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S-Allyl cysteine reduces eosinophilic airway inflammation and mucus overproduction on ovalbumin-induced allergic asthma model

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ABSTRACT

S-Allyl cysteine (SAC) is an active component in garlic and has various pharmacological effects, such as anti-inflammatory, anti-oxidant, and anti-cancer activities. In this study, we explored the suppressive effects of SAC on allergic airway inflammation induced in an ovalbumin (OVA)-induced asthma mouse model. To induce asthma, BALB/c mice were sensitized to OVA on days 0 and 14 by intraperitoneal injection and exposed to OVA from days 21 to 23 using a nebulizer. SAC was administered to mice by oral gavage at a dose of 10 or 20 mg/kg from days 18 to 23. SAC significantly reduced airway hyperresponsiveness, inflammatory cell counts, and Th2 type cytokines in bronchoalveolar lavage fluid induced by OVA exposure, which was accompanied by reduced serum OVA-specific immunoglobulin E. In histological analysis of the lung tissue, administration of SAC reduced inflammatory cell accumulation into lung tissue and mucus production in airway goblet cells induced by OVA exposure. Additionally, SAC significantly decreased MUC5AC expression and nuclear factor-κB phosphorylation induced by OVA exposure. In summary, SAC effectively suppressed allergic airway inflammation and mucus production in OVA-challenged asthmatic mice. Therefore, SAC shows potential for use in treating allergic asthma.

1. Introduction

Asthma is a chronic airway inflammatory disease that exhibits airway hyperresponsiveness (AHR), mucus hypersecretion, and airway remodeling [1,2]. Asthma mainly occurs in childhood and its development is associated with genetic and environmental factors [3,4]. Eosinophils are considered as important mediators of asthma development. These cells are recruited by various stimuli such as cytokines, chemokines, and reactive oxygen species produced by allergens, air pollutants, and toxic materials [5,6]. Activated eosinophils induce airway allergic responses such as airway inflammation, mucus secretion, and AHR by releasing large amounts of histamine, serotonin, reactive oxygen species, and proinflammatory mediators via degranulation [5,6]. In a clinical study, eosinophils increased the number of $CD4^+$ cells that produce Th2 type cytokines such as interleukin (IL)-5 and IL-13 as detected in bronchoalveolar lavage fluid (BALF) and mucosal biopsies [7]. IL-5 and IL-13 are essential for the development and maturation of eosinophils. Previous studies reported that suppression of IL-5 production can reduce eosinophilia caused by an allergic challenge and IL-13 is involved in mucus production with MUC5AC [7–9].

Excessive mucus secretion induces plugging of the lower respiratory tract airways, which has been associated with increased morbidity and mortality of asthma patients because of airflow limitation [10,11]. Airway mucus mainly consists of MUC5AC and MUC5B which are involved in the pathogenesis of various respiratory diseases and contribute to increasing airway inflammation [11–13]. In asthmatic conditions, the airway undergoes goblet cell hyperplasia, eventually overproducing MUC5AC in the airway and resulting in airflow limitation [14]. Therefore, inhibition of MUC5AC production may be an effective treatment strategy for controlling asthmatic responses.

S-Allyl cysteine (SAC) is a compound present in garlic extract and

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has long been used as a traditional medicine [15]. SAC has shown many biological effects in various experimental studies. Particularly, SAC exhibited anti-inflammatory properties by down-regulating the nuclear factor (NF)-κB signaling pathway [16–18]. SAC reduced pro-inflammatory cytokines such as tumor necrosis factor-α and IL-6 released by the NF-κB signaling pathway in an acute ethanol-induced gastric damage rat model [15]. Additionally, in an Ultraviolet B (UVB)-induced wrinkle model, SAC decreased induced nitric oxide synthase, cyclooxygenase-2, and matrix metalloproteinase-9 expression via NF-κB signaling [16]. NF-κB plays a central role in regulating inflammatory responses by inhibiting cytokine production and gene expression in asthma models [17–19]. However, no studies have examined the anti-inflammation effects of SAC in an allergic asthma model.

In this study, we examined whether SAC can attenuate inflammation caused by ovalbumin (OVA)-induced asthma. To investigate the therapeutic mechanism of SAC, we evaluated the inflammatory cell count, AHR, cytokine production, protein expression, and histopathology.

2. Materials and method

2.1. Animals

Specific pathogen-free BALB/c mice (female, 19–21 g, 6–8 weeks old) were purchased from Koatech Co. (Pyeongtaek, Korea). Mice were maintained in groups ($n = 8$ per group) under controlled standard conditions (12-h-light/dark cycle, humidity 55 ± 5%, temperature 22 ± 2 °C) with free access to food and water. Mice were randomly divided into 5 groups: normal control group (NC; PBS challenge + non-treated group), OVA group (OVA; OVA challenge + PBS treatment), dexamethasone-treated group (DEX; OVA challenge + 3 mg/kg dexamethasone treatment), and SAC treated group (SAC10 and 20; OVA challenge + 10 and 20 mg/kg SAC treatment, respectively). The experimental design was approved by the Institutional Animal Care and Use Committee of Chonnam National University.

2.2. Experimental allergic asthma model and drug treatment

To prepare the experimental allergic asthma model, mice were immunized via intraperitoneal injection of OVA (20 μg/mouse) with aluminum hydroxide (2 mg/mouse) on days 0 and 14. On days 21–23, mice exposed 1% (w/v) OVA solution for 1 h using a nebulizer (Omron, Tokyo, Japan). SAC was supplied by Namhae Garlic Research Institute. SAC (10 and 20 mg/kg) and dexamethasone (3 mg/kg) were administrated to animals by oral gavage 1 h before the OVA challenge on days 18–23. AHR was measured following methacholine aerosolization for 3 min by whole-body plethysmography (Allmedicus, Seoul, Korea) on day 24. Methacholine doses were 0, 5, 10, and 15 mg/mL in phosphate-buffered saline (PBS). The results are shown as the dimensionless parameter enhanced pause (Penh). The BALF, serum, and lung tissue were collected 24 h after AHR.

2.3. BALF and serum analysis

BALF was obtained via tracheostomy and ice-cold PBS (0.7 mL) was infused into the lung twice (total volume of 1.4 mL). Total inflammatory cell numbers were determined with a hemocytometer and cells were counted in at least five squares. The differential cell count was determined using Diff-Quik staining reagent (Sysmex, Kobe, Japan) according to the manufacturer's instructions. Cytokine levels in the BALF were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols. Serum was obtained from blood collected from the caudal vena cava. Total Ig E and allergen-specific Ig E levels in the serum were measured by ELISA.

2.4. Western blot

The lung tissues were homogenized and protein concentration was determined using Bradford reagent (Bio-Rad, Hercules, CA, USA). The proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were incubated with 5% skim milk blocking solution for 1 h and then exposed to primary antibodies overnight at 4 °C. The following primary antibodies and dilutions were used: anti-β-actin (1:2000 dilution; Cell Signaling, Danvers, MA, USA), anti-pNF-κB (1:1000 dilution; Abcam, Cambridge, UK), anti-NF-κB (1:1000 dilution; Abcam), and anti-MUC5AC (1:1000 dilution; Abcam). The membranes were incubated with horseradish peroxidase-conjugated secondary goat IgG for 1 h. The bands were visualized using an ECL kit using ChemiDoc (Bio-Rad).

2.5. Histological study

After obtaining the BALF, lung tissues were obtained and fixed in 10% (v/v) paraformaldehyde. Lung tissues were embedded in paraffin, section to 4 μm, and stained with hematoxylin and eosin solution and periodic acid-Schiff to estimate inflammation and mucus production.

For protein expression measurement, lung tissues were subjected to immunohistochemistry analysis. Slides were deparaffinized, dehydrated, and washed in 0.05% Tween 20 in PBS. Washed slides were blocked to prevent nonspecific staining using goat serum for 20 min incubation at room temperature. After nonspecific staining, the slides were incubated with 1:200 diluted primary mouse anti-mouse MUC5AC antibody for 2 h at room temperature. The slides were washed and incubated with a biotinylated secondary antibody and biotin-peroxidase complex for 1 h at room temperature. Incubated slides were washed and incubated with diaminobenzidine for an additional 5 min. Stained slides were examined using a microscope.

2.6. Statistical analysis

The data are expressed as the means ± standard deviation (SD). Statistical significance was determined by analysis of variance followed by a multiple comparison test with Dunnett's adjustment. P values < 0.05 were considered significant.

3. Results

3.1. SAC attenuated AHR in OVA challenge mice

The OVA-challenged group showed a greater increase in AHR than the NC group with increasing methacholine concentrations (Fig. 1). The dexamethasone-treated group showed lower AHR compared to the OVA-challenged group. Additionally, the SAC treatment groups showed reduced AHR compared to the OVA-challenged group. Specifically, the SAC treatment group showed significantly decreased AHR compared to the OVA-challenged group with 40 mg/mL methacholine.

3.2. SAC reduces inflammatory cells in OVA challenge mice

The OVA-challenged group showed increased inflammatory cells, particularly eosinophils and macrophages, in the BALF compared to the NC group (Fig. 2). The dexamethasone-treated group showed reduced inflammatory cell counts compared to the OVA-challenged group. Additionally, the SAC-treated groups showed significantly reduced inflammatory cell counts compared to the OVA-challenged group.

Consistent with the results of inflammatory cell counting, the OVA-challenge group showed elevated inflammatory cell infiltration into the lung tissue compared to the NC group. The dexamethasone-treated group showed reduced inflammatory cell infiltration into the lung tissue compared to the NC group. Additionally, the SAC-treated groups

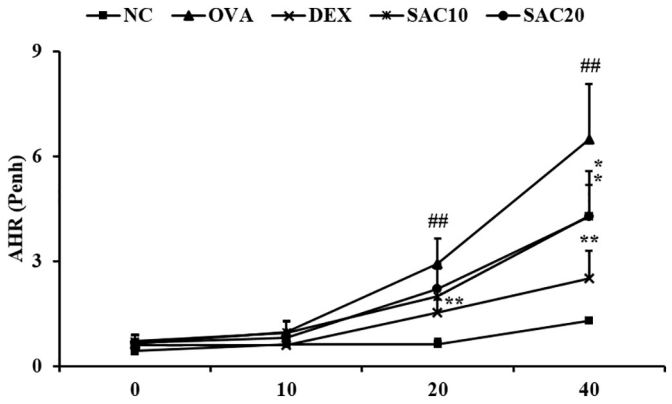

Fig. 1. SAC decreased airway hyperresponsiveness. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

showed effective attenuation of inflammatory cell infiltration induced by OVA challenge (Fig. 3).

3.3. SAC decreases Th2 type cytokines and Ig E levels in BALF and serum

IL-5 and IL-13 levels in the BALF were elevated in the OVA-challenged group compared to in the NC group (Fig. 4). The dexamethasone-treated group showed decreased cytokine levels compared to the OVA-challenged group. The SAC-treated group showed significantly reduced levels of cytokines induced by OVA challenge. Additionally, the OVA-challenged group showed elevated Ig E and OVA-specific Ig E in the serum compared to the NC group. However, the dexamethasone-treated group showed lower levels of Ig E and OVA-specific Ig E in the serum compared to the OVA-challenged group (Fig. 5). The SAC-treated groups showed decreased Ig E and OVA-specific Ig E in the serum-induced by OVA challenge. Particularly, 20 mg/kg of SAC markedly reduced Ig E and OVA-specific Ig E levels in the serum compared to in the OVA-challenged group.

3.4. SAC reduced p65 phosphorylation and MUC5AC expression in lung tissue challenged by OVA

Increased the phosphorylation of p65 was observed in the lung tissue of the OVA-challenged group compared to the NC group (Fig. 6A). However, in the SAC-treated group, phosphorylation of p65 in the lung tissue was significantly decreased compared to in the OVA-challenged group. In addition, MUC5AC expression was increased in the OVA-challenged group compared to in the NC group. In contrast, the SAC-treated groups showed reduced MUC5AC expression in the lung tissue induced by OVA challenge (Fig. 6B).

3.5. SAC decreased mucus production and MUC5AC activity in OVA challenge mice

The OVA-challenged group showed markedly increased mucus production compared to the NC group (Fig. 7). However, the dexamethasone-treated group showed decreased mucus production induced by OVA challenge. Additionally, the SAC-treated groups showed reduced mucus production compared to the OVA-challenged group. Consistent with the results of periodic acid-Schiff staining, MUC5AC expression in the lung tissue and BALF was increased in the OVA-challenged group compared to in the NC group (Fig. 8A and B). However, the SAC-treated groups showed decreased MUC5AC expression in the lung tissue and BALF induced by OVA challenge.

4. Discussion

Allergic asthma is a chronic inflammatory airway disease that afflicts approximately 300 million people worldwide [20]. Many studies have attempted to develop new therapeutic agents for asthma. In this study, we evaluated the therapeutic effects of SAC on allergic inflammatory responses in a murine model of OVA-induced asthma. SAC treatment inhibited the elevation in inflammatory cell counts in the BALF and reduced AHR in OVA-challenged mice. SAC also suppressed the increased IL-5, IL-13, Ig E, and OVA-specific Ig E levels induced by OVA challenge. In histological analysis, SAC decreased inflammatory cell infiltration into the lung tissue and mucus production with decreased MUC5AC expression in OVA-challenged mice. Additionally, SAC suppressed p65 phosphorylation induced by OVA challenge.

In allergic asthma, eosinophilia is considered as an important feature and is associated with the production of Th2 cytokines and allergen-specific Ig E [21]. Exposure to various factors such as allergens, air pollutants, toxic chemicals, and infection agents stimulate alveolar macrophages, airway epithelial cells, and dendritic cells [22,23]. These responses activate T cells, B cells, smooth muscle cells, and goblet cells [24]. Th2 cytokines such as IL-5 and IL-13 are involved in the progression of immune signal transduction and induce the production of Ig

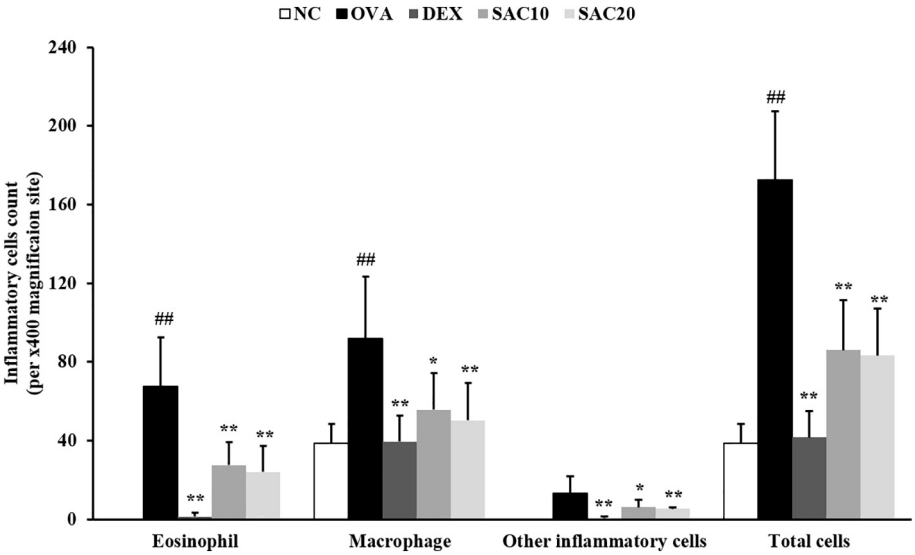

Fig. 2. SAC decreased inflammatory cell counts in the BALF. (A) Total inflammatory count, (B) eosinophil count. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values are shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

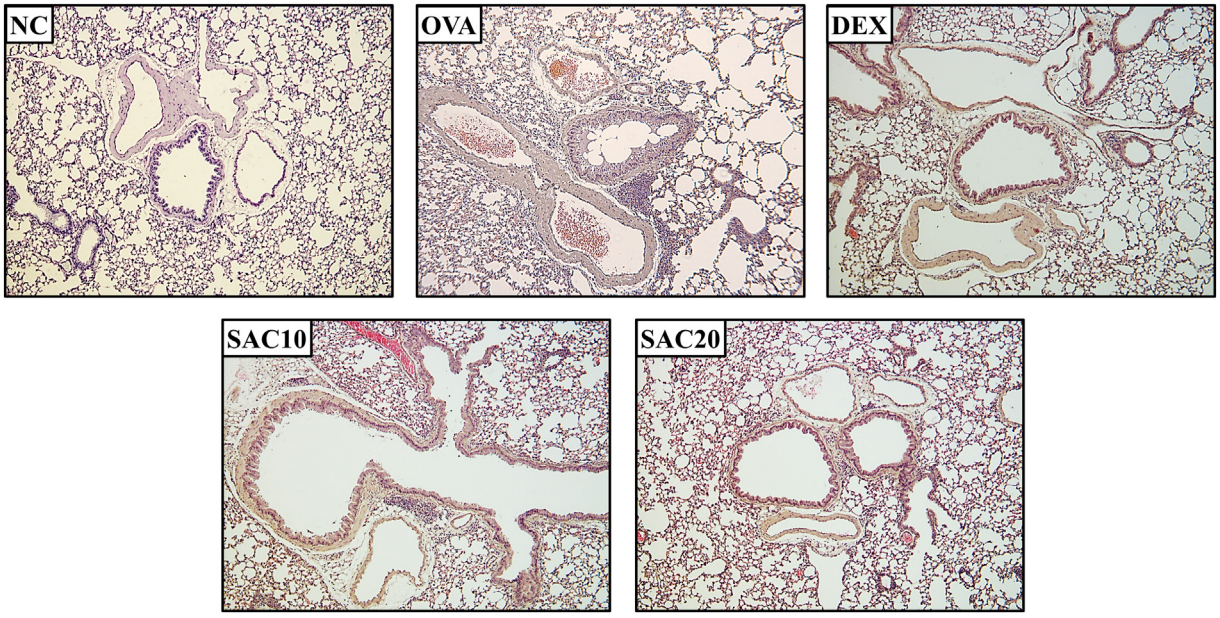

Fig. 3. SAC decreased inflammatory cell infiltration detected by hematoxylin and eosin staining. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge.

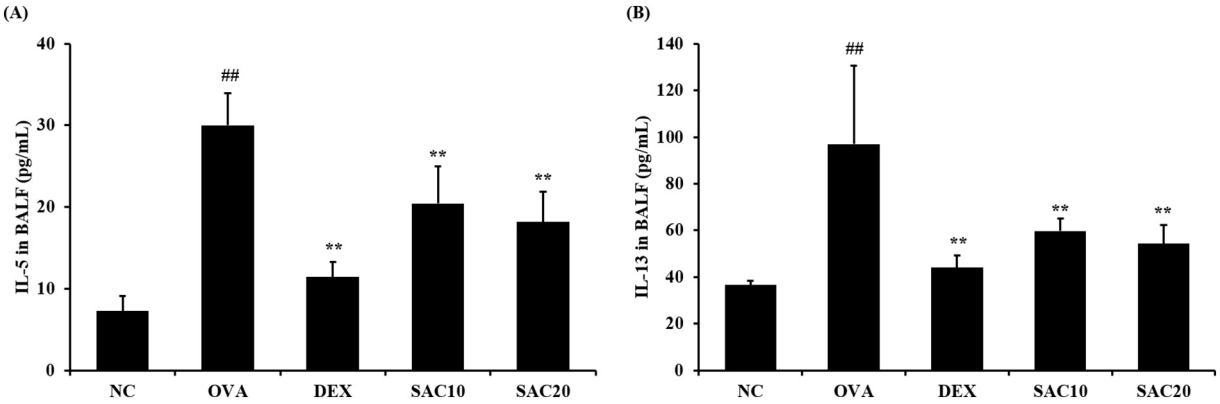

Fig. 4. SAC reduced Th2 cytokines in the BALF. (A) Measurement of IL-5, (B) Measurement of IL-13. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values are shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

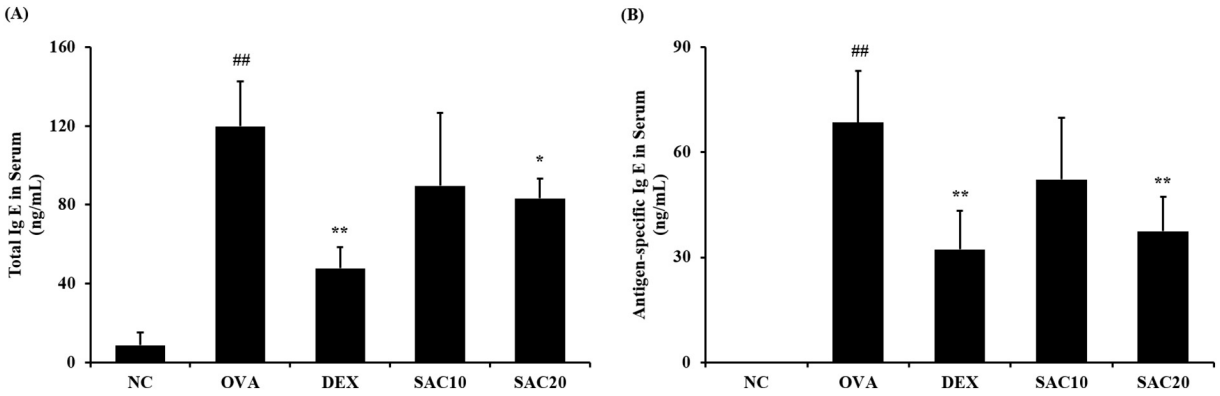

Fig. 5. SAC inhibited Ig E production in the serum. (A) Total Ig E production, (B) allergen-specific Ig E production. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values are shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

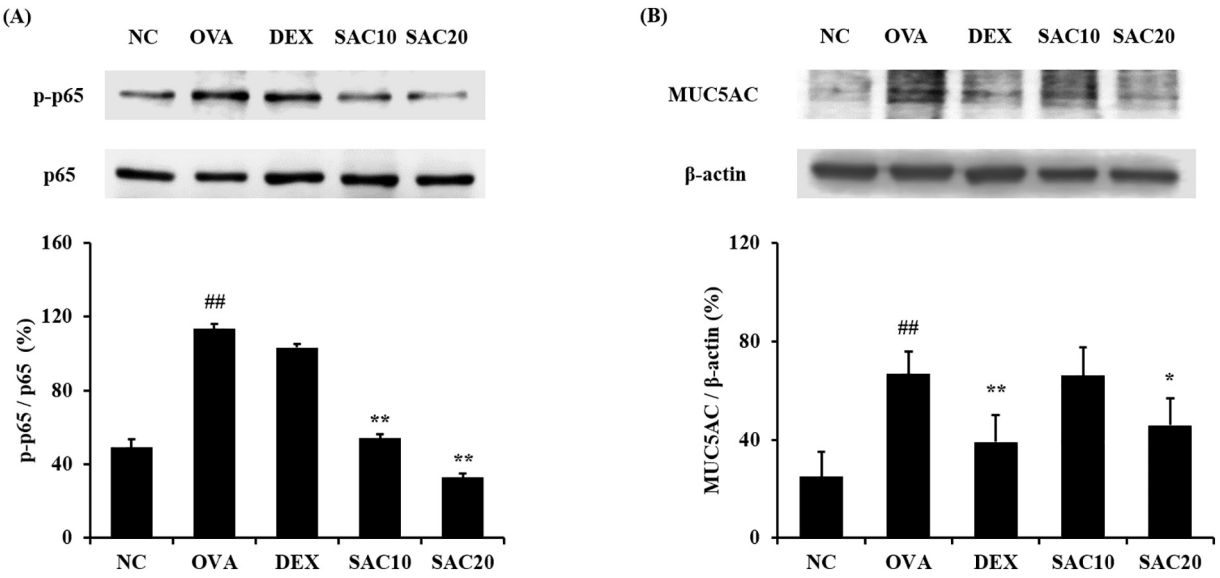

Fig. 6. SAC reduced p65 phosphorylation and MUC5AC expression in lung tissue. (A) p65 phosphorylation, (B) MUC5AC expression. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values are shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

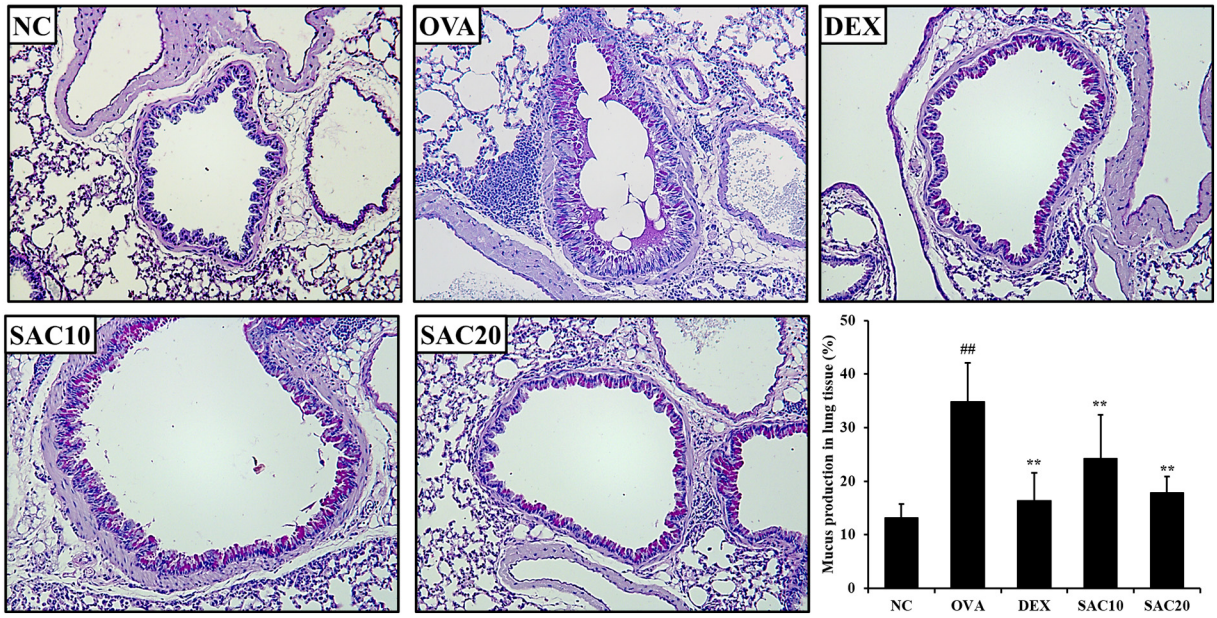

Fig. 7. SAC attenuated mucus production in periodic acid-Schiff staining. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values are shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

E and eosinophilia [25,26]. In this study, SAC treatment significantly inhibited dose dependently the elevation of inflammatory cells, IL-5 and IL-13 in the BALF induced by OVA challenge and reduced Ig E and OVA-specific Ig E levels in the serum. These results indicate that the anti-asthmatic effects of SAC are associated with reduced production of Th2 cytokines.

Th2 cytokines are closely involved in AHR and mucus production [27]. Th2 cytokines activate mast cells and eosinophils, which produce autacoids such as histamine and leukotrienes [28]. These mediators lead to contraction of airway smooth muscle, resulting in AHR [29–31]. Additionally, Th2 cytokines elevate the mucus production of airway goblet cells by activating inflammatory signaling pathway molecules such as mitogen-activated protein kinases, NF-κB, and signal transducer and activator of transcription [32]. MUC5AC is a major component of airway mucus in the respiratory tract layer [33]. During asthma development, MUC5AC is markedly increased in goblet cells by elevated Th2 cytokines, leading to airway limitation [34,35]. In this study, SAC significantly reduced AHR and mucus production, which may be closely related to decreases in Th2 cytokine production.

Activation of NF-κB signaling is regarded as an important factor in inducing allergic responses such as airway inflammation and mucus production [36–38]. Phosphorylation of NF-κB leads to its translocation into the nucleus and transcription of inflammatory proteins and MUC5AC by binding to promoter regions as a transcription factor [36]. NF-κB aggravates allergic responses in asthma via these reactions [38]. Thus, suppressing NF-κB phosphorylation is a potential therapeutic

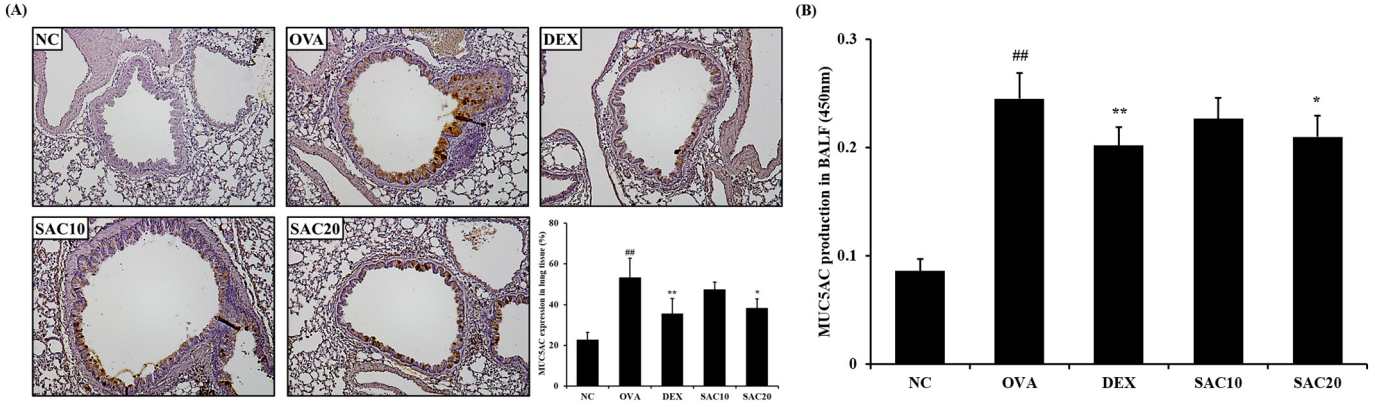

Fig. 8. SAC reduced MUC5AC expression and production. (A) MUC5AC activity in lung tissue. (B) Measurement of MUC5AC production in the BALF. NC: non-treatment and non-OVA challenge; OVA: OVA challenge; DEX: dexamethasone 3 mg/kg per day and OVA challenge; SAC10: S-allyl cysteine 10 mg/kg per day and OVA challenge; SAC20: S-allyl cysteine 20 mg/kg per day and OVA challenge. The values are shown as the means ± SD. $^{\#}P < 0.05$ vs NC; $^{\#\#}P < 0.01$ vs NC; $^{*}P < 0.05$ vs OVA; $^{**}P < 0.01$ vs OVA.

target for treating asthma. In this study, OVA-challenged mice showed increased phosphorylation of p65 and increased AHR and MUC5AC expression. However, SAC treatment effectively inhibited dose dependently p65 phosphorylation, resulting in reduced AHR and MUC5AC expression. These results indicate that SAC protects against allergic responses via decreases in NF-κB pathway activities.

Garlic is a common food consumed widely worldwide and is used as a folk medicine for curing various diseases [39]. SAC is one of the major organic garlic compounds and exerts anti-inflammatory and anti-oxidant effects [40]. SAC alleviates inflammatory mediators such as inflammatory cytokine, nitric oxide, matrix-metalloproteinase, and cyclooxygenase-2 [39–41]. The properties of SAC are closely associated with inhibition of NF-κB activation [40]. Therefore, our results are strongly supported by those of previous studies.

In conclusion, SAC suppressed the increase in allergic lung inflammatory responses in an OVA-induced allergic asthma model. SAC effectively inhibited elevations in Th2 cytokines, IgE, inflammatory cell counts, AHR, and mucus production, which were associated with suppressed NF-κB activation. Thus, our results indicate that SAC can be used as a protective agent to alleviate allergic asthma.

Acknowledgements

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