as by reduction of inflammatory cytokines such as TNF- α and IL-1 β [12].

Both Th2 and Th1 reactions are involved in allergic inflammation in AR, and Th2 cytokines in particular play a central role in the pathogenesis of airway inflammation [29,30]. In this study, we demonstrated that KOB03 significantly reduced the release of the Th2 cytokine, IL-4, compared with ketotifen or montelukast alone. In addition, KOB03 significantly increased the release of the Th1 cytokine, IFN- γ , compared with ketotifen and montelukast. These results suggest that KOB03 could control the balance of Th1/Th2 cytokine in allergic response and may explain the apparent therapeutic potential of KOB03 in AR.

The nasal mucosa symptoms in AR involve infiltration of inflammatory cells like eosinophils and damage to the submucosal edema and to the nasal epithelium. In particular, histopathological changes of nasal mucosa commonly observed in AR are the loss of ciliated cells in nasal epithelium, the hypertrophy of the olfactory ganglia, the proliferation of goblet cells and the infiltration of eosinophils [31]. In this study, KOB03 treatment reduced these histopathological changes in the nasal mucosa similar to ketotifen or montelukast treatment, suggesting that KOB03 has good potency in suppressing the nasal destruction by rhinitis.

AR is a complicated inflammatory disease and nasal exposure to allergens leads to infiltration of inflammatory cells, such as activated eosinophils and Th2 cells, into the nasal mucosa by chemoattractant factors including IL-5, chemical mediators including CysLTs and chemokines including eotaxin [32]. Edema of the nasal mucosa develops as a secondary reaction with inflammatory cells. Therefore, antiallergic and antiinflammatory medications such as nasal glucocorticosteroids are thought to be the most effective treatment

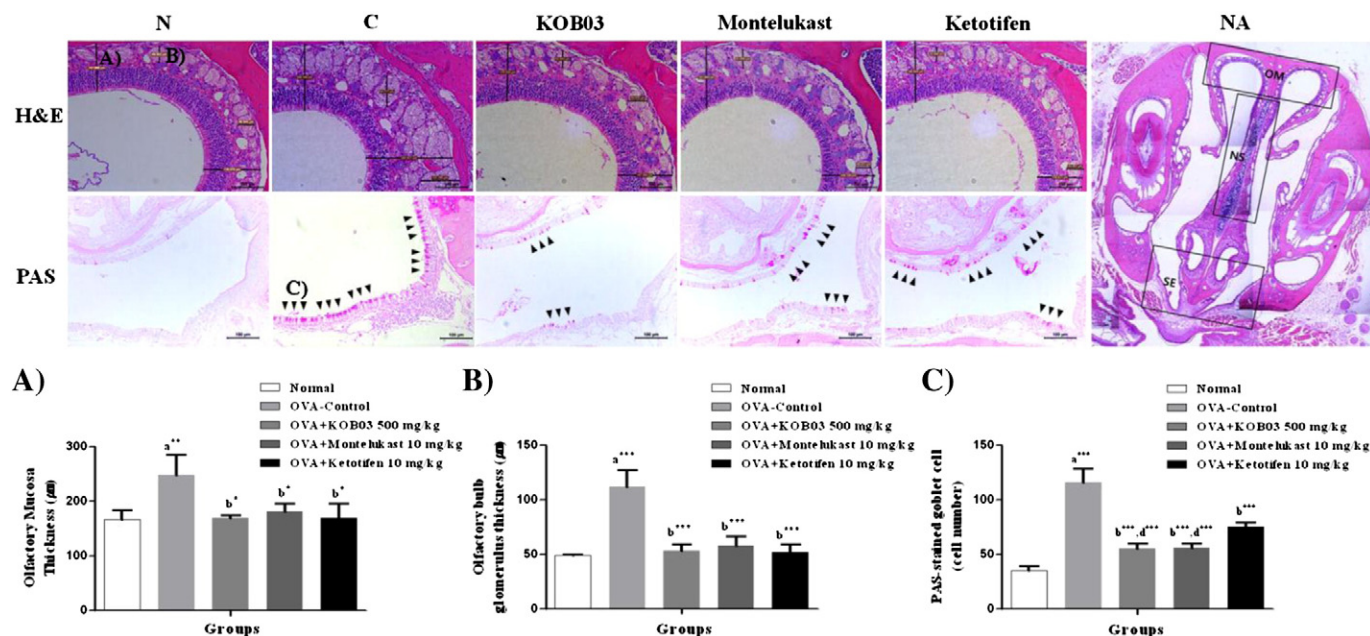


Fig. 5. Effects of KOB03 on the histopathological changes of nasal mucosa in OVA-sensitized/challenged mice. The histological changes of nasal mucosa were observed by microscope after H&E (x200) and PAS (x200) staining. N, normal; C, OVA-control group; KOB03, KOB03 (500 mg/kg)-treated group; Montelukast, montelukast (10 mg/kg)-treated group; and Ketotifen, ketotifen (10 mg/kg)-treated group. NA, the histology of nasal anatomy; OM, olfactory mucosa; NS, cartilage of nasal septum; and SE, nasal squamous epithelium. A) The data shown represent the thickness of olfactory mucosa, B) olfactory bulb glomerulus, and C) the counts of PAS-positive goblet cells with mucin release in mucous membrane. Arrows indicate PAS-stained goblet cells. Independent experiments were performed (n = 3 per group), and the data indicate the means $\pm$ S.E. A difference was considered statistically significant when *P < 0.05, **P < 0.01 and ***P < 0.001 vs. normal (a), OVA-control group (b), montelukast-treated group (c) and ketotifen-treated group (d).

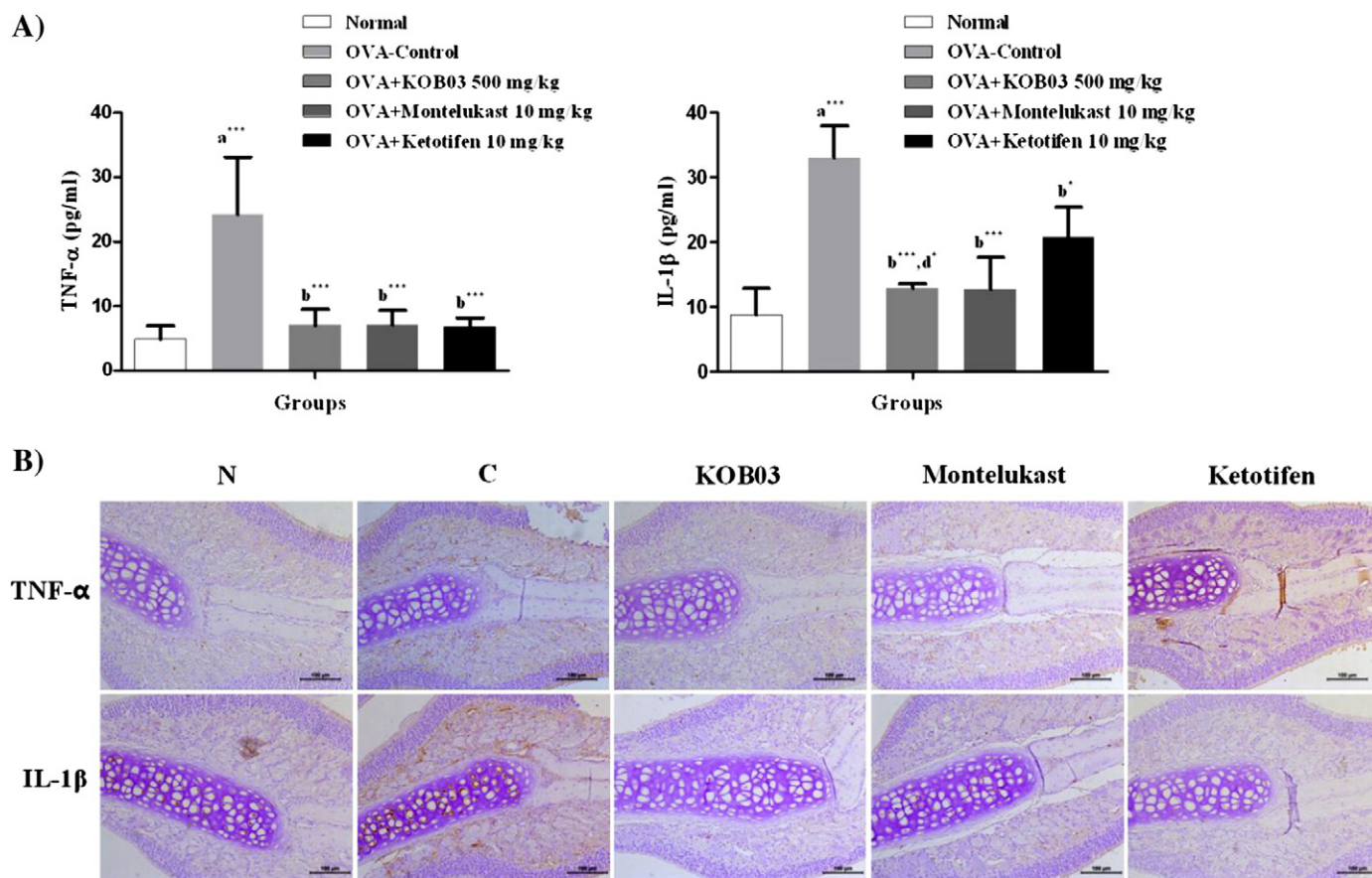


Fig. 6. Effects of KOB03 on the expression of TNF- α and IL-1 β in nasal septum of OVA-sensitized/challenged mice. The levels of TNF- α and IL-1 β were measured in the sera by ELISA (A). The expression of TNF- α and IL-1 β in the nasal mucosa was analyzed by immunohistochemistry (B; $\times 200$). Independent experiments were performed ($n = 6$ per group), and the data indicate the means $\pm$ S.E. A difference was considered statistically significant when $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. normal (a), OVA-control group (b), montelukast-treated group (c) and ketotifen-treated group (d). N, normal; C, OVA-control group; KOB03, KOB03 (500 mg/kg)-treated group; Montelukast, montelukast (10 mg/kg)-treated group; and Ketotifen, ketotifen (10 mg/kg)-treated group.

for controlling the symptoms and inflammatory mechanisms of AR. Proinflammatory cytokines such as TNF- α and IL-1 β play an important role in the pathogenesis of AR [33] and are produced by the IgE-mediated activation of mast cells and eosinophils. In this study, KOB03 strongly inhibited the expression of TNF- α and IL-1 β in the nasal mucosa of OVA sensitization/challenge-induced AR mice. Its effect was similar to that of montelukast's, though with much higher levels of IL-1 β compared with ketotifen treatment. These results suggest that KOB03 can attenuate allergic inflammation by suppressing the expression of TNF- α and IL-1 β in AR.

Our study demonstrated that KOB03 has an inhibitory efficacy in AR, improving the symptoms, inhibiting the allergic/inflammatory mediators and regulating the Th1/Th2 balance in OVA-induced AR mice. These effects were somewhat stronger than those of ketotifen or montelukast, suggesting that KOB03 may have multiple modes of action in AR.

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