

Saikosaponin A ameliorates nasal inflammation by suppressing IL-6/ROR- γ t/STAT3/IL-17/NF- κ B pathway in OVA-induced allergic rhinitis

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ABSTRACT

Allergic rhinitis (AR) is a type I hypersensitivity immune response and is a common chronic allergic respiratory disorder characterized by one or more nasal symptoms. Despite many studies on AR therapy, the drugs of treatment for AR remain limited in effect. In the present study, we aimed to elucidate the effects of saikosaponin A (SSA) on nasal inflammation, T helper (Th)2 and Th17 cytokines, retinoic acid-related orphan nuclear receptor (ROR)- γ t, signal transducer and activator of transcription (STAT)3, and nuclear factor (NF)- κ B signalings in ovalbumin (OVA)-induced AR mice model. OVA-induced AR mice exhibited increase in nasal symptoms, histological alteration, OVA-specific immunoglobulin (Ig)E/IgG1, ROR- γ t, STAT3 and NF- κ B signalings. However, the administration of SSA significantly decreased allergic symptoms including nasal rubbing and sneezing. Additionally, histological alterations such as mucosa layer thickness, goblet cell hyperplasia, eosinophils and mast cell infiltration in nasal tissues dramatically improved by treatment with SSA. Also, SSA treatment decreased the levels of OVA-specific IgE/IgG1 in serum and the levels of Th2 and Th17 cytokines such as interleukin (IL)-6 and IL-17 in nasal lavage fluid (NALF). Moreover, SSA inhibited the activation of transcription factor ROR- γ t, STAT3 and phosphorylated STAT3 in both NALF and lung. Further, SSA could also significantly inhibit the expressions of NF- κ B p65 and phosphorylated NF- κ B p65 in NALF and lung. These present results suggested that SSA may attenuate OVA-induced allergic rhinitis through regulating the expression of IL-6/ROR- γ t/STAT3/IL-17/NF- κ B signaling. The results indicate that SSA may be used as a therapeutic candidate for allergic rhinitis disease.

1. Introduction

Allergic rhinitis (AR) is one of the most common chronic allergic respiratory disorders worldwide that usually coexists with asthma. The pathological and mechanistic characteristics are similar to those of allergic asthma [1]. AR is an IgE-mediated inflammation typified by one or more nasal symptoms including sneezing, itching, rhinorrhea and nasal blockage [2,3]. The Korean international studies of asthma and allergies indicate that the prevalence of AR in children was 20.8% and most of them are suffering from atopic allergy, allergic conjunctivitis and eczema as well [4]. These inflammatory symptoms are triggered after allergen-specific IgE and T cells recognize inhalant allergen in the environment, and its inflammatory process involves many different inflammatory cells such as eosinophils and mast cells, cytokines, and

other regulatory molecules [5]. And then IgE-stimulated mast cells and inflammatory cells secrete allergic mediators such as histamine, interleukin (IL)-1 β , IL-4, IL-6, and tumor necrosis factor (TNF)- α [6]. An increase in IL-1 β , IL-6, and TNF- α indicates the presence of inflammation [7,8]. These cytokines play serious roles in the inflammatory responses, along with the activation of T cells and the improvement of B cell survival [9]. IL-6 can induce naive CD4⁺ T cells into T-helper (Th) 17 cells by strengthening the expression of signal transducer and activator of transcription-3 (STAT-3) [10,11]. Both IL-6 and STAT3 have recently emerged as major regulators in the differentiation and function of Th17 cells, via a positive loop enhancing expression and/or activation IL-6 itself, IL-17 and STAT3 [12]. In addition, retinoic acid receptor-related orphan receptor (ROR)- γ t is crucial to the differentiation of Th17 cells. Th17 cells produce IL-17, IL-21 and

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Abbreviation

AR	allergic rhinitis
Dex	dexamethasone
ELISA	Enzyme-linked immunosorbent assay
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
I.P.	intraperitoneal
HE	hematoxylin and eosin
NALF	nasal lavage fluid
NF-κB	Nuclear factor-kappaB

OVA	ovalbumin
PAS	periodic acid-Schiff
p-NF-κB	phosphorylated NF-κB
P.O.	per oral
p-YSTAT3	tyrosine phosphorylated STAT3
ROR-γt	retinoic acid-related orphan nuclear receptor-γt
SSA	Saikosaponin A
STAT3	signal transducer and activator of transcription 3
TB	toluidine blue
Th	T-helper
TNF	Tumor necrosis factor

IL-22 which play a role in mucosal inflammation [13] and are responsible for triggering the nuclear factor (NF)-κB [14]. NF-κB regulates many cellular pathways and processes, including inflammation, immune response, apoptosis, and proliferation. Persistent activation of NF-κB has been observed in allergic airway inflammation [15,16]. NF-κB regulates the differentiation of CD4⁺ T cells and the transcriptional process of Th2 cytokines IL-4 and IL-13 [17]. Targeted inhibition of the NF-κB signaling pathway could suppress the airway inflammation and the production of Th2 cytokines in ovalbumin (OVA)-sensitized/challenged mice [18,19].

Even though various agents including antihistamine, corticosteroids, nasal decongestants and mast cell stabilizers are currently used to treat AR [20], the rates of side effects and recurrence are increasing by these drugs treatment for a long time [21]. Consequently, needs to new or alternative approaches to improve allergic symptoms are becoming an increase and complementary or alternative medicines have got an increasingly appealing component of standard medical care in Western and Oriental countries. It has been recognized natural products as sources of new drugs and demonstrated their pharmacological effects during decades of research [22]. Also, drugs from natural products have been known to cause less side effects than other synthetic drugs [23].

Bupleurum falcatum is well known for the protective effects of neurotoxicity and neuroinflammation via the regulation of nuclear receptor-related 1 protein or NF-κB [24,25]. In recent years, several different saikosaponins have been isolated from *Radix Bupleuri* [26]. Saikosaponin A (SSA, PubChem CID: 4483645, Fig. 1) is one of the triterpenoid saponins and the most important active saikosaponins purified from *Bupleurum falcatum* [27], which induces apoptotic mechanisms in human breast cancer cell lines [28–30]. *In vivo*, SSA has been reported to protect against experimental sepsis via inhibition of NOD2-mediated NF-κB activation [31] and to play a critical anti-inflammatory effect through inhibition of NF-κB and NLRP3 signaling pathways in LPS-induced acute lung injury [32]. Although SSA is appeared as a new therapeutic agent to target inflammatory process [33], the inhibitory effect of SSA on the IL-6/ROR-γt/STAT3/IL-17/NF-κB pathways in OVA-induced AR is not well understood. So, we examined the effects of SSA on the nasal mucosa inflammation and the productions of Th2 and Th17 cytokines in OVA-induced AR mice. We next investigated the effect of SSA on the serum levels of OVA-specific IgE/IgG, and the activation of ROR-γt, STAT3 and NF-κB pathways in OVA-induced AR mice.

2. Materials and methods

2.1. Materials

Compound 48/80, OVA (grade VI), and SSA were purchased from Sigma-Aldrich (St, Louis, MO, USA). Aluminum hydroxide adjuvant was purchased from Pierce (Imject® Alum, Rockford, IL, USA) and dexamethasone (Dex) was purchased from Innovative Research of America (Toledo, Ohio, USA).

2.2. Animals

The 6-week-old inbred male BALB/c male mice were purchased from Damool Science (Daejeon, Korea). These animals were housed for 7 days under standard laboratory conditions before experiments and had freely access to OVA-free food and water. All experiments were performed in accordance with the Guidelines which were approved by the Institutional Animal Care and Use Committee of Chonbuk National University Laboratory Animal Center (CBN 2016–37).

2.3. Induction of allergic rhinitis model

For the experimental group, mice were randomly divided into 5 groups according to treatment: control, OVA, 2 mg/kg body weight (BW)/day SSA, 10 mg/kg BW/day SSA, and 2.5 mg/kg BW/day Dex groups as reference drug for positive control in OVA-induced AR mice. Mice of OVA, 2 mg/kg BW/day SSA, 10 mg/kg BW/day SSA, and 2.5 mg/kg BW/day Dex groups were sensitized on days 0, 7 and 14 by intraperitoneal injection of 50 μg OVA with 1 mg of aluminum hydroxide adjuvant in a total volume of 200 μl, while the control group was injected with alum in PBS on the same schedule. Next, on 5 consecutive days from 21 to day 25, these sensitized mice were intranasally challenged with OVA (10 mg/ml, 20 μl/nostril). On days 15–25, 2 mg/kg BW/day SSA, 10 mg/kg BW/day SSA and 2.5 mg/kg BW/day Dex groups were also orally treated once daily with SSA or Dex (Fig. 2A), while OVA group was received the only saline by oral administration. Animals were sacrificed 24 h after the last challenge, and nasal tissues and NALF were obtained for analysis to investigate the inhibitory effect of SSA on OVA-induced AR.

2.4. Clinical symptoms

Nasal symptoms were evaluated by counting the frequencies of nasal rubbing and sneezing. After 400 μg of OVA intranasal challenge on days 21–25, the numbers of sneezing and nasal rubbing were

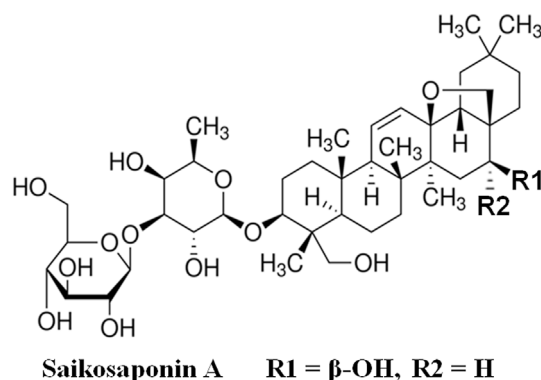


Fig. 1. Chemical structure of Saikosaponin A.

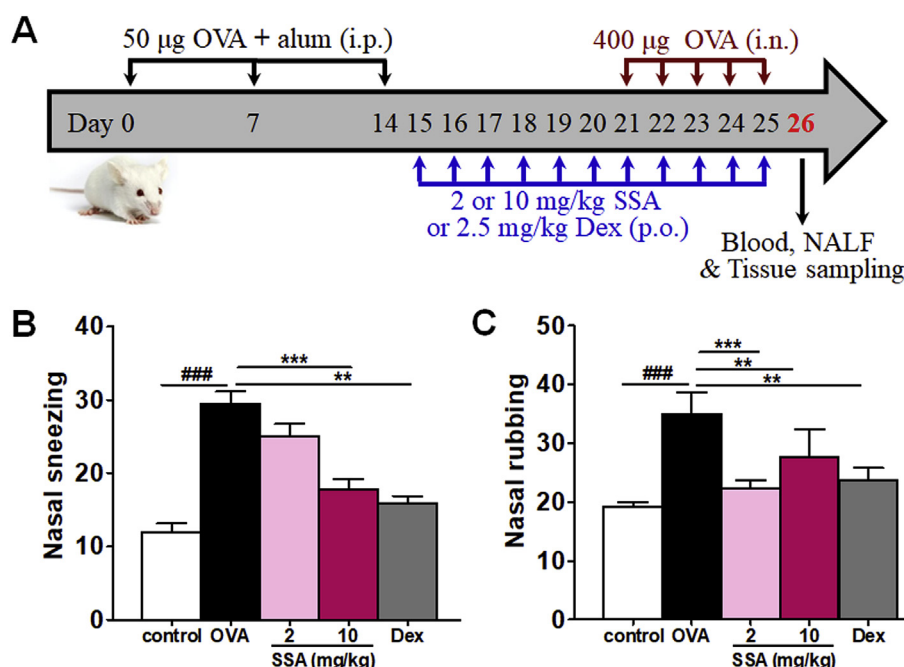


Fig. 2. Experimental protocol for AR and effect of SSA on nasal symptoms of OVA-induced AR mice. (A) Experimental protocol for allergic rhinitis model. The frequencies of (B) total sneezing and (C) nasal rubbing during 5 days for OVA challenge were evaluated. Values represent the mean $\pm$ SD ($n = 6$ per group). ### $P < 0.001$ versus the control group. ** $P < 0.01$, *** $P < 0.001$ versus the OVA group.

recorded during a 20-min period to count by two blinded observers.

2.5. Collection of blood sample

Twenty-four hours after the final OVA challenge, blood specimens were collected from retro-orbital plexus. Serum was obtained by centrifugation $1000 \times g$ for 10 min at 4°C (Centrifuge 5403; Eppendorf, Hamburg, Germany), and then serum was stored at -80°C until used.

2.6. Collection and analysis of nasal lavage fluid (NALF)

After anesthesia, NALF was collected by cannulating the upper part of the trachea into the nasal cavity direction and lavaging with 1 ml saline. Supernatants were collected for measurement of cytokines and stored at -80°C until used. Differential cell counts of NALF were performed after cytopspin (Centrifuge 5403; Eppendorf, Hamburg, Germany) and staining by Diff Quik stain (Sysmex Co., Kobe, Japan) in accordance with the manufacturer's protocols.

2.7. Histopathologic analysis

Tissue samples were immediately fixed in 10% neutral formalin for 2 days at room temperature and decalcified in Calci-Clear Rapid histological decalcifying agent (National Diagnostics, Atlanta, GA) for 3 days and then embedded in paraffin. The section ($4\ \mu\text{m}$ thickness) was stained with hematoxylin-eosin (for general structure), Giemsa (for eosinophils), periodic acid-Schiff (for goblet cells and mucus), and Toluidine blue (for mast cells) solution.

2.8. Measurement of Th1, Th2, Th17 cytokines, OVA-specific IgE and IgG1, NF- κB p65 and p-NF- κB p35, STAT3 and p-YSTAT3, and ROR- γt

The levels of Th1 cytokines (IFN- γ (R&D Systems, MN, USA, Cat. No. MIF00) and IL-12 (R&D Systems, MN, USA, Cat. No. M1270)), Th2-related cytokines (TNF- α (R&D Systems, MN, USA, Cat. No. MTA00B), IL-1 β (R&D Systems, MN, USA, Cat. No. MLB00B), IL-5 (R&D Systems, MN, USA, Cat. No. M5000), IL-6 (R&D Systems, MN, USA, Cat. No. M6000B) and IL-13 (CUSABIO, Hubei, China, Cat. No. CSB-E0462m)), and Th17 cytokine (IL-17A, R&D Systems, MN, USA, Cat. No. M1700) in NALF from each mouse were measured according to the

manufacturer's protocols using the appropriate ELISA. Also, the serum levels of OVA-specific IgE (Biolegend, CA, USA, Cat. No. 439807) and IgG1 (Cayman, MI, USA, Cat. No. 500830) and the levels of NF- κB p65 (Cell signaling, MA, USA, Cat. No. 7174C) and p-NF- κB p35 (Cell signaling, MA, USA, Cat. No. 7173C) in NALF were measured using ELISA kits according to the manufacturer's instructions. In addition, the levels of STAT3 (Lifespan BioSciences, WA, USA, Cat. No. LS-F38419) and tyrosine phosphorylated STAT3 (p-YSTAT3, Invitrogen, CA, USA, Cat. No. 7300CA), and ROR- γt (Finetest, Hubei, China, Cat. No. EM8104) in NALF were measured using ELISA kit.

2.9. Statistical analysis

Results were expressed as the mean $\pm$ standard deviation (SD) for the number of experiments. Statistical analysis was adjusted to Student's t -test and ANOVA test. Significance was considered as P -values of less than 0.05.

3. Results

3.1. Effect of SSA on nasal symptoms of OVA-induced AR mice

Sneezing, itching and/or nasal congestion are the main symptoms of AR [2,3]. To determine the effect of SSA on nasal symptoms, after OVA intranasal challenge on days 21–25, the frequency of sneezing and nasal rubbing behaviors were recorded during 20 min to count by two blinded observers. AR mice induced by OVA showed meaningful increases in sneezing and nasal rubbing compared to control mice (Fig. 2B). SSA oral administration (2 or 10 mg/kg BW/day) tended to reduce the number of sneezing (Fig. 2B). Similar results were also obtained from nasal rubbing (Fig. 2C). SSA treatments considerably improved nasal symptoms dose-dependently compared with those of OVA-induced AR mice (Fig. 2B).

3.2. Effect of SSA on the infiltration of eosinophils and other inflammatory cells in NALF of OVA-induced AR mice

To investigate the underlying mechanism of SSA's inhibitory effect on nasal symptoms of OVA-induced AR mice, the number of total cells and differential inflammatory cells in NALF were counted. OVA-

induced AR mice exhibited the increases in total cells and inflammatory cells in NALF than the control group (Fig. 3A). However, Oral administration of 10 mg/kg BW/day SSA or 2 mg/kg BW/day Dex remarkably decreased the number of total cells, eosinophils, other inflammatory cells, detached epithelial cells in NALF (Fig. 3B).

3.3. Effect of SSA on histopathological changes of nasal mucosal tissues and lungs of OVA-induced AR mice

To confirm the inhibitory effect of SSA on nasal symptoms and inflammatory cell infiltration in NALF of OVA-induced AR mice, we examined the histopathological changes of nasal mucosal tissues including mucosal thickness, eosinophil and mast cell infiltration and goblet cell hyperplasia from mice of each group. As shown in Fig. 4A, histopathological analysis exhibited OVA challenges caused remarkably histopathological alterations (Fig. 4A) such as epithelial disruption, interstitial edema (Fig. 4B), infiltration of inflammatory cells including eosinophils (blue arrows, Fig. 4A,C), goblet cell hyperplasia (black arrows, Fig. 4A,D) and mast cells (red arrows, Fig. 4A,E), and patchy hemorrhage. Also, the mucosal thickness of nasal septum increased in OVA-induced AR mice compared to control mice. In contrary, SSA oral administration notably mitigated these histopathological changes in a dose-dependent manner, such as SSA treatment improved epithelial disruption and interstitial edema (Fig. 4A and B), and inhibited the infiltration of eosinophils (Fig. 4C) and the hyperplasia of goblet cells (Fig. 4D) and mast cells (Fig. 4E). These improved effects were more obviously in 10 mg/kg BW/day of SSA.

3.4. Effect of SSA on the serum levels of OVA-specific IgE and IgG1 of OVA-induced AR mice

IgE and other immunoglobulin classes such as IgG1 play pivotal roles in basic regulatory mechanisms of allergic inflammation have recently stimulated research into the therapeutic potential of directly targeting these antibodies [34,35]. In order to investigate the effect of SSA on the progression of allergic reactions, we examined the serum levels of OVA-specific IgE and IgG1 in OVA-induced AR mice. As shown in Fig. 5, both levels of OVA-specific IgE and IgG1 in OVA-induced AR mice were significantly higher than those in mice of control group. However, SSA oral treatment significantly inhibited OVA-specific IgE (Fig. 5A) and IgG1 productions (Fig. 5B).

3.5. Effect of SSA on the levels of Th1, Th2 and Th17 cytokines in NALF of OVA-induced AR mice

To identify the regulatory effect of SSA on the Th1/Th2/Th17

immune responses, the levels of secreted cytokines (TNF- α , IL-4, IL-5, IL-6, IL-13, IL-17, IFN- γ and IL-12) in NALF were assessed. AR mice induced by OVA showed increased levels of TNF- α (Fig. 6A), IL-5 (Fig. 6B) and IL-13 (Fig. 6C) in NALF, respectively. However, SSA significantly diminished these cytokine levels (Fig. 6A–C), respectively. AR mice induced by OVA resulted in increasing in IL-17 (Fig. 6D). Treatment with SSA significantly diminished IL-17 production both in NALF (Fig. 6D). In addition, the anti-inflammatory cytokines such as IFN- γ (Fig. 6E) and IL-12 (Fig. 6F) cytokines were assayed. However, there is no significance between them (Fig. 6E and F). These results were consistent with the decrease in inflammatory cells infiltrating into both NALF and nasal tissues of SSA-treated mice via modulating the Th2/Th17 cytokines.

3.6. Effect of SSA on activation of ROR- γ t, STAT3 and NF- κ B p65 pathway in NALF of OVA-induced AR mice

Based on the knowledge that NF- κ B plays an important role in allergic inflammation of the lung by inducing the transcription of various pro-inflammatory mediators [36], we hypothesized that SSA would perform protective effect by suppressing NF- κ B activation. To confirm the effects of SSA on NF- κ B pathway, the levels of NF- κ B and p-NF- κ B were performed with NALF. As shown in Fig. 7, the levels of NF- κ B (Fig. 7A) and p-NF- κ B (Fig. 7B) in NALF of OVA-induced AR mice were significantly increased than those of control mice. However, the increases were decreased by treatment of SSA (Fig. 7). Many inflammatory cytokines induced by STAT3 or NF- κ B can provide positive feedback to activate STAT3 and NF- κ B. IL-6 mediated by NF- κ B activates ROR- γ t and STAT3, thereby increasing the production of IL-17. To prove the inhibitory effect of SSA on the activation of ROR- γ t, STAT3 and p-STAT3, the levels of ROR- γ t, STAT3 and p-STAT3 in NALF were checked. The levels of ROR- γ t (Fig. 7C), STAT3 (Fig. 7D) and p-STAT3 (Fig. 7E) in the NALF of OVA-induced AR mice were significantly higher than those of control group. However, increased the levels of STAT3 and p-STAT3 in the NALF were diminished by SSA oral treatment (Fig. 7).

4. Discussion

SSA, a bioactive ingredient isolated from *Bupleurum falcatum*, has been known to exhibit anti-inflammatory effect [37]. SSA inhibited carrageenan-induced paw edema in rats and acetic acid-induced vascular permeability in mice [37]. Furthermore, SSA has been reported to inhibit oxidative stress and inflammation in LPS-stimulated human umbilical vein endothelial cells [38]. However, the inhibitory effect of SSA on the IL-6/ROR- γ t/STAT3/IL-17/NF- κ B pathways in OVA-induced

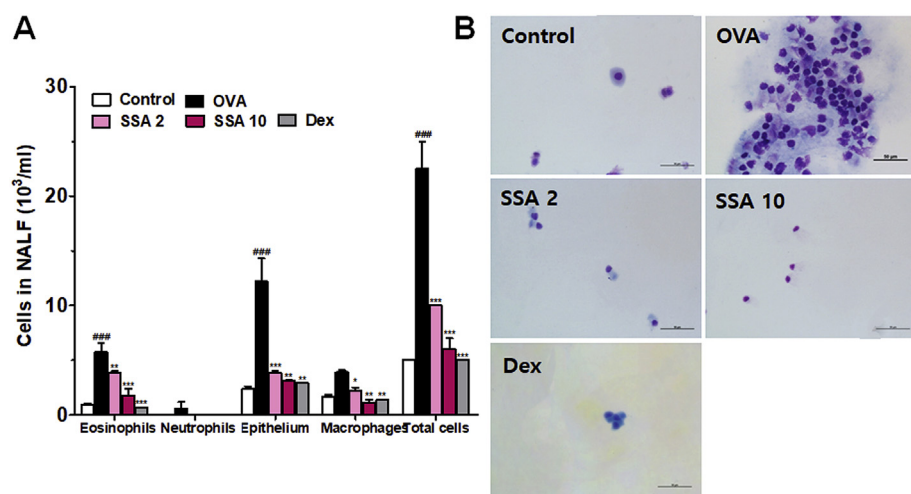


Fig. 3. Effect of SSA on infiltration of inflammatory cells into NALF of OVA-induced AR mice. (A) Total and differential cell numbers were counted using a hemocytometer. (B) Cytospin cell preparations were made by NALF and staining with Diff-Quik. Scal bar = 100 μ m. Values represent the mean $\pm$ SD (n = 6 per group). ###P < 0.001 versus the control group. *P < 0.05, **P < 0.01, ***P < 0.001 versus the OVA group.

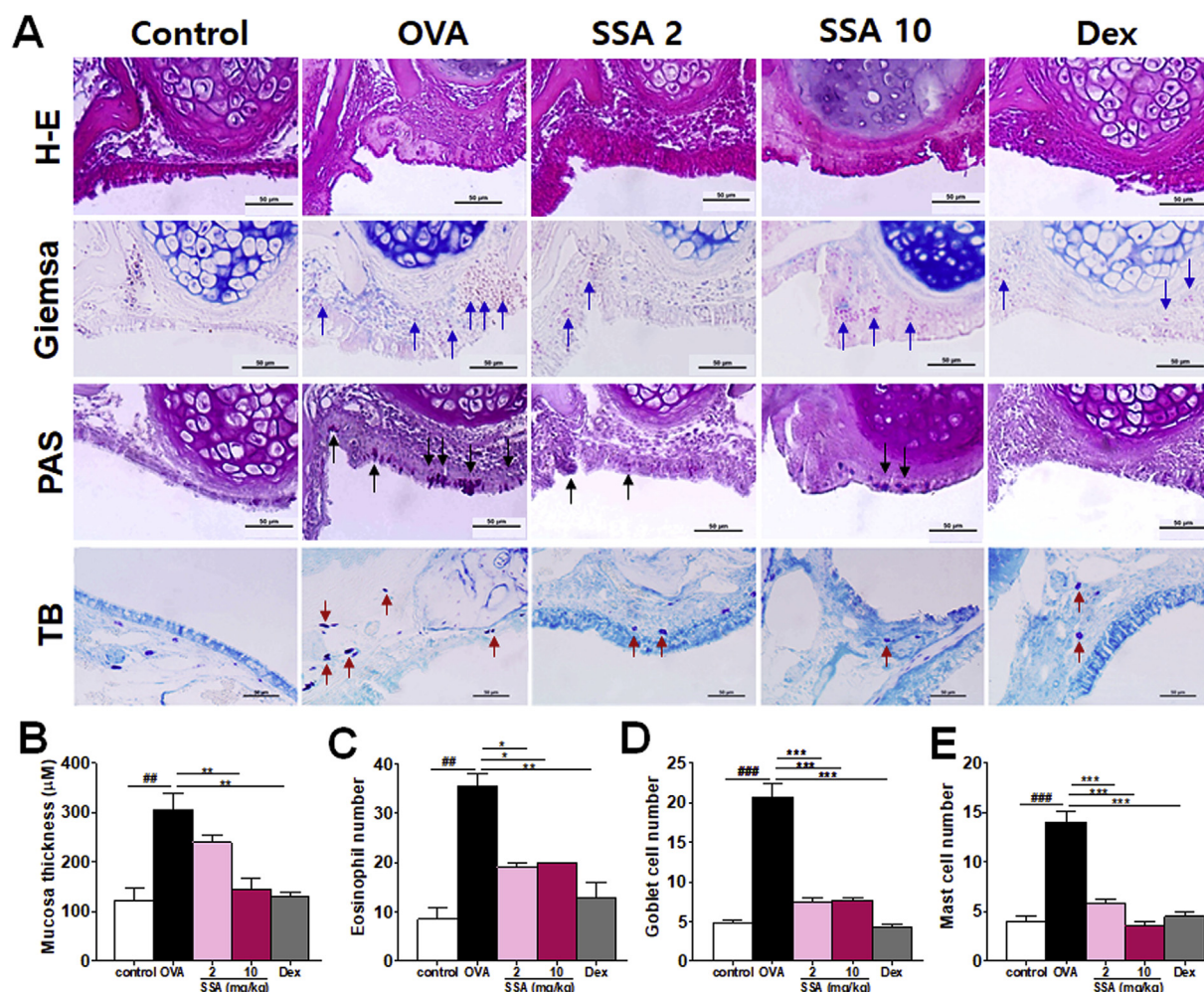


Fig. 4. Effect of SSA on histological changes in nasal and lung tissues of OVA-induced AR mice. (A) The general structure of nasal mucosa, (B) epithelium thickness of nasal mucosa, Hematoxylin-eosin (H-E) staining; (C) Infiltration of the eosinophils (Blue arrows), Giemsa staining; (D) Goblet cells (Black arrows) hyperplasia. Periodic acid-Schiff (PAS) staining; (E) Mast cells (Red arrows) immigration to the epithelium. Toluidine blue (TB) staining; Scale bar = 50 μm in the nasal tissues and Bar = 100 μm in the lungs. Values represent the mean ± SD (n = 6 per group). ##P < 0.01, ###P < 0.001 versus the control group. *P < 0.05, **P < 0.01, ***P < 0.001 versus the OVA group.

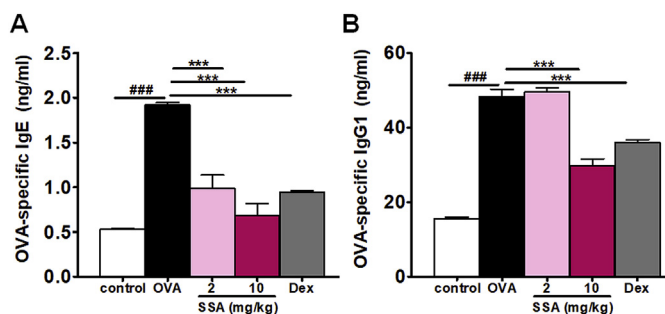


Fig. 5. Effect of SSA on OVA-specific IgE/IgG expressions in OVA-induced AR mice. The serum levels of (A) OVA-specific IgE and (B) OVA-specific IgG1 were measured. Values represent the mean ± SD (n = 6 per group). ###P < 0.001 versus the control group. *P < 0.05, **P < 0.01, ***P < 0.001 versus the OVA group.

AR is not well understood. The aim of this study was to assess the inhibitory mechanism of SSA on nasal inflammation of OVA-induced AR mouse model. In the present study, our results demonstrated that SSA significantly inhibited inflammatory response in OVA-induced AR mice. SSA significantly inhibited the OVA-induced AR immune responses in serum. Moreover, SSA significantly inhibited OVA-induced NF-κB

activation and inflammatory cytokine productions in NALF. Many inflammatory cytokines induced by STAT3 or NF-κB can provide positive feedback to activate STAT3 and NF-κB. IL-6 mediated by NF-κB activates STAT3 and ROR-γt, thereby increasing the production of IL-17. So, dual suppression of STAT3 and NF-κB is attractive treatment strategy to treat inflammatory diseases. These results suggest that SSA may be a potential agent for the treatment of AR.

NF-κB, a nuclear transcription factor, has an intimate relativity with several physiologically and pathologically processes such as inflammatory response, immune reaction, cell proliferation, transformation, and apoptosis [39]. Previously, glucocorticoids were reported to suppress immune inflammation by interacting with NF-κB [40]. Dexamethasone-induced anti-inflammatory responses are associated with the inhibition of NF-κB [41]. Our results had been reported that the NF-κB p65 pathway was activated in the OVA-induced AR model. However, after treatment with SSA, the NF-κB p65 pathway was inhibited, the productions of NF-κB p65 and p-NF-κB p65 were down-regulated along with the production of IL-6 and TNF-α. Therefore, these results supported the fact that the anti-inflammatory effect of SSA is regulated through the inhibition of NF-κB P65 pathway.

Gene transcription of correlated inflammatory cytokines is activated by NF-κB pathway, which is involved in the initiation of inflammatory response through inducing the up-regulation of cytokines including TNF-α, IL-5, IL-6 and IL-13 [42]. A complex cytokine network mediates

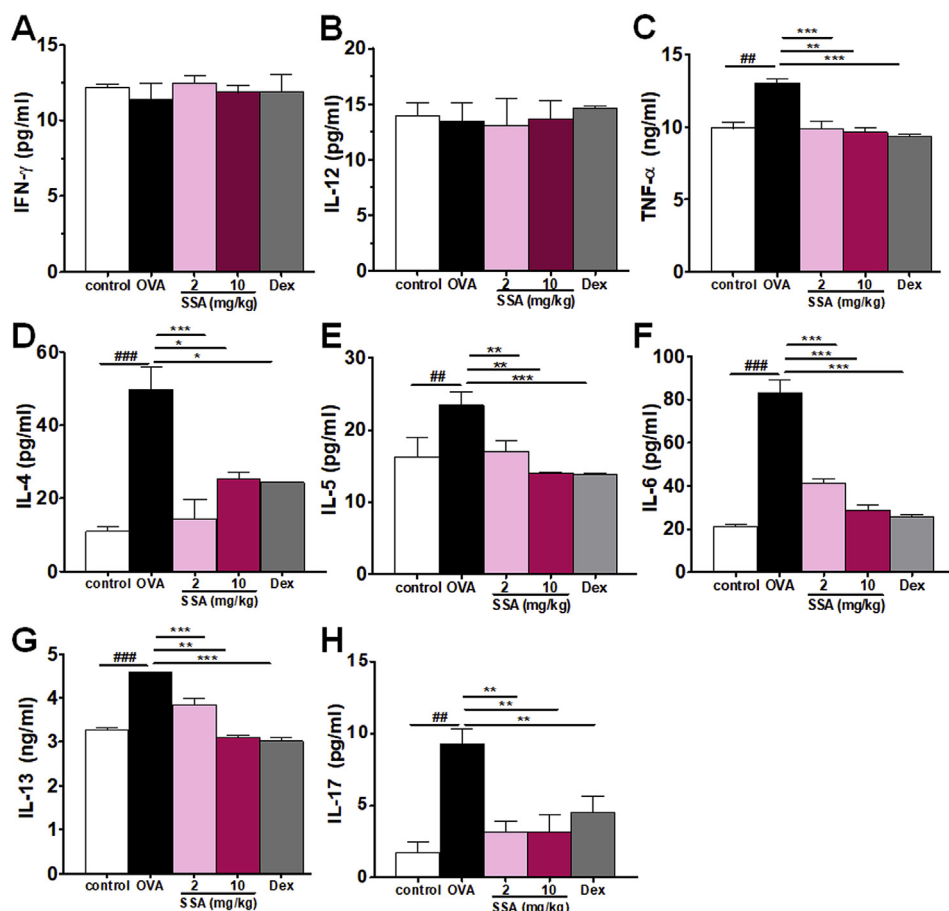


Fig. 6. Effect of SSA on Th1, Th2 and Th17 cytokines levels in OVA-induced AR mice. The levels of Th1 cytokines (A) IFN- γ and (B) IL-12; Th2 cytokines (C) TNF- α , (D) IL-4, (E) IL-5, (F) IL-6 and (G) IL-13; Th17 cytokine (H) IL-17 were determined in NALF. Values represent the mean $\pm$ SD (n = 6 per group). ##P < 0.01, ###P < 0.001 versus the control group. ***P < 0.001 versus the OVA group.

the inflammatory response in OVA-induced AR. IL-6 and TNF- α contained in the granules of mast cell stimulates adaptive factor that stimulates the expression of pro-inflammatory cytokines [43]. In a previous study, SSA inhibited chemical-induced liver inflammation and fibrosis in rats [44]. In our results revealed that the levels of TNF- α , IL-5, IL-6 and IL-13 in NALF were up-regulated in OVA-induced AR group, but the administration of SSA markedly down-regulated these cytokines. The recruitment of eosinophils in allergic rhinitis has been suggested to be initiated by Th2 cytokines [45]. AR is an IgE-mediated

inflammation characterized by the Th2 type of allergic reaction and involves eosinophil infiltration, goblet cell hyperplasia, and mast cell accumulation in the nasal mucosa [46]. The present results demonstrated that inflammatory cells such as mast cells, eosinophils infiltration, and goblet cell hyperplasia were decreased in SSA treated mice as compared with OVA-induced AR mice. The preventing effects of SSA on inflammatory cells infiltration into the nasal tissue may be attributed to the inhibition of Th2 cytokines. These results suggest that SSA exerts an anti-inflammatory effect through the regulation of pro-inflammatory

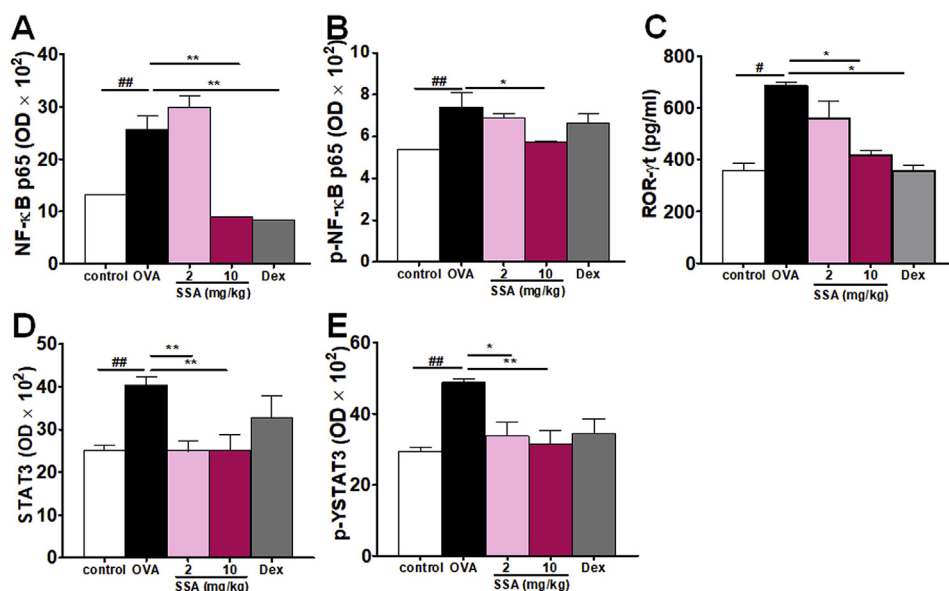


Fig. 7. Effect of SSA on the levels of NF- κ B/ROR- γ t/STAT3 pathway. (A) NF- κ B p65, (B) p-NF- κ B p65, (C) ROR- γ t, (D) STAT3, and (E) p-STAT3 were analyzed in NALF. Values represent the mean $\pm$ SD (n = 6 per group). ##P < 0.01, ###P < 0.001 versus the control group. *P < 0.05, **P < 0.01, ***P < 0.001 versus the OVA group.

cytokine levels.

IL-6 is a pleiotropic cytokine with various biological activities in immune regulation, hematopoiesis, inflammation, and oncogenesis. IL-6 activates two transcription factors, both STAT-3 and ROR- γ t which have been known as direct downstream of IL-6 and major regulators in the differentiation and function of Th17 [47–49]. Interestingly, STAT3-deficient CD8⁺ T cells failed to produce IL-17 [50], and ROR- γ deficient mice have reduced Th17 differentiation and are less susceptible to experimental autoimmune encephalomyelitis, indicating that this transcription factor is important with respect to autoimmunity and inflammation [51]. In present study, the productions of ROR- γ t, STAT3 and p-STAT3 were activated in the NALF of OVA-induced AR model. However, increased the levels of ROR- γ t, STAT3 and p-STAT3 in the NALF were diminished by SSA oral treatment, the productions of ROR- γ t, STAT3 and p-STAT3 were down-regulated along with the productions of IL-6, NF- κ B p65 and p-NF- κ B p65. Therefore, these results supported the fact that the anti-inflammatory effect of SSA is regulated through the inhibition of ROR- γ t/STAT3 pathway.

IL-17 is a key proinflammatory cytokine in the Th17 pathway and contributes to the pathogenesis of such inflammatory diseases as asthma and rheumatoid arthritis [46]. Th17 cells can upregulate Th2 cell-mediated eosinophilic airway inflammation and IL-17 can enhance airway smooth muscle contraction and proliferation, and epithelial permeability of the airways to allergen [52]. Th17-deficient mice displayed less airway hyper-responsiveness and airway remodeling in response to allergen challenge [53]. IL-17 increased mucous expression of airway epithelial cells in human and mouse, and activated mucin 5AC gene expression and protein production through NF- κ B dependent pathway [54]. We found that Th17 responses were greater in AR mice than in control mice, but were down-regulated with SSA treatment, suggesting that SSA can suppress the Th17 reactions.

In summary, the treatment of SSA provides significant inhibitory effect on allergic inflammation of nasal mucosa in OVA-induced AR mice through the regulation of Th2 and Th17 responses and the inhibition of IL-6/STAT3/ROR- γ t and NF- κ B pathway. These findings indicate that SSA can be a viable therapeutic agent in AR suppression.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbi.2019.108874>.

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