

Suppressive effect of verproside isolated from *Pseudolysimachion longifolium* on airway inflammation in a mouse model of allergic asthma

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Abstract

Allergic inflammation of the airways has a critical role in asthma development. We investigated a suppressive effect of verproside (3,4-dihydroxy catalpol) isolated from the extract of *Pseudolysimachion longifolium* on asthmatic parameters—such as immunoglobulin E (IgE) level, cytokine release, eosinophilia, airway hyperresponsiveness and mucus hypersecretion—in an OVA-sensitized/challenged mouse model. Verproside significantly inhibited the increase of total IgE and the cytokines IL-4 and IL-13 in plasma and bronchoalveolar lavage fluid, and also effectively suppressed airway hyperresponsiveness, eosinophilia and mucus hypersecretion in OVA-induced asthmatic mice. The efficacy of verproside was comparable to montelukast, an anti-asthmatic drug that is currently available. These results suggest that verproside could be a major marker in herbal medicines that are used for asthma treatment, and could also act as a lead for anti-asthmatic drugs.

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1. Introduction

Asthma is regarded as a complex syndrome of the airways that is characterized by airflow obstruction, acute or chronic inflammation, airway hyperresponsiveness (AHR) and structural remodeling [1]. With regards

to pathophysiology, asthma is recognized by T-helper 2 (Th2)-cell-driven chronic inflammation, and a variety of inflammatory mediators (such as cytokines, chemokines, signalling molecules, adhesion molecules and growth factors) from immune cells and structural cells in the airways are involved in various stages of asthma [2]. In numerous studies using knockout mice models and clinical research, the critical observations in asthma could fall into several characteristic parameters, such as immune responses, eosinophilia, AHR and structural remodeling [3,4]. Each of the parameters seems not to

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have direct correlations with one another; however, IgE-mediated immune responses and eosinophilia are prominent symptoms in the airways of allergic asthma [5,6], and the produced cytokines (such as IL-4, IL-5 and IL-13) in the allergic process also play an important role in AHR development and airway remodeling [7]. Indeed, asthma is a result of orchestrated inflammatory events, many of which involve specific inhibitors that act on the pathways of asthma—for example, histamine H1 antagonists, thromboxane antagonists, platelet-activating-factor antagonists, cyclooxygenase inhibitors, nitrogen monooxygenase inhibitors and prostaglandin inhibitors—have been tried but have failed in clinical trials [3]. In contrast, glucocorticoids, which suppress the progenitor levels of inflammatory cells to baseline by widespread inhibition of cytokine synthesis and cytokine mediated immune-cell survival, have so far been used to manage the symptoms of asthmatic patients over a period of 30 years [8]. These reports suggest that the therapeutic approach for asthma management should focus on restoring the balance of asthmatic parameters rather than searching for potent inhibitors of specific pathways of the asthmatic process.

We have searched anti-asthmatic herbal compounds that have been used for the alleviation of symptoms in asthma, with ethnopharmaceutical evidence coming from Korea. *Pseudolysimachion longifolium* (Korean name; Kin-san-kkori-pul, Scrophulariaceae, previously known as *Veronica longifolia* [9]) is a perennial herb distributed in Korea, China, Russia and Europe. With *P. linearifolium* in same genus, it has been used for a traditional phytomedicine for maladies such as bronchitis, cough and asthma in Korea and China [10]. This study describes the suppressive effect of verproside

(3,4-dihydroxy catalpol) isolated from *P. longifolium* on asthmatic parameters, such as IgE level, cytokine release, and eosinophilia, AHR and mucus hypersecretion in a OVA-sensitized/challenged mouse model. The expectation of this study is to be able to contribute to asthma management.

2. Materials and methods

2.1. Isolation of verproside

P. longifolium was collected in Kong-Ju, Korea, in August of 2003. The voucher specimen (PEB 01569) was deposited in the Plant Extract Bank of Korea Research Institute of Bioscience and Biotechnology (KRIBB), in Daejeon, Korea. The dried whole plants of *P. longifolium* (1.1 kg) were extracted with methanol (5 L) twice at room temperature to get an extract (100.5 g). This extract was suspended in water (1 L) and partitioned with an equal volume of *n*-buthanol. The *n*-buthanol soluble fraction (80.0 g) was then subjected to a silica gel column chromatography (70–230 mesh, 8.5×65 cm) and eluted with a chloroform–methanol mixture (methanol 0–100%, step gradient), and yielded six fractions (Fr. 1–Fr. 6). The Fr. 3 (18.0 g, between chloroform–methanol 7/3–6/4, v/v) was subjected to repeated column chromatography using a normal phase silica column (silica gel, 230–400 mesh, 6.0×60 cm, chloroform–methanol mixture, methanol 10–50% step gradient) and a reversed phase silica gel column (RP-18, YMC Gel ODS-A, 6.0×60 cm, methanol/water, 1/4, v/v), and yielded verproside (686.0 mg). The structure was confirmed by NMR (¹H, ¹³C, DEPT, HMQC, HMBC), EI-MS and optical rotation with those reported previously [11] and the purity of verproside was analyzed as more than 99.5% by HPLC system (Shimadzu SCL-10A with SPD-M 10A vp PDA detector, column; Phenomenex Synergi 4 um Fusion RP-80, 4.6×150 mm, elution: MeOH/DW, 35/65, v/v, 0.8 ml/min) (Fig. 1).

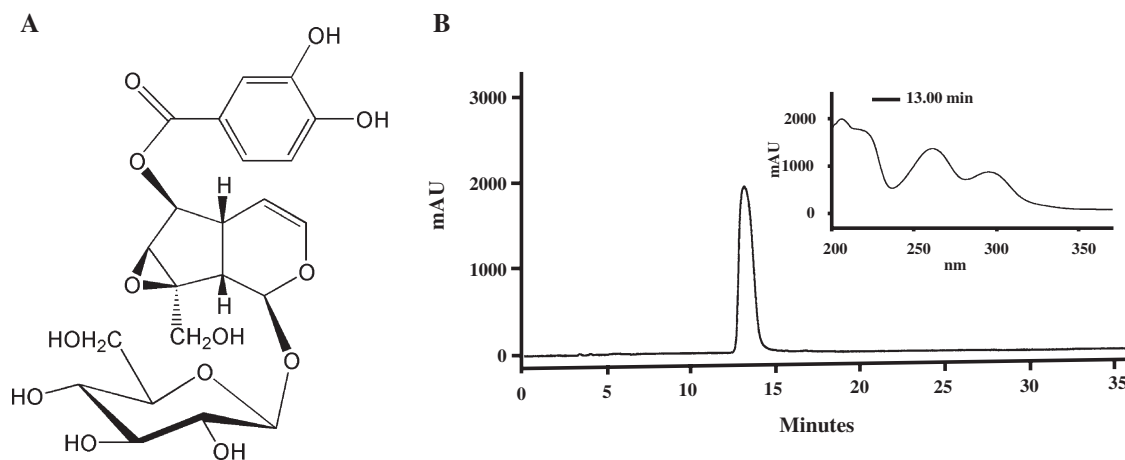


Fig. 1. Structure (A) and HPLC chromatogram (B) of verproside isolated from *Pseudolysimachion longifolium*. Analytical condition for HPLC is mentioned in the text.

2.2. Mice

Specific pathogen-free female BALB/c mice, aged 8–10 weeks, which were routinely screened serologically for relevant respiratory pathogens, were purchased from ORIENT Co. (Seoul, Korea). The mice were maintained in the animal facility under standard laboratory conditions for 1 week prior to the experiments [12], and they were provided water and standard chow ad libitum. All experimental procedures were carried out in accordance with the National Institutes of Health Guidelines for the care and use of laboratory animals, and were executed according to the National Animal Welfare Law.

2.3. Animal sensitization and airway challenge

Groups of mice ($n=6$ or 7) were studied; they received the following treatment: (1) sham-sensitization plus challenge with phosphate-buffered saline (PBS; ipNeb); (2) sensitization plus challenge with OVA (ovalbumin: Sigma A5503; Sigma, St. Louis, MO) (ipNeb); (3) sensitization with OVA (i.p.) plus challenge with OVA (Neb) and samples (verproside or montelukast, p.o.). Briefly, mice were sensitized by intraperitoneal injection of 20 μ g OVA, which was emulsified in 2 mg aluminum hydroxide in 100 μ l of PBS buffer (pH 7.4) on days 0 and 14. The mice were challenged through the airways with OVA (1% in PBS) for 20 min using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on days 28, 29 and 30 after the initial sensitization. The mice were sacrificed 48 h after the last challenge (day 32) to characterize the suppression effect of verproside on the airways of allergic asthma.

2.4. Sample treatment

Verproside (30 mg/kg) was suspended in PBS and administered intragastrically using a 25-gauge stainless steel blunt feeding needle 1 h before each challenge, and control animals were exposed only on the PBS solution. As a positive control, montelukast (30 mg/kg; MSD Korea Ltd., Seoul, Korea) was treated with the same procedure for verproside in the experiment.

2.5. Measurement of airway hyperresponsiveness (AHR)

AHR was measured using a whole-body plethysmography (OCP3000; Allmedicus, Korea) 24 h after the final aerosol challenge [13]. Each mouse was placed in a brometric plethysmographic chamber and challenged with aerosolized PBS, followed by increasing concentrations of aerosolized methacholine (12.5, 25 and 50 mg/ml) for 3 min. Bronchoconstriction was recorded for an additional 5 min at each concentration. The highest Penh value of each sample was obtained during each methacholine challenge, and expressed as a percentage of a basal Penh value in response to control (PBS) challenge.

2.6. Inflammatory cell counts in bronchoalveolar lavage fluid

The mice were sacrificed with an overdose of pentobarbital (Sigma P3761) 48 h after the last challenge, and a tracheotomy was performed. After ice-cold PBS (0.5 ml) was instilled into the lungs, bronchoalveolar lavage fluid (BALF) was obtained by aspiration three times (total 1.5 ml) via tracheal cannulation [9]. BALF was centrifuged and supernatants were collected and stored at -70°C before use.

The total inflammatory cell number was assessed by the counting of cells in at least five squares of a hemocytometer after excluding dead cells by staining with trypan blue [14]. 100 μ l of BALF was loaded onto a slide and centrifuged ($200\times g$, 4°C , 10 min) to fix the cells onto the slide using a cytospin machine (Hanil Science Industrial, Korea). The cell pellets was suspended in 0.5 ml of PBS, and 100 μ l of the solution was spun onto each slide. After slides were dried, the cells were fixed and stained by Diff-Quik[®] Stain reagents (B4132-1A; Dade Behring Inc. Deerfield, IL) according to the manufacturer's instructions.

2.7. Measurement of total IgE and cytokines in plasma and BALF

Complementary capture and detection antibody pairs for mouse IgE antibodies were purchased from PharMingen (San Diego, CA), and the IgE enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturer's directions. Duplicate samples in plasma were diluted to 1:100. IgE levels in each sample were measured from optical density readings at 450 nm, and IgE concentrations were calculated from a standard curve that was generated using recombinant IgE (5–2000 ng/ml). The amount of IL-4 and IL-13 contained in BALF was measured with a specific mouse ELISA kit (R&D Systems; Minneapolis, MN). The detection limit of the assays was 250 pg/ml.

2.8. Lung histology

The lung tissue was fixed for 24 h in 10% neutral-buffered formalin. After being embedded in paraffin, then made into 4- μ m thickness sections, the tissue was stained with H&E solution (hematoxylin; Sigma MHS-16 and eosin, Sigma HT110-1-32), and periodic acid-Schiff (PAS) stain (IMEB Inc.; San Marcos, CA) for assessment of mucus production. Subsequently, the stained tissue was mounted and cover-slipped with Dako-mounting medium (Dakocytomation; Denmark Carpinteria CA). The degree of cell infiltration in the airway was scored in a double-blind screen by two independent investigators [15]. The peri-bronchiole and perivascular inflammation was evaluated by using a scoring of 0–5, 0, no cells; 1, a few cells; 2, a ring of cells 1 cell-layer deep; 3, a ring of cells 2–4 cells deep; 4, a ring of cells; 5, cells deep. Goblet-cell hyperplasia in the airway epithelium was quantified based on a five-point system: 0, no goblet cells; 1, <25% of the epithelium; 2, 25–50% of the epithelium; 3, 50–75% of

the epithelium; 4, >75% of the epithelium. For each mouse, five airway sections that were randomly distributed throughout the left lung were analysed, and their average scores were calculated. Quantitative analysis of mucus production was performed using an image analyzer (Leica Microsystem Imaging solution Ltd., Cambridge, UK).

2.9. Image capture and photomicrographs

Photomicrographs were captured using a Photometric Quanix digital camera and montages were assembled in Adobe Photoshop 7.0. The images were then cropped and corrected for brightness and contrast, but otherwise were not manipulated.

2.10. Statistical analysis

Data were expressed as the mean $\pm$ standard error of the mean (S.E.M.). Statistical significance was determined by Student's two-tailed *t*-test for independent means, using the Microsoft Excel program. The critical level for significance was set at $P < 0.05$.

3. Results

3.1. Effect of verproside on AHR

The AHR was evaluated by calculation Penh values (enhanced pause) 24 h after the final OVA challenge. The Penh value of the OVA-treated group was significantly higher than that of the PBS control group ($P < 0.05$) at any concentration of 12.5–50.0 mg/ml of methacholine. In the verproside+OVA-challenged group, the Penh value was significantly reduced compared with that of the OVA-treated group ($P < 0.05$). A positive control, montelukast, which has been widely used as an anti-asthmatic drug, showed a similar decrease of AHR with verproside (Fig. 2).

3.2. Effect of verproside on total IgE and cytokine levels

IgE level in plasma and BALF were measured by ELISA in each experimental group, 48 h after the last challenge. The IgE levels were found to be greatly increased in the OVA-treated mice: 85.6 ± 17.3 μ g/ml (plasma, $P < 0.05$) and 59.4 ± 38.4 (BALF, $P < 0.005$) compared with the PBS-treated mice (16.9 ± 23.9 μ g/ml in plasma, 1.0 ± 0.1 ng/ml in BALF). The IgE levels of verproside-treated mice were significantly reduced to 40.2 ± 13.2 μ g/ml (plasma, $P < 0.005$) and 21.5 ± 11.2 ng/ml (BALF, $P < 0.05$). In the case of montelukast, the IgE levels were much lower as 31.4 ± 14.2 μ g/ml (plasma, $P < 0.005$) and 3.8 ± 0.7 ng/ml (BALF, $P < 0.05$) (Fig. 3A and B).

To determine the effect of verproside on cytokine release in the OVA-induced asthmatic mice, the levels of cytokines (IL-4 and IL-13) in BALF were measured using ELISA 48 h after the last challenge. OVA challenge induced a significant elevation of the cytokines to 14.1 ± 6.1 pg/ml (IL-4) and

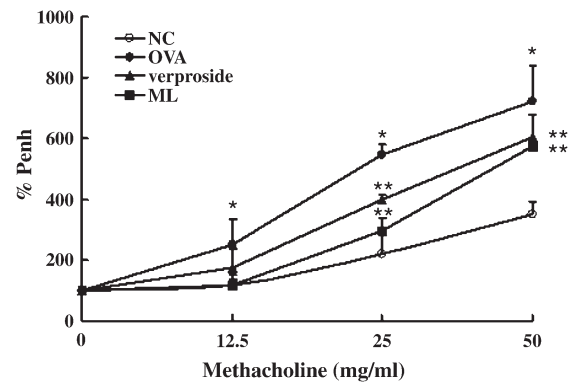


Fig. 2. Effect of verproside on airway hyperresponsiveness (AHR). AHR was measured using plethysmography 24 h after the last ovalbumin (OVA)-challenged mice by increments of methacholine (12.5–50 mg/ml). NC, normal control exposed PBS only; OVA, OVA-sensitized/challenged mice; Verproside, verproside (30 mg/kg)+OVA-sensitized/challenged mice; ML, montelukast (30 mg/kg)+OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ S.E.M. *Significant difference from NC and **significant difference from OVA, $P < 0.05$.

178.5 ± 96.4 pg/ml (IL-13) in the BALF compared with the control (IL-4, 0.1 ± 0.5 pg/ml; IL-13, 0.1 ± 1.0 pg/ml). In the verproside-treated group, the cytokines were significantly suppressed; $64.5 \pm 27.7\%$ decrease in IL-4 ($P < 0.05$) and $74.9 \pm 15.5\%$ in IL-13 ($P < 0.005$) from a OVA-challenged group. Montelukast also showed a significant reduction in both of IL-4 ($69.5 \pm 22.0\%$ decrease, $P < 0.05$) and IL-13 ($84.5 \pm 8.2\%$ decrease, $P < 0.05$) from the control. These results demonstrate that verproside reduced the IL-4 and IL-13 concentrations in the BALF of the asthmatic model as montelukast did (Fig. 3C and D).

3.3. Effect of verproside on OVA-induced eosinophilia in BALF and lung tissue

To evaluate the suppression of verproside on the eosinophilia in OVA-challenged mice, the recruited cells in BALF were counted 48 h after the last challenge. OVA caused a marked influx of leucocytes into the BALF from a PBS control group. The total cells were counted as $40.5 \pm 16.4 \times 10^4$ cells/mouse ($P < 0.001$) compared with the PBS-treated control ($2.3 \pm 0.6 \times 10^4$ cells/mouse). Eosinophils were found to be less than 5% of total cells in the PBS-treated mice, however, these were increased dramatically to be more than 75% of total leukocytes in the BALF of OVA-challenged mice. In the verproside-treated mice, the cell migration was significantly attenuated; $79.3 \pm 13.1\%$ decrease in total cells ($P < 0.005$) and $86.2 \pm 7.2\%$ in eosinophils ($P < 0.001$) from an OVA-treated control group. A positive control, montelukast, showed a similar suppressive effect of leukocyte influx in BALF as $78.3 \pm 12.1\%$ decrease in total cells ($P < 0.005$) and $80.7 \pm 11.1\%$ decrease in eosinophils ($P < 0.005$) (Fig. 4).

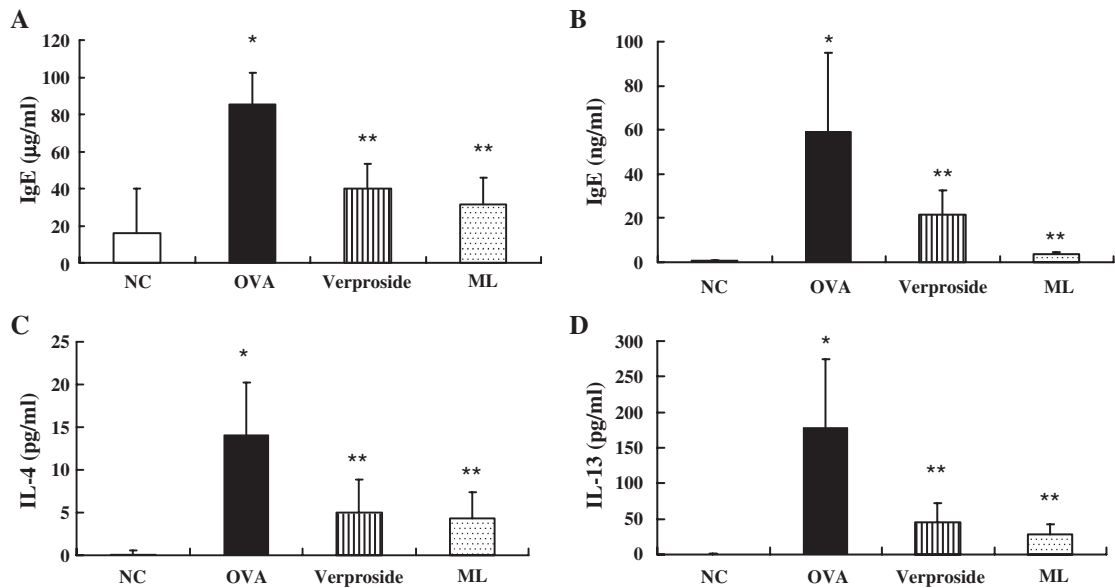


Fig. 3. Effect of verproside on total IgE levels and cytokine levels in plasma and bronchoalveolar lavage fluid (BALF). Plasma and BALF were collected 48 h after the last ovalbumin (OVA)-challenged mice. Each level was analyzed using ELISA ($n=5$ or 6). (A) Total immunoglobulin E (IgE) level in plasma, (B) total IgE level in BALF, (C) interleukin (IL)-4 level in BALF, (D) IL-13 level in BALF. NC, normal control mice treated with PBS only; OVA, OVA-sensitized/challenged mice; Verproside, verproside (30 mg/kg)+OVA-sensitized/challenged mice; ML, montelukast (30 mg/kg)+OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ S.E.M. ($n=6$ /group). *Significant difference from NC, $P<0.005$, and **significant difference from OVA, $P<0.05$.

To estimate the suppressive effect of verproside on the eosinophilia, lung tissues were collected 48 h after the last challenge. In the OVA-challenged mice, leukocytes were found to be infiltrated into the peri-bronchiole and perivascular connective tissue; of these leukocytes, eosinophilia

was mainly observed (Fig. 5A-II, $P<0.05$). In the verproside+OVA-challenged mice, the infiltration of eosinophil-rich leukocytes was significantly attenuated compared with the OVA-treated mice (Fig. 5A-III and B, $P<0.05$).

3.4. Effect of verproside on mucus production

Mucus hypersecretion is a characteristic of airway remodeling. To evaluate the effect of suppression of verproside on mucus overproduction caused by goblet-cell hyperplasia, we stained lung sections with Periodic acid Schiff (PAS) stain, and scored the stained area in each experimental group. In the OVA-treated mice, mucus overproduction was clearly observed as a violet colour in the bronchial airways compared with the PBS-treated mice (vs. Fig. 6A-I and A-II). In contrast, mucus was markedly diminished in the verproside+OVA-challenged mice (Fig. 6A-III). The mucus area was scored as 3.60 ± 0.64 in the OVA-treated mice compared with PBS-treated mice ($P<0.05$) and it was significantly decreased to 1.43 ± 0.23 in the verproside+OVA-treated mice ($P<0.05$), which was even lower than the positive reference, montelukast (1.53 ± 0.24 , $P<0.05$) (Fig. 6B). This result demonstrated that verproside reduced goblet-cell hyperplasia and mucus hypersecretion significantly in the airway remodeling process.

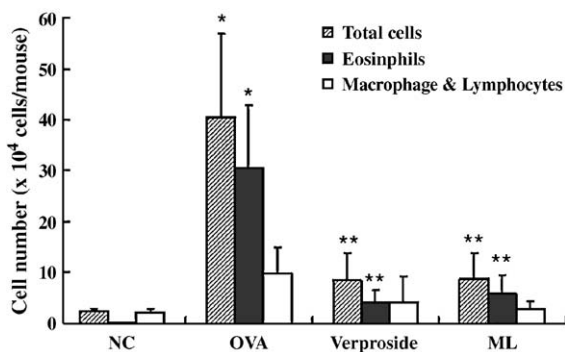


Fig. 4. Effect of verproside on the recruitment of inflammatory cells in bronchoalveolar lavage fluid (BALF) of mice 48 h after the last OVA-challenge. Cells were isolated by cytopspin and stained with Diff-Quick. The cell number was assessed by counting cells directly on a light microscope in at least five squares of a hemocytometer after excluding dead cells using trypan blue. NC, normal control mice treated PBS only; OVA, OVA-sensitized/challenged mice; Verproside, verproside (30 mg/kg)+OVA-sensitized/challenged mice; ML, montelukast (30 mg/kg)+OVA-sensitized/challenged mice. Values are expressed as mean $\pm$ S.E.M. ($n=6$ /group). *Significant difference from NC, $P<0.001$ and **significant difference from OVA, $P<0.005$.

4. Discussion

Verproside is an iridoid, which belongs to catapol analogues, and is commonly isolated in the Scrophulariaceae

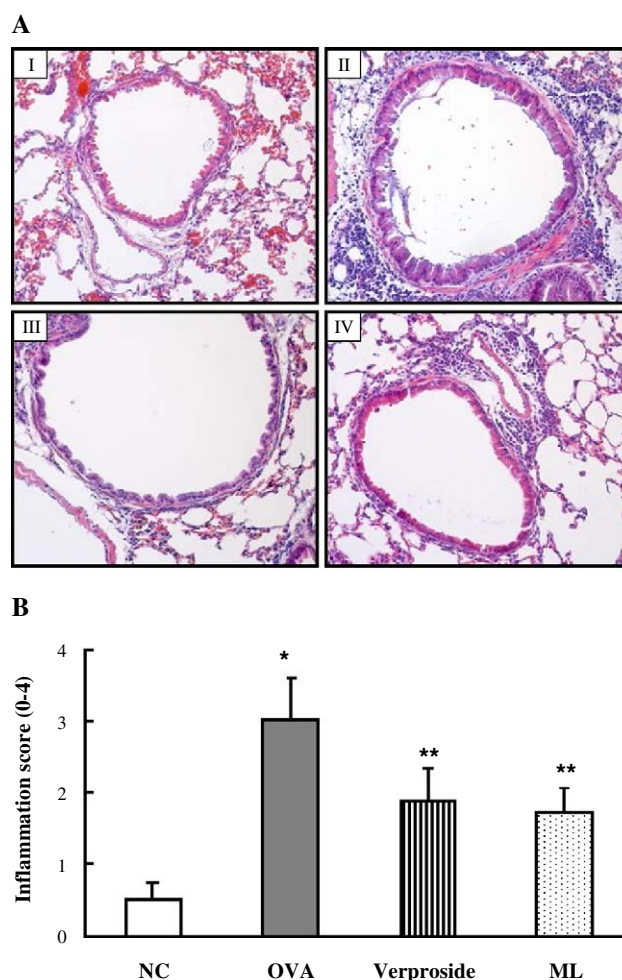


Fig. 5. Effects of verproside on the recruitment of leukocytes in lung tissue. (A) Histological examination of lung tissue was performed 48 h after the last OVA challenge. Lung tissues were fixed, sectioned at 4 μ m thickness, and stained with H&E solution. (magnification $\times 200$) (B) Scoring the extent of inflammation by quantitative analysis of inflammatory-cell infiltration in lung sections were performed based on the methods of Myou et al. [15]. I and NC, normal control mice; II and OVA, OVA-sensitized/challenged mice; III and Verproside, verproside (30 mg/kg)+OVA-sensitized/challenged mice; IV and ML, montelukast (30 mg/kg)+OVA-sensitized/challenged mice. *Significant difference from NC, $P < 0.005$ and **significant difference from OVA, $P < 0.05$.

family. Catalpol-related compounds (catalposide, verproside, verminoside and picoside II) have been isolated from *P. linarifolium*, however, only the aglycones of flavonoids (apigenin, luteolin, and 6-hydroxyluteolin) were reported to be the active ingredients in the traditional cough remedy [16]. From *P. longifolium*, catalpol has been previously isolated with aucubin [17], but verproside was investigated for the first time in this study. Iridoids have been associated with a variety of biological functions, including antimicrobial, antitumoral and hepatoprotective effects [18]. Recently, there have been several reports about iridoids that show anti-inflammatory activities in vitro. Aucubin and its hydrolyzed product were reported to exert anti-inflammatory activities by the inhibition of

tumor necrosis factor- α (TNF- α) [19], cytokines production in RAW264.7 macrophages [20], and the inhibition of LTC₄ release in peritoneal macrophages [21]. Catalposide has been determined to inhibit the production of TNF- α , interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) and NF- κ B activation in lipopolysaccharide-activated RAW 264.7 cells [22]. Besides, loganic acid from *Lonicera implexa* and catapol derivative mixture from *Scrophularia auriculata* showed anti-inflammatory activities in carrageenan-induced mouse paw edema and TPA-induced mouse ear edema [23]. However, there has been no report for the iridoids on the anti-asthmatic effect so far. The present study has demonstrated that verproside exhibited an anti-asthmatic effect by the suppression of elevated IgE, IL-4

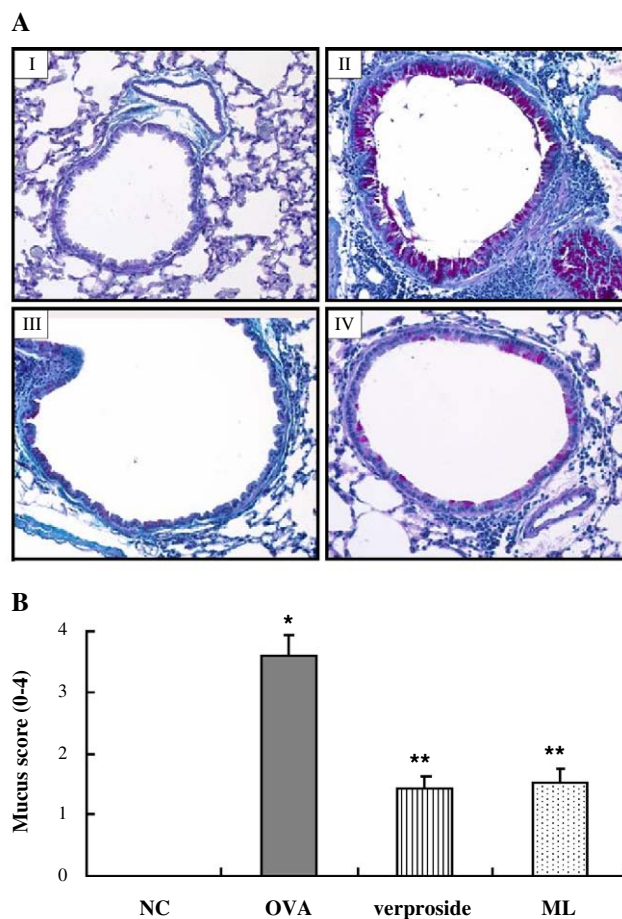


Fig. 6. Effects of verprosides on mucus production. (A) Histological examination of mucus secretion in lung tissue was measured 48 h after the last OVA-challenged mice. Lung tissues were fixed, sectioned at 4 μ m thickness, and stained with periodic acid Schiff (PAS) for mucus production. (magnification $\times 200$). (B) Scoring of mucus production in lung sections were performed based on the methods of Myou et al. [15]. I and NC, normal control mice; II and OVA, OVA-sensitized/challenged mice; III and Verprosides, verprosides (30 mg/kg)+OVA-sensitized/challenged mice; IV and ML, montelukast (30 mg/kg)+OVA-sensitized/challenged mice. * Significant difference from NC, $P < 0.005$ and ** significant difference from OVA, $P < 0.05$.

and IL-13 levels and eosinophilia in the plasma and BALF, and mucus overproduction in the lung tissues in an OVA-induced asthmatic mouse model.

Th2-derived cytokines, such as IL-4 and IL-13, are produced by resident cells in the airways; these cells include alveolar macrophages, mast cells, epithelial cells, as well as recruited eosinophils and lymphocytes [24]. In the knockout murine model, these cytokines have been implicated in the processes of sensitization and allergic responsiveness to increase the IgE level [25] and eosinophilia into the airways [26]. Verprosides significantly reduced the levels of total IgE and IL-4 and IL-13 in the plasma and BALF of the OVA-induced mouse model; therefore, verprosides could be an effective compound to suppress the early stages of allergic asthma.

AHR was measured to indicate the bronchial constriction that is commonly found in asthmatics. In cytokine-knockout murine models, it has been shown that the cytokines IL-4, IL-13 and other mediators have overlapping roles on AHR, as well as eosinophilia [7], although both parameters are not directly correlated with each other [27]. The finding that AHR was significantly reduced by verprosides could be explained, in part, by the suppression of overexpressed IgE and cytokines. In addition, AHR is claimed to correlate with subepithelial fibrosis, which is observed in the airway wall thickening during the remodeling process [28].

In this study, verprosides showed a significant reduction in the goblet-cell hyperplasia and mucus production of OVA-treated mice. Mucus hypersecretion is reported to be a typical symptom in remodeling. IL-4

and IL-13 were suggested to reduce the mucus production, as shown by the fact that mucus was significantly reduced by IL-4-receptor treatment or IL-4-receptor- α -deficient mice [29], and in IL-13 $^{-/-}$ mice [26]. Infiltrating eosinophils into the airway also had an effect on the mucus secretion of epithelial goblet cells [30]. Our findings implied that mucus hypersecretion was attenuated by the suppressive effects of verproside on the cytokine production and eosinophilia.

Cysteinyl-leukotriens have been shown to mediate many of features of asthma such as bronchoconstriction, edema, eosinophilia, AHR and mucus secretion [31]. Montelukast was developed as a cysteinyl leukotriene (cys-LT)-1 receptor antagonist [32], in which the clinical responses in patients with aspirin-sensitive asthma, nocturnal exacerbations of asthma and allergic asthma led to montelukast being successfully introduced into the market [33]. In this study, montelukast exerted a significant effect against elevated IgE and IL-4 and -13 levels, eosinophilia, AHR and mucus production. When we tried to find the effects of verproside on the arachidonic pathway, however, it failed to show significant suppressions against cysteinyl leukotrienes (LTC₄ and LTD₄) or prostaglandin (PGD₂ and PGE₂) production in a bone-marrow mast-cell assay and a rat basophilic leukemia-cell assay (data not shown). Further study should be required to assess the precise mechanisms of verproside in the asthmatic process.

In summary, we determined that verproside effectively suppressed the elevated IgE and cytokines levels, eosinophilia, AHR and mucus overproduction in OVA-induced asthmatic mice. Our results suggest that verproside could be an efficacious marker that induces immunological and pharmacological modulation of allergic asthma. Verproside has real potential to be a major marker in herbal medicines that are used in asthma treatment, and also is a strong candidate for use in anti-asthmatic drugs.

Acknowledgments

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