



Bamboo salt reduces allergic responses by modulating the caspase-1 activation in an OVA-induced allergic rhinitis mouse model

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ABSTRACT

Bamboo salt (BS) is a specially processed salt according to the traditional recipe using sun-dried salt (SDS) and bamboo in Korea. The present study investigated the effects and mechanism of BS, SDS, NaCl, or mineral mixture (containing zinc, magnesium, and potassium) on ovalbumin (OVA)-induced allergic rhinitis (AR) animal model. The increased number of rbs was inhibited by the oral administration of BS, SDS, NaCl, mineral mixture, or nose inhalation of BS. The increased levels of IgE, histamine, and interleukin (IL)-1 β in serum were reduced by BS. The level of interferon- γ was increased, whereas the level of IL-4 was reduced on the spleen tissue of BS-treated mice. In the BS-treated mice, the number of eosinophils and mast cells infiltration increased by OVA-sensitization were also decreased. Protein levels of inflammatory cytokines were reduced by BS or NaCl administration in the nasal mucosa of the AR mice. In addition, BS inhibited caspase-1 activity in the nasal mucosa tissue. In activated human mast cells, BS significantly inhibited the production of IL-1 β and thymic stromal lymphopoietin and activation of caspase-1. Our data indicate that BS has anti-allergic and anti-inflammatory effects by regulating of caspase-1 activation in AR mice and in vitro models.

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1. Introduction

Allergic rhinitis (AR) is broadly defined as inflammation of the nasal mucosa. It is a common disorder that affects up to 40% of the population (Small et al., 2007). Although not life-threatening, AR can deteriorate the quality of life and can be a major risk (Oh et al., 2012). Most of the clinical symptoms of patients with AR, including rhinorrhea, nasal itching, sneezing, and nasal congestion, cause a lot of pain (Stokes et al., 2011). AR is frequently involved in acute and chronic airway diseases such as bronchial asthma, which is also caused by hypersensitivity to antigens, resulting in greater local inflammation as well as bronchoconstriction, vasomotor change, and mucus hypersecretion (Smolensky et al., 2007). Among immune cells, mast cells play critical roles in the pathogenesis of allergic responses in AR. Activated mast cells can release a wide variety of inflammatory mediators, including interleukin (IL)-1 β , and thymic stromal lymphopoietin (TSLP) (Moon and Kim, 2011).

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T helper (Th)1 and Th2 are two of many distinct subsets of Th lymphocytes, as defined by various functions and cytokine characterizations (Mosmann and Coffman, 1989; Paul and Seder, 1994). An imbalance of Th1/Th2 is thought to contribute to the pathogenesis of autoimmune diseases and important for autoimmune AR (Kirmaz et al., 2005). Th1 cells and Th2 cells has been considered important for homeostasis of the immune system in the whole body (Mosmann et al., 1986). Various diseases caused by an imbalance between Th1 and Th2. Especially, Th2 cells may play some important roles in the development of AR, and the suppression of Th2 lymphocytes could have the potential to be new therapeutic targets for the treatment of AR (Shirasaki et al., 2011). AR is an IgE-mediated inflammatory disease of the nasal mucous membranes due to the interaction of allergen characterized by a Th2-immunologic pattern with mast cells and an inflammatory infiltrate made up of eosinophils, which release several mediator, chemokines and cytokines, such as IL-1 β (Pawankar et al., 2011). Mast cells contribute to the induction and/or maintenance of eosinophilic inflammation by a variety of mechanisms, including IgE-dependent and IgE-independent processes (Pawankar et al., 2007). Chemotactic and activation signals in mast cells induced the recruitment of these cells to inflammatory sites (Bournazou et al., 2010). Mast cells release pro-inflammatory cytokines that

induce expression of adhesion molecules in endothelium and recruit of leukocytes, which is essential to the pathogenesis of inflammatory diseases (Zhang et al., 2011). Especially, the expression of intercellular adhesion molecule-1 (ICAM-1) in the epithelial cells of the mucosa exposed to the allergen in the absence of clinical symptoms (Montoro et al., 2007). Furthermore, macrophage-inflammatory protein-2 (MIP-2) is a potent chemoattractant for immune cells (Gupta et al., 1996).

Several mechanisms control the production and activity of IL-1 β , including the processing of the 31-kDa inactive IL-1 β precursor form into the bioactive 17-kDa IL-1 β , and the release from secretory lysosomes through K⁺-dependent mechanisms (Wilson et al., 1994; Andrei et al., 2004; Perregaux and Gabel, 1994). Processing of bioactive IL-1 β depends on activation of caspase-1 by protein complexes termed the inflammasomes (Martinon et al., 2002). Caspase-1 is activated within inflammasome, a large cytosolic protein complex that is induced by a growing number of endogenous, microbial, chemical or environmental stimuli (Stutz et al., 2009).

Bamboo salt (BS) is created by eliminating the harmful ingredients with firing at minimum 800 °C for eight times and melting at minimum 1500 °C. Moreover, the nutritious ingredients from bay salt, pine trees, bamboos, loess and pine resin are included into bamboo salt through these firing and melting processes. It is known to have therapeutic effects for diseases such as viral diseases, dental plaque, gastropathy, diabetes, circulatory organ disorders, cancer, and anti-inflammatory disorders (Shin et al., 2003, 2004; Yang et al., 1999; Huh et al., 2001; Sharma et al., 2001). Thus, we hypothesized that BS may improve the symptoms of AR model without side effect. This present study investigated the beneficial effects of BS on OVA-induced AR mice.

2. Materials and methods

2.1. Materials

Ovalbumin (OVA), phorbol 12-myristate 13-acetate (PMA), calcium ionophore (A23187), O-phthalaldehyde, avidin peroxidase, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) tablets substrate, bicinchoninic acid (BCA), antidinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), and other reagents were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), Iscove's modified Dulbecco's medium, and streptomycin were purchased from Invitrogen (Carlsbad, CA). Anti-mouse IgE/IL-1 β [mature form detection antibody (Ab)]/IL-4/IFN- γ MIP-2/ICAM-1 Ab, biotinylated anti-mouse IgE/IL-1 β /IL-4/IFN- γ MIP-2/ICAM-1 Ab, recombinant mouse IgE/IL-1 β /IL-4/IFN- γ MIP-2/ICAM-1, anti-human IL-1 β TSLP Ab, biotinylated anti-human IL-1 β TSLP Ab, and recombinant human IL-1 β TSLP Ab were purchased from BD Biosciences Pharmingen (San Diego, CA). Ab for procaspase-1, caspase-1 and α -tubulin were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The mouse OVA-specific IgE kit was purchased from DS Pharma Biomedical Co. Ltd. (Osaka, Japan). The caspase-1 assay kit was supplied by R&D Systems, Inc. (Minneapolis, MN).

2.2. Preparation of BS and mineral mixture

BS was provided by Tae-sung Food Inc. (Jeonbuk, Republic of Korea). It was processed by a special technique, various times processing at extremely high temperature reaching about 1500 °C with SDS, bamboo, pine tree firewood, pine resin, and yellow earth water, etc. It contains mostly sodium chloride (about 91.7% of BS) along with potassium, calcium, iron, copper, manganese, sulfur, zinc, and magnesium in elementary quantities. Powdered BS was prepared by dissolving with distilled water (DW) then filtered through 0.22- μ m syringe filter. Dilutions of BS were made in DW. Kim et al. (1998) reported the contents of zinc, magnesium, and potassium in the BS was higher compared with crude salt. In order to compare with the ingredient except the salt of BS, we made a mineral mixture. Composition of mineral mixture consists of three minerals: zinc (65.2%), magnesium (23.6%), and potassium (11.2%) (Kim et al., 1998).

2.3. OVA-induced AR animal model

We maintained 6-week-old female BALB/c (Charles River Laboratories, Inc., Wilmington, MA) mice under pathogen-free conditions. Mouse care and experimental procedures were performed under the approval from the animal care committee of Kyung Hee University [Approval No. KHUASP (SE)-11-037]. The mice were sensitized on days 1, 5, and 14 by intraperitoneal injection of 100 μ g of OVA emulsified

and 20 mg of aluminum hydroxide (Sigma-Aldrich) in a 100 μ l of phosphate-buffered saline (PBS) and challenged intranasally with 2 μ l of 1.5 mg of OVA or PBS. The mice were challenged intranasally with PBS in a similar manner for the negative control. BS (0.01, 0.1, and 1 g/kg), SDS (1 g/kg), or a control vehicle (DW) was administered orally for 10 days before the intranasal OVA challenge. Nasal symptoms were only evaluated by counting the number of nasal rubs that occurred in the 10 min after OVA intranasal provocation at the 10 day mark after the challenge. We measured OVA-specific IgE as the relevant endpoint for AR. The number of mice in each group was 6. OVA-primed mice displayed a significant increase in sneezing behavior when challenged intranasally with OVA (Bain et al., 2011). In our study, although the frequency of sneezing was increased by the OVA challenge, there were no appreciable differences in the appearance of the mice between the OVA-unsensitized and OVA-sensitized groups (data not shown).

2.4. Procedure for the homogenization of spleen and mucosa tissue for mouse cytokine determination

Frozen tissue samples were weighed and homogenized (100 mg tissue per ml of homogenization buffer). The homogenization buffer contained 1 mM PMSF, 1 μ g/ml pepstatin A, 1 μ g/ml aprotinin, and 1 μ g/ml leupeptin in phosphate buffered saline, pH 7.2, with 0.05% sodium azide and 0.5% Triton X 100. The samples were homogenized with a polytron and then subjected to one round of freeze thaw, and then sonicated for 10 min, and then incubated at 4 °C for 1 h. The homogenate was centrifuged at 120,000g. The supernatants were used for cytokine measurements. The data are expressed at pg/mg protein, with protein concentration determined using a bicinchoninic acid protein assay method.

2.5. Human mast cell line culture

The human mast cells (HMC-1) were grown in Iscove's modified Dulbecco's medium supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% heat-inactivated FBS at 37 °C 5% CO₂ and 95% humidity. HMC-1 cells (3×10^5 cells/ml) were treated with BS (0.01, 0.1, and 1 mg/ml) or SDS (1 mg/ml) for 1 h before stimulation with PMA and calcium ionophore A23187 (PMACI) was incubated for 2 or 8 h.

2.6. Histamine assay

The histamine was measured from serum according to the manufacturer's specifications using the histamine assay kit supplied by Oxford Biomedical Research (Oxford, MI).

2.7. Enzyme-linked immunosorbent assay (ELISA)

HMC-1 cells (3×10^5 cells/ml) were treated with BS (0.01, 0.1, and 1 mg/ml) or SDS (1 mg/ml) for 1 h before stimulation with PMACI incubated for 8 h. Cytokines of serum, mucosa, spleen tissue, and supernatant were measured by an ELISA (Oh et al., 2012). The OVA-specific IgE was measured from the serum according to the manufacturer's specifications using an OVA-specific IgE kit. Cytokine levels in the spleen and nasal mucosa were divided according to the total protein. The protein was estimated using the BCA method with the BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). This method combines the reduction of cupric ions to cuprous ions by the protein in an alkaline medium and then the subsequent reaction of the cuprous ions with two molecules of BCA to give an intense purple color read at 560 nm.

2.8. Reverse transcription-polymerase chain reaction

HMC-1 cells (3×10^6) were treated with BS (0.01, 0.1, and 1 mg/ml) or SDS (1 mg/ml) for 1 h before stimulation with PMACI incubated for 6 h. The total RNA was isolated from the cells according to the manufacturer's specification using an easy-BLUE RNA extraction kit (iNtRON Biotechnology, Kyunggi-do, Korea). Total RNA (2 μ g) was heated at 65 °C for 10 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 90 min at 37 °C using a cDNA synthesis kit (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). The polymerase chain reaction (PCR) was performed with the following primers for the human IL-1 β (5'-GGG GTA CCT TAG GAA GAC ACA AAT TG-3'; 5'-CCG GAT CCA TGG CAC CTG TAC GAT CA-3'), TSLP (5'-TAT GAG TGG GAC CAA AAG TAC CG-3'; 5'-GGG ATT GAA GGT TAG GCT CTG G-3'), and human GAPDH (5'-CCT GCT TCA CCA CCT TCT TG-3'; 5'-CAA AAG GGT CAT CAT CTC TG-3'). GAPDH was used to verify whether equal amounts of RNA were used for reverse transcription (RT) and PCR amplification from different experimental conditions. Saturation curves for PCR were obtained from various experimental conditions (RNA concentrations, annealing temperatures, and PCR cycle numbers). We determined the optimal amplification conditions (annealing temperature and PCR cycle number) of primers for the PCR. The annealing temperature was 50 °C for human IL-1 β , 62 °C for human TSLP, and 60 °C for human GAPDH, respectively. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

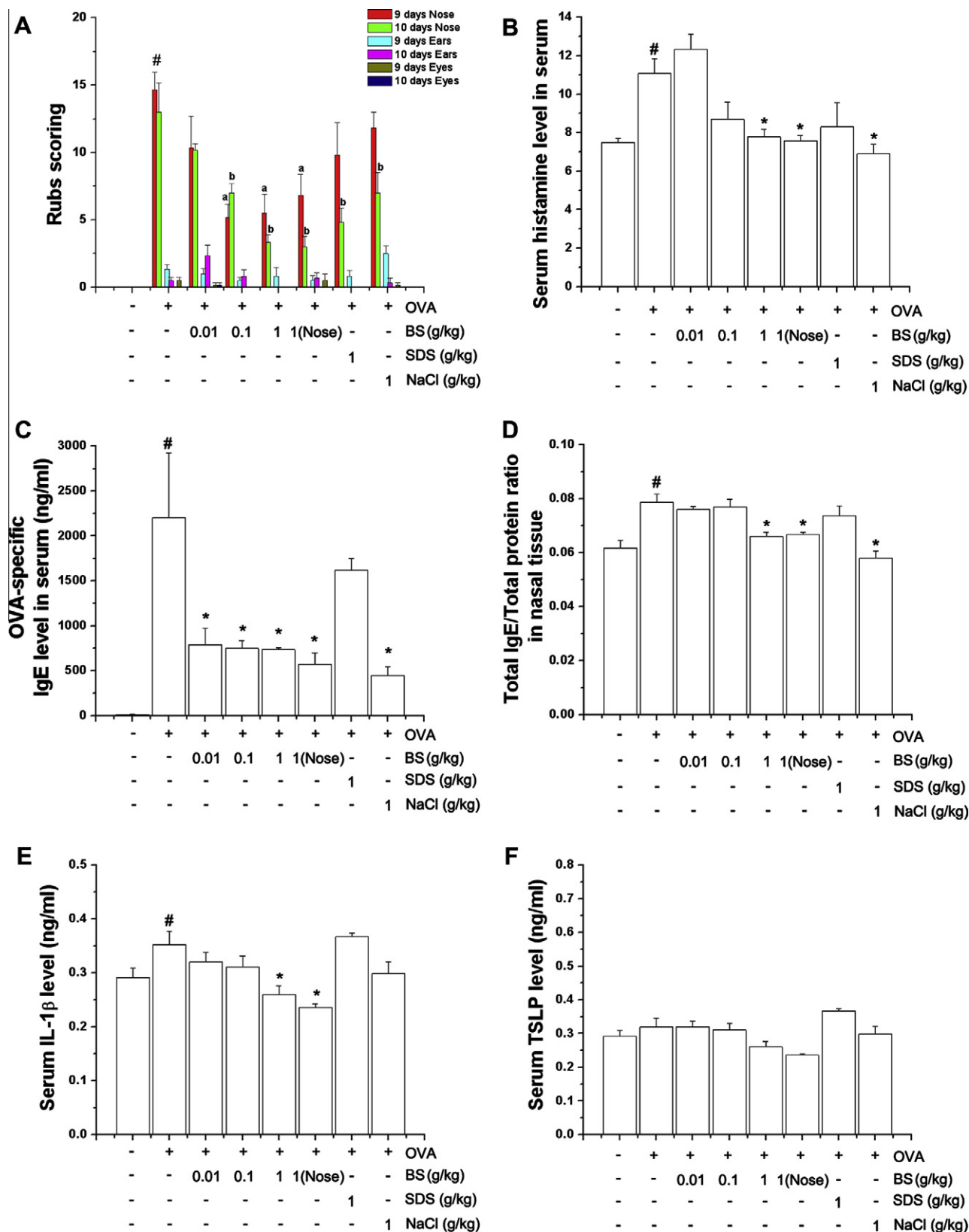


Fig. 1. Effects of BS on clinical symptoms, IgE, IL-1 β , and TSLP levels in the AR model. AR mice were administered with BS (0.01, 0.1, and 1 g/kg), SDS (1 g/kg), or NaCl (1 g/kg). 1 (nose) Means direct nasal inhalation of BS. (A) The number of the nasal rube that occurred in the 10 min after the OVA intranasal provocation. (B) Serum was isolated from blood to measure histamine. OVA-specific IgE (C), IL-1 β (E), and TSLP (F) in serum and total IgE in nasal mucosa tissue (D) of AR mice were measured by ELISA method. [#] $P < 0.05$, significantly different from the OVA-unsensitized mice. ^a $P < 0.05$, significantly different from the OVA-sensitized mice on 9 days. ^b $P < 0.05$, significantly different from the OVA-sensitized mice on 10 days. ^{*} $P < 0.05$, significantly different from the OVA-sensitized mice; $n = 6$.

2.9. Western blot analysis

HMC-1 cells (3×10^6) were treated with BS (0.01, 0.1, and 1 mg/ml) or SDS (1 mg/ml) for 1 h before stimulation with PMACI incubated for 2 h. Protein expressions were measured by Western blot analysis (Oh et al., 2012).

2.10. Histological analysis

Tissue samples were immediately fixed with 10% formaldehyde and embedded in paraffin. The sections of the nasal mucosa sample were 4 μ m thick. Each section was stained with hematoxylin and eosin (H&E, for eosinophils), alcian blue and safranin O (A&S, for mast cells), or immunohistochemical stain (for IL-1 β) before dewaxing and dehydration. The numbers of eosinophils, mast cells, and IL-1 β on both sides of the septal mucosa were counted. Sections were coded and randomly analyzed by two blinded observers.

2.11. Caspase-1 assay

The caspase-1 assay used nasal mucosa tissue and cell extracts. Caspase-1 activity was measured according to the manufacturer's specifications using a caspase assay kit (R&D Systems, Inc.). Equal amounts of the total protein were quantified by a BCA protein quantification kit (Sigma–Aldrich) in each lysate. Catalytic activity of caspase-1 from the cell lysate was measured by the proteolytic cleavage of YVAD-pNA (BioVision, Inc., San Francisco, CA) for 4 h or various times at 37 °C. The plates were read at 405 nm.

2.12. Statistical analysis

The experiments shown are a summary of the data from at least three experiments, and statistical analyses were performed using SPSS statistical software (SPSS 11.5; SPSS Inc., Chicago, IL). Treatment effects were analyzed by one-way analysis of variance, offered by Tukey's multiple range tests, and $P < 0.05$ was used to indicate significance.

3. Results

3.1. Effects of BS on clinical symptoms, histamine, IgE, IL-1 β , and TSLP levels in the AR model

To determine the inhibitory effects of BS in the AR model, we sensitized mice on days 1, 5, and 14 by intraperitoneal injections of 100 μ g of OVA emulsified in 20 mg of aluminum hydroxide and the challenged mice with 1.5 mg of OVA. We first assessed the effect of BS, SDS, and NaCl (major component of BS and SDS) on clinical symptoms of the OVA-sensitized mice. The numbers of nasal after the OVA challenge in the OVA-sensitized mice were significantly higher than those in the OVA-unsensitized mice.

Treatments with BS, SDS, or NaCl on mice, rub scores were significantly inhibited at 10 days (Fig. 1A). Histamine levels in the serum were decreased by the BS or NaCl treatment (Fig. 1B). In the OVA sensitized mice, levels of IgE in the serum and nasal mucosa tissue increase significantly than the OVA-unsensitized mice. Serum OVA-specific IgE and total IgE in the nasal mucosa tissue were inhibited significantly by the treatment BS or NaCl. Especially, these inhibitory effects show not only oral administration but also direct inhalation on the nose in mice (Fig. 1C and D). To identify the relationship of the inflammatory cytokines and the effects of BS on AR mice, we measured IL-1 β and TSLP levels in the serum of AR mice. As shown Fig. 1E, IL-1 β levels in serum of OVA-sensitized mice were increased more than normal mice. BS treatment on AR mice significantly inhibited the levels of IL-1 β compared with control group (Fig. 1E). However, protein levels of TSLP did not affect by the treatment of BS (Fig. 1F). However, SDS did not affect the various allergic factors on AR mice. This indicates that NaCl is an active component of BS. Inhibitory effect of BS in rubs scoring and serum IL-1 β was higher than that of NaCl.

3.2. Effects of BS on IFN- γ and IL-4 levels in the spleen of the AR models

Next, to investigate the Th1/Th2 immune reaction in BS-treated mice, we analyze IFN- γ and IL-4 levels in the spleen. Levels of IFN- γ in the AR mice were significantly decreased compared with those in the OVA-unsensitized mice. IFN- γ levels were significantly increased in the BS-treated AR mice (Fig. 2A). Furthermore, the levels of IL-4 in the AR mice were significantly increased compared with normal mice. But, IL-4 levels were significantly decreased by the treatment of BS or SDS treatment on AR mice (Fig. 2B). NaCl did not affect the levels of IFN- γ and IL-4 in the spleen.

3.3. Effects of BS on eosinophil and mast cell infiltration and inflammatory cytokines expression in the AR nasal mucosa tissue

The numbers of inflammatory cells (eosinophil and mast cell) in the nasal mucosa in the AR mice were significantly higher than those in the OVA-unsensitized mice. These increasing numbers of inflammatory cells were decreased by BS. Furthermore, immunohistochemical analysis of the nasal mucosa sections in the AR mice demonstrated that the expressions of IL-1 β are significantly

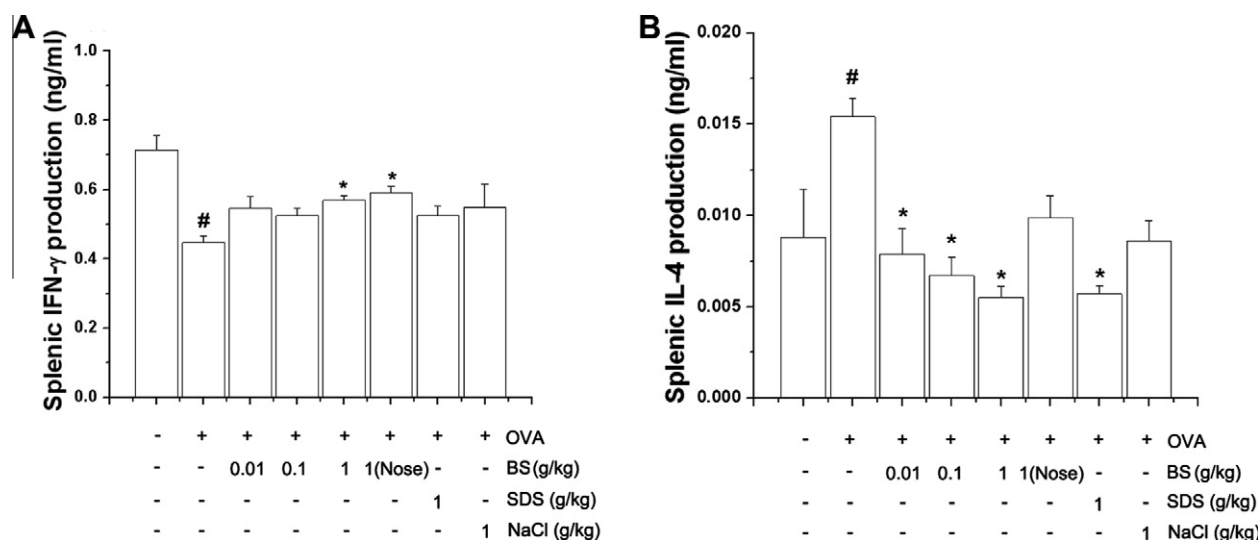


Fig. 2. Effects of BS on IFN- γ and IL-4 levels in the spleen of the AR mice. AR mice were administered with BS (0.01, 0.1, and 1 g/kg), SDS (1 g/kg), or NaCl (1 g/kg). 1 (nose) Means direct nasal inhalation of BS. IFN- γ (A) and IL-4 (B) levels in the spleen of the AR mice were measured by ELISA method. [#] $P < 0.05$, significantly different from the OVA-unsensitized mice. ^{*} $P < 0.05$, significantly different from the OVA-sensitized mice; $n = 6$.

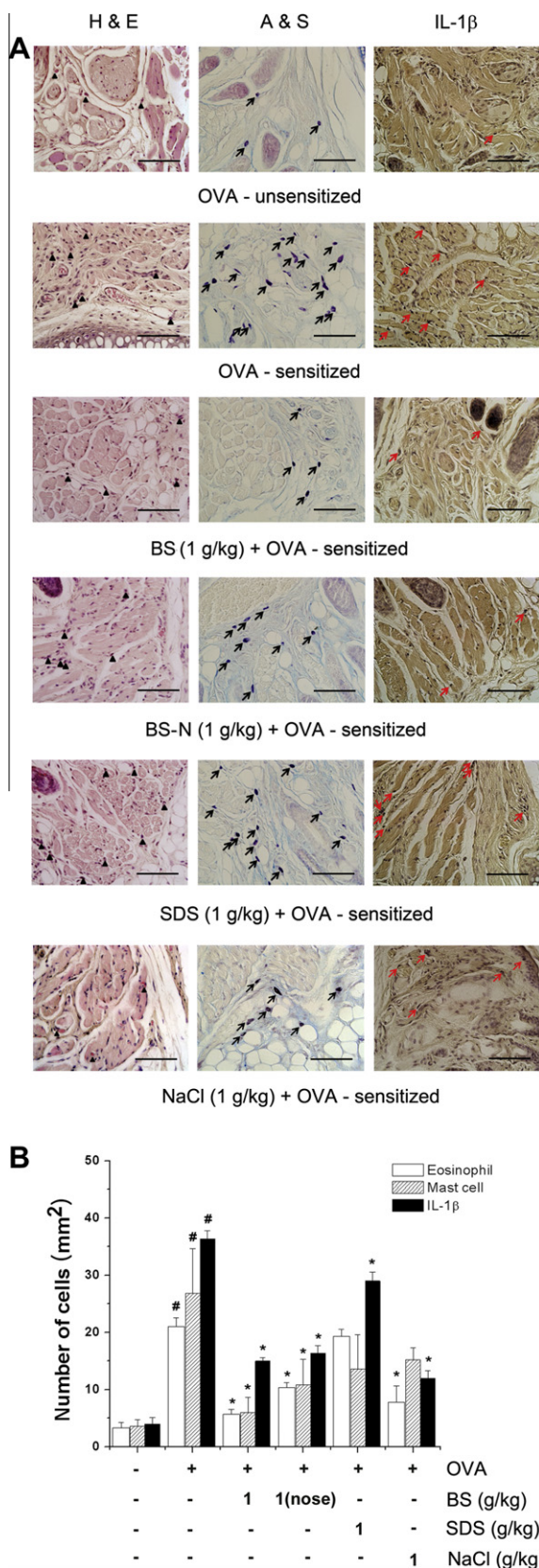


Fig. 3. Effects of BS on inflammatory cell infiltration and inflammatory cytokine expression in the AR nasal mucosa tissue. (A) Nasal mucosa stained with hematoxylin and eosin [(H&E) for eosinophils, arrowhead], Alcian blue and safranin O [(A&S) for mast cells, black arrow], and immunohistochemical diaminobenzidine stain (for IL-1 β , red arrow). (B) Eosinophil, mast cell, and IL-1 β were counted by two individuals. After five randomly selected tissue sections per mouse were counted. [#] $P < 0.05$, significantly different from the OVA-unsensitized mice. * $P < 0.05$, significantly different from the OVA-sensitized mice (original magnification 400 $\times$).

increased, whereas in the BS, SDS, or NaCl treated mice, it is decreased (Fig. 3A and B). Inhibitory effect of BS in cell infiltration was higher than that of NaCl.

3.4. Effects of BS on IL-1 β , TSLP, ICAM-1, and MIP-2 levels of the nasal mucosa tissue of AR models

To evaluate the anti-inflammatory effect of BS on AR model, we measured the protein levels of IL-1 β , TSLP, MIP-2, and ICAM-1 in the AR mice. The protein levels of IL-1 β , ICAM-1, and MIP-2 in the nasal mucosa tissue increased in the AR mice compared with those levels in the normal mice (Fig. 4A, C, and D). The protein levels of IL-1 β , ICAM-1, and MIP-2 were significantly decreased by the treatment of BS or NaCl. However, protein levels of TSLP were not reduced by the treatment of BS or NaCl (Fig. 4B). SDS had no effect on the levels of inflammatory mediators.

3.5. Effects of BS on caspase-1 activation in the nasal mucosa of the AR mice

Caspase-1 acts a crucial role in inflammatory responses by cleaving pro-IL-1 β into secreted pro-inflammatory cytokines (Wilson et al., 1994). To determine whether BS inhibited caspase-1 activation, we performed caspase-1 assay with the nasal mucosa tissues. BS (1 g/kg) or NaCl (1 g/kg) also inhibited OVA-induced caspase-1 activation (Fig. 5). However, SDS had no effect on the caspase-1 activation.

3.6. Effects of BS on PMACI-induced IL-1 β , TSLP, and caspase-1 activation in HMC-1 cells

Mast cells play a major role in the inflammatory response of AR model. And IL-1 β and TSLP is major inflammatory cytokine released from mast cells after allergic responses. We investigated the effects of BS on the expression of IL-1 β and TSLP in HMC-1 cells. BS, SDS, and NaCl had no effect on the cell viability (Fig. 6A). The protein and mRNA levels of IL-1 β were significantly inhibited by BS (1 mg/ml) treatment (Fig. 6B and D). In addition, increasing TSLP levels were significantly decreased by the treatment of BS or NaCl (Fig. 6C). However, the mRNA level of TSLP was not changed by BS (Fig. 6D). To evaluate whether BS inhibited the caspase-1 activation, we performed a Western blot analysis. PMACI-induced caspase-1 activation was inhibited by the treatment of BS or NaCl but not SDS (Fig. 6E and F).

3.7. Effects of mineral mixture on clinical symptoms, histamine, and IgE levels in the AR model

In order to investigate the effects of ingredient except of salt of BS, we performed the rubs scoring and measured the serum histamine and IgE levels. Oral administration with mineral mixture on mice, rub scores was significantly inhibited at 10 days (Fig. 7A). Histamine levels in the serum were decreased by the mineral mixture treatment (Fig. 7B). Furthermore, in the OVA-sensitized mice, levels of IgE in the serum were decreased by the mineral mixture treatment (Fig. 7C). This indicates that mineral mixture is also active component of BS.

4. Discussion

These findings herein show for the first time that BS inhibits the OVA-sensitized AR mice. In this study, we demonstrated that BS inhibited the allergic and inflammatory reaction in the AR mice model.

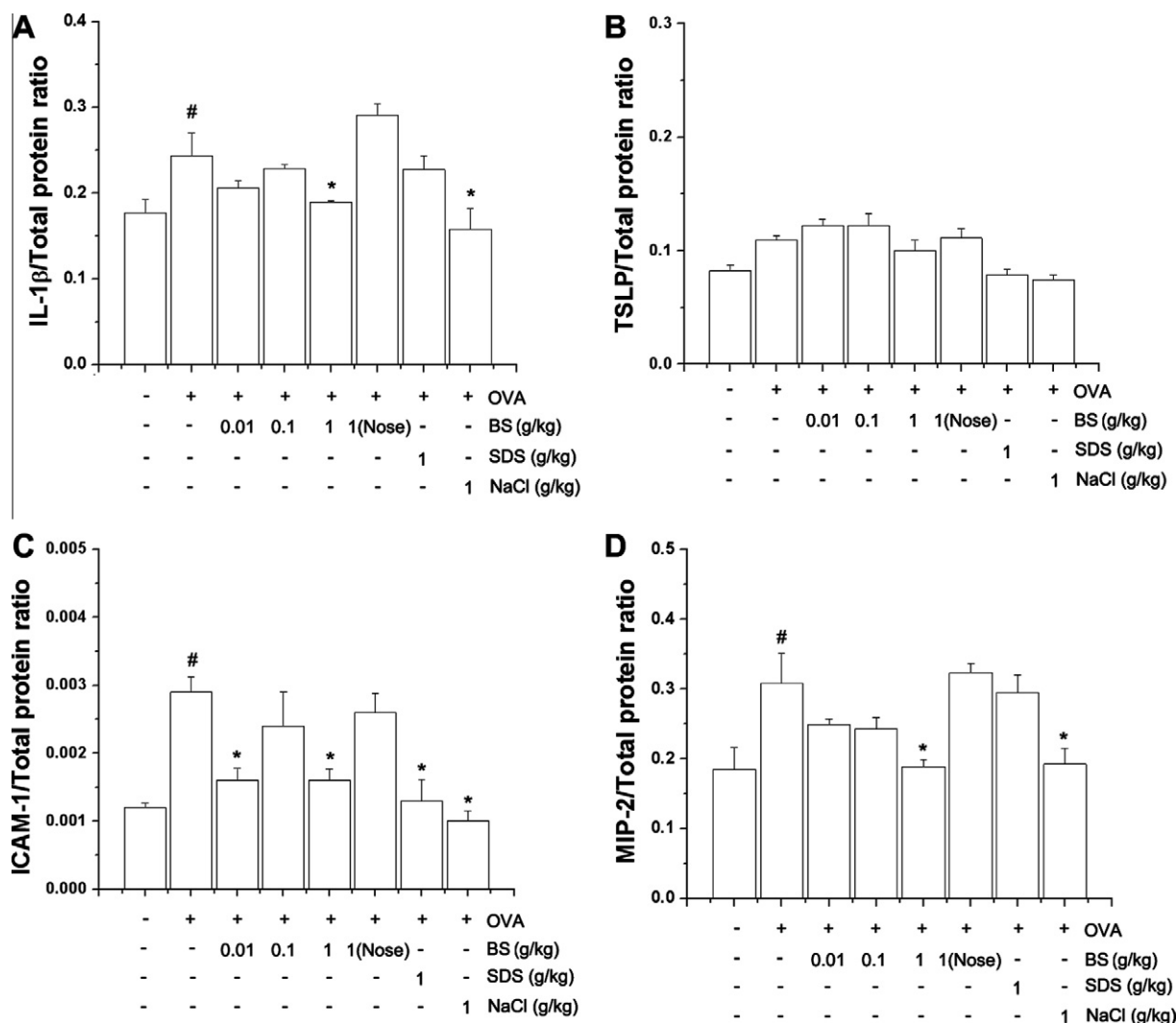


Fig. 4. Effects of BS on IL-1 β , TSLP, ICAM-1, and MIP-2 levels in the nasal mucosa tissue of the AR mice. AR mice were administered with BS (0.01, 0.1, and 1 g/kg), SDS (1 g/kg), or NaCl (1 g/kg). 1 (nose) means direct nasal inhalation of BS. IL-1 β (A), TSLP (B), ICAM-1 (C), and MIP-2 (D) levels in the nasal mucosa tissue of the AR mice were measured by ELISA method. BS-N, nasal inhalation. [#] P < 0.05, significantly different from the OVA-unsensitized mice. ^{*} P < 0.05, significantly different from the OVA-sensitized mice; n = 6.

Allergic diseases are traditionally referred to as immediate or type 1 hypersensitivity reactions, with IgE as a critical factor. IgE is involved in allergic inflammation, especially in early-phase response, but it may also be involved in the late-phase allergic response (Rosenwasser, 2011). The inflammatory response in the nasal mucosa in subjects with allergic rhinitis challenged intranasally with an allergen includes a late-phase response characterized by recruitment of eosinophils, basophils, and T cells expressing Th2 cytokines including IL-4 (Broide, 2010). Recruitment of inflammatory cells, including eosinophils, mast cells, basophils, and T cells, results in the further release of histamine and leukotrienes, as well as in the release of other compounds, including pro-inflammatory cytokines, which sustain the allergic response and promote the late phase response (Fuentes-Beltrán et al., 2009; Fukui et al., 2009). Generally, glucocorticosteroids (GC) are known to inhibit allergic inflammation (Leung et al., 2008). GC inhibits the infiltrating of inflammatory cells and their recruitment into the nasal mucosa. Moreover, GC-treated mast cells exhibit biochemical and functional properties different from immature untreated cells (Pitton et al., 1988). In this study, we investigated that BS inhibited the clinical symptoms, IgE production, and inflammatory cytokine production. Therefore, we can also suggest that the BS may have anti-allergic effects.

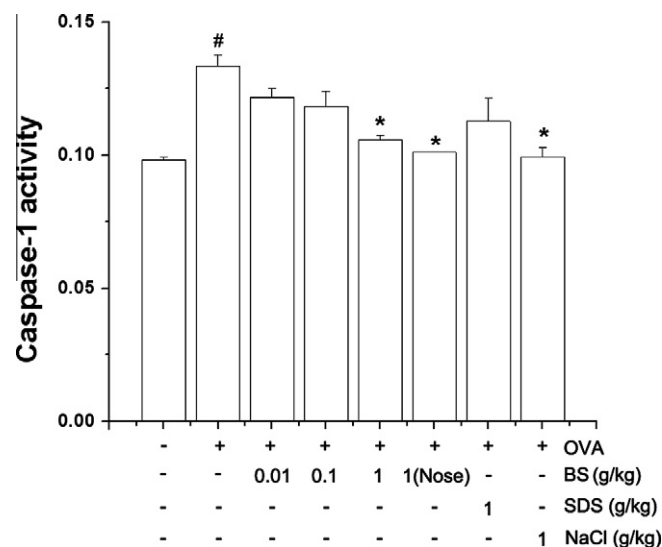


Fig. 5. Effects of BS on caspase-1 activation in the nasal mucosa of the AR mice. AR mice were administered with BS (0.01, 0.1, and 1 g/kg), SDS (1 g/kg), or NaCl (1 g/kg). 1 (nose) means direct nasal inhalation of BS. Caspase-1 activity was assayed. [#] P < 0.05, significantly different from the OVA-unsensitized mice. ^{*} P < 0.05, significantly different from the OVA-sensitized mice; n = 6.

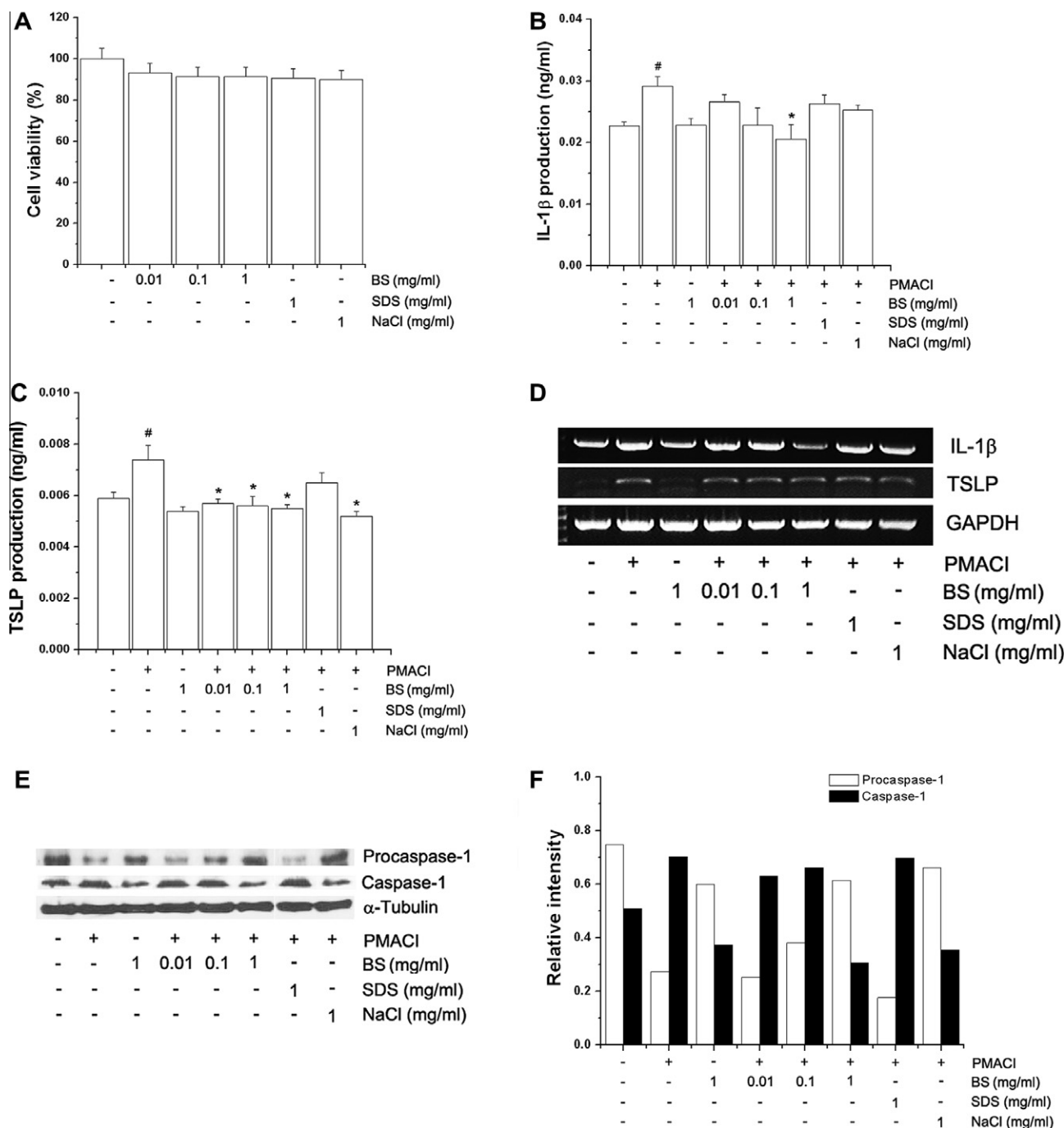


Fig. 6. Effects of BS on PMACI-induced IL-1 β , TSLP, and caspase-1 expression in HMC-1 cells. (A) Cell viability was evaluated by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay. (B and C) HMC-1 cells were treated with BS (0.01, 0.1, and 1 mg/ml), SDS (1 mg/ml), or NaCl (1 mg/ml) for 1 h and then stimulated with PMACI for 8 h. IL-1 β and TSLP were measured by ELISA method. (D) The mRNA was measured using the RT-PCR method. (E) Caspase-1 protein expression was evaluated by using Western blot analysis. (F) The levels of protein expression were quantified by densitometry. Expression was normalized to α -tubulin expression. [#] $P < 0.05$: significantly different from the unstimulated cells. ^{*} $P < 0.05$: significantly different from the stimulated cells.

Before an eosinophil reaches to the target tissue, a whole cascade of events is necessary including the chemoattraction by cytokines or chemokines and the endothelial up-regulation of adhesion molecules like ICAM-1 (Kleinjan et al., 2010). Furthermore, MIP-2 mRNA was strongly up-regulated by treatment of allergen. MIP-2 is strongly chemotactic for neutrophils, induced their activation and degranulation and could be secreted by epithelial cells through their activation by TNF- α , leading to an amplification of the inflammatory response (Havaux et al., 2005). We showed that the levels of ICAM-1 and MIP-2 in the OVA-sensitized mice were significantly higher than those in the OVA-unsensitized

mice. Up-regulation of ICAM-1 and MIP-2 by OVA-sensitized AR mice was markedly reduced by the treatment of BS. TSLP, which activates myeloid dendritic cells (DCs) to produce OX40 ligand (OX40L), which triggers a Th2 inflammatory response (Li et al., 2011). Compelling evidence demonstrates that TSLP represents one of the master switches initiating allergic inflammation at the interface between epithelial cells and DCs and has a determinant role in allergic diseases (Li et al., 2011). In this study, we showed that BS inhibited the mast cell-induced TSLP production but not AR animal model. Therefore, we can presuppose that TSLP plays an important role as an initiator of early phase on AR.

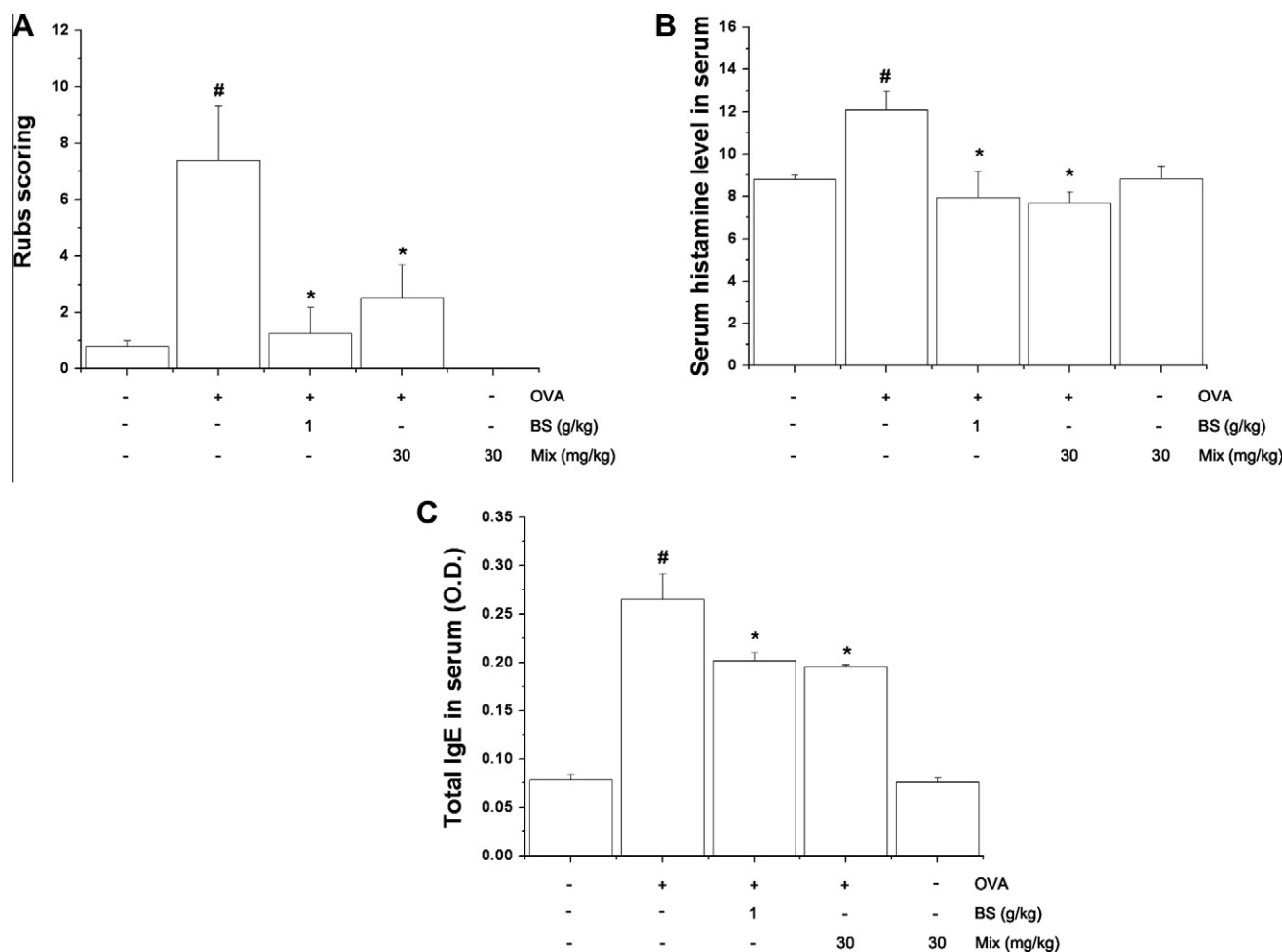


Fig. 7. Effects of mineral mixture on clinical symptoms, histamine, and IgE levels in the AR model. AR mice were orally administered with BS or mineral mixture (30 mg/kg). (A) The number of the nasal rube that occurred in the 10 min after the OVA intranasal provocation. (B) Serum was isolated from blood to measure histamine. (C) Total IgE in the serum of AR mice were measured by ELISA method. [#] $P < 0.05$, significantly different from the OVA-unsensitized mice. ^{*} $P < 0.05$, significantly different from the OVA-sensitized mice; $n = 5$. Mix, mineral mixture.

Caspase-1 is initially expressed as an inactive precursor, which is activated in large complexes called inflammasomes (Martinon et al., 2009). Caspase-1 activity is required for the activation of proIL-1 β , but also for the unconventional secretion of proIL-1 α and of many other proteins involved in inflammation, repair and cytoprotection (Keller et al., 2008; Nickel and Rabouille, 2009). Besnard et al. have reported that inflammasome activation leading to IL-1 β production contributes to the control of allergic asthma (Besnard et al., 2011). Caspase-1 activity was increased in patients with AR (Jeong et al., 2011a,b). In this study, we demonstrated that caspase-1 was activated in the AR mice. BS inhibited IL-1 β production and caspase-1 activity. Therefore, we postulate that BS might be inhibits the inflammatory cytokine production via the regulation of caspase-1 activation.

In this study, we showed that various allergic and inflammatory phenotypes were ameliorated by the treatment with BS or NaCl. However, too much salt causes the side effects. The most well-known side effect of too much salt intake is a high blood pressure. An excess of salt may lead to constrict the blood vessels, which is dangerous because the heart is required to work harder to pump blood throughout the body (American Heart Association Nutrition Committee et al., 2006). A technical report produced by WHO and the Food and Agriculture Organization of the United Nations (FAO) recommended the consumption of less than 5 g NaCl (or 2 g sodium) per day as a population nutrient intake goal, while ensuring that the salt is iodized (WHO, 2003). However, Bang et al.

reported that blood pressures were not changed in 5 volunteers who took 10 g of BS (Bang et al., 2002). Zhao reported the difference of crystal structure among the SDS, NaCl, and BS by using SEM micrographs (Zhao, 2011). So, these differences may affect to efficacy of NaCl or BS on human body. Therefore, we suggested that BS could be included among the new therapeutic options for the treatment of patient suffering from AR etc.

BS contains abundant minerals such as natural sodium, potassium, calcium, chloride, magnesium, manganese, iron, zinc, and copper etc. The contents of calcium, iron, zinc, potassium, and manganese in the BS were higher whereas the sulfate content was lower compared with SDS (Shin et al., 2004). Zinc ions are an important anti-inflammatory factor (Novick et al., 1997). Maintenance of ion concentration gradients is essential for the function of many organs. Various mineral ions also play a crucial role in many cell functions such as cell proliferation, energy metabolism, protein and DNA syntheses, cytoskeleton activation, and ROS scavenging activity (Jeong et al., 2011a,b). In our study, mineral mixture also decreased on clinical symptoms and histamine and IgE levels in the serum. Therefore, these results suggest that combination of various other beneficial mineral ions has a synergistic effect in AR.

5. Conclusion

This study demonstrated that BS inhibited the allergic and inflammatory responses. Our results showed that the inhibitory

effect of BS may be direct or indirect through the regulation of inflammatory cell infiltration and cytokine production in the nasal mucosa. Furthermore, our data indicates that BS inhibited the allergic and inflammatory responses via the blockade of caspase-1 pathways. Therefore, our results provide evidence that BS will be beneficial in the regulation of AR.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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