

Hydrogen sulfide diminishes the levels of thymic stromal lymphopoietin in activated mast cells

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Abstract Bamboo salt (BS) is a Korean traditional type of salt and has been reported to have therapeutic effects on allergic inflammation. Thymic stromal lymphopoietin (TSLP) aggravates inflammation in the pathogenesis of allergic reactions, such as allergic rhinitis (AR). To confirm an active compound of BS, we investigated the effect of sulfur, a compound of BS, on the levels of TSLP in a human mast cell line, HMC-1 cells and a mouse model of AR using hydrogen sulfide (H₂S) donor, sodium hydrosulfide (NaSH). We treated NaSH or BS in HMC-1 cells and activated the HMC-1 cells with phorbol myristate acetate and calcium ionophore A23187 (PMACI). ELISA for the production measurement of TSLP, PCR for the mRNA expression measurement of TSLP, and western blot analysis for the expression measurement of upstream mediators were performed. Mice were treated with NaSH and sensitized with ovalbumin (OVA). The levels of TSLP were measured in serum and nasal mucosa tissue in an OVA-induced AR mouse model. NaSH or BS diminished the production and mRNA expression of TSLP as well as interleukin (IL)-6 and tumor necrosis factor (TNF)- α in the PMACI-activated HMC-1 cells. NaSH or BS diminished the level of

intracellular calcium in the PMACI-activated HMC-1 cells. NaSH or BS reduced the expression and activity of caspase-1 in the PMACI-activated HMC-1 cells. And NaSH or BS inhibited the expression of receptor interacting protein-2 and the phosphorylation of extracellular signal-regulated kinase in the PMACI-activated HMC-1 cells. The translocation of NF- κ B into the nucleus as well as the phosphorylation and degradation of I κ B α in the cytoplasm were diminished by NaSH or BS in the PMACI-activated HMC-1 cells. Furthermore, NaSH inhibited the production of TSLP, IL-6, and IL-8 in TNF- α -activated HMC-1 cells. Finally, the administration of NaSH showed a decrease in number of rubs on mice with OVA-induced AR. And the levels of immunoglobulin E and TSLP in the serum and the level of TSLP in the nasal mucosa tissue of the OVA-induced AR mice were reduced by NaSH. In conclusion, these findings show that H₂S, as an active compound of BS is a potential agent to cure allergic inflammation.

Keywords Calcium · Caspase-1 · Hydrogen sulfide · Mast cell · NF- κ B · Thymic stromal lymphopoietin · Allergic rhinitis

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Introduction

Bamboo salt (BS) which is a Korean traditional type of salt, is produced by inserting solar salt in a thick bamboo stub, plugging with mud on sides, and repeatedly baking them various times. In the process of baking, the BS has higher contents of minerals or nutrients from the solar salt, bamboo, and mud [37]. BS has been reported to have therapeutic effects on various diseases, such as allergic rhinitis (AR) [35], cancer [75], or diabetes [63]. BS contains various minerals such as potassium, zinc, sulfur, etc. [37]. Potassium

supplementation reduced renal inflammation [71]. Zinc supplementation inhibited chronic hepatitis or respiratory tract infection [57]. Sulfur supplementation is required for immune function in humans [18]. The deficiency of sulfur triggers abnormalities, such as decline in normal intestinal mucosal growth or antioxidant function [4]. Sulfur turns into hydrogen sulfide (H_2S) by sulfur bacteria and it may occur naturally in water [27]. H_2S plays opposite roles in many tissues. H_2S was reported to have many lethal and sublethal effects of on humans and animals [70]. However, H_2S improved atherosclerosis [73], myocarditis [26], airway hyper-responsiveness, or inflammation in mice [74].

Mast cells found in mucosal tissues are a major effector cell in allergic inflammatory reactions, including AR [1]. Mast cell degranulation is induced by calcium influx and then the mast cell produces various cytokines, such as thymic stromal lymphopoietin (TSLP), interleukin (IL)-6, or tumor necrosis factor (TNF)- α [39, 66]. TSLP is a critical factor in allergic inflammation and allergy-related diseases, including AR [5]. In our previous study, TSLP was produced through the receptor interacting protein2 (RIP2)/caspase-1/nuclear factor (NF)- κ B signaling which is regulated by the intracellular calcium in mast cells [20]. Caspases, a family of cysteine proteases, play critical roles in apoptosis and inflammation [11]. Caspase-1 is recognized as an inflammatory caspase [45] and a regulator of NF- κ B signaling, which plays an important role in inflammation and immunity [64]. RIP2, a caspase recruitment domain-containing kinase, interacts with caspase-1 and plays a critical role in NF- κ B activation [61]. RIP2 triggers the phosphorylation of I κ B α , separation of I κ B α from NF- κ B, and I κ B α degradation. The liberated NF- κ B is translocated into the nucleus and binds specific DNA sequences, triggering the synthesis of pro-inflammatory mediators such as TSLP, TNF- α , IL-6, or IL-8 [36, 49, 67]. RIP2 also leads to phosphorylation of extracellular signal-regulated kinase (ERK), a mitogen-activated protein (MAP) kinase [43] which is required for NF- κ B activation [16].

The effect of H_2S , a compound of BS, on the levels of TSLP in mast cells has not yet been clarified. This study provides evidences that H_2S diminishes the levels of TSLP through blockade of caspase-1 signaling in phorbol myristate acetate and calcium ionophore A23187 (PMACI)-activated mast cells and the levels of TSLP in an ovalbumin (OVA)-induced AR mouse model.

Materials and methods

Chemicals

We purchased sodium hydrosulfide (NaSH, H_2S donor) from Samchu Chemical (#C/N = S3666, purity = 70 %, Pyeongtaek, Republic of Korea) and BS from Hongik-biotec (Damyang, Republic of Korea). NaSH and BS were dissolved with distilled water. We purchased Isocove's Modified Dulbecco's Medium (IMDM) and fetal bovine serum (FBS) from Gibco BRL (Grand Island, NY, USA); PMA, A23187 (Calcimycin; $C_{29}H_{37}N_3O_6$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid acetoxymethyl ester (BAPTA-AM), and Fura-2/AM from Sigma Chemical Co (St. Louis, MO, USA); TSLP antibodies and a caspase-1 assay kit from R&D Systems (Minneapolis, MN, USA); IL-6 antibodies, TNF- α antibodies, IL-8 antibodies, recombinant human TNF- α , and immunoglobulin E (IgE) antibodies from Pharmingen (San Diego, CA, USA); caspase-1, RIP2, ERK, phosphorylation of ERK (pERK), NF- κ B, poly-ADP-ribose polymerase (PARP), phosphorylation of I κ B α (pI κ B α), I κ B α , actin, and tubulin antibodies from Santa Cruz Biotechnology (Dallas, TX, USA).

Pyeongtaek, Republic of Korea) and BS from Hongik-biotec (Damyang, Republic of Korea). NaSH and BS were dissolved with distilled water. We purchased Isocove's Modified Dulbecco's Medium (IMDM) and fetal bovine serum (FBS) from Gibco BRL (Grand Island, NY, USA); PMA, A23187 (Calcimycin; $C_{29}H_{37}N_3O_6$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid acetoxymethyl ester (BAPTA-AM), and Fura-2/AM from Sigma Chemical Co (St. Louis, MO, USA); TSLP antibodies and a caspase-1 assay kit from R&D Systems (Minneapolis, MN, USA); IL-6 antibodies, TNF- α antibodies, IL-8 antibodies, recombinant human TNF- α , and immunoglobulin E (IgE) antibodies from Pharmingen (San Diego, CA, USA); caspase-1, RIP2, ERK, phosphorylation of ERK (pERK), NF- κ B, poly-ADP-ribose polymerase (PARP), phosphorylation of I κ B α (pI κ B α), I κ B α , actin, and tubulin antibodies from Santa Cruz Biotechnology (Dallas, TX, USA).

HMC-1 cells cultures

HMC-1 cells were incubated in IMDM supplemented with penicillin (100 units/ml), of streptomycin (100 μ g/ml), and 10 % FBS at 37 °C in 5 % CO_2 with 95 % humidity.

Cytokines assay

The levels of TSLP, IL-6, TNF- α , and IL-8 from HMC-1 cells, the levels of IgE and TSLP in the serum, and the level of TSLP in the nasal mucosa tissues were examined using a sandwich ELISA according to manufacturer's instructions (R&D System Inc, Pharmingen).

Real-time polymerase chain reaction (PCR) analysis

SYBR Green master mix was used for quantitative real-time PCR. The detection of mRNA was analyzed using an ABI StepOne real time PCR System (Applied Biosystems, Foster City, CA, USA). Primer sequences for GAPDH, TSLP, IL-6, and TNF- α were as follows: GAPDH (5'TCG ACA GTC AGC CGC ATC TTC TTT3'; 5'ACC AAA TCC GTT GAC TCC GAC CTT3'); TSLP (5'TAT GAG TGG GAC CAA AAG TAC CG3'; 5'GGG ATT GAA GGT TAG GCT CTG G3'); IL-6 (5'AAA TTC GGT ACA TCC TCG ACG GCA3'; 5'AGT GCC TCT TTG CTG CTT TCA CAC3'); TNF- α (5'AGG ACG AAC ATC CAA CCT TCC CAA3' R, 5'TTT GAG CCA GAA GAG GTT GAG GGT3'). Typical profile times used were the initial step, 95 °C for 10 min followed by a second step at 95 °C for 10 s for 40 cycles with a melting curve analysis. The level of target mRNA was normalized to the level of GAPDH

and compared with the control. Data were analyzed using the $\Delta\Delta\text{CT}$ method.

MTT assay

HMC-1 cells (1×10^5) were pretreated with NaSH and BS for 2 h and then activated with PMACI for 7 h. Cell aliquots were incubated with 500 μl of IMDM and 50 μl of a MTT solution (5 mg/ml) for 4 h at 37 °C under 5 % CO_2 and 95 % humidity. After washing out the supernatant, the insoluble formazan product was dissolved in dimethyl sulfoxide. Then, optical density of 96-well plates was measured at 540 nm using an ELISA reader (Molecular Devices Corp., Sunnyvale, CA).

Fluorescent measurements of intracellular calcium

HMC-1 cells (1×10^5) were pretreated with Fura-2/AM in IMDM containing 10 % FBS for 30 min and then were harvested. After washing twice with calcium free medium (containing 0.5 mM EGTA, extracellular calcium chelator), the cell suspension were placed into a 96-well plate and were pretreated with NaSH, BS, or BAPTA-AM for 20 min. The kinetics of the intracellular calcium were recorded every 10 s for 500 s at 440 nm (excitation at 360 nm) in a spectrofluorometer after PMACI stimulation.

Western blot analysis

The samples were prepared according to lysis procedures. The samples were heated at 95 °C for 5 min and cooled on ice. Following the centrifugation at 15,000g for 5 min, the proteins in the cell lysates were separated by 10 % SDS-PAGE and transferred to nitrocellulose paper. The membrane was blocked with 5 % skim milk in PBS containing tween-20 for 1 h at room temperature and incubated with primary and secondary antibodies. Finally, the protein bands were visualized by enhanced chemiluminescence procedures (Amersham Biosciences Corp, Piscataway, NJ, USA) according to the manufacturer's instructions.

Caspase-1 activity assay

Caspase-1 activity was examined according to the manufacturer's instructions using a caspase-1 assay kit (R&D system Inc.).

Preparation of nuclear protein extraction and cytoplasmic protein extracts

The nuclear protein extraction and cytoplasmic protein extracts were prepared as previously described [20].

OVA-induced AR mouse model

Four-week-old female BALB/c mice were purchased from the Dae-Han Experimental animal center (Eumsung, Chungbuk, Republic of Korea). All protocols for mice were approved by the institutional animal care committee of Kyung Hee University [KHUASP (SE)-12-019]. The experimental procedure for the mouse model of AR was performed according to a previous report [31]. In brief, mice were sensitized intraperitoneal (i.p.) injection of 100 μg of OVA emulsified in 20 mg of aluminum hydroxide (Sigma-Aldrich) on days 1, 5, and 14. NaSH (1 mg/kg) or dexamethasone (DEX) was orally administered before intranasal (i.n.) OVA challenge every day for 10 days. DEX was prepared at a dose of 3 mg/kg according to a previous report [40]. Nasal symptom was evaluated by counting the number of rubs that occurred in 10 min following OVA i.n. provocation. After the mice were euthanized by cervical dislocation, blood samples and tissue specimens were collected.

Statistical analysis

All results are representative of three independent experiments and expressed as the mean $\pm$ SEM. The statistical results were performed by an ANOVA with a Tukey post hoc test and an independent *t* test and using SPSS statistical software. A value of $p < 0.05$ was believed significant result. The graphs were made using originPro7.5 software.

Results

Inhibitory effect of NaSH on the production of TSLP from PMACI-activated HMC-1 cells

First, we examined the effect of NaSH on the production and mRNA expression of TSLP as a new molecular target of NaSH in PMACI-activated HMC-1 cells. NaSH dose-dependently diminished the production and the mRNA expression of TSLP in PMACI-activated HMC-1 cells ($p < 0.05$, Fig. 1a, b). BS also significantly diminished the production and the mRNA expression of TSLP in this study as the report of Kim et al. [35] ($p < 0.05$, Fig. 1a, b). We examined the additional regulatory effects of NaSH or BS on the levels of IL-6 and TNF- α . NaSH or BS significantly diminished the production and mRNA expression of IL-6 and TNF- α in PMACI-activated HMC-1 cells ($p < 0.05$, Fig. 1c, d). And we examined cell viability of NaSH or BS in HMC-1 cells with references [14, 25, 35]. We confirmed that cytotoxicity did not show at doses of 0.01, 0.1, and 1 mM of NaSH and 1 mg/ml of BS (Fig. 1c).

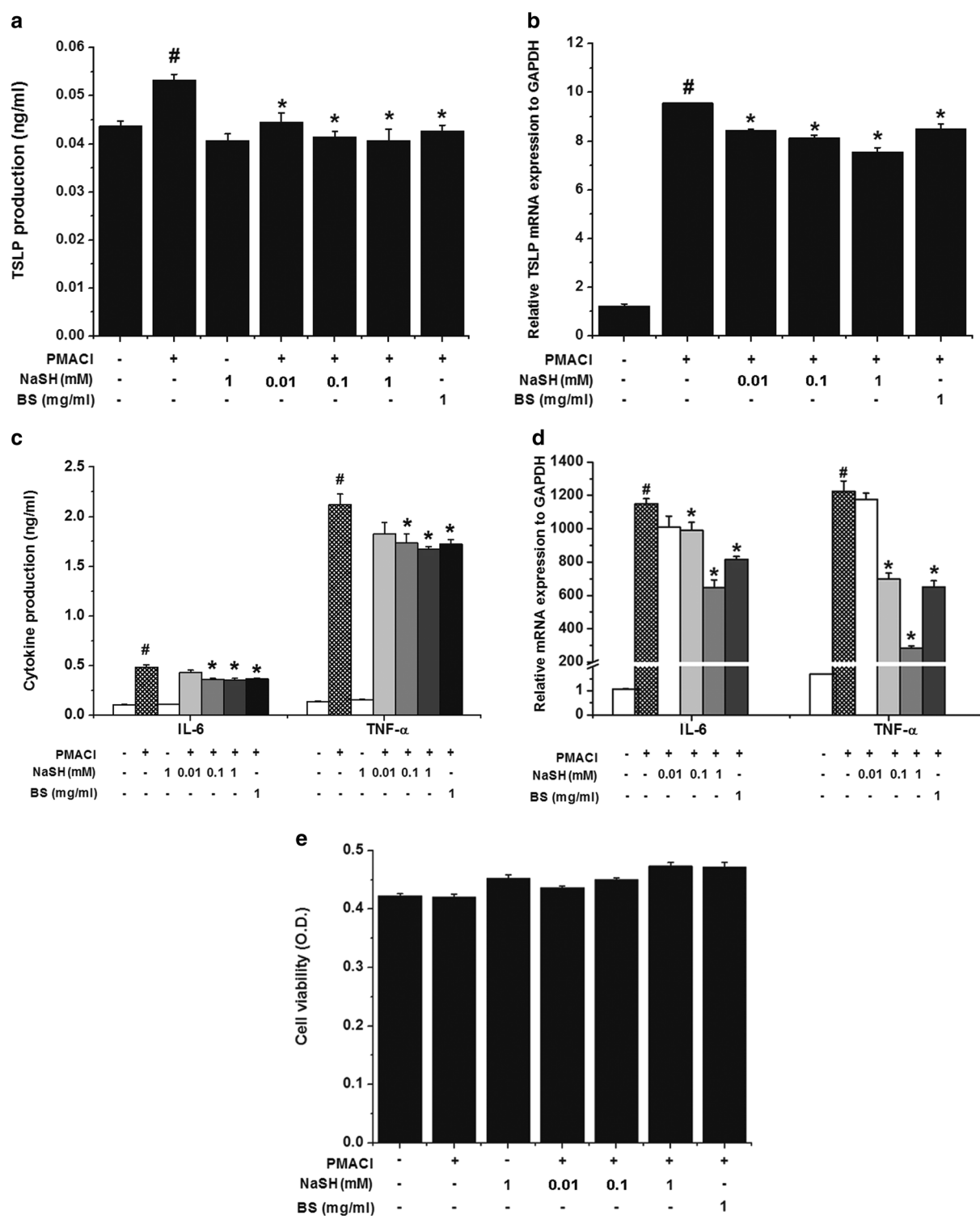


Fig. 1 Inhibitory effect of NaSH on the production of TSLP from activated HMC-1 cells. **a, c** HMC-1 cells (3×10^5) were pretreated with NaSH or BS for 2 h and then activated with PMACI for 7 h. The production of TSLP, IL-6, and TNF- α was analyzed with ELISA method. **b, d** HMC-1 cells (1×10^6) were pretreated with NaSH or

BS for 2 h and then activated with PMACI for 5 h. The mRNA expression of TSLP, IL-6, and TNF- α was analyzed with a real time-PCR analysis. [#] $p < 0.05$; significantly different from inactivated cells. ^{*} $p < 0.05$; significantly different from PMACI-activated cells. **e** Cell viability of NaSH or BS was determined with an MTT assay

Inhibitory effect of NaSH on the level of intracellular calcium in PMACI-activated HMC-1 cells

Because it is clear that the levels of TSLP are regulated by NaSH or BS in activated mast cells, the level of intracellular calcium which is an upstream effector of TSLP production is investigated. When HMC-1 cells are activated with PMACI, the levels of intracellular calcium were increased. However, NaSH or BS diminished the levels of intracellular calcium in activated HMC-1 cells (Fig. 2). The regulatory effect of NaSH (1 mM) on the level of intracellular calcium is similar to that of BAPTA-AM (10 μ M, calcium chelator).

Inhibitory effect of NaSH on the level of caspase-1 in PMACI-activated HMC-1 cells

To sequentially understand the effect of NaSH or BS on down-regulation of TSLP, the activation of caspase-1, a downstream effector of intracellular calcium, is examined in PMACI-activated HMC-1 cells. The expression of caspase-1 was diminished by NaSH or BS in PMACI-activated HMC-1 cells (Fig. 3a). And NaSH or BS significantly diminished the caspase-1 enzymatic activity ($p < 0.05$, Fig. 3b).

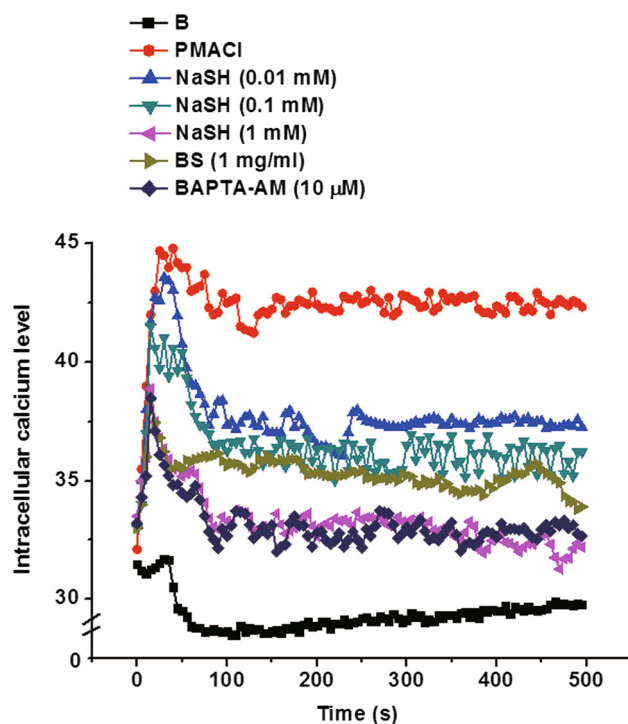


Fig. 2 Inhibitory effect of NaSH on the level of intracellular calcium in activated HMC-1 cells. HMC-1 cells were pretreated with NaSH, BS, or BAPTA-AM for 20 min and then activated with PMACI

Inhibitory effect of NaSH on the levels of RIP2, ERK, and NF- κ B in PMACI-activated HMC-1 cells

Furthermore, because the levels of TSLP are regulated through the expression of various upstream proteins including caspase-1, we examined the effects of NaSH or BS on the expression of RIP2 and the phosphorylation of ERK MAP kinase in PMACI-activated HMC-1 cells. PMACI increased the expression of RIP2 and the phosphorylation of ERK MAP kinase in HMC-1 cells. However, NaSH or BS diminished the expression of RIP2 and the phosphorylation of ERK MAP kinase in PMACI-activated HMC-1 cells (Fig. 4a, b). Finally, we examined the effects of NaSH or BS on the translocation of NF- κ B into the nucleus and degradation of I κ B α in the cytoplasm that are the downstream signal of RIP2/caspase-1/ERK MAP kinase. PMACI increased the translocation of NF- κ B into the nucleus, whereas decreased the levels of NF- κ B in the cytoplasm. And PMACI increased phosphorylation of I κ B α and degradation of I κ B α in the cytoplasm. However, NaSH or BS diminished the translocation of NF- κ B into the nucleus and phosphorylation and degradation of I κ B α in the cytoplasm. NaSH or BS increased the levels of NF- κ B in the cytoplasm (Fig. 4c).

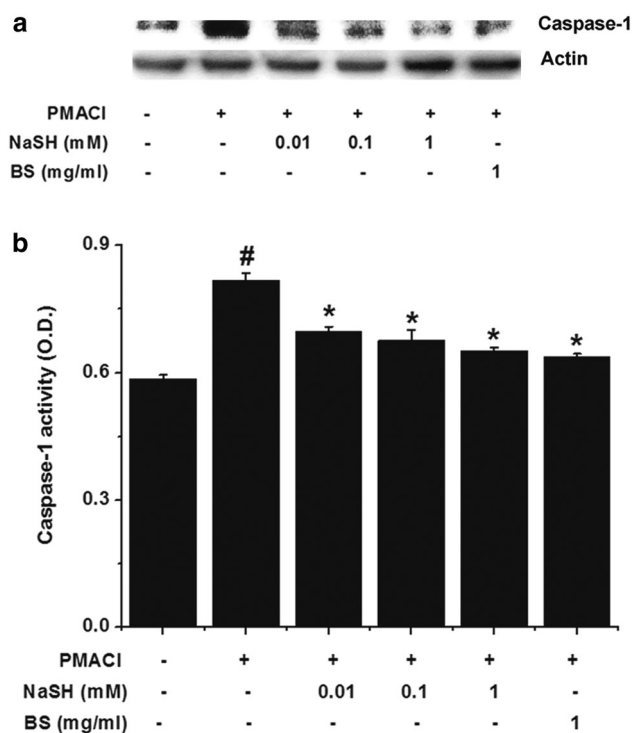


Fig. 3 Inhibitory effect of NaSH on the level of caspase-1 in activated HMC-1 cells. **a** HMC-1 cells (3×10^6) were pretreated with NaSH or BS for 2 h and then activated with PMACI for 30 min. The expression of caspase-1 was analyzed with a Western blot analysis. **b** The caspase-1 activity was measured with a caspase-1 assay kit

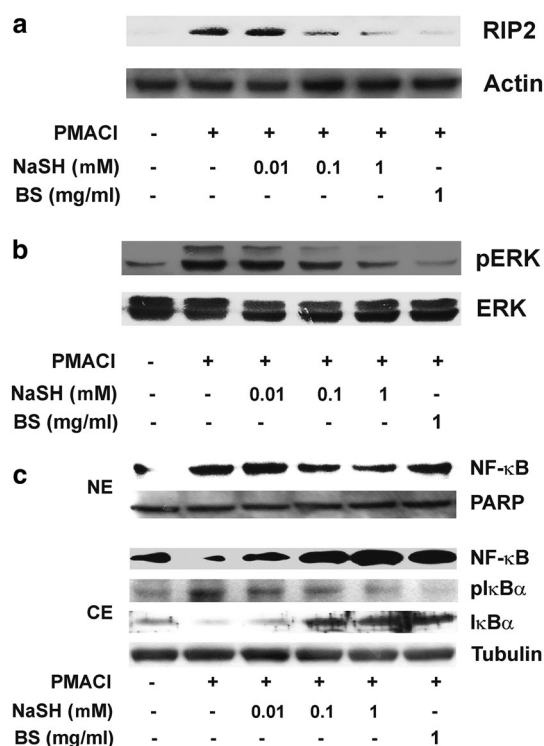


Fig. 4 Inhibitory effect of NaSH on the levels of RIP2, ERK, and NF- κ B in activated HMC-1 cells. **a**, **b** HMC-1 cells (3×10^6) were pretreated with NaSH or BS for 2 h and then activated with PMACI for 30 min. The expression of RIP2 and phosphorylation of ERK MAP kinase were analyzed with a western blot analysis. **c** HMC-1 cells (3×10^6) were pretreated with NaSH or BS for 2 h and activated with PMACI for 2 h. The translocation of NF- κ B and phosphorylation and degradation of I κ B α were analyzed with a western blot analysis. **NE** nuclear extract, **CE** cytoplasmic extract

Inhibitory effect of NaSH on the production of TSLP from TNF- α -activated HMC-1 cells

Further, we investigated the regulatory effect of NaSH on the production of TSLP induced by a specific activator in HMC-1 cells. TNF- α is a pleiotropic regulatory pro-inflammatory cytokine that mediates the production of various cytokines including IL-6 via binding to the membrane-bound TNF- α receptor [68]. The stimulation with TNF- α induced inflammatory response in HMC-1 cells [72]. Thus, we treated TNF- α for 24 h in NaSH-pretreated HMC-1 cells according to a previous report [24]. The stimulation with TNF- α significantly increased the production of TSLP from HMC-1 cells ($p < 0.05$, Fig. 5a). However, NaSH significantly decreased this increase from HMC-1 cells ($p < 0.05$, Fig. 5a). And NaSH significantly decreased the production of IL-6 and IL-8 from TNF- α -activated HMC-1 cells ($p < 0.05$, Fig. 5b, c). NaSH did not induce the production of TSLP, IL-6, and IL-8 in inactivated HMC-1 cells (Fig. 5).

Inhibitory effect of NaSH on the levels of TSLP in an AR mouse model

Finally, we investigated the inhibitory effect of NaSH in an AR mouse model. The OVA-sensitized mice showed significant increases in number of rubs compared with the OVA-unsensitized mice ($p < 0.05$). However, the increased number of rubs was significantly inhibited by treatment with NaSH ($p < 0.05$, Fig. 6a). The serum IgE level increased by OVA sensitization was significantly reduced by treatment with NaSH ($p < 0.05$, Fig. 6b). NaSH significantly reduced the level of TSLP in the serum of OVA-sensitized mice ($p < 0.05$, Fig. 6c). Also, OVA sensitization significantly increased the level of TSLP in the nasal mucosa tissue. However, the increased TSLP level in the nasal mucosa tissue of OVA-sensitized mice was significantly decreased by treatment with NaSH ($p < 0.05$, Fig. 6d). DEX was used as a reference drug in this study [62]. DEX significantly inhibited the number of rubs, the levels of IgE and TSLP in the serum, and the level of TSLP in the nasal mucosa tissue in this study ($p < 0.05$, Fig. 6).

Discussion

Recent studies have suggested that an intake of salt is a major cause of various diseases, such as hypertension [53], cardiovascular disease [69], or diabetes [10]. However, in case of BS, it has been reported to have therapeutic effects on various diseases [35, 63, 75]. Sulfur, a component of BS, was also reported to be effective for the treatment of allergy or pain syndromes [56]. Furthermore, we establish that the treatment with BS or H₂S diminishes the levels of TSLP through the blockade of caspase-1 signaling in activated mast cells and the levels of TSLP in an OVA-induced AR mouse model in this study.

Mast cells are effector cells of adaptive immune responses and principal to initiate an early inflammatory reaction [23]. TSLP is an important mediator of allergic inflammation and a potent activator of human mast cells [15]. TSLP amplifies Th2 cytokine production from mast cells [6]. In addition, TSLP which was expressed in mast cells aggravated atopic dermatitis (AD) in mice [21]. Ginsenoside Rg1 decreased the levels of TSLP in mast cells and regulated AR in mice [32]. Although the levels of TSLP protein presented in this study were detected low, H₂S or BS significantly diminished the production and mRNA expression of TSLP in PMACI-activated HMC-1 cells. Redhu et al. [58] reported that a low level of TSLP produced via NF- κ B pathway in airway cells is sufficient to initiate or perpetuate the allergic inflammation as a critical

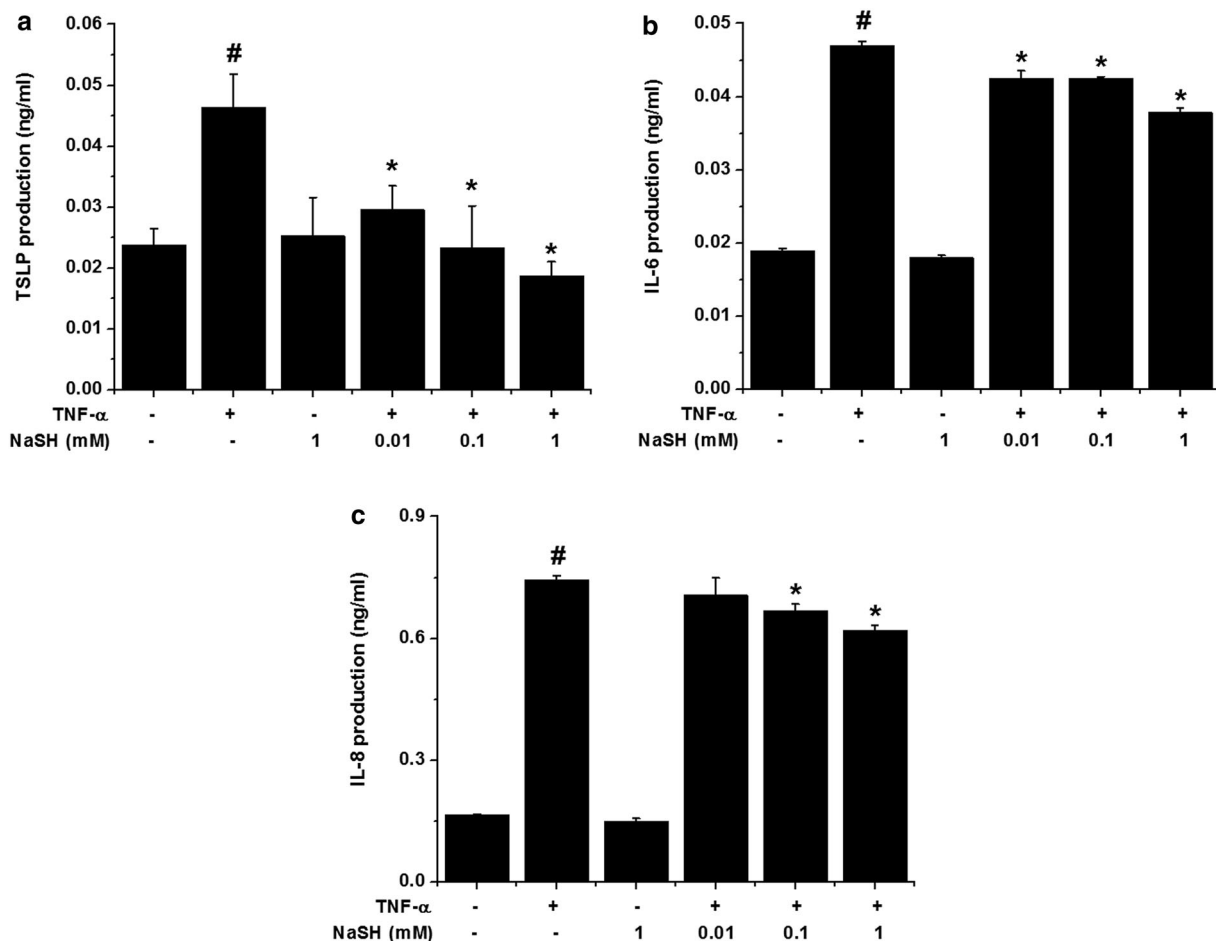


Fig. 5 Inhibitory effect of NaSH on the production of TSLP from TNF- α -activated HMC-1 cells. HMC-1 cells (3×10^5) were pre-treated with NaSH or BS for 2 h and then activated with TNF- α (10 ng/ml) for 24 h. The production of **a** TSLP, **b** IL-6, and **c** IL-8

was analyzed with ELISA method. # $p < 0.05$; significantly different from inactivated cells. * $p < 0.05$; significantly different from TNF- α -activated cells

proallergic cytokine. The low level of TSLP correlates with severity in patients with AD and in animal models of AD [47, 50]. The low production of TSLP in airway epithelial cells facilitated the development and/or exacerbation of Th2-type airway inflammation [41]. Thus, we infer that H₂S has a substantial regulatory effect on the TSLP production from mast cells. In addition, H₂S or BS decreased the levels of IL-6 and TNF- α which are considered as a biomarker of ongoing inflammation [33] in PMACI-activated HMC-1 cells. Also, H₂S inhibited the production of TSLP, IL-6, and IL-8 induced by TNF- α in HMC-1 cells. We can deduce that TNF- α might reveal a putative inducer of the release of TSLP in an autocrine manner in PMACI-activated mast cells. Therefore, these results suggest that H₂S or BS may regulate the allergic inflammations through the suppression of levels of TSLP as well as inflammatory cytokines. However, although HMC-1 cells are the only established human mast cell line and have been widely

used for in studies of human mast cell biology, HMC-1 cells have kit-mutations [65]. Therefore, further work may be necessary to clarify how kit-mutations affect the TSLP production by PMACI stimulation in mast cells.

An allergen exposure triggers the calcium influx and induces the degranulation of mast cells [22]. The PMA, a PKC activator is a substitute for diacylglycerol and A23187 is a mostly used calcium ionophore. PMACI increased the level of intracellular calcium in mast cells. However, H₂S or BS diminished the level of intracellular calcium in PMACI-activated HMC-1 cells, similar to BAPTA-AM. Although sodium sulfite induced the degranulation of mast cells [9], Liu et al. [44] reported that H₂S decreased the degranulation of mast cells from rat tissue. BAPTA-AM diminished the levels of lipopolysaccharide-induced inflammatory mediators in peritoneal macrophages [55]. In addition, we reported BAPTA-AM inhibited the levels of TSLP through the RIP2/caspase-1/

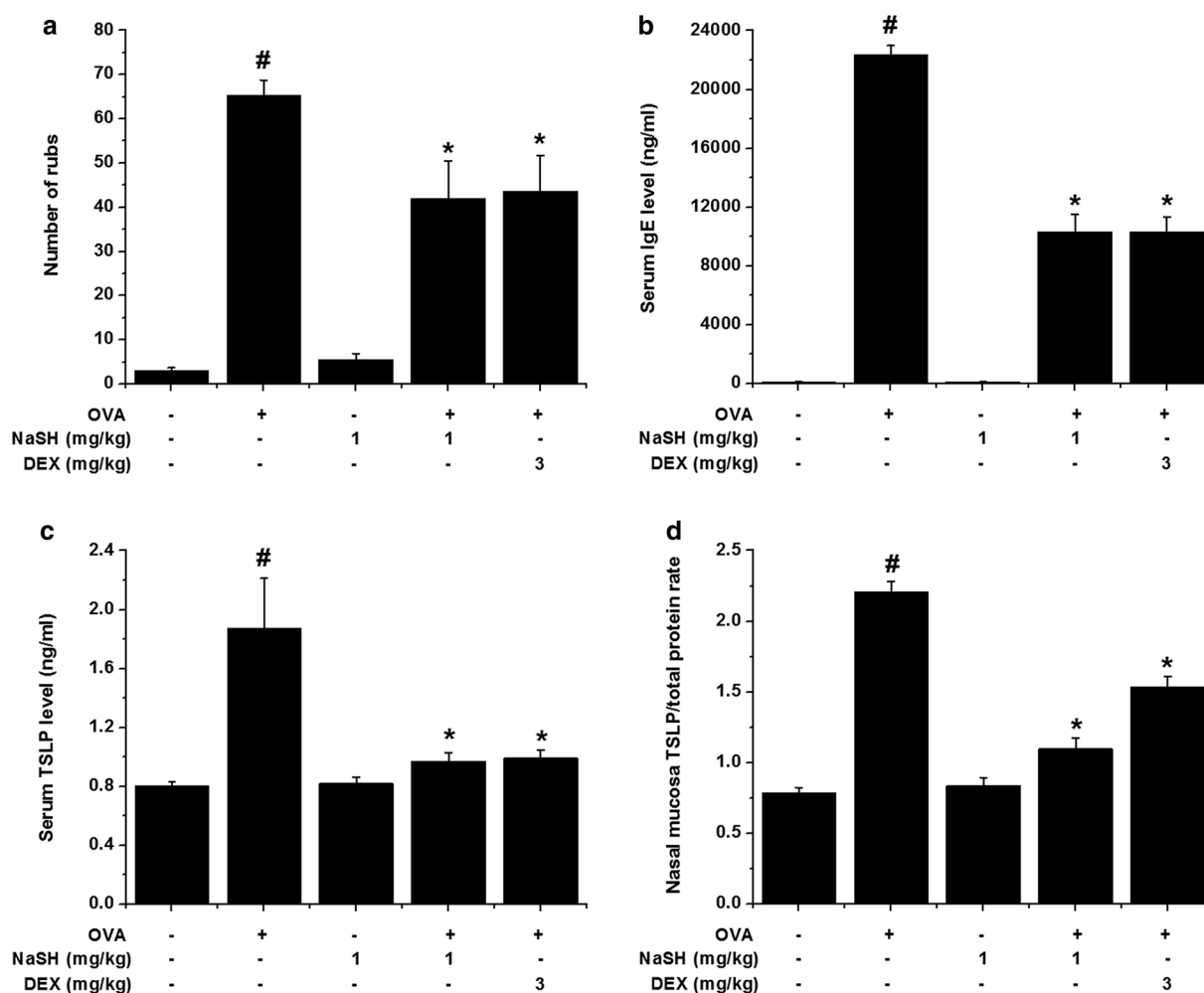


Fig. 6 Inhibitory effect of NaSH on the levels of TSLP in an AR mouse model. Mice were orally treated with NaSH (1 mg/kg) or DEX (3 mg/kg) for 10 days prior to the i.n. OVA challenge. **a** The number of rubs that occurred in 10 min after the OVA i.n. provocation was counted for 10 min. The levels of **b** IgE and **c** TSLP in the serum were measured by ELISA method. **d** The protein level of TSLP in the

nasal mucosa tissue homogenates was measured using the ELISA method. This was presented as a ratio to the total protein level in the tissue. $n = 5$. [#] $p < 0.05$; significantly different from OVA-unsensitized mice. ^{*} $p < 0.05$; significantly different from OVA-sensitized mice

NF- κ B signaling [66]. We thus postulated that H₂S or BS would also regulate the levels of TSLP through the blockade of the calcium signaling.

Calcium is necessary to activate caspase-1 [12]. Caspase-1 activation mediates inflammation and allergic reactions [2]. RIP2 which is an upstream activator of caspase-1, was up-regulated in allergic airway inflammation [17]. RIP2 is required for NF- κ B activation, and it induces immune responses [60]. A defect in RIP2 activation or caspase-1 activation induced less production of inflammatory cytokines [61]. H₂S or BS also decreased the expression of RIP2 and activation of caspase-1 in activated HMC-1 cells. Thus, it is conceivable that H₂S or BS would inhibit the allergic inflammation.

MAP kinase signaling plays a central role in the initiation of inflammatory responses [48]. RIP2 is involved in

MAP kinases-signaling pathways [52]. Phosphorylation of ERK which controls the release of allergic mediators in mast cells is responsible for caspase-1 activation [19]. Phosphorylation of ERK increases transcriptional activity of NF- κ B [7]. Inflammatory cytokines are expressed to rely on the activation of NF- κ B, and it requires the phosphorylation of I κ B α [13, 54]. NF- κ B activation promoted allergic sensitization in mice [3]. Glucocorticoids inhibited the proinflammatory activities of ERK MAP kinase and NF- κ B [34]. Likewise, H₂S or BS attenuated the ERK MAP kinase/NF- κ B/I κ B α signaling pathway in activated HMC-1 cells. These results demonstrate that the anti-inflammatory effects of H₂S or BS might derive from down-regulation of the ERK/NF- κ B signaling.

AR can result from the activation of mucosal mast cells beneath the nasal epithelium [29] and IgE-mediated

inflammation of the nose upon contact of the nasal mucosa with allergens [30]. TSLP is important in allergic diseases inducing AR and plays a role as a potential therapeutic target [8]. The expression of TSLP was increased in the nasal epithelium of biopsies from patients with AR [38]. TSLP pathways are essential for evoking systemic IgE responses and allergy development [51]. OVA is a common allergen to clarify the pathogenetic mechanisms underlying AR as well as establish novel therapeutic targets [42]. Blocking TSLP inhibited AR development in the OVA-induced mouse model of AR [59]. BS was reported to regulate the levels of TSLP and reduce AR reactions [35]. TNF- α is closely involved in the AR initiation and AR development [28]. Mast cell-derived inflammatory cytokines including TNF- α generated at the site of AR play an important role for the induction of TSLP expression in the nasal epithelium [46]. Redhu et al. [58] reported that TNF- α -induced TSLP expression via NF- κ B pathway can be a prime target for anti-inflammatory drugs. In this study, H₂S inhibited the levels of TSLP in the serum and the nasal mucosa tissue of OVA-induced mouse model of AR. H₂S down-regulated the serum IgE level in OVA-sensitized mice. In addition, H₂S inhibited the production of TSLP induced by TNF- α in mast cells. Therefore, we can conclude that H₂S inhibits the development of AR through the regulation of TSLP.

Taken together, we establish for the first time that H₂S, as an active compound of BS, diminishes the levels of TSLP through the down-regulation of intracellular calcium/RIP2/caspase-1/ERK MAP kinase/NF- κ B signaling in mast cells and in an OVA-induced AR mouse model. These data clarify that H₂S might have therapeutic potential to cure allergic reactions aggravated by TSLP.

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Compliance with ethical standards

Conflict of interest None to report.

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