



Skullcapflavone II inhibits ovalbumin-induced airway inflammation in a mouse model of asthma

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ABSTRACT

Skullcapflavone II is a flavonoid derived from *Scutellaria baicalensis*, a widely used herbal medicine in anti-inflammatory and anticancer therapy in Korea. Skullcapflavone II antagonized the bradykinin receptor more potently than any of the other flavonoids derived from this plant. Here, we were investigated its therapeutic effects in a mouse model of ovalbumin (OVA)-induced allergic asthma. Administration of skullcapflavone II significantly reduced airway hyperresponsiveness (AHR), airway eosinophilia, Th2 cytokine production, and increased transforming growth factor- β 1 (TGF- β 1) levels in bronchoalveolar lavage (BAL) fluids and lungs from OVA-sensitized and -challenged mice. Skullcapflavone II administration also significantly suppressed subepithelial collagen deposition and goblet cell hyperplasia, elevated Smad7 expression and suppressed pSmad2/3 levels. Collectively, these findings indicate that skullcapflavone II, a potential bradykinin antagonist, reduced the major pathophysiological features of allergic asthma, at least in part by acting on TGF- β 1/Smad signaling pathways. Thus, skullcapflavone II may have therapeutic potential for the treatment of allergic asthma.

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1. Introduction

Bronchial asthma is characterized by reversible airway obstruction in response to allergen, chronic airway inflammation, and nonspecific airway hyperresponsiveness (AHR). The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall by mast cells, lymphocytes and eosinophils [1], and is associated with the increased expression of various inflammatory mediators, cytokines, and adhesion molecules [2,3]. The Th2 cytokines including interleukins (IL)-4, IL-5, and IL-13, produced by activated CD4+ T cells, play a central role in the pathogenesis of asthma [4,5] by controlling the key process of immunoglobulin E (IgE) production, the growth of mast cells and the differentiation and activation of mast cells and eosinophil [6–8].

TGF- β 1 is a multifunctional cytokine that is known to be involved in the pathophysiology of asthma. Its expression is increased in human chronic lung diseases like asthma [9] and the increased levels have been correlated with disease severity and the degree of peribronchial fibrosis [10]. The TGF- β 1 signaling transduction pathways typically include the activation of TGF- β 1 type I and II receptors and the subsequent phosphorylation and translocation of the intracellular effectors, Smad2 and Smad3, to the nucleus where they regulate gene transcription. Another involved factor, Smad7, is an intracellular inhibitor

that is rapidly induced by TGF- β family members and provides a negative feedback loop [11–13]. Thus, blockade of TGF- β /Smad signaling in mature T cells by expression of Smad7 enhanced airway inflammation and airway reactivity, suggesting that regulation of T cells by TGF- β was crucial for negative regulation of the inflammatory response [14].

Scutellaria baicalensis Georgi (*S. baicalensis*) is an important medicinal herb that has been widely used for the treatment of various diseases, such as viral hepatitis, pancreatitis, bronchitis, and (more recently) tumors [15,16]. Traditional Chinese Medicine considers *S. baicalensis* Georgi to be a detoxification and damp-removing agent capable of unblocking the lung and stomach meridians. Therefore it can be used to treat diseases related to the respiratory and digestive systems [17,18]. Skullcapflavone II, one of the flavonoids isolated from *S. baicalensis*, has been reported to inhibit bradykinin-induced contractions in the rat ileum and uterus, as well as bradykinin-induced plasma extravasation, endotoxic shock, and acetic acid induced writhing in mice [19]. However, there is no report regarding the anti-asthmatic effect of skullcapflavone II. Here, we investigated the effect of skullcapflavone II, on airway inflammation, AHR, and TGF- β 1/Smad signaling in an ovalbumin (OVA)-induced mouse model of allergic asthma.

2. Materials and methods

2.1. Experimental regimen

Skullcapflavone II was isolated from the methanol extracts of the dried roots of *S. baicalensis* Georgi according to the previous reports [19]. The chemical structure of skullcapflavone II is shown in Fig. 1.

Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay; TGF- β 1, transforming growth factor- β 1; OVA, ovalbumin; PAS, periodic acid-Schiff; SAL, saline.

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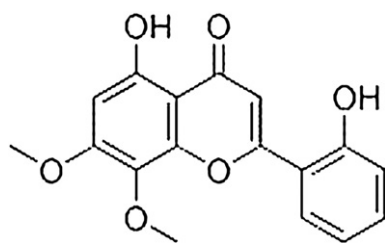


Fig. 1. The chemical structure of skullcapflavone II; molecular formula, $C_{17}H_{14}O_6$; molecular weight, 314.29.

2.2. Animals

Female BALB/c mice, 6 weeks of age (Koatech Co., Korea), were used. All protocols were approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology.

2.3. Experimental protocol

Specific-pathogen-free female BALB/c mice were sensitized on days 1 and 14 by intraperitoneal injection of 20 μ g OVA (Sigma A5503; Sigma-Aldrich, St. Louis, MO, USA) emulsified in 1 mg of aluminum hydroxide (Sigma A8222, Sigma-Aldrich, St. Louis, MO, USA) in a total volume of 200 μ l. Between days 21 and 27 after initial sensitization, the mice were challenged for 20 min with an aerosol of 1% (wt/vol) OVA in saline using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan). Dexamethasone (3 mg/kg body weight/day; Sigma-Aldrich, St. Louis, MO, USA), montelukast (30 mg/kg body weight/day), or skullcapflavone II (10 or 30 mg/kg body weight/day) were dissolved in saline and administered by oral gavage on days 21–27, before each challenge. As a control, mice were sensitized, challenged, and administered with saline alone, using the same protocol. Bronchoalveolar lavages were performed 48 h after the last challenge.

2.4. Measurement of airway responsiveness

Airway responsiveness was indirectly assessed 24 h after the last challenge using single-chamber, whole body plethysmography (Allmedicus, Seoul, Korea). Each conscious mouse was challenged with increasing concentrations of methacholine aerosol (6.25–50 mg/ml in saline) for 3 min. After each methacholine challenge, enhanced pause (penh) values were calculated for 3 min.

2.5. Inflammatory cell counts in bronchoalveolar lavage (BAL) fluid

BAL was performed as described previously [20]. In brief mice were anesthetized with 50 mg/kg of pentobarbital (Hanlim Pharm., Seoul, Korea) and BAL fluids were obtained by lavaging the lungs with saline delivered via a tracheal cannula. The BAL fluids were then deposited onto cytospin slides and stained with Diff-Quik (Dade Behring Inc., Deerfield, IL, USA). Differential cell counts were performed by two independent investigators.

2.6. Measurement of total IgE and OVA-specific IgE levels in serum

Serum levels of total and OVA-specific IgE were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated with 100 μ l/well IgE (2 μ g/ml; Serotec, Oxford, UK) or OVA (10 μ g/ml; Sigma-Aldrich, St. Louis, MO, USA) in PBS/Tween-20 and antibodies in serum were detected using isotype-specific secondary antibodies (anti-mouse IgE; Serotec, Oxford, UK). The plates were washed four times, 200 μ l of *o*-phenylenediamine dihydrochloride (Sigma-Aldrich) was added to each well, the plates were incubated for 10 min in the dark, and absorbance was measured at 450 nm. Total and

OVA-specific IgE concentrations were calculated from a standard curve generated from a 250 ng/ml solution of recombinant IgE (Serotec, Oxford, UK).

2.7. Measurement of Th2 cytokines and TGF- β 1 in BAL fluids

The levels of IL-4, IL-13, and TGF- β 1 were quantified from BAL fluid supernatants using the appropriate enzyme immunoassays (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions.

2.8. Histological analysis

Lungs were fixed with 4% paraformaldehyde and embedded in paraffin. Sections (4 μ m thick) were stained with hematoxylin and eosin for assessment of inflammatory cell infiltration. The degrees of peribronchial and perivascular inflammation were evaluated on a subjective scale of 0 to 3, as described previously [21]. A value of 0 was assigned when no inflammation was detectable; 1 was assigned when occasional cuffing with inflammatory cells was observed; 2 was assigned when most bronchi or vessels were surrounded by a thin layer (one to five cells) of inflammatory cells; and a value of 3 was assigned when most bronchi or vessels were surrounded by a thick layer (>5 cells) of inflammatory cells. Samples were scored in a blinded fashion. Goblet cell hyperplasia was assessed by periodic acid-Schiff (PAS) staining. The mucus levels in the airway were quantified by counting PAS-positive and PAS-negative epithelial cells in each bronchiole, as described previously [22]. The results are expressed as the percentage of PAS-positive cells per bronchiole, calculated as the number of PAS-positive epithelial cells per bronchiole divided by the total number of epithelial cells in each bronchiole.

2.9. Western blot analysis

Lung tissues were homogenized in the presence of protease inhibitors to yield lung protein extracts. Protein concentrations were determined using the Bradford reagent (Bio-Rad Inc., Hercules, CA) and samples (30 μ g protein per lane) were resolved by 10% SDS-PAGE. The separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA) by the wet transfer method (250 mA, 90 min). Nonspecific binding was blocked with 5% nonfat milk in Tris-buffered saline Tween 20 (TBST; 25 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 2 h, and the blots were incubated overnight at 4 $^{\circ}$ C with anti-pSmad3, Smad7, or TGF- β 1 antibodies (Cell Signaling Technology Inc., Beverly, MA, USA). Anti-rabbit horseradish peroxidase-conjugated IgG was used as the secondary antibody, and the results were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech) followed by exposure to photographic film.

2.10. Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

To assess liver function, serum ALT and AST levels were determined using commercial kits (Beckman Coulter, Inc., Fullerton, CA, USA) and an auto-analyzer (Beckman CX4, CA, USA).

2.11. Densitometric analyses and statistics

All immunoreactive signals were analyzed by Image J Software from the National Institute of Health. The data are expressed as means $\pm$ SE. The SPSS for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Differences between groups were determined by one-way ANOVA. A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of skullcapflavone II on airway responsiveness

Airway responsiveness was assessed by monitoring penh values in response to increasing doses of methacholine. The penh values produced by methacholine administration (at doses from 12.5 to 50 mg/ml) were significantly increased in the OVA-sensitized and -challenged mice compared with control mice (Fig. 2A). However, skullcapflavone II significantly and dose-dependently decreased the penh values of OVA-sensitized and -challenged mice at the levels comparable to that observed in the dexamethasone and montelukast-treated group. These results indicate that dexamethasone, montelukast, and skullcapflavone II treatment reduces OVA-induced AHR.

3.2. Effect of skullcapflavone II on infiltration of inflammatory cells in BAL fluids

The numbers of total cells, lymphocytes, neutrophils, and eosinophils in OVA-challenged mice were significantly increased in BAL fluids 48 h after the last challenge as compared with the levels in control mice (Fig. 2B). These increases in the numbers of total cells,

lymphocytes, neutrophils, and eosinophils were significantly and dose-dependently reduced by the administration of skullcapflavone II. Dexamethasone and montelukast also significantly reduced inflammatory cells in BAL fluids.

3.3. Effect of skullcapflavone II on pathological changes

Histological analyses revealed the typical pathological features of asthma in OVA-sensitized and -challenged mice. Numerous inflammatory cells had infiltrated around the bronchiole, the airway epithelium was thickened, and mucus and debris had accumulated in the lumen of bronchioles (Fig. 3B) in OVA-challenged mice compared to control mice (Fig. 3A). However, mice treated with skullcapflavone II showed marked reductions in the thickening of the airway epithelium, the infiltration of inflammatory cells in the peribronchiolar region, and the amount of debris in the airway lumen (Fig. 3E and F). In OVA-sensitized and -challenged mice, the peribronchial and perivascular lung inflammation scores determined 48 h post-challenge were significantly increased than those of the control mice (Fig. 3G). This increased peribronchial and perivascular lung inflammation was significantly and dose-dependently decreased by the administration of skullcapflavone II. Dexamethasone and montelukast also significantly decreased peribronchial and perivascular lung inflammation (Fig. 3C and D).

3.4. Effect of skullcapflavone II on mucus gland hypertrophy and goblet cell hyperplasia

Mucus hypersecretion, pathological feature of asthma that contributes significantly to airflow limitation, is accompanied by mucous gland hypertrophy and goblet cell hyperplasia. The percentage of the airway epithelium that stained positively with PAS was significantly greater in OVA-sensitized and -challenged mice (Fig. 4B) than in control mice (Fig. 4A). The administration of skullcapflavone II significantly and dose-dependently reduced the percentage of airway epithelium staining positively with PAS in OVA-sensitized and -challenged mice, which was similar to the reduction in montelukast treated mice (Fig. 4G).

3.5. Effect of skullcapflavone II on peribronchial collagen deposition

The trichrome staining areas were significantly increased in OVA-challenged mice (Fig. 5B) compared with control mice (Fig. 5A). The administration of skullcapflavone II significantly and dose-dependently reduced the levels of peribronchial fibrosis (Fig. 5E and F). Dexamethasone and montelukast also significantly reduced the levels of peribronchial fibrosis (Fig. 5C and D).

3.6. Effect of skullcapflavone II on the serum levels of total IgE and OVA-specific IgE

Enzyme immunoassays showed that the serum levels of total IgE and OVA-specific IgE (Fig. 6A and B) were significantly increased in OVA-challenged mice at 48 h after the last OVA challenge, compared with control mice. These OVA-induced increases in the levels of total IgE and OVA-specific IgE were significantly and dose-dependently reduced by the administration of skullcapflavone II. Dexamethasone and montelukast also significantly reduced the levels of total IgE and OVA-specific IgE in serum.

3.7. Effect of skullcapflavone II on liver function

Assessment of serum AST and ALT levels revealed that skullcapflavone II (10 and 30 mg/kg) did not affect the activities of AST and ALT, indicating that normal liver function was preserved in skullcapflavone II-treated mice (Fig. 7).

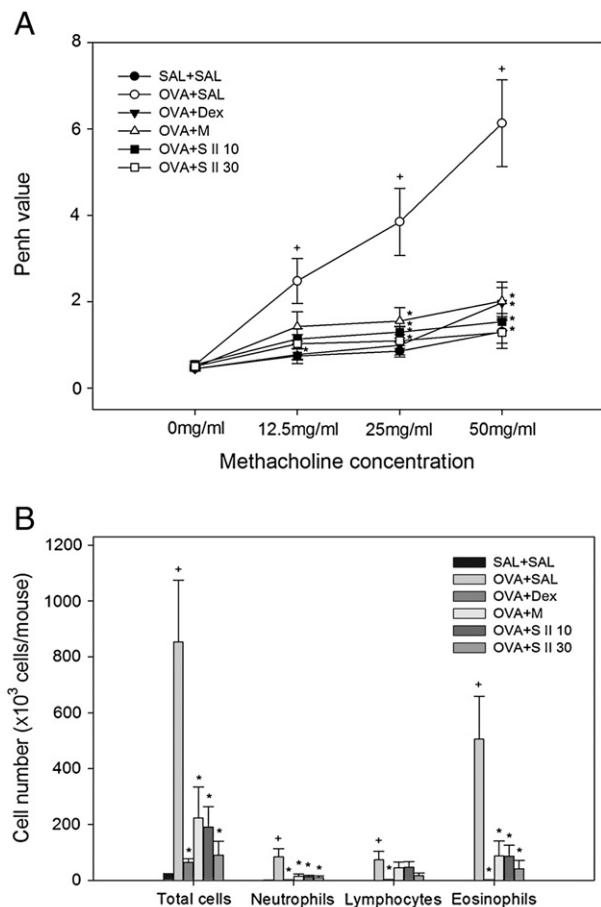


Fig. 2. A, Effect of skullcapflavone II on airway responsiveness. Skullcapflavone II administration significantly attenuates development of AHR in OVA-sensitized and challenged mice. Airway responsiveness was measured 24 h after the last challenge in control mice challenged and administered saline (SAL + SAL), OVA challenged mice administered saline (OVA + SAL), OVA challenged mice administered dexamethasone (OVA + Dex), OVA challenged mice administered montelukast (OVA + M), OVA challenged mice administered 10 mg/kg skullcapflavone II (OVA + SII10), and OVA challenged mice administered 30 mg/kg of skullcapflavone II (OVA + SII30). B, Effect of skullcapflavone II on total cells and differential cellular components in BAL fluids. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

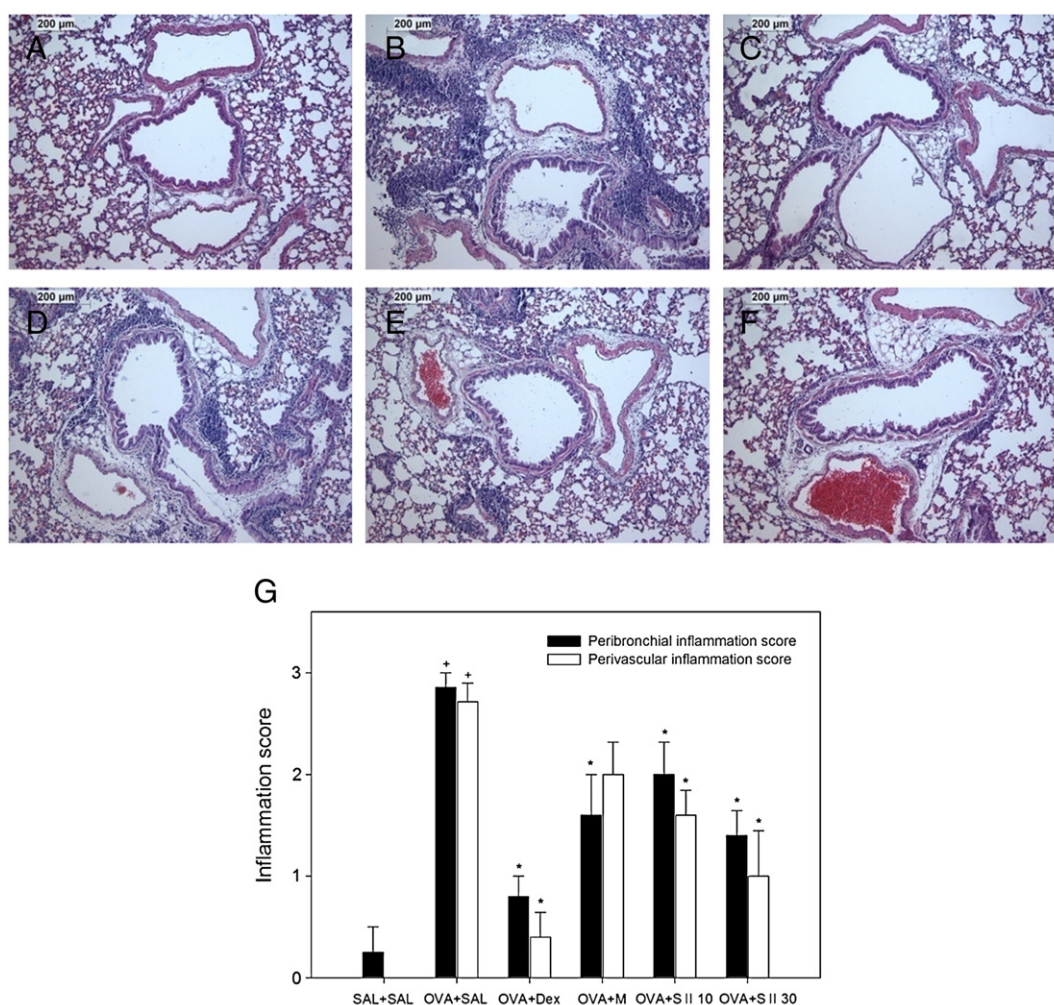


Fig. 3. Effect of skullcapflavone II on lung inflammation of OVA-challenged mice. A–F, H&E stained lung sections of control mice (A), OVA challenged mice administered saline (B), OVA challenged mice administered dexamethasone (C), OVA challenged mice administered montelukast (D), OVA challenged mice administered 10 mg/kg of skullcapflavone II (E), OVA challenged mice administered 30 mg/kg of skullcapflavone II (F). G, Lung inflammation was measured, and total lung inflammation was defined as the average of the inflammation scores. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

3.8. Effect of skullcapflavone II on the levels of IL-4, IL-5, and IL-13 in BAL fluids

Enzyme immunoassays showed that levels of IL-4 (Fig. 8A), IL-5 (Fig. 8B), and IL-13 (Fig. 8C) in BAL fluids were significantly increased in OVA-challenged mice at 48 h after the last OVA challenge, compared with control mice. The increased levels of these cytokines were significantly and dose-dependently reduced by the administration of skullcapflavone II. Dexamethasone and montelukast also significantly reduced the levels of IL-4, IL-5, and IL-13 in BAL fluids.

3.9. Effect of skullcapflavone II on TGF- β_1

Western blot analysis showed that TGF- β_1 protein levels in lung tissues were significantly increased in OVA-challenged mice at 48 h after the last OVA challenge, compared with the levels in control mice (Fig. 9A). These increases in TGF- β_1 levels were significantly and dose-dependently reduced by the administration of skullcapflavone II (Fig. 9B). Similarly, enzyme immunoassays revealed that the levels of TGF- β_1 in BAL fluids were significantly increased in OVA-challenged mice at 48 h after the last OVA challenge, compared with the levels in control mice (Fig. 9C), but increased TGF- β_1 levels were significantly and dose-dependently reduced by the administration

of skullcapflavone II manner. Dexamethasone and montelukast also significantly reduced the levels of TGF- β_1 in BAL fluids and lung tissues.

3.10. Effect of skullcapflavone II on Smad expression

We investigated the levels of phospho-Smad2/3 (pSmad2/3) and Smad7 in the lungs of OVA-challenged mice following administration of skullcapflavone II. Increased pSmad2/3 levels were observed after 7 days of OVA challenge (Fig. 10A) and these levels were considerably decreased by administration of skullcapflavone II or dexamethasone (Fig. 10C). In contrast, the levels of Smad7 were markedly increased in OVA-sensitized and -challenged mice treated with skullcapflavone II and dexamethasone compared to mice that received OVA-sensitized and -challenged mice (Fig. 10B). No significant difference was seen in skullcapflavone II- vs. dexamethasone-treated mice in terms of their pSmad2/3 and Smad7 levels.

4. Discussion

In this study, we show that administration of skullcapflavone II, one of the flavonoid from *S. baicalensis*, attenuated OVA-induced airway inflammation, Th2 cytokines levels, IgE and OVA-specific IgE levels, the number of mucus-producing cells in the airway, peribronchial fibrosis

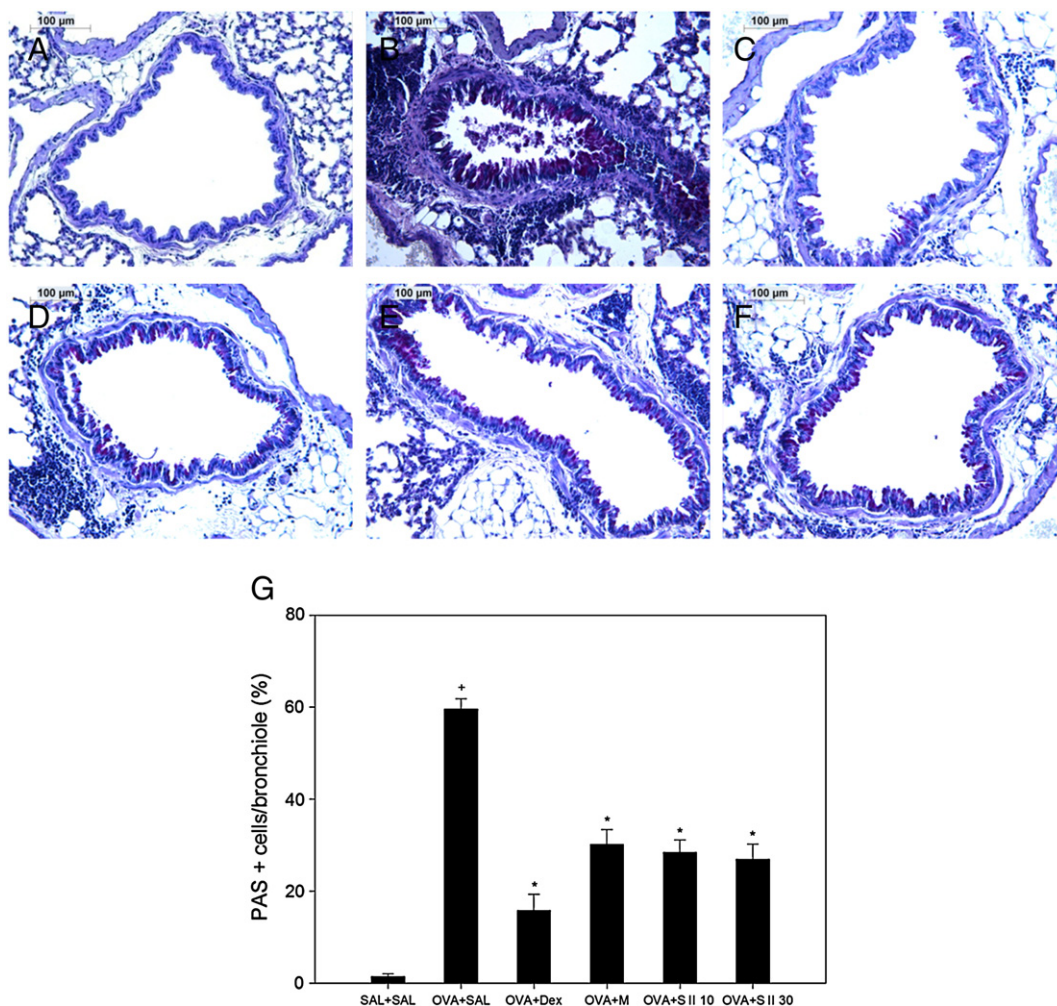


Fig. 4. Effect of skullcapflavone II on airway mucus expression. A–F, PAS-stained lungs sections of control mice (A), OVA challenged mice administered saline (B), OVA challenged mice administered dexamethasone (C), OVA challenged mice administered montelukast (D), OVA challenged mice administered 10 mg/kg of skullcapflavone II (E), and OVA challenged mice administered 30 mg/kg of skullcapflavone II (F). G, Quantitation of airway mucus expression. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

and AHR. Furthermore, we show that these effects were associated with changes in the expression levels of signaling molecules from the TGF- β 1/Smad pathway.

The pathogenesis of asthma is associated with increased infiltration of inflammatory cells and excessive mucus secretion into the airway [23]. OVA-induced allergic asthma is recognized as a disease that results from chronic airway inflammation characteristically associated with the infiltration of lymphocytes, eosinophils, macrophages, and neutrophils into the bronchial lumen. Eosinophils are markers of allergic airway inflammation in asthma and play a central role in the pathogenesis of asthma [23–25]. In the OVA-induced model of allergic asthma, skullcapflavone II resulted in a significant decrease in the number of eosinophils, neutrophils, lymphocytes, and macrophages in the BAL fluids. Moreover, histopathological analysis revealed that skullcapflavone II administration suppressed the infiltration of inflammatory cells and the hyperplasia of goblet cells. This effect was more effective than the effects of montelukast which is currently available in anti-asthmatic drugs. Many clinical studies also reported a correlation between pulmonary eosinophilia and asthma, as well as a correlation with the level of eosinophils in the BAL fluids [24–26]. Thus, the suppression of pulmonary eosinophilia by skullcapflavone II could be explained by the inhibition of the differentiation and migration of eosinophils. An aberrant Th2-type response to allergens characterized by the overproduction of IL-4, IL-5, and IL-13, which

are critical for the maintenance of ongoing IgE-mediated eosinophilic inflammation [26]. IL-4 is the Th2 cytokine that is most important for the induction of isotype switching to IgE in B lymphocytes [27]. IL-13 can also regulate the recruitment of eosinophils to the airways, via its various effects on epithelial and smooth muscle cells [28]. We showed here that administration of skullcapflavone II opposed the effects of OVA challenge on IL-4 and IL-13, reducing both the levels of secreted cytokines in the BAL fluids. Elevated serum IgE level, which is a hall marker of the Th2 immune response, was also reduced by skullcapflavone II administration. These results indicate that skullcapflavone II provides relief against airway inflammation by reducing the levels of Th2 cytokines, such as IL-4 and IL-13. Consistent with the reduction of the levels of these cytokines, the recruitment of inflammatory cells, especially of eosinophils and macrophages, was suppressed markedly in the BAL fluids after skullcapflavone II administration.

It is believed that inflammation play a critical role in AHR development [29]. It has been established that IL-5 is important in some animal models and almost certainly participate in the events leading to the eosinophilic inflammation and AHR in asthma. In addition, there is a close relationship between eosinophils, IL-4, IL-5, and IL-13 in the mechanism regulating AHR in the asthmatic lung [29]. Moreover, IgE-mediated mast cell activation may contribute to AHR by producing a wide array of inflammatory mediators and

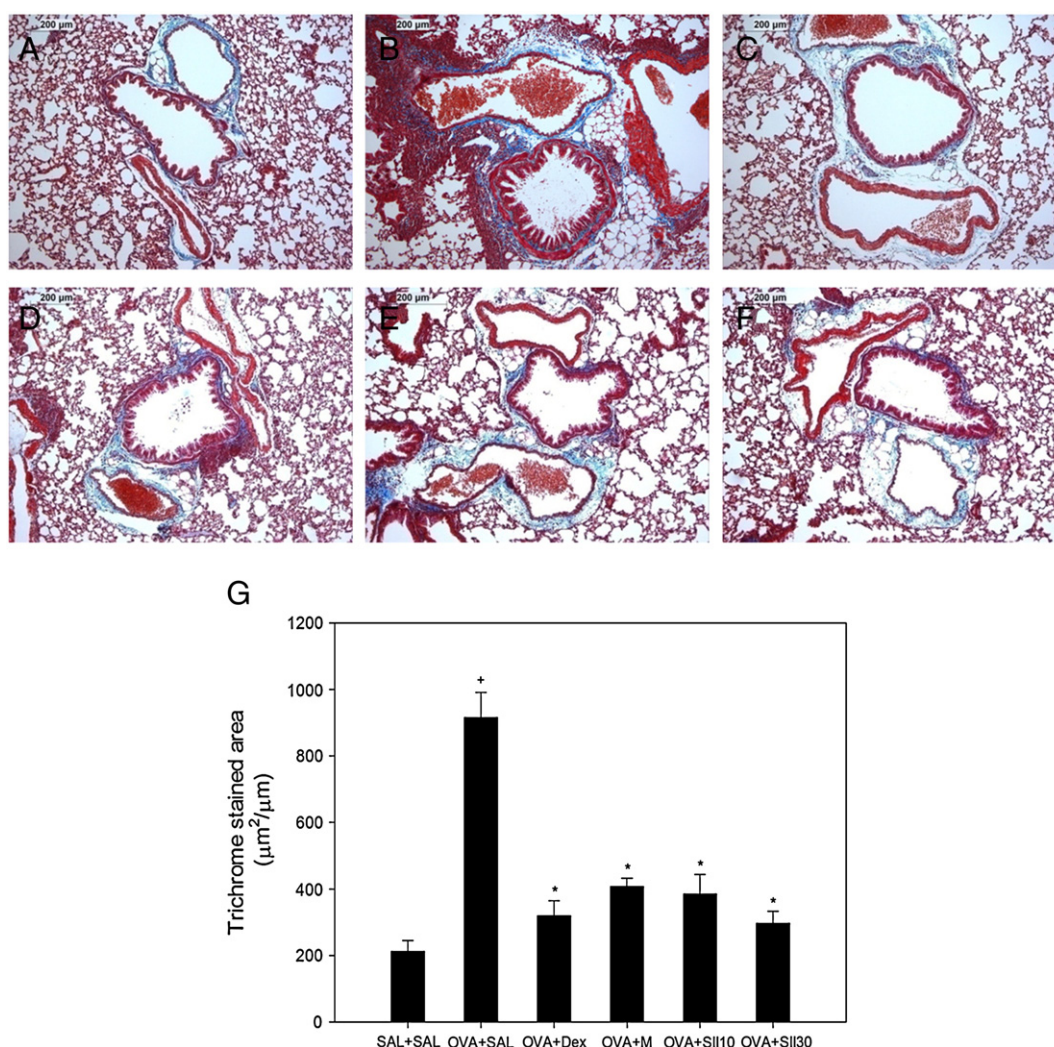


Fig. 5. Effect of skullcapflavone II on peribronchial fibrosis in lung tissues. A–F, Trichrome stained lung sections of control mice (A), OVA challenged mice administered saline (B), OVA challenged mice administered dexamethasone (C), OVA challenged mice administered montelukast (D), OVA challenged mice administered 10 mg/kg of skullcapflavone II (E), OVA challenged mice administered 30 mg/kg of skullcapflavone II (F). G, Quantitation of peribronchial fibrosis. ⁺ $p < 0.05$ vs. SAL + SAL; ^{*} $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

cytokines [30]. Administration of skullcapflavone II was significantly decreased the penh values in response to increasing doses of methacholine. Although recent studies have suggested that there are problems with penh system, it has been widely used for measuring nonspecific bronchial hyperresponsiveness in murine model of allergic airway inflammation [31–33]. Thus it could be explained that administration of skullcapflavone II was significantly suppressed AHR and it may be associated with the reduction in Th2 cytokine production, tissue eosinophilia and serum OVA-specific IgE level.

TGF- β 1 is a potent fibrotic factor responsible for the induction of myofibroblast activation and synthesis of the extracellular matrix, and numerous recent studies have examined the relationship between TGF- β 1 and airway remodeling [11,13,20,34]. Therefore, inhibiting production of TGF- β 1 or blocking its bioactivity to depress collagen formation in airway is considered as one of the potential ways for the treatment of airway remodeling [10,13]. Members of the TGF- β 1 superfamily initiate their cellular action by binding to serine/threonine kinase receptors that directly regulate the intracellular Smad pathway. The Smads are a unique family of signal transduction molecules that can transmit signals directly from cell surface receptors to the nucleus [12]. A previous study showed that blockade of

TGF- β /Smad signaling in mature T cells by expression of Smad7 enhanced airway inflammation and airway reactivity, suggesting that regulation of T cells by TGF- β 1 was crucial for negative regulation of the inflammatory response [14]. Here, we investigated the expression of TGF- β 1 signaling by detecting the expression of the intracellular effectors Smads. Crude extracts of *S. baicalensis* as well as its pure compounds, baicalin, baicalein and chrysin were previously shown to dose-dependently up-regulate TGF- β 1 gene expression in RAW 264.7 cells. However, another flavonoid of *S. baicalensis*, wogonin, did not exhibit up-regulation of TGF- β 1 gene or protein expression in RAW 264.7 cells [35]. In this study, we found that administration of skullcapflavone II reduced the expression of TGF- β 1 and modulated its signaling partners in the airways, as demonstrated by decreased pSmad2/3 levels and increases in the levels of the inhibitory molecules, Smad7. Therefore, our findings suggest that skullcapflavone II may prevent collagen deposition at least part by modulating the activity of the TGF- β 1 signaling pathway.

Several flavonoids isolated from *S. baicalensis* have been reported to inhibit bradykinin-induced contractions in the rat ileum and uterus, as well as bradykinin-induced plasma extravasation, endotoxin shock, and acetic acid induced writhing in mice. Among the flavonoids from *S. baicalensis*, skullcapflavone II exhibits most potent antagonistic

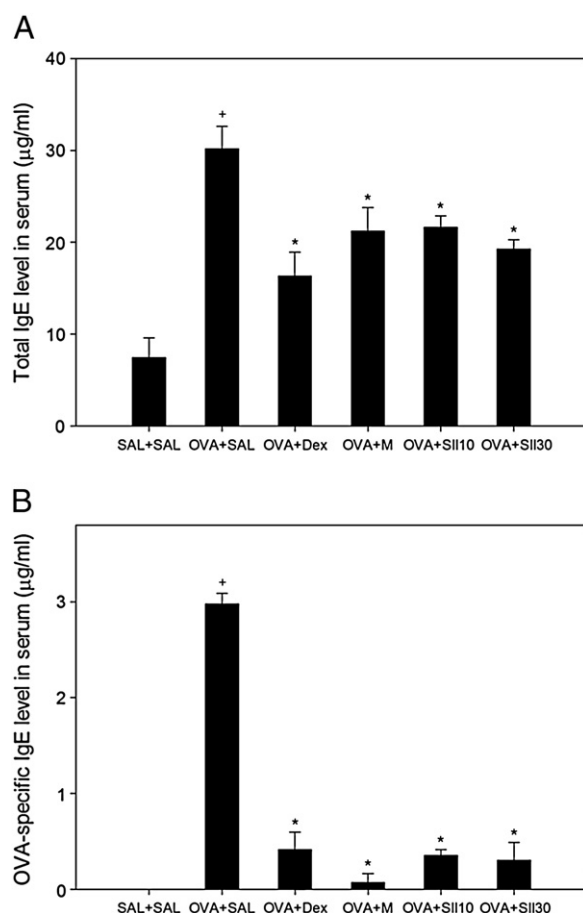


Fig. 6. Effect of skullcapflavone II on IgE and OVA-specific IgE levels in serum. A–B, Enzyme immunoassay of IgE in serum (A), OVA-specific IgE in serum (B). * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

activity against the bradykinin receptor [19]. Bradykinin has been implicated in the pathogenesis of asthma in that elevated kinin levels have been detected in plasma and BAL fluids of asthmatic patients [36,37]. Thus, it was reported that various bradykinin antagonists prevent airway inflammation and antigen-induced hyperresponsiveness [38–40]. In addition, TGF- β and VEGF, cytokines are known to be involved in airway remodeling in asthma, were produced in a mouse model of allergic asthma and that the cytokines production was suppressed by the bradykinin antagonist. Skullcapflavone II, a potential bradykinin antagonist, inhibits OVA-induced airway inflammation,

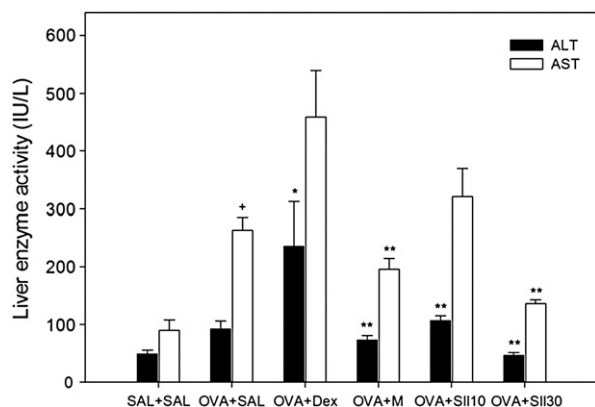


Fig. 7. Effect of skullcapflavone II on AST and ALT levels in serum. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

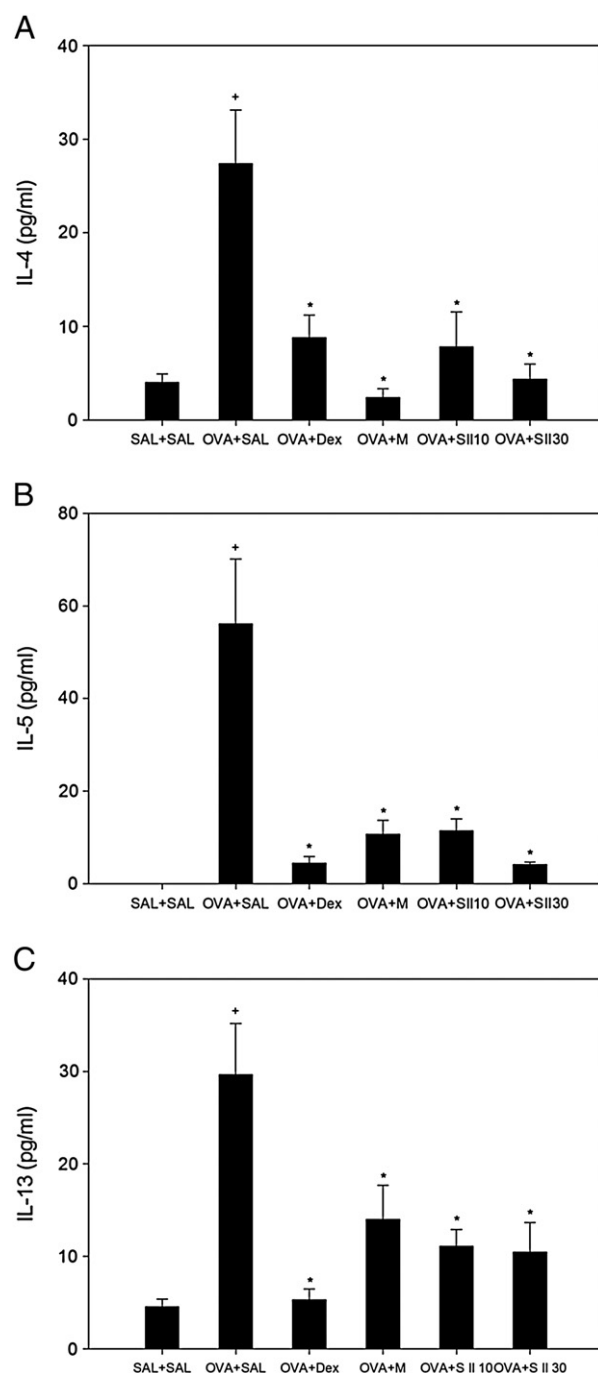


Fig. 8. Effect of skullcapflavone II on the levels of Th2 cytokines in BAL fluids. A–C, Enzyme immunoassay of IL-4 (A), IL-5 (B), and IL-13 (C) in BAL fluids. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

AHR, and early stage airway remodeling via decreased production of TGF- β 1. However, further study will be needed to investigate solid mechanism of skullcapflavone II involving TGF- β /Smad pathway.

Serum ALT and AST level was investigated to confirm the liver toxicity of skullcapflavone II before investigating the effects of skullcapflavone II in a mouse model of asthma. Both ALT and AST, which are known indicators of hepatocellular damage, were markedly increased by the oral administration of dexamethasone. However, oral administration of montelukast and skullcapflavone II significantly reduced the serum level of ALT and AST than dexamethasone. This data indicate that montelukast and skullcapflavone II used in the present study had no cytotoxic effect on the mice.

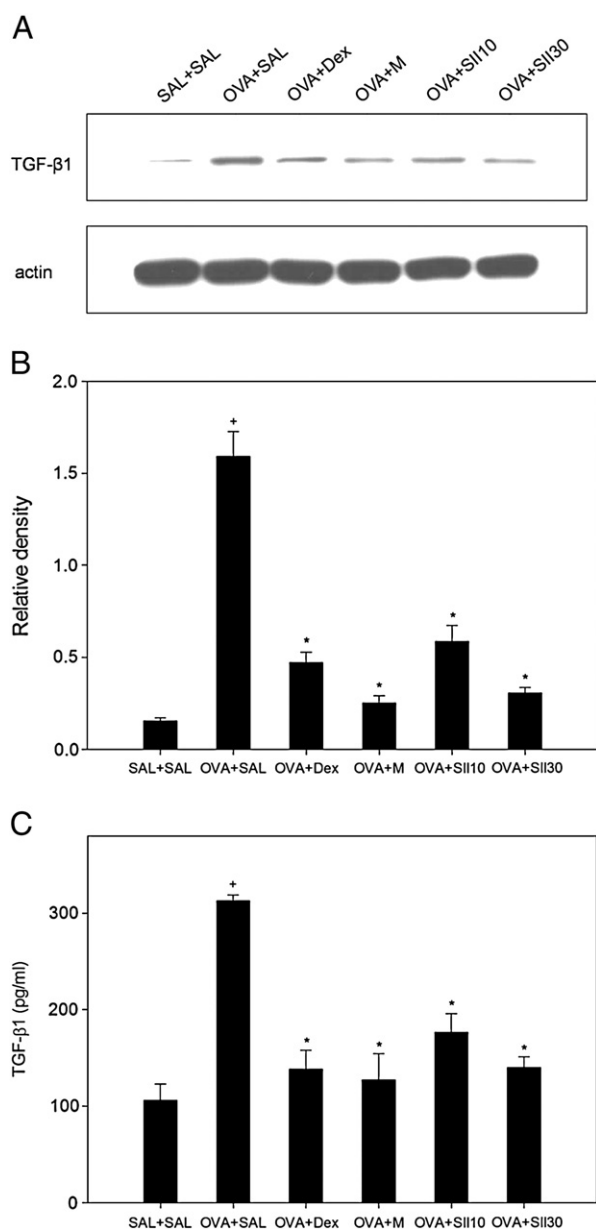


Fig. 9. Effect of skullcapflavone II on TGF- β 1 expression in lung tissues and in BAL fluids. A, Western blotting of TGF- β 1. B, Densitometric analyses are presented as the relative ratio of TGF- β 1 to actin. C, Enzyme immunoassay of TGF- β 1 in BAL fluids. $^+p < 0.05$ vs. SAL + SAL; $^*p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

In conclusion, we herein demonstrate that skullcapflavone II inhibits OVA-induced airway inflammation, AHR, and early stage airway remodeling via decreased production of TGF- β 1 and modulation of its downstream signaling components in the lung. In this experiment both dexamethasone, a glucocorticoid, and montelukast, a leukotriene C4 receptor antagonist, were used as positive controls. Despite of the strong effect of glucocorticoids on anti-inflammation at lung tissue, it has some defects to use for a longer time to asthma patients. In case of montelukast, the efficacy by itself is not so strong as to recover efficiently when treated to asthma patients. The anti-asthmatic effects of skullcapflavone II was comparable to that of montelukast and dexamethasone, widely available anti-asthmatic drugs, hence, skullcapflavone II may have therapeutic potential for the treatment of allergic asthma.

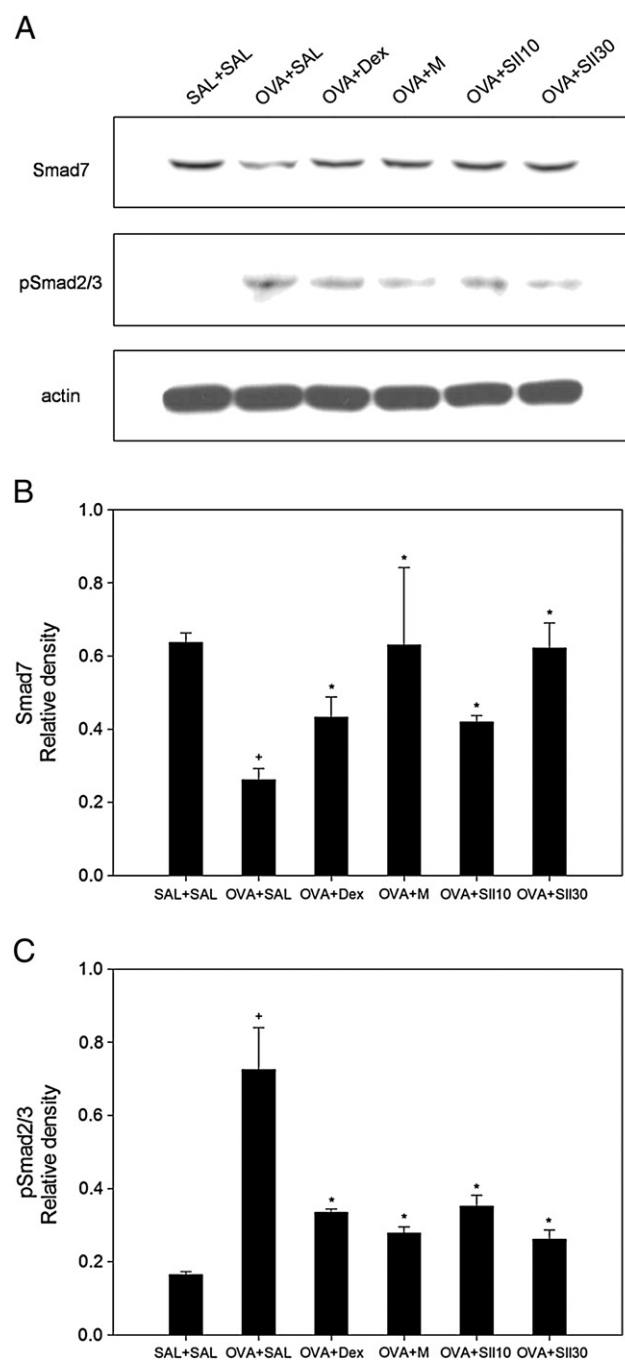


Fig. 10. Effect of skullcapflavone on Smad protein expression. A, Western blotting of pSmad2/3 and Smad7 in lung tissues. B, Densitometric analyses are presented as the relative ratio of pSmad2/3 to actin. C, Densitometric analyses are presented as the relative ratio of Smad7 to actin. $^+p < 0.05$ vs. SAL + SAL; $^*p < 0.05$ vs. OVA + SAL. Results are presented as means $\pm$ SEM; $n = 5$.

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