Endogenous production of hydrogen sulfide in human sinus mucosa and its expression levels are altered in patients with chronic rhinosinusitis with and without nasal polyps

Jae Woong Hwang, M.D., Young Joon Jun, M.D., Se Jin Park, M.D., Tae Hoon Kim, M.D., Ki Jeong Lee, M.D., Soo Min Hwang, M.D., Seung Hoon Lee, M.D., Heung Man Lee, M.D., and Sang Hag Lee, M.D.

ABSTRACT

Background: Chronic rhinosinusitis with nasal polyps (CRSwNPs) or CRS without nasal polyps (CRSsNPs) is characterized by persistent inflammation of sinonasal mucosa. No one causative factor fully explains for the pathological manifestations of CRS. Endogenous hydrogen sulfide (H2S) has been shown to participate in inflammatory diseases, functioning as an inflammatory mediator in various organs. We analyzed the contents and synthesis activity of H2S, the expression and distribution pattern of H2S-generating enzymes, cystathione β-synthase (CBS), and cystathione γ-lyase (CSE) in CRSwNPs and CRSsNPs. The effects of H2S on the expression of CRS-relevant cytokines and the effects of cytokines on the expression of CBS and CSE were assessed in an in vitro experiment.

Methods: The contents and synthesis activity of H2S and the expression and distribution pattern of CBS and CSE in sinus mucosa were evaluated using spectrophotometry, real-time polymerase chain reaction, Western blot, and immunohistochemistry. Cultured epithelial cells were used to elucidate the effects of H2S donor, sodium hydrosulfide (NaHS), on the expression of CRS-relevant cytokines and the effects of cytokines on H2S-generating enzymes expression.

Results: The contents and synthesis activity of H2S were increased in CRSwNPs and CRSsNPs. CBS and CSE were localized to the superficial epithelium and submucosal glands, but CSE was also found in vascular endothelium. NaHS induced increased expression of IL-4, IL-5, interferon-γ, and TNF-α. CBS and CSE expression in cultured cells was up-regulated by CRS-relevant cytokines.

Conclusion: H2S levels are increased in CRS, contributing to increased production of cytokines. These results suggest that H2S may function as an inflammatory mediator in CRS.

In recent studies, hydrogen sulfide (H2S) has also been identified as a member of the novel family of gaseous mediators. Furthermore, endogenous H2S, along with NO and CO, has been positioned as the third endogenous signaling gasotransmitter in various organs, including the respiratory system.12–13 Endogenous H2S is produced in many tissues by two H2S-generating enzymes, cystathione β-synthase (CBS) and cystathione γ-lyase (CSE).12,13 H2S is involved in a number of pathophysiological processes of the respiratory system such as chronic obstructive pulmonary disease (COPD) and asthma.14–16 Serum levels of H2S have been shown to be significantly higher in patients with stable COPD than in control subjects.15 In a chronic COPD rat model, endogenous H2S has been shown to play a protective role, both as an anti-inflammatory agent and a bronchodilator.17 Significantly higher H2S levels were recently observed in the synovial fluid of patients with rheumatoid arthritis than osteoarthritis, and its levels significantly correlated with inflammatory scores.18 The expression and activity of CSE in human articular chondrocytes, one of H2S-synthesizing enzymes, was induced by treatment with IL-1β, TNF-α, IL-6, or lipopolysaccharide (LPS).19 Chronic kidney disease is associated with a significant reduction in plasma H2S concentrations, diminished H2S-producing capacity, and down-regulation of the H2S-producing enzymes, suggesting that its deficiency may contribute to progression of chronic kidney disease.20 In a colitis animal model, H2S appeared to act as an anti-inflammatory molecule (summarized in Table 1). In this respect, it is hypothesized that alterations in the level of endogenous H2S may be involved in the pathogenesis of CRS.

Therefore, to clarify the role of H2S in the pathogenesis of CRS, the present study was undertaken to measure the tissue contents and synthesis activity of H2S and the expression levels and the distribution pattern of CBS and CSE in ethmoid sinus mucosa of healthy controls and patients with CRSwNPs and CRSsNPs. Furthermore, we elucidated the impact of H2S on the expression of cytokines relevant.
Increased H2S formation
findings, and the findings of computed tomography scan were scored
CRSwNPs
§The data of CRSwNPs are significantly higher than those of control and

American Journal of Rhinology & Allergy 13
The diagnosis of CRS and the presence or absence of NPs were made
during endoscopic reduction in patients with a blowout fracture. During
the operation, the normal-appearing ethmoid sinus mucosa, not
injured by the fracture, was considered as a normal control. Sample
of inflammatory ethmoid sinus mucosa lining the interior surface of
the ethmoid sinus cells were collected from patients undergoing
diagnosis surgery for CRSwNPs and CRSsNPs.
Tissue samples were divided into three portions; The first and
second portion were frozen in liquid nitrogen and stored at −80°C for
subsequent RNA isolation and protein isolation. For immunochemistry,
the third portion was embedded in paraffin wax. A part of normal
and inflammatory sinus mucosa was used for epithelial culture
Sections stained with hematoxylin and eosin were examined
to obtain a general impression of the typical pathological features.
The number of total inflammatory cells, eosinophils, mononuclear
and plasma cells in the subepithelial layer was counted at high
g population magnification (×400), and five high-power fields were
randomly selected.

Measurement of Tissue H2S Content and H2S
Synthesis in Sinus Mucosa
H2S content in the sinus mucosa was measured by zinc-trap spec-
photometry as described previously.18,25 This method has been
widely used for the measurement of H2S in tissues.26,27 Frozen tissues
(50 mg) were respectively homogenized in a mixture of 0.5 mL of zinc
acetate (1%) and 0.5 mL of borate buffer (pH 11). Then, 0.5 mL of
N,N,N-dimethyl-p-phenylenediamine sulfate (20 mM, in 7.2 M of HCl)
and 0.5 mL of FeCl3 (300 mM) were added to tissue homogenate.
Reaction tubes were immediately sealed and incubated at 37°C for
30 minutes with shaking. The absorbance of the resulting solution was
measured at 670 nm with a spectrophotometer. The H2S concentration
was calculated according to the calibration curve of standard H2S
solutions.
The capacity for H2S synthesis was measured as previously
described.28 Briefly, frozen tissue was homogenized in 50 mM of ice-cold
phosphate buffered saline (pH 7.4) containing protease and phosph-
ate inhibitors. The tissue homogenates were mixed with L-cysteine
(5 mM) and pyridoxal 5'-phosphate (2 mM) at the final volume of 0.25
mL and incubated at 37°C for 90 minutes. The reaction was stopped
by adding 0.125 mL of 50% trichloroacetic acid and 0.125 mL of zinc
acetate and 0.5 mL of borate buffer. The samples were then
incubated at 37°C for 60 minutes. The reaction mixtures were mixed with 0.5 mL of
N,N,N-dimethyl-p-phenylenediamine sulfate and 0.02 mL of FeCl3
at 37°C for an additional 30 minutes and then centrifuged for 5 minutes.
The supernatants were then measured at 670 nm. The capacity for H2S
synthesis was calculated by subtracting the H2S concentration con-
taining L-cysteine by those without L-cysteine.

Isolation and Culture of Epithelial Cells from
Normal and Inflammatory Sinus Mucosa
Normal and inflammatory sinus mucosa were treated with 0.5%
Dispase in a 1:1 mixture of DMEM/F12 supplemented with penicillin
G sodium (50 IU/mL) (Lonza Walkersville Inc., Walkersville, MD)
and streptomycin sulfate (50 μg/mL) overnight at 4°C. Epithelial cells
were then separated from the sinus mucosa by using a sterile nasal

### Table 1 Diseases associated with changes in H2S generation

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>Chen et al.14</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Chen et al.15</td>
</tr>
<tr>
<td>Colitis</td>
<td>Whitman et al.16</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>Park et al.17</td>
</tr>
<tr>
<td>Decreased H2S formation</td>
<td>Chen et al.18</td>
</tr>
</tbody>
</table>

### Table 2 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CRSwNPs</th>
<th>CRSsNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age (yr) mean range</td>
<td>29.5 (18–35)</td>
<td>37.6 (17–40)</td>
<td>35.7 (20–45)</td>
</tr>
<tr>
<td>No. of female/male patients</td>
<td>5/20</td>
<td>7/18</td>
<td>8/17</td>
</tr>
<tr>
<td>Asthma history</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Skin test</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Smoking</td>
<td>5/25</td>
<td>6/25</td>
<td>7/25</td>
</tr>
<tr>
<td>No. of sinus surgeries</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SNOT-20*</td>
<td>2.5 ± 1.6</td>
<td>27.9 ± 2.5</td>
<td>22.5 ± 3.7</td>
</tr>
<tr>
<td>CT grade*</td>
<td>0.0</td>
<td>18 ± 1.1</td>
<td>7.6 ± 1.7</td>
</tr>
<tr>
<td>Endoscopy scores*</td>
<td>0</td>
<td>9.6 ± 0.9</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>No. of sinus surgeries</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total inflammatory cells#</td>
<td>11.7 ± 5.8</td>
<td>77.5 ± 11.8</td>
<td>72.8 ± 9.6</td>
</tr>
<tr>
<td>Eosinophils§</td>
<td>1.0 ± 0.2</td>
<td>4.9 ± 1.5</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td>Mononuclear cells#</td>
<td>15.5 ± 6.9</td>
<td>45.3 ± 12.1</td>
<td>34.7 ± 15.2</td>
</tr>
<tr>
<td>Plasma cells§</td>
<td>1.6 ± 1.3</td>
<td>5.2 ± 1.3</td>
<td>2.8 ± 1.3</td>
</tr>
</tbody>
</table>

*The data of CRSwNPs and CRSsNPs are significantly higher than those of controls, with difference between CRSwNPs and CRSsNPs.
#The data of CRSwNPs and CRSsNPs are significantly higher than those of controls, with difference between CRSwNPs and CRSsNPs. Total number of inflammatory cells includes the number of other inflammatory cells (i.e., neutrophils, basophils, etc.) found in tissues, in addition to the number of eosinophils, mononuclear cells, and plasma cells.
§The data of CRSwNPs are significantly higher than those of control and CRSsNPs, without difference between CRSwNPs and controls.
CRSwNPs = chronic rhinosinusitis with nasal polyps; CRSsNPs = chronic rhinosinusitis without nasal polyps; CT = computed tomography; SNOT20 = 20-item Sino-Nasal Outcome Test.

to CRS and determined the effect of CRS-relevant cytokines on the expression levels of CBS and CSE using cultured epithelial cells.

## METHODS

### Subjects

Twenty-five patients with CRSwNPs, 25 patients with CRSsNPs, and 25 controls were enrolled. Patients undergoing endoscopic re-
duction because of blowout fracture were selected as control subjects. The diagnosis of CRS and the presence or absence of NPs were made
g according to the current “European Position Paper on Rhinosinusitis and Nasal Polyps 2012.” Patients symptoms, endoscopic physical
findings, and the findings of computed tomography scan were scored as described previously.19-24 None of the control subjects or patients with CRSwNPs and CRSsNPs had either a history of asthma or a

Copyright (c) Oceanside Publications, Inc. All rights reserved.
For permission to copy go to https://www.oceansidepubl.com/permission.htm
cytology brush, agitated, and collected into a conical tube. The cells were cultured under air–liquid interface in serum-free bronchial epithelial growth medium (BEGM; Lonza Walkersville, Inc., Walkersville, MD). Cultured epithelial cells were stimulated with IL-4, IL-5, IL-1β, IL-13, transforming growth factor (TGF) β1, TNF-α, or interferon (IFN) γ at a concentration of 10 and 30 ng/mL, respectively, for 12 and 24 hours. These cytokines were purchased from PeproTech (Rocky Hill, NJ). The cultured cells stimulated with cytokines were harvested and analyzed for CBS and CSE mRNA and proteins using real-time polymerase chain reaction (PCR) and Western blot. To evaluate the effect of sodium hydrosulfide (NaHS) on the cytokine expression levels, which are relevant for pathogenesis of CRS, the activity of H2S synthesis was measured in sinus mucosa of patients with CRSwNPs and CRSsNPs. As shown in Fig. 2, the expression levels of CBS and CSE mRNA were significantly higher in sinus mucosa of patients with CRSwNPs and CRSsNPs, including H2S levels and the data evaluated by one-way ANOVA, and Bonferroni post hoc correction was applied. ANOVA test followed by post hoc analysis with Bonferroni correction was also performed to establish statistically significant differences in values obtained from healthy controls and the patients with CRSwNPs and CRSsNPs, including H2S levels and the data evaluated by real-time PCR, Western blot analysis, and ELISA. The level of significance was defined at p < 0.05.

**RESULTS**

**H2S Contents and the Capacity for H2S Synthesis in Normal and Inflammatory Sinus Mucosa**

As illustrated in Fig. 1, H2S contents in sinus mucosa were significantly higher in patients with CRSwNPs and CRSsNPs than in healthy controls. However, these levels were not significantly different between patients with CRSwNPs and CRSsNPs (Fig. 1 A). To determine whether the capacity for H2S synthesis is altered in inflammatory sinus mucosa, we measured the generation of H2S from normal and inflammatory sinus mucosa. As illustrated in Fig. 2, the activity of H2S synthesis was increased in inflammatory sinus mucosa of patients with CRSwNPs and CRSsNPs in the presence of l-cysteine. The activity of H2S synthesis was increased in inflammatory sinus mucosa of patients with CRSwNPs and CRSsNPs. However, there were no significant differences in the activity of H2S synthesis between patients with CRSwNPs and CRSsNPs (Fig. 1 B).
CBS and CSE with respect to β-actin was significantly higher in inflammatory sinus mucosa of patients with CRSwNPs and CRSsNPs than healthy control subjects, indicating that the expression levels of CBS and CSE protein increased in inflammatory sinus mucosa of patients with CRSwNPs and CRSsNPs when compared with that in controls. There were no differences in the expression levels of CBS and CSE protein between inflammatory sinus mucosa of patients with CRSwNPs and CRSsNPs (Fig. 2, C and D).

The distribution pattern of CSE and CBS in normal and inflammatory sinus mucosa was analyzed using immunohistochemistry. Immunohistochemical staining showed a homologous pattern in sinus mucosa from healthy controls and patients with CRSwNPs and CRSsNPs (Fig. 2 E). CBS in healthy and inflammatory sinus mucosa showed stronger immunoreactivity in the superficial epithelium and submucosal glands. CSE was localized to the vascular endothelium, in addition to the superficial epithelium, and submucosal gland (Fig. 2 E).

Effects of NaHS on the Expression of Cytokines and the Effects of Cytokines on the Expression of CBS and CSE in Cultured Epithelial Cells

To determine the possible effects of H2S on cytokine expression in the sinus mucosa, expression levels of IL-4, IL-5, IL-1α, IFN-γ, TNF-α, and TGF-β1 were examined using real-time PCR and ELISA after stimulation of cultured normal and inflammatory epithelial cells with the H2S donor, NaHS. As shown in Fig. 3 A, NaHS increased the expression of IL-4, IL-5, IFN-γ, and TNF-α mRNA. However, the expression levels of IL-1β, IL-13, and TGF-β1 mRNA did not change after stimulation with NaHS. The same results were also obtained when cultured epithelial cells derived from inflammatory sinus mucosa were used (data not shown). To further confirm the effects of NaHS, we performed ELISA to detect protein levels of each cytokine in cultured cells (Fig. 3 B). These results mirrored the mRNA data.

The effect of each cytokine on the expression levels of CBS and CSE mRNA and protein was also evaluated using cultured epithelial cells. Epithelial cells derived from normal and inflammatory sinus mucosa were cultured in the presence of cytokines for 12 or 24 hours, and the expression levels of CBS and CSE mRNA and protein were then determined. Both CBS and CSE were up-regulated in response to IL-4, IL-5, IL-1α, IL-13, IFN-γ, and TNF-α, whereas TGF-β1 had no effect on enzymes expression (Fig. 4). The same results were also obtained when cultured epithelial cells derived from inflammatory sinus mucosa were used (data not shown).

DISCUSSION

In our study, we found that H2S is produced in normal sinus mucosa and is up-regulated in the inflammatory sinus mucosa of patients with CRS, irrespective of presence of NPs. Increased activity of H2S synthesis was noted in the inflammatory sinus mucosa of CRS patients, compared with normal sinus mucosa. Immunohistochemical analysis showed that CSE was localized to submucosal glands, vascular endothelium, and superficial epithelium in normal and inflammatory sinus mucosa, whereas CBS was mainly distributed in submucosal glands and superficial epithelium. Expression levels of these H2S-synthesizing enzymes, CBS and CSE, increased in inflammatory sinus mucosa and was modulated in cultured epithelial cells after stimulation with CRS-relevant cytokines. Furthermore, the H2S donor, NaHS, differentially increased expression levels of cytokines that have been shown to be related to the pathogenesis of CRS. These data provide evidence that the up-regulation of H2S in CRSwNPs and CRSsNPs may play an important role in the pathophysiology of CRS and contribute to increased production of cytokines. This is the first study to compare the expression levels and distribution pattern of H2S and the H2S-synthesizing enzymes, CSE and CBS, which may be helpful in understanding the pathogenesis of CRS.

Endogenous H2S is generated by two pyridoxal-5′-phosphate-dependent enzymes, CBS and CSE, with l-cysteine used as a major substrate. These enzymes are abundantly expressed in various organs, and the enzymic pathways for H2S production are tissue specific.26 In some tissues, CSE and CBS are both required for H2S synthesis. In other tissues, tissue-specific differences in the relative CBS and CSE expression levels result in one or the other enzyme being the more prominent source of H2S generation.12 CBS expression is significant in the brain as the primary physiological source of H2S in the central nervous system. In other tissues, such as cardiovascular system, respiratory system, testes, adrenal, and spleen, CBS expression is rare or absent. CSE is expressed abundantly in the mammalian cardiovascular system and respiratory system and also appears to be the main H2S-forming enzyme in liver, kidney, uterus, and placenta, as well as pancreatic islets.29 Small intestine and stomach tissues express low amounts of CSE.60 Previously, transcripts of the H2S-synthesizing enzyme CSE and CBS have been evaluated in the nasal mucosa of the guinea pig, where more expression of CSE mRNA, but not of CBS mRNA, was detected, suggesting that CSE is the major H2S-producing enzyme in the nasal mucosa of the guinea pig.31 Our current data show that both CBS and CSE are present in normal human sinus mucosa, suggesting that they are both needed for the generation of H2S in human sinus mucosa. Interestingly, in human sinus mucosa, CBS was mainly localized to the submucosal glands and superficial epithelium, whereas CSE was exclusively expressed in vascular endothelium, superficial epithelium, and submucosal glands. These results are in accordance with those of our previous results evaluated in human nasal mucosa.32 These observations imply that in human nasal and sinus mucosa, CBS may be the main H2S-producing enzyme in superficial and submucosal glandular epithelium, and CSE may
mainly contribute to the production of H$_2$S in vascular tissues, superficial epithelium, and submucosal glands. In the present study, H$_2$S content increased in inflammatory sinus mucosa of CRS, irrespective of the presence of NPs, and was accompanied by up-regulation of both CBS and CSE. This observation points to the importance of CBS and CSE as a major source of H$_2$S in inflammatory sinus mucosa.

Figure 2. (A and B) Real-time polymerase chain reaction (PCR) and (C and D) Western blot analysis of (A and C) cystathione $\beta$-synthase (CBS) and (B and D) cystathione $\gamma$-lyase (CSE) expression in normal sinus mucosa of (C) control subjects ($n = 25$) and inflammatory sinus mucosa of chronic rhinosinusitis with nasal polyps (CRSwNP; $n = 25$) and chronic rhinosinusitis without nasal polyps (CRSsNP; $n = 25$). Asterisk indicates significant difference in the expression levels of CBS and CSE between normal control and CRSwNP or CRSsNP ($p < 0.05$). The panels of Western blot show representative data. Results are expressed as mean $\pm$ SD. (E) Immunohistochemical localization of CBS$^1$-$^3$ and CSE$^4$-$^6$ in normal sinus mucosa of control subjects$^4$-$^6$ and inflammatory sinus mucosa of CRSwNP$^3$-$^5$ and CRSsNP$^3$-$^6$. Arrow head indicates superficial epithelium; arrow indicates vascular endothelium; G indicates submucosal glands (original magnification: $\times$100).
We also examined the effect of H$_2$S substrate, l-cysteine on endogenous levels of H$_2$S and observed that l-cysteine significantly increased the level of endogenous H$_2$S in inflammatory sinus mucosa, compared with that in normal sinus mucosa. There is evidence from several studies that l-cysteine increases H$_2$S production in various tissues.\textsuperscript{28,33,34} This observation points to the concordance between progression of CRS and increased H$_2$S-producing capacity of the inflammatory sinus mucosa. Additional studies are required to measure the expression/activity of H$_2$S using pharmacologic inhibitors of CBS and CSE in sinus mucosa.

Evidence is emerging for the involvement of H$_2$S in human chronic inflammatory diseases, including asthma, COPD, and colitis.\textsuperscript{14–16,21} Altered expression of CBS or CSE and endogenous H$_2$S levels have been shown to be responsible for inflammation. Significant increases in H$_2$S production and up-regulation of CSE expression have been observed in studies of rodent models of acute pancreatitis and endotoxemia.\textsuperscript{28,35} The serum levels of H$_2$S decreased in subjects with stable and severe acute asthma exacerbation compared with healthy subjects.\textsuperscript{16} In animal models of ovalbumin-treated asthma, serum and tissue H$_2$S levels were decreased, in association with decreased CSE expression levels and activity.\textsuperscript{16} Although the expression pattern and extent of H$_2$S-producing enzymes in the lung and in airway tissues are variable depending on the species and cell type, human smooth muscle cells and primary fibroblasts have been shown to express CSE and CBS proteins.\textsuperscript{36,37} Endogenous H$_2$S concentration increased in patients with stable COPD and decreased in patients with acute exacerbation of COPD.\textsuperscript{15} However, these studies did not report whether the expression levels of both enzymes were changed. In colitis, the mRNA levels of CSE and CBS as well as the H$_2$S content in the colonic mucosa increased.\textsuperscript{21} Our previous study also showed that increased expression of H$_2$S is associated with increased CBS and CSE expression in allergic rhinitis.\textsuperscript{32} However, there is only limited information about the regulation of CBS and CSE expression responsible for the endogenous synthesis of H$_2$S in chronic inflammatory diseases. CBS expression is known to be up-regulated by epidermal growth factor, TGF-$\alpha$, cAMP, and dexamethasone in reactive astrocytes.\textsuperscript{38} CSE is up-regulated by the NO donor such as S-nitroso-N-acetyl penicillamine.\textsuperscript{39} Another study showed that LPS induced CSE expression in macrophages and this effect was inhibited by glucocorticoids, suggesting a role of CSE regulation in inflammatory processes.\textsuperscript{40} In addition, inflammatory cytokines such as TNF-$\alpha$, IL-1$\beta$, and IL-6 induced the expression of CSE in primary human articular chondrocytes.\textsuperscript{19} Based on these results, we used an epithelial culture system to evaluate the possible effects of CRS-relevant cytokines on the expression of CBS and CSE in sinus mucosa.

Figure 3. The expression levels of cytokines analyzed by (A) real-time polymerase chain reaction (PCR) and (B) ELISA in cultured epithelial cell derived from normal sinus mucosa after stimulation with sodium hydrosulfide (NaHS; 0.1 or 1 mM) for 12 or 24 hours. Black bar indicates stimulation for 12 hours. White bar indicates 24-hour stimulation. Results are expressed as mean ± SD. Asterisk indicates statistically significant difference in cytokine expression levels.
with CRSwNPs and CRSsNPs, thereby contributing to increased production of H$_2$S in inflammatory sinus mucosa.

The question of whether H$_2$S is beneficial or detrimental in inflammatory processes has been controversial because data are so far exclusively limited to animal and cellular models. Some studies have indicated that endogenous H$_2$S is anti-inflammatory. For example, the administration of H$_2$S donor, NaHS, to animals increases peak expiratory flow, indicating an alleviation of airway obstruction.\textsuperscript{16} Furthermore, histological analysis has indicated that NaHS administration inhibits airway inflammation and airway remodeling.\textsuperscript{16} Inhibition of H$_2$S synthesis resulted in inflammation and mucosal injury in the small intestine and colon along with down-regulation of cyclooxygenase-2 expression and prostaglandin synthesis, and H$_2$S donors also diminish colitis in rats.\textsuperscript{21} H$_2$S donors have also been shown to inhibit the synthesis of proinflammatory mediators in LPS-stimulated murine macrophages and IL-8 secretion by primary human pulmonary airway smooth muscle cells in vitro.\textsuperscript{28,37} In contrast, a proinflammatory action of H$_2$S has also been described. H$_2$S stimulates the activation of human monocytes with the generation of proinflammatory cytokines, and this response is through the extracellular-regulated kinase-mediated NF-kB signaling pathway.\textsuperscript{41} In pancreatic acinar cells, the H$_2$S donor, NaHS, up-regulated monocyte chemotactic protein 1, macrophage-inflammatory protein-1a, MIP-2, and RANTES release, suggesting that the proinflammatory effect of H$_2$S is partially mediated by the release of chemokines.\textsuperscript{46} On the other hand, CRSwNPs is characterized by a Th1 polarization with high levels of IFN-$\gamma$ and TGF-$\beta$-1, and CRSwNPs shows Th2 polarization with high levels of IL-4, IL-5, and IL-13.\textsuperscript{5} In this study, we focused on the effects of NaHS on the expression of cytokines relevant to the pathogenesis of CRS, using cultured epithelial cells. The current data showed increased production of IL-4, IL-5, IFN-$\gamma$, and TNF-$\alpha$ in sinus mucosa after stimulation with IL-4, IL-5, IL-1$\beta$, IL-13, interferon (IFN) $\gamma$, TNF-$\alpha$, and transforming growth factor (TGF) $\beta$1, respectively, for 12 (n = 6) and 24 hours (n = 6). Black bar indicates stimulation for 12 hours. White bar indicates 24-hour stimulation. Results are expressed as mean ± SD. Asterisk indicates statistically significant difference in CBS and CSE expression levels.

**REFERENCES**
