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Involvement of endogenous hydrogen sulfide in cigarette smoke-induced changes in airway responsiveness and inflammation of rat lung

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ABSTRACT

Hydrogen sulfide (H_2S), recently considered the third endogenous gaseous transmitter, may have an important role in systemic inflammation. We investigated whether endogenous H_2S may be a crucial mediator in airway responsiveness and airway inflammation in a rat model of chronic exposure to cigarette smoke (CS). Rats randomly divided into control and CS-exposed groups were treated with or without sodium hydrosulfide (NaHS, donor of H_2S) or propargylglycine (PPG, inhibitor of cystathionine-γ-lyase [CSE], an H_2S-synthesizing enzyme) for 4-month exposure. Serum H_2S level and CSE protein expression in lung tissue were higher, by 2.04- and 2.33-fold, respectively, in CS-exposed rats than in controls ($P < 0.05$). Exogenous administration of NaHS to CS-exposed rats alleviated airway reactivity induced by acetylcholine (Ach) or potassium chloride (KCl) by 17.4% and 13.8%, respectively, decreased lung pathology score by 32.7%, inhibited IL-8 and TNF- α concentrations in lung tissue by 34.2% and 31.4%, respectively, as compared with CS-exposed rats (all $P < 0.05$). However, blocking endogenous CSE with PPG in CS-exposed rats increased airway reactivity induced by Ach or KCl, by 24.1% and 24.5%, respectively, and aggravated lung pathology score, by 44.8%, as compared with CS-exposed rats (all $P < 0.01$). Incubation *in vitro* with NaHS, 1–3 mmol/L, relaxed rat tracheal smooth muscle precontracted by Ach or KCl. However, the NaHS-induced relaxation was not blocked by glibenclamide (10^{-4} mol/L), L-NAME (10^{-4} mol/L), or ODQ (1 μmol/L) or denudation of epithelium. Endogenous H_2S may have a protective role of anti-inflammation and bronchodilation in chronic CS-induced pulmonary injury.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction due to chronic bronchitis or emphysema. Cigarette smoking is the major risk factor of COPD [1]. It induces an inflammatory response in the airways that might play a key role in the pathogenesis of COPD. Furthermore, multi-center clinical trials showed that current smokers with functional evidence of early COPD have airway hyper-responsiveness [2,3]. Similarly, a dose-dependent effect of cigarette smoking on airway responsiveness was reported [4]. Airway hyper-responsiveness is an independent predictor of mortality in COPD patients [4]. However, the mechanisms of such hyper-responsiveness in COPD are less understood than are those in bronchial asthma.

The major effects of hydrogen sulfide (H_2S), long recognized as a toxic gas with a strong odor of rotten eggs in water pollution and industrial air pollution, are intoxication of the central nervous system and inhibition of the respiratory system [5]. Recently, H_2S was found endogenously generated in various mammalian tissues and may be a functional regulator in nervous and cardiovascular systems. H_2S is synthesized endogenously in various mammalian tissues by two pyridoxal-5′-phosphate-dependent enzymes responsible for metabolizing L-cysteine: cystathionine β-synthease (CBS) and cystathionine γ-lyase (CSE). The substrate of CBS and CSE, L-cysteine, can be derived from alimentary sources or can be liberated from endogenous proteins. It can also be synthesized endogenously from L-methionine through the trans-sulphuration pathway, with homocysteine being an intermediate in the process. Because of its endogenous metabolism and physiological functions, H_2S is well positioned in the novel family of endogenous gaseous transmitters and, in addition to nitric oxide (NO) and carbon monoxide (CO),

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might be the third endogenous signaling gasotransmitter [5]. Pulmonary tissue is rich in the H_2S synthase, cystathionine γ-lyase (CSE), and generates endogenous H_2S. Our previous study showed endogenous H_2S concentration increased in patients with stable COPD and decreased in patients with acute exacerbation of COPD; therefore, endogenous H_2S may be involved in the pathogenesis of airway inflammation and airflow obstruction in COPD [6,7].

In this study, we investigated whether endogenous H_2S may be a crucial mediator in airway hyper-responsiveness and airway inflammation in a chronic cigarette smoke (CS)-exposed rat model.

2. Methods

2.1. Chronic cigarette smoke-exposed rat model

All animal care and experimental protocols were in compliance with the PR China Animal Management Rule (documentation 55, 2001, Ministry of Health of PR China) and the Third Hospital, Peking University Guide for the Care and Use of Laboratory Animals.

Male Sprague–Dawley rats weighing 200–250 g were supplied by the Animal Center, Health Science Center, Peking University, and randomly divided into six groups (each $n = 6$) for treatment: control, CS, sodium hydrosulfide (NaHS; an H_2S donor), NaHS + CS, propargylglycine (PPG; CSE inhibitor), and PPG + CS. The rats in the CS groups were exposed to whole-body mainstream CS generated from 20 commercial unfiltered cigarettes, which yielded 1.2 mg nicotine, 17 mg tar each, in a dynamic smoke exposure box (diameter 700 mm, Tianjin Hope Corp., Tianjin, China) for 4 h/day, 7 days/week. The PM 2.5 levels were 25 mg/m^3 after the exposure. Freshly prepared NaHS (14 μmol/kg; Sigma) and PPG (37.5 mg/kg, Sigma) were intraperitoneally administered 30 min before CS exposure in the NaHS + CS and PPG + CS groups, respectively. The control group underwent an identical schedule for sham exposure, but received air rather than smoke. The NaHS and PPG groups underwent the same schedule as NaHS + CS and PPG + CS groups, respectively, but received air rather than smoke for sham exposure. Rats were anesthetized by intraperitoneal injection of 20% (w/v) urethane (5 mL/kg) 24 h after 4-month exposure.

2.2. Measurement of airway responsiveness [8]

The trachea of rats was removed and put into ice-cold Krebs-Henseliet (K-H) solution of the following composition (mmol/L): 110 NaCl, 25 $NaHCO_3$, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 D(+) glucose, 2.5 $CaCl_2$, pH 7.4. After removing adhering fat and connective tissue, the trachea was cut into several 3-cartilage segments.

Tissue was then suspended in a 10-mL organ bath containing K-H solution. The K-H solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tension of 0.5 g and allowed to equilibrate for at least 1 h, during which the K-H solution was changed every 15 min.

Concentration–effect curves were obtained by exposing the preparation to cumulative increasing concentrations of acetylcholine (Ach) or potassium chloride (KCl). Airway responsiveness was measured by use of a Powerlab polygraph (AD Instruments, Australia).

2.3. Histology analysis

The left lung tissues were fixed with being injected 2 mL paraformaldehyde into the bronchi, and then put into paraformaldehyde solution and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E), periodic acid-Schiff, and Masson trichrome. The following nine parameters were evaluated as described [9], with modification: constriction or occlusion of the small airway lumen, abscission or ulceration, squamous cell metaplasia and goblet cell proliferation of epithelium, inflammatory cell infiltration, pigment deposits, connective tissue proliferation and smooth muscle hypertrophy in the airway wall, and emphysema of lung tissue. Three membranous bronchioles of each rat were selected at random for evaluation. Scores from 0 (normal) to 3 (most abnormal) were assigned to each pathologic feature. The total pathologic score is the sum of the score for all nine individual parameters. Scoring was performed by two experienced pathologists blinded to testing. Two scores were assigned, and the mean value was recorded.

2.4. Measurement of IL-8 and TNF-α concentrations in lung tissue

Lung tissues were homogenized on ice in 1:10 (w/v) 0.01 mol/L of ice-cold potassium phosphate buffer (pH 7.2) with use of a glass homogenizer. The homogenates were centrifuged at 4000 rpm for 10 min. The liquid supernatants were obtained for testing IL-8 and TNF-α concentrations. Cytokine level was assayed using double-antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA), following the manufacturer's instructions. ELISA kits for detection of IL-8 and TNF-α were purchased from R&D systems (Minneapolis, MN, USA). They were expressed by pg/g lung tissue.

2.5. Measurement of H_2S content in plasma and lung tissue

H_2S content in plasma was measured with use of a sulfide-sensitive electrode (PXS-270; Shanghai). Briefly, sulfide antioxidant buffer (SAOB II) was added into the standard or sample at a ratio of 1:1, then stirred thoroughly. Electrodes were rinsed in distilled water, blotted dry and placed into the standard and sample. When a stable reading was displayed, the mV value was recorded. The H_2S concentration was calculated against the calibration curve of the standard H_2S solution [6].

The H_2S content in lung tissue was measured as described previously, with modification [10]. Briefly, lung tissues were homogenized in a 10-fold volume (w/v) of ice-cold potassium phosphate buffer (pH 6.8). The reaction was performed in a 25 mL Erlenmeyer flask. Cryovial test tubes (2 mL) were used as the center wells, each containing 0.5 mL of 1 mol/L NaOH. The reaction mixture contained lung tissue homogenate and 1 mol/L HCl at a ratio of 1:5. The flasks containing reaction mixture and central wells were flushed with N_2 for 30 s before being sealed with a double layer of parafilm. The reaction was initiated by transferring the flask from ice to a 37 °C shaking water bath. After incubation at 37 °C for 4 h, the contents of the central wells were transferred to 10-mL beakers, each containing 0.5 mL antioxidant solution. Then the solution was analyzed by use of sulfide-sensitive electrodes (PXS-270; Shanghai), and the lung tissue H_2S content was calculated against the calibration curve of the standard H_2S solution. The H_2S concentration was expressed as nanomoles per milligram protein.

2.6. Protein expression of CSE

Tissue from the right middle lung was homogenized in 50 mmol/L Tris/HCl (pH 7.4) containing 0.25 mmol/L EDTA, 0.5 mmol/L PMSF, 5 μg/mL aprotinin, 5 μg/mL leupeptin, and 5 μg/mL benzamidine. The proteins were solubilized by boiling in SDS–PAGE sample buffer [0.0625 mmol/L Tris/HCl (pH 6.8) containing 10% (v/v) glycerol, 1% (w/v) SDS, 1% (w/v) β-mercaptoethanol, and 0.01% (w/v) bromophenol blue], then proteins (10 μg per lane) were resolved by electrophoresis in 12% SDS–polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked overnight at 4 °C in 10% (w/v) dried milk protein in phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween-20. The blots were washed in

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PBS containing 0.05% (v/v) Tween-20 and incubated for 1 h in the presence of monoclonal anti-CSE (1:2000) (Abnova; Taiwan), washed extensively, then incubated for 1 h with horseradish peroxidase-conjugated anti-mouse IgG (1:4000) (Santa Cruz, USA). The bands were visualized by use of an enhanced chemiluminescence kit following the manufacturer's instructions (Applygen Technologies; Beijing). The expression of the housekeeping protein β-actin was measured with use of an anti-β-actin antibody (Santa Cruz Biotechnology), and results were expressed as a ratio of the expression of the measured protein to that of β-actin [10].

2.7. Effect of NaHS on airway tension in vitro

The tracheae in normal rats ($n = 6$) were prepared as described previously. To denude epithelium, the epithelium of the trachea was removed by inserting a moistened cotton wire into the lumen of trachea and gently rubbing with a corkscrew motion 5–6 times. The removal of epithelium was confirmed by H&E staining. The effects of cumulatively applied NaHS were observed in resting preparations and in preparations precontracted with Ach, 10^{-4} mol/L, or KCl, 80 mmol/L. Glybenclamide, 10^{-4} mol/L (ATP-sensitive K^+ channel [K_{ATP}] inhibitor); L-NAME, 10^{-4} mol/L (inhibitor of nitric oxide synthase [NOS]), and ODQ, 1 μmol/L (inhibitor of soluble guanylyl cyclase [sGC]) were applied to the bath solution 15 min before the addition of Ach (11,12). To examine whether the epithelium was involved in the relaxant effect of NaHS, only animals in the epithelium-denuded group were denuded of epithelium. To quantify the essential NaHS-induced inhibition of muscle contraction, concentration–effect curves for Ach and KCl were calculated after preparations were pre-incubated with NaHS for 30 min. The relaxant activity of NaHS in the preparations precontracted with Ach or KCl was represented as a percentage of the initial tension before the addition of NaHS.

To investigate whether NaHS at this concentration had toxic effects on the trachea, tissues were prepared for histological evaluation. Briefly, tissues were fixed in 4% paraformaldehyde, embedded in paraffin and cut into 5-μm sections and stained with H&E.

2.8. Statistical analysis

Data are expressed as mean ± SD. Comparisons among groups involved one-way ANOVA followed by Student–Newman-Keuls test. Comparisons between two groups involved unpaired Student's t test. SPSS v10.0 (SPSS, Chicago, IL, USA) was used for analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Chronic exposure to CS induced H_2S/CSE upregulation

As compared with controls, the NaHS-alone and NaHS + CS groups showed increased plasma levels of H_2S, by 2.70-fold and 3.29-fold, respectively, but the PPG-alone group showed decreased level, by 77.1% (all $P < 0.01$) (Fig. 1A). Exogenous administration of NaHS in NaHS + CS group increased the plasma level of H_2S, by 61.4%, whereas blockade of endogenous CSE with PPG in the PPG + CS group decreased the level, by 50.9%, as compared with the CS-alone group(all $P < 0.01$). Although the H_2S level of lung tissue in each group did not significantly differ ($P > 0.05$) (Fig. 1B), plasma H_2S level and CSE protein expression in lung tissue were higher, by 2.04- and 2.33-fold, respectively, for the CS than control group (all $P < 0.05$) (Fig. 1C and D).

3.2. Administration of NaHS alleviated and PPG aggravated lung injury and airway hyper-responsiveness in rats exposed to CS

In our study, CS induced necrosis and erosion of bronchial epithelium, metaplasia of airway goblet cells, inflammatory cell infiltration, proliferation of airway smooth muscles and fibrous connective tissues of airway wall, and emphysema to different degrees. The lung pathology score for the CS group was 2.63-fold higher that for the control group ($P < 0.01$, Figs. 2 and 3). Controls and the NaHS-alone group did not differ in lung pathology score ($P > 0.05$), whereas the score was 3.29-, 5.26-, and 2.44-fold higher, respectively, in the PPG-alone, PPG + CS, and NaHS + CS groups

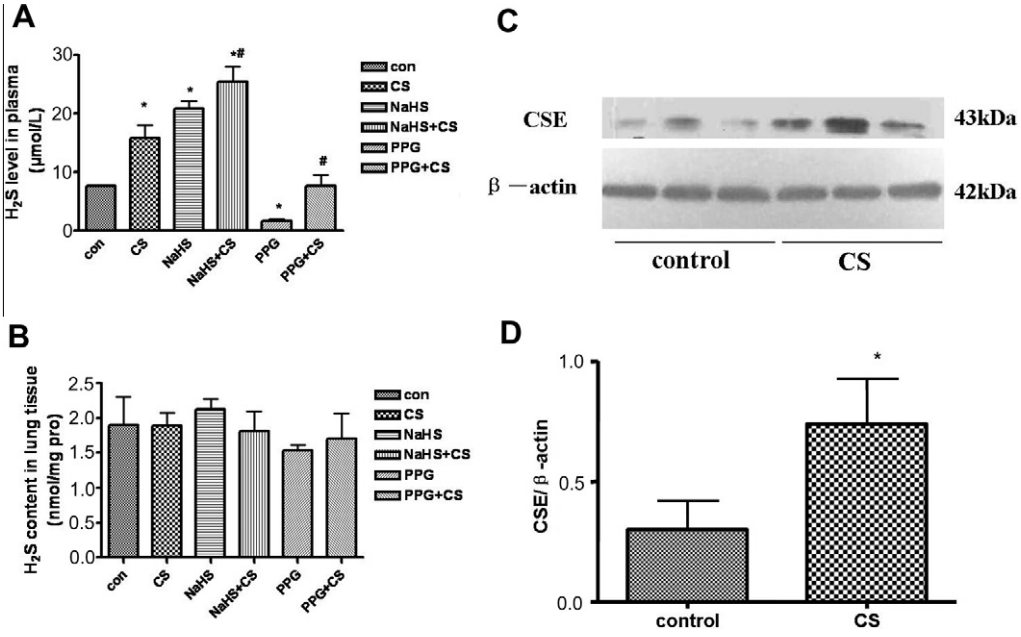

Fig. 1. Endogenous H_2S/CSE pathway was upregulated in rats chronically exposed to CS. (A) plasma H_2S levels in lung ($n = 4$–5); and (B) endogenous H_2S levels in lung ($n = 4$). (C) and (D) Western blot analysis of CSE protein expression in lung tissues from control and CS groups and relative intensity normalized to the expression of β-actin ($n = 3$); $^*P < 0.01$ versus control, $^\#P < 0.01$ versus CS group.

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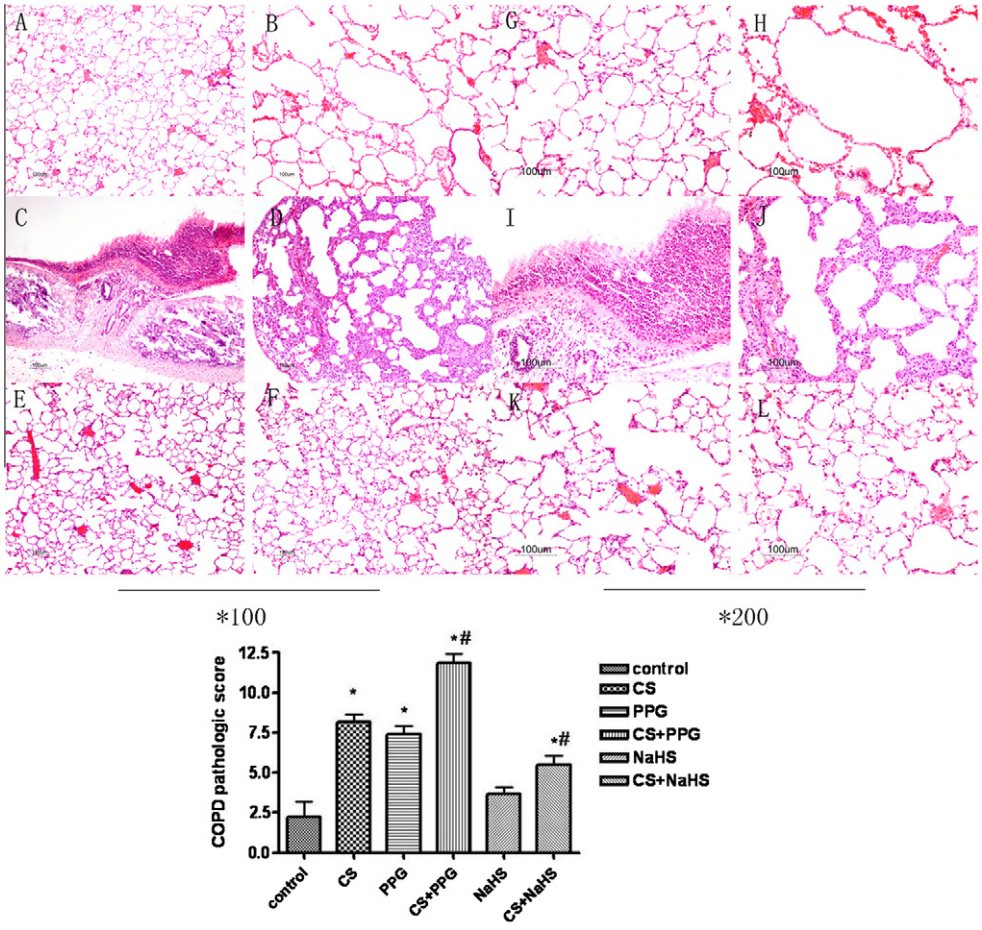

Fig. 2. Lung pathology in rats chronically exposed to cigarette smoke (CS) with or without NaHS or proparglyglycine (PPG). Lung tissue was obtained from rats chronically exposed to air or CS with or without NaHS or PPG, stained with hematoxylin and eosin and examined on light microscopy. Magnification A-F HE × 100, G-L HE × 200. A and G, control group; B and H, CS group; C and I, PPG group; D and J, PPG + CS group; E and K, NaHS group; F and L NaHS + CS group. $^{*}P < 0.01$ compared with controls. $^{\#}P < 0.01$ compared with CS group ($n = 4$–6).

than control group (all $P < 0.01$) (Fig. 2). The lung pathology score was lower by 32.7% with exogenous administration of NaHS in the NaHS + CS group, whereas blockade of endogenous CSE by PPG in the PPG + CS group increased the score by 44.8% than in the CS group (all $P < 0.01$).

IL-8 concentration in lung tissue in CS group was higher by 60.2% than control group ($P < 0.05$) (Table 1). IL-8 and TNF-α concentrations in lung tissue were lower by 34.2% and 31.4%, respectively, in NaHS + CS group than in CS group (all $P < 0.05$). Both pro-inflammatory cytokines were lower by 39.5% and 34.3%, respectively, in NaHS + CS group than in PPG + CS group (all $P < 0.05$) (Table 1).

Trachea from rats in the CS group showed significantly higher Ach or KCl-induced contraction than for controls ($P < 0.05$). The contraction induced by 3×10^{-4} mol/L Ach was 1.49 ± 0.13 g and 2.24 ± 0.27 g for the control and CS groups, respectively ($P < 0.05$). Similarly, the contraction induced by 75 mmol/L KCl was 1.12 ± 0.14 g and 1.96 ± 0.15 g, respectively ($P < 0.05$) (Fig. 4). Airway hyper-responsiveness induced by Ach or KCl did not differ between the NaHS-alone and control groups (all $P > 0.05$), but was increased by 20.8% and 24.1%, respectively, in the PPG-alone group (all $P < 0.05$) (Fig. 4). Exogenous administration of NaHS in the NaHS + CS group alleviated airway hyper-responsiveness induced by Ach or KCl, by 17.4% and 13.8%, respectively, as compared with CS group (all $P < 0.05$), and blockade of endogenous CSE with PPG in the PPG + CS group increased airway responsiveness induced by Ach or KCl by 24.1% and 24.5%, respectively (all $P < 0.01$).

3.3. Relaxing effect of NaHS on airway smooth muscle in vitro

In rat tracheal rings without precontraction, incubation with the H_2S donor NaHS, applied cumulatively in a range of 10^{-6}–10^{-2} mol/L, did not affect muscle tension. The medium pH values after incubation for the NaHS and control groups were 7.368 ± 0.010 and 7.398 ± 0.011, respectively ($P > 0.05$). However, when the preparations were precontracted with Ach, 10^{-4} mol/L, or KCl, 80 mmol/L (the level of force generated by Ach and KCl was 1.833 ± 0.053 g and 1.498 ± 0.049 g, respectively), NaHS, (1–3) × 10^{-3} mol/L, produced marked relaxant activity in a concentration-dependent manner (Fig. 5A). Pre-exposure of the tracheal ring to NaHS, 1×10^{-3} or 3×10^{-3} mol/L, did not alter the contractions induced by Ach or KCl (Fig. 5B). NaHS, 3×10^{-3} mol/L, incubation had no effect on histological structure (Fig. 5C).

The concentration–response curve for the relaxant effect of NaHS in the rat trachea was not affected by pre-incubation with glybenclamide, 10^{-4} mol/L; L-NAME, 10^{-4} mol/L; or ODQ, 1 μmol/L (Fig. 6). To test whether the relaxation effect of H_2S was mediated by epithelium, epithelium was removed from isolated trachea. However, the relaxant effect was not affected in such trachea (Fig. 6).

4. Discussion

The present study suggested that chronic exposure to CS can induce rat pulmonary injury and airway hyper-responsiveness.

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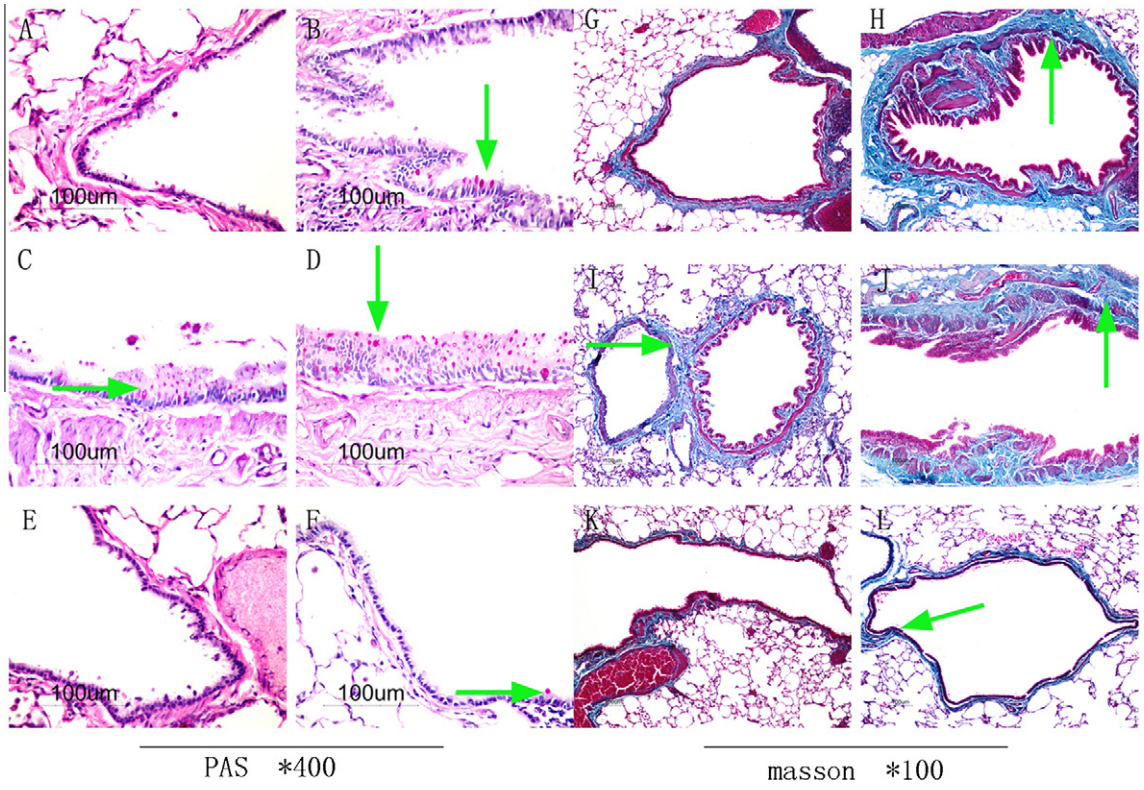

Fig. 3. Lung pathology in rats chronically exposed to CS with or without NaHS or PPG. Lung tissue was obtained from rats chronically exposed to air or CS with or without NaHS or PPG, stained with periodic acid-Schiff or Masson trichrome, and examined on light microscopy. (A–F: PAS, magnification × 400; G–L: Masson trichrome, magnification × 100) A and G, control group; B and H, CS group; C and I, PPG group; D and J, PPG + CS group; E and K, NaHS group; F and L, NaHS + CS group. The arrows in A–F show goblet cell; the arrows in G–L show collagen.

Table 1
IL-8 and TNF-α concentrations in lung tissue in rats chronically exposed to cigarette smoke (CS) with or without NaHS or proparglyglycine (PPG) (mean ± SD).

Groups	IL-8 (pg/g lung tissue)	TNF-α (pg/g lung tissue)
Control	38.52 ± 8.63	2371 ± 282
CS	61.71 ± 12.31*	2758 ± 637
PPG	53.36 ± 6.25	2453 ± 563
NaHS	46.43 ± 7.23	2433 ± 688
PPG + CS	67.07 ± 4.68*	2880 ± 480
NaHS + CS	40.61 ± 14.20#,+	1891 ± 446#,+

* $P < 0.05$, compared with control;
\# $P < 0.05$, compared with CS;
\+ $P < 0.05$, compared with CS + PPG.

Endogenous H_2S generation was upregulated in the CS group. Administration of NaHS alleviated and PPG, inhibitor of CSE, aggravated lung injury and airway hyper-responsiveness in the CS group. The NaHS-induced relaxation in the rat tracheal was not blocked by inhibitors of K_{ATP}, NOS, sGC, or denudation of epithelium.

H_2S has been generally considered a toxic gas found in the contaminated environmental atmosphere. In recent years, however, increasing studies have suggested that endogenous H_2S in physiological concentration is a gasotransmitter. H_2S was found to be endogenously generated by two pyridoxal-5′-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and CSE, with L-cysteine used as a major substrate. The endogenous H_2S/CSE pathway was altered during hypoxic pulmonary hypertension and high pulmonary blood flow-induced pulmonary hypertension in rats, and H_2S relaxed pulmonary artery smooth muscle cells and inhibited their proliferation [11–15]. Our previous study showed increased endogenous H_2S concentration in patients with stable COPD and decreased concentration in those with acute exacerbation of COPD; therefore, endogenous H_2S may be involved in the pathogenesis of airway inflammation and airflow obstruction in COPD [6]. CSE was mainly expressed in airway and vascular

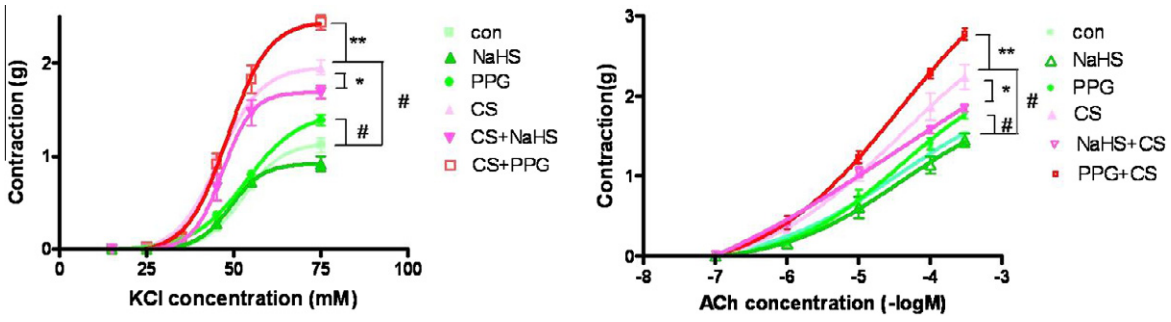

Fig. 4. Airway responsiveness to acetylcholine (Ach; right panel) or potassium chloride (KCl; left panel) in rats chronically exposed to CS with or without NaHS or PPG. Compared with control, $^{\#}P < 0.05$; compared with CS group, $^{*}P < 0.05$; $^{**}P < 0.01$ ($n = 4$).

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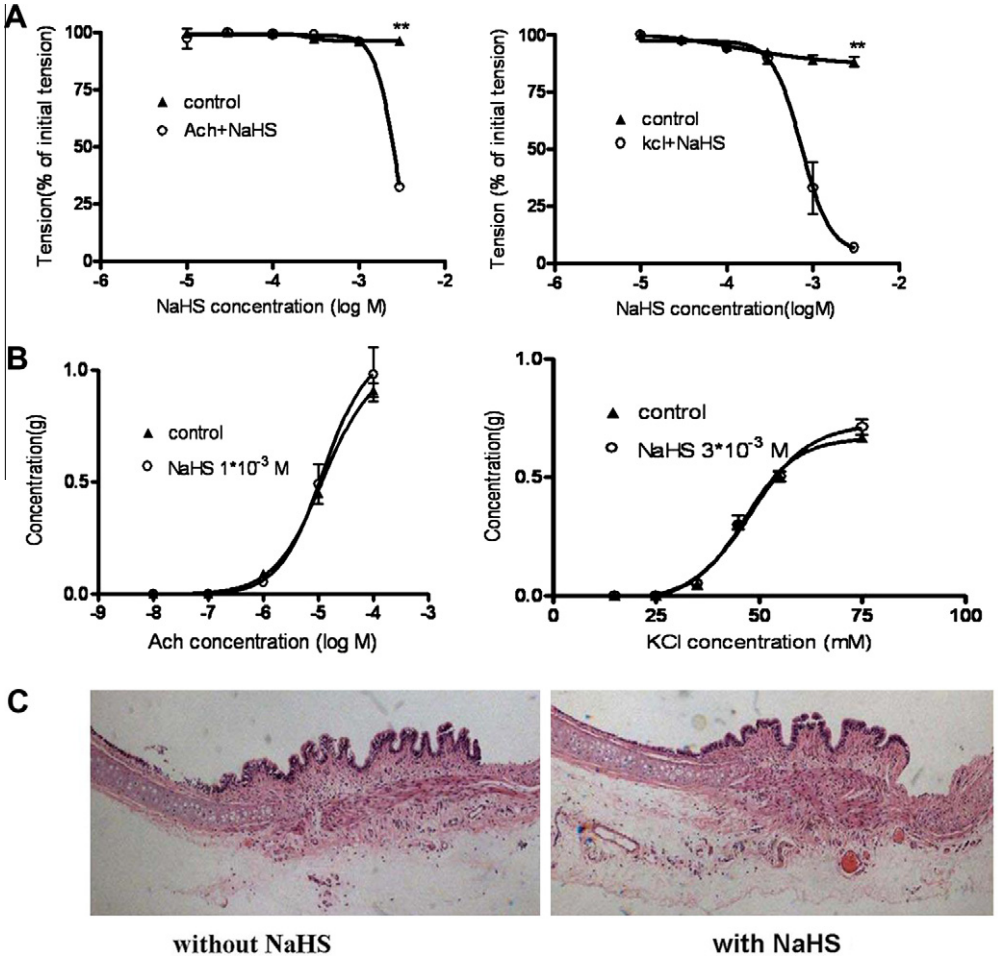

Fig. 5. Effect of NaHS on rat tracheal smooth muscle precontracted with Ach or KCl and effect of pre-exposure to NaHS on airway tension and pathology. (A) NaHS, $(1–3) \times 10^{-3}$ mol/L, produced marked relaxant activity on precontraction with Ach (left panel) or KCl (right panel) in a concentration-dependent manner ($n = 6$). $^{**}P < 0.01$ compared to controls. (B) Effect of pre-exposure of the tracheal ring to NaHS, 1×10^{-3} or 3×10^{-3} mol/L, on contractions induced by Ach or KCl ($n = 6$). (C) Effect of incubation with NaHS, 3×10^{-3} mol/L, on tracheal pathology.

smooth muscle cells in rat lung tissue [10]. Levels of endogenous H_2S were decreased in pulmonary tissue in ovalbumin (OVA)-treated rats [10]. Exogenous administration of NaHS alleviated airway inflammation and airway remodeling: peak expiratory flow increased, goblet cell hyperplasia and collagen deposition score decreased, and decreased number of total cells recovered from bronchoalveolar fluid treatment with influx of eosinophils and neutrophils. The H_2S levels of serum and lung tissue were positively correlated with peak expiratory flow and negatively correlated with the level of eosinophils and neutrophils in bronchoalveolar lavage fluid, score of lung pathology. NaHS treatment significantly attenuated pulmonary inducible nitric oxide synthase (iNOS) activation in OVA-treated rats. These results suggest that the H_2S/CSE pathway plays an anti-inflammatory and anti-remodeling part in asthma pathogenesis and could be a novel target in prevention and treatment of asthma [10]. However, whether endogenous H_2S is involved in CS-induced changes in the structure and function of rat lung was unknown.

Cigarette smoking is the major risk factor of COPD. Here we found that chronic CS exposure can induce necrosis and erosion of bronchial epithelium, metaplasia of airway goblet cells, airway lumen occlusion by mucus and cells, inflammatory cell infiltration, proliferation of airway smooth muscle and fibrous connective tissues of the airway wall, and different degrees of emphysema, all characteristics of COPD. IL-8 concentration in lung tissue was increased in CS group. The airway responsiveness induced by Ach or KCl was increased in CS group. These results were similar to those in the literature [16]. CSE protein expression in lung tissues and the H_2S levels of plasma were increased in the CS group. These results suggest that the H_2S/CSE pathway in lung tissues was upregulated after chronic CS exposure in rats. It is known that the H_2S level of lung tissue may be influenced by many factors, including CSE activity, CSE protein expression, and mRNA expression. Further study need to be done to illustrate the factors influencing the generation of CSE/H_2S pathway in lung tissues.

In the present study, the lung pathology score was not affected with NaHS-alone treatment but was increased with the CSE inhibitor PPG alone as compared with controls. Exogenous administration of NaHS reduced the lung pathology score, and blockade of endogenous CSE with PPG increased the score, as compared with CS group. IL-8 and TNF-α concentrations in lung tissue were lower in NaHS + CS group than in CS group. These results indicated that H_2S may have provided pulmonary protection in terms of lung morphology and pro-inflammatory cytokines after chronic CS exposure.

One of the main functions of H_2S in mammalian peripheral tissues is to modulate smooth muscle tone. For example, H_2S can relax rat aortic tissues and mesenteric artery beds [17–19]. However, the role of H_2S in airway responsiveness in a rat model of chronic CS exposure was unknown. We investigated the responsiveness of airway smooth muscles to Ach and KCl and found that

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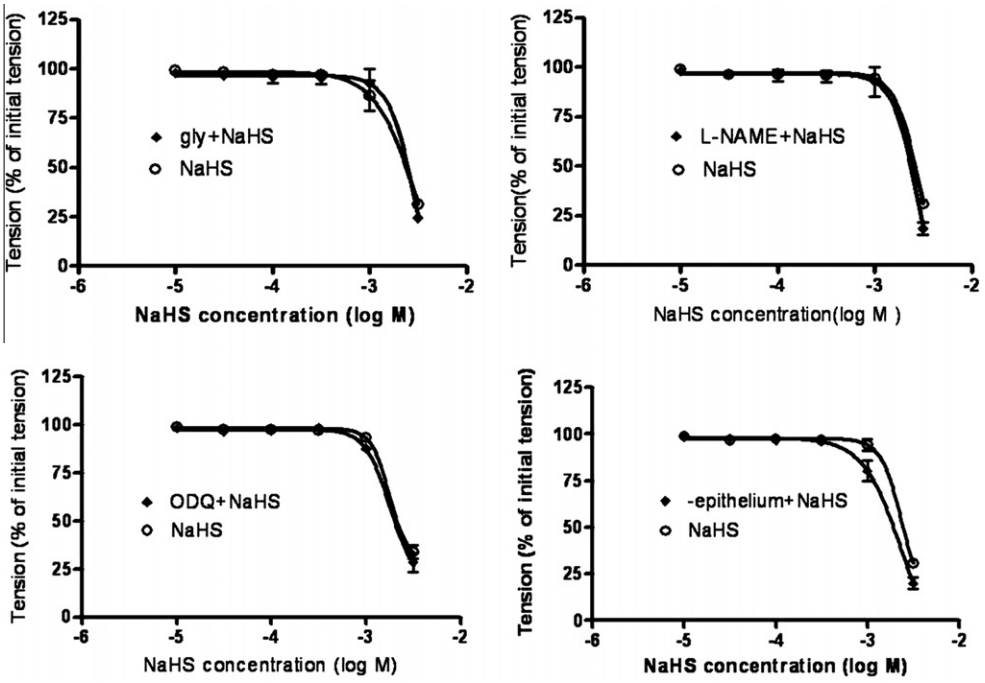

Fig. 6. The underlying mechanism of NaHS-induced relaxant response in rat trachea. The NaHS-induced relaxation in the rat trachea on treatment with K_{ATP} inhibitor glibenclamide (10^{-4} mol/L); NOS inhibitor L-NAME (10^{-4} mol/L); soluble guanylyl cyclase inhibitor ODQ (1 μmol/L); or denudation of epithelium. Values were percentage of initial Ach (10^{-4} mol/L)-induced tension ($n = 6$). Gly: glibenclamide; epithelium: removal of epithelium.

after CS exposure, the responsiveness of airway smooth muscle with Ach and KCl treatment was significantly augmented; the hyper-responsiveness of airway smooth muscle was decreased with NaHS treatment but increased with PPG treatment as compared with CS group. Therefore, H_2S may act as a bronchodilator in chronic CS exposure rat model.

The mechanism of endogenous H_2S on chronic CS-induced pulmonary injury is not fully understood. The role of endogenous H_2S in systematic inflammation is controversial: it may have a pro- or an anti-inflammatory effect under different conditions [20,21]. Recent study showed that H_2S can be an inhibitor of hypochloric acid-mediated oxidative damage in the brain, a scavenger of endogenous peroxynitrite in nervous tissues, and an antagonist of lipid peroxidation induced by oxygen-free radicals in the heart [22–24]. H_2S donors induced neutrophil apoptosis and suppressed expression of some leukocyte and endothelial adhesion molecules [25]. In lipopolysaccharide-stimulated microglia and astrocytes, H_2S had an anti-inflammatory effect, which may be due to inhibition of iNOS and p38 mitogen-activated protein kinase signaling pathways [26]. These findings have important implications for anti-inflammatory drug development. Our study found that a marked infiltration of inflammatory cells into the sub-epithelial and smooth muscle layers was found in the CS group, while administration of NaHS can alleviate such changes significantly, which indicates that endogenous H_2S may have an anti-inflammatory role in COPD.

To explore whether H_2S has an effect on airway smooth muscle in the physiological condition, we investigated the effect of H_2S on airway smooth muscle *in vitro*. NaHS did not impair airway smooth muscle functionally or histologically but exhibited marked relaxant activity in a concentration-dependent manner when the preparations were precontracted with Ach and KCl. However, whether this effect is physiologically relevant is unclear. The great differences between airway structure and vascular structure might account for the tissue-specific response to NaHS. Previous study showed that NaHS evoked strong bronchodilation in mice but only slight relaxation in guinea-pig rings [27].

The mechanism of bronchodilation triggered by H_2S is unclear. Previous study showed that H_2S at relatively high concentrations relaxes vascular smooth muscle, seems to involve a small, variable component of NO-related mechanisms, and have a K_{ATP}-channel opening and possibly an endothelium-dependent hyperpolarizing factor-like effect. H_2S also induces intracellular acidification via activation of $Cl^-/HCO3^-$ exchanger, which is responsible, at least in part, for H_2S-mediated vasorelaxation. H_2S-mediated vasorelaxation also depends in part on calcium entry. H_2S facilitates erectile function in part through the cyclic adenosine monophosphate pathway [15,18,19,28,29]. In this study, the concentration–response curve for the relaxant effect of NaHS in the rat trachea was not affected by the K_{ATP} inhibitor glybenclamide, the NOS inhibitor L-NAME, or the sGC inhibitor ODQ. To test whether the relaxation effect of H_2S was mediated by epithelium, epithelium was removed from isolated trachea, but the relaxant effect was not affected in such trachea. Obviously, the mechanism for trachea response to H_2S is not clear. Differences in distributions of H_2S targets, K_{ATP} channel densities, sensitivities of voltage-dependent Ca^{2+}channels to membrane potential change, and sensitivities of contractile proteins to intracellular calcium levels may be mechanisms for further exploration. More in-depth analyses to elucidate the mechanisms in bronchodilation triggered by H_2S are in progress.

In conclusion, our study showed that exogenous administration of NaHS could alleviate airway hyper-responsiveness, lung pathology changes and pro-inflammatory cytokines expression induced by chronic exposure to CS in rats, and those changes were aggravated when endogenous H_2S was inhibited. *In vitro*, NaHS relaxed the rat tracheal smooth muscle precontracted with Ach or KCl. We suggest a possible protective role of anti-inflammation and bronchodilation for H_2S in the pathogenesis of COPD due to chronic CS exposure.

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