HYDROGEN SULFIDE: A NOVEL PLAYER IN AIRWAY DEVELOPMENT, PATHOPHYSIOLOGY OF RESPIRATORY DISEASES AND ANTIVIRAL DEFENSES

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Abstract

Hydrogen sulfide (H$_2$S) is a biologically relevant signaling molecule in mammals. Together with the volatile substances nitric oxide (NO) and carbon monoxide (CO), H$_2$S is defined as a gasotransmitter, playing a physiological role in a variety of functions such as synaptic transmission, vascular tone, angiogenesis, inflammation and cellular signaling. The generation of H$_2$S is catalyzed by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3 mercaptopyruvate sulfurtransferase (3-MST). The expression of CBS and CSE is tissue-specific with CBS being expressed predominantly in the brain and CSE in peripheral tissues, including lungs. CSE expression and activity are developmentally regulated and recent studies suggest that CSE plays an important role in lung alveolarization during fetal development. In the respiratory tract, endogenous H$_2$S has been shown to participate in the regulation of important functions such as airway tone, pulmonary circulation, cell proliferation or apoptosis, fibrosis, oxidative stress, and inflammation. In the past few years, changes in the generation of H$_2$S have been linked to the pathogenesis of a variety of acute and chronic inflammatory lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Recently, our laboratory has made the critical discovery that cellular H$_2$S exerts a broad-spectrum antiviral activity both in vitro and in vivo, in addition to an independent anti-inflammatory activity. These findings have important implications for the development of novel therapeutic strategies for viral respiratory infections, as well as other inflammatory lung diseases, especially in light of recent significant efforts to generate controlled-release H$_2$S donors for clinical therapeutic applications.
Keywords

Hydrogen sulfide; H₂S donors; antiviral
Text

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Introduction

Hydrogen sulfide (H$_2$S) is colorless gas, toxic in high concentrations (1), that plays an important role in vital body functions by acting as a gaseous molecular mediator, similar to nitric oxide (NO) and carbon monoxide (CO) (2). It is synthesized in mammalian cells via two pyridoxal-5’-phosphate-dependent enzymes responsible for metabolism of L-cysteine: cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), and a third pathway that involves the combined action of 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT) [reviewed in (3;4)]. To mitigate the toxic effects of H$_2$S, scavenging mechanisms exist in mammalian cells. Free H$_2$S can be oxidized in mitochondria by the enzyme sulfide quinone reductase (SQR) (5), or methylated in the cytoplasm using thiol-S-methyltransferase (6). Free H$_2$S can also be bound by methemoglobin and by molecules with metal- or disulfide bonds and excreted with biological fluids (7) (Figure 1). Cells in different tissues usually express all enzymes at various levels; however CSE is the dominant enzyme responsible for the production of H$_2$S in cardiovascular system (8,9) and CBS in nervous system (10;11). The respiratory system has detectable levels of expression of both CSE and CBS (12). Recently, Madurga et al used laser capture microdissection followed by PCR to show that CBS is predominantly expressed in airway vessels and epithelial cells, while CSE is expressed in mouse lung parenchyma (13).

Since its discovery as a gasotransmitter, H$_2$S has gained attention as a powerful anti-inflammatory, cytoprotective and vasoactive agent. Hydrogen sulfide has been shown to have anti-inflammatory and antioxidant activity, to modulate ion channel functions and cellular signaling by inhibiting or activating a multitude of signaling pathways [reviewed in (14;15)]. Although, there is no clear consensus on the mechanism of H$_2$S action on cellular signaling, H$_2$S
has been shown to act as a scavenger for reactive oxygen species (ROS), due to its reducing and nucleophilic nature, and to modify proteins by S-sulfhydration, which affects protein function, cellular localization, stability and resistance to oxidative damage [reviewed in (16;17)].

In the respiratory tract, endogenous H$_2$S has been shown to participate in the regulation of important physiological functions such as airway tone, pulmonary circulation, cell proliferation and apoptosis, and to modulate lung fibrosis, oxidative stress, and inflammation (Figure 2). In the sections below, we describe the role of H$_2$S in physiological and pathological lung conditions, and the potential therapeutic use of H$_2$S donors in various lung diseases.

**Role of H$_2$S in lung physiology and development**

H$_2$S generation has been show to play a pivotal role in maintaining several respiratory functions. Studies *in vitro*, using brainstem slices of neonatal rats, have shown that H$_2$S is involved in the central control of rhythmic respiration (18;19). In those studies, the authors found that exogenous H$_2$S could affect the respiratory activity in a diphasic mode, with a decrease in the respiratory frequency in the initial stage followed by its increase in the later stage. They also found that endogenous H$_2$S could be produced through the CBS pathway and it was involved in the control of rhythmic respiration of the slices, and that the two opposite stages of effects of H$_2$S on the respiratory activity might be induced by opening K$_{ATP}$ channels and activating the cAMP pathway, respectively. Studies *in vivo* using H$_2$S donors and CBS inhibitors also suggest that H$_2$S likely plays a role in regulation of respiratory rhythm (20). Different concentrations of sodium hydrosulfide (NaHS) and cysteine (Cys) were used to explore whether H$_2$S can affect respiratory activity in adult rats. The authors found that low concentrations of NaHS did not significantly
affect the rhythmic discharge of the diaphragm, whereas low concentrations of Cys increased it. In addition, moderate concentration of NaHS and Cys could induce biphasic respiration responses, resulting in changes in rhythm, while high concentrations could irreversibly suppress the diaphragmatic discharge.

Exogenous application of H$_2$S has been shown to trigger electrolyte absorption by respiratory epithelium [reviewed in (21)]. This effect is achieved via inhibition of Na$^+$/K$^+$-ATPase and K$_{Ca}$ channels (22-24) and results in increased mucociliary clearance, which could be beneficial by enhancing elimination of pathogenic microorganisms (24). Most importantly, H$_2$S has been shown to be involved in lung vascular development and alveolarization (13;25). Systemic administration of the H$_2$S slow-releasing donor GYY4137 partially restored arrested alveolarization in an experimental model of bronchopulmonary dysplasia (25). The same group later showed that the H$_2$S-generating enzymes CBS and CSE are also important for normal development of the lung (13). Using pharmacological and functional ablation of expression of these enzymes, they observed a significant decrease in both vascular and alveolar development both in vitro and in vivo (13). In support of a protective role of H$_2$S in the development of bronchopulmonary dysplasia, CSE expression and activity seem to be developmentally regulated, as demonstrated by studies in human premature infants, newborns and infants in their first year of life, in which this enzyme has been measured and found to be delayed in maturation (26;27). Lower CSE expression and activity could also play an important pathogenic role in the context of respiratory viral infections occurring in the first year of life, for which an intact H$_2$S-generating pathway seems to be an important protective mechanism against disease (see section below).
Role of Endogenous Hydrogen Sulfide In Airway Diseases

Acute and chronic respiratory diseases constitute a major health burden in both children and adults. It is estimated that chronic respiratory illnesses such as chronic obstructive pulmonary disease (COPD) and asthma are the third leading cause of death accounting for almost 150,000 cases in 2015 in US only (28). Similarly, acute viral respiratory illnesses due to paramyxovirus and influenza virus infections are associated with high morbidity and increased mortality among populations at risk such as infants, elderly and immunocompromised patients (29-31). In the past few years, the role of endogenous H$_2$S has been investigated in the pathophysiology of several respiratory diseases, including asthma, COPD, rhinitis and viral infections, as summarized in the paragraphs below.

*Asthma.* In a rat model of asthma, levels of endogenous H$_2$S were decreased in pulmonary tissue in ovalbumin-treated rats. Serum levels of H$_2$S were positively correlated with H$_2$S levels in lung tissues and both positively correlated with peak expiratory flow and negatively with proportion of eosinophils and neutrophils in bronchoalveolar lavage fluid (BALF), inflammatory cells airway infiltration, goblet cell and airway smooth muscle hyperplasia (32). In a mouse model of asthma, lack of CSE expression resulted in increased airway hyperresponsiveness (AHR) following methacholine challenge and increased lung inflammation (33).

Several human studies have shown a decrease in serum and exhaled breath H$_2$S levels both in adult and pediatric asthmatic populations (34-36). Lower H$_2$S levels were shown to correlate with abnormal pulmonary lung function tests and severity of asthma (34-36). Only one study, performed in adult asthmatic patients using sputum samples, showed an opposite correlation,
with higher H$_2$S levels associated with more severe disease (37), possibly due to changes in H$_2$S-producing bacteria in the oral cavity (38). Taken together, these data provide evidence for an important protective role of H$_2$S in allergic airway disease.

**COPD.** In a mouse model of tobacco smoke-induced emphysema, 12 to 24 week smoke exposure was associated with decreased CSE and CBS expression in the lungs (39). In a rat model of chronic smoke exposure, blocking endogenous CSE with propargylglycine (PAG) increased airway reactivity induced by methacholine and significantly aggravated epithelial damage and emphysema, indicating a protective role of endogenous H$_2$S production in the development of the disease (40).

In human studies, serum H$_2$S level was significantly higher in patients with stable COPD than that in patients with acute exacerbation of COPD (AECOPD) and they were significantly lower in smokers than nonsmokers, in both the AECOPD patient pool and healthy control group (41). In terms of patients with stable COPD, serum H$_2$S levels were significantly lower in those with stage III obstruction, versus stage I (41). This correlated positively with the percentage of predicted forced expiratory volume in one second (FEV1) values and negatively with neutrophils present in induced sputum in patients with acute exacerbation (41). Patients with COPD also display lower levels of CSE protein and CBS mRNA (42) and lower exhaled H$_2$S levels were found in COPD patients with significantly eosinophilia and sputum eosinophils, worse lung function and more frequent exacerbations (43).
**Allergic Rhinitis.** In guinea pigs model of ovalbumin-induced allergic rhinitis, the levels of serum H$_2$S and CSE mRNA expression in nasal mucosa were significantly reduced and the clinical disease was more severe with the administration of the CSE inhibitor PAG (44,45). However, two human studies of nasal mucosal samples isolated from patient with allergic rhinitis showed higher levels of H$_2$S, CSE and CBS mRNA and protein expression (46), with no correlation with either severity of disease or presence and absence of polyps (47).

**Cystic fibrosis.** Analysis of H$_2$S levels in sputum from cystic fibrosis patients showed that levels of H$_2$S inversely correlated with the amount of sputum produced (48). Thus, high levels of H$_2$S were associated with lower amount of sputum and also correlated with decreased likelihood of inpatient hospitalization and need for supplemental oxygen, suggesting that hydrogen sulfide could be a biomarker of good health in cystic fibrosis patients (48). However, this study comprised of only 21 patients and thus no clear statistical correlation between H$_2$S and changes in disease progression could be drawn.

**Viral infections.** Recent investigations in our laboratory have identified a novel antiviral role for H$_2$S. Respiratory Syncytial Virus (RSV) infection of airway epithelial cells was associated with decreased CSE mRNA and protein expression, reduced ability to generate intracellular H$_2$S, and increased SQR expression, leading to increased H$_2$S degradation in viral-infected cells (49). Inhibition of CSE with propargylglycine (PAG) significantly increased production of cytokines and chemokines in response to RSV infection and was associated with increased viral infectious particle formation (49). These latest findings were replicated in a mouse model of RSV infection,
using mice lacking CSE expression. CSE knock-out mice exhibited enhanced clinical disease, assessed by body weight loss and a validated clinical illness score, increased airway hyperresponsiveness (AHR) in response to methacholine challenge, assessed by total body plethysmography, enhanced viral replication and enhanced lung inflammation, compared to wild type mice (50). These data altogether indicate that endogenous H$_2$S plays an important role in controlling viral replication and lung disease in response to RSV infection.

**Hydrogen sulfide donors for treatment of respiratory diseases**

As noted above, it is becoming increasingly clear that a number of airway diseases are associated with a state of H$_2$S deficiency; therefore the use of H$_2$S donor molecules may provide a possible therapeutic approach by replenishing and increasing H$_2$S tissue levels. A number of H$_2$S donors exist which include inorganic salts, compounds with organic backbones, amino-acids and naturally occurring compounds [reviewed in (51)], as well as H$_2$S-releasing chimeras [reviewed in (52)]. Some of these have made progress into clinical trials (a list of these trials is available at [www.clinicaltrials.gov](http://www.clinicaltrials.gov)). In the following section we discuss the evidence in support of the potential use of H$_2$S donor molecules for therapeutic applications in respiratory diseases (Table 1).

**Inorganic salts**

Inorganic salts, such as sodium hydrosulfide (NaHS), are the H$_2$S donors most commonly used to investigate the therapeutics effects of H$_2$S administration *in vitro* and *in vivo*, and have been
tested in a variety of respiratory diseases. They are the most cost-effective but least controllable
H$_2$S donors available, due to an immediate release of H$_2$S in solution or biological fluid, creating
challenges due to potentially toxic effects on the studied system. They showed a protective effect
in numerous in vivo models of lung diseases, as illustrated below. Most of the described studies
used concentrations up to 50 µmol/kg of body weight given intraperitoneally with no reported
toxicity.

Exogenous supplementation of H$_2$S using NaHS resulted in improved symptoms in several
animal models of asthma (33;53;54). In mice with ovalbumin-induced asthma, administration of
NaHS thirty minutes before and eight hours after challenge was associated with lower AHR in
response to methacholine challenge, reduced recruitment of inflammatory cells (eosinophils and
neutrophils) in bronchial lavage (BAL) fluid, and lower levels of Th2 cytokines including IL-5,
IL-13 and eotaxin, and decreased mast cell degranulation and neutrophil recruitment to the lungs
(33;53;54). The decrease in GSH/GSSG ratio, an indicator of cellular oxidative stress, present in
ovalbumin-induced asthma was also restored by exogenous NaHS treatment (54). Similar results
were obtained in an ovalbumin-induced rat model of asthma, which additionally showed a
decrease in goblet cell hyperplasia and reduced collagen deposition in NaHS treated animals
(32).

NaHS treatment in a model of ozone exposure, which induces symptoms similar to asthma, was
also associated with a better disease outcome. The positive effects of both pre- and post-exposure
NaHS treatment included reduction of cellular infiltration, decreased pro-inflammatory cytokine
secretion and decreased AHR in response to methacholine challenge (37). NaHS treatment also
restored CSE and CBS mRNA and protein levels, which were significantly reduced in ozone-
exposed mice (37).
In rodent models, exogenous H₂S administration consistently showed positive effects on several aspects of COPD. In a mouse model of emphysema induced by cigarette smoke, administration of NaHS daily, 5 days per week for 24 weeks, was able to improve lung morphology and reduce inflammatory cell recruitment in BAL fluid (39). NaHS also reduced pro-inflammatory cytokine levels and reduced bronchial wall thickness caused by cigarette smoke (39). Similarly, in a rat model of cigarette smoke exposure, NaHS treatment decreased AHR and reduced cytokine and chemokine secretion (40). Emphysema caused by ozone was also improved by NaHS treatment. Pre-treatment of mice with NaHS prevented the increase in forced residual capacity and decrease in total lung capacity and prevented decrease in ratio of forced expiratory volume in the first 25 and 50 milliseconds (FEV25 and FEV50) to forced vital capacity (FVC) (55). It also decreased lymphocytes, neutrophils and eosinophil recruitment and decreased bronchial wall remodeling. Post-treatment also provided partial protection against ozone-induced lung injury and emphysema (55).

Administration of NaHS has been shown to decrease lung fibrosis and type I collagen deposition in a rat model of pulmonary fibrosis induced by cigarette smoke (56). Additionally, it attenuated the increase in malondialdehyde (MDA), a marker of oxidative injury, caused by cigarette smoking, and raised levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase (56). Smoking raised levels of inflammatory mediators C-reactive protein (CRP), TNF-α, IL-1β, and IL-6, which were also decreased by NaHS administration (56).

In a rat model of bleomycin-induced lung fibrosis, administration of NaHS attenuated the increase in hydroxyproline, a marker of lung collagen deposition, ameliorated alveolar wall thickening, honeycombing, and collagen deposition. Plasma levels of H₂S, which decreased with bleomycin, were also rescued by NaHS treatment (57). In a similar mouse model of bleomycin-
induced sclerosis, NaHS treatment reduced levels of pro-inflammatory cytokines ED1, MCP-1 and TGF-β1, lung cellular infiltration and fibrosis, evidenced by the reduction of collagen deposition, prevention of alveolar collapse and thickening of alveolar septa (58).

Finally, in a guinea pig model of allergic rhinitis, exogenous administration of NaHS was able to alleviate both the disease symptoms and the underlying inflammatory parameters (44).

**Phosphorodithioate-based donors**

In 2008, Li et al reported GYY4137, a Lawesson’s reagent derivative, as a water-soluble H₂S donor which showed a slow (in the micromolar range when used at millimolar concentrations) and controllable release of H₂S by hydrolysis in a pH-dependent manner (59). Since then, this compound has been widely used and it has shown anti-inflammatory properties in cultured cells and in a variety of animal models of inflammation *in vivo* (60). GYY4137 has been tested in some models of lung diseases, as illustrated below, with results similar to the inorganic salts.

A recent study by Zhang and colleagues showed a beneficial effect of GYY4137 treatment in a mouse model of LPS-induced acute lung injury (61). Intraperitoneal administration of 50 mg/kg of GYY4137 right before challenge resulted in decreased secretion of LPS-induced pro-inflammatory cytokines such as IL-6 and IL-8, and increased production of the anti-inflammatory cytokine IL-10. GYY4137 treatment was associated with a strong anti-oxidative effect, evidenced by reduced levels of H₂O₂ and MDA and restored activity of the antioxidant enzymes superoxide dismutase and catalase in lung tissues, leading to normalization of the GSH/GSSG ratio (61). Although GYY4137 has not been used in animal models of COPD, GYY4137 treatment showed improvement of COPD-related parameters in an *in vitro* cell culture
model of smoke exposure (42). Alveolar macrophages isolated from cigarette smoke exposed rats and U937 cells exposed to cigarette smoke showed a reduction in pro-inflammatory mediator secretion upon treatment with the compound (42).

In agreement with the recent findings that endogenous H$_2$S plays an important antiviral and anti-inflammatory role in RSV infection, treatment of airway epithelial cells with GYY4137 reduced RSV-induced proinflammatory mediator production and significantly reduced viral replication, even when administered several hours after viral adsorption. GYY4137 also significantly reduced replication and inflammatory chemokine production induced by human metapneumovirus (hMPV) and Nipah virus (NiV) in infected airway epithelial cells, suggesting a broad inhibitory effect of H$_2$S on paramyxovirus infections. GYY4137 treatment had no effect on RSV genome replication, viral mRNA and protein synthesis, but it inhibited syncytia formation and virus assembly/release. GYY4137 inhibition of proinflammatory gene expression occurred by modulation of activation of the key transcription factors Nuclear Factor (NF)-κB and Interferon Regulatory Factor (IRF)-3 at a step subsequent to their nuclear translocation (49).

Importantly, intranasal delivery of 50 mg/kg of GYY4137 to RSV-infected mice during the first 24h post-infection (p.i.) significantly reduced viral replication and markedly improved clinical disease parameters and pulmonary dysfunction. Administration of GYY4137 up to 200 mg/kg was not associated with significant toxicity, but did not result in increased benefit. The protective effect of GYY4137 was associated with significant reduction of viral-induced proinflammatory mediators and lung cellular infiltrates in vivo (50).

In addition to paramyxoviruses, GYY417 showed a similar antiviral effect in an in vitro model of infection with other highly pathogenic RNA viruses, including influenza A and B, Ebola virus, Far-eastern subtype tick-borne flavivirus, Rift Valley fever virus and Crimean-Congo
hemorrhagic fever virus (62). Administration of GYY4137 up to 6 hours p.i. dramatically reduced virus replication and production of proinflammatory cytokines, likely due to the observed inhibition of NF-κB and IRF-3 signaling pathways, similar to what was found in RSV infection. Differently from paramyxovirus infection, GYY4173 treatment was associated with decreased influenza A RNA and protein expression (62).

**Thiol-Activated H₂S Donors**

This is a group of recently synthesized thiol-activated H₂S donors that release H₂S in the presence of thiols, such as cysteine and GSH. This group includes N-mercapto-based donors, S-arylthiooximes, perthiol-based donors, dithioperoxyanhydrides, thioamide- and aryl isothiocyanate-based donors and gem-Dithiol-based donors [reviewed in (51)]. Preliminary data suggest that a member of the gem-dithiol-based donor family, called TAGDD-1, has a similar antiviral activity of GYY4173 when tested in an *in vitro* model of RSV and influenza virus infection (Casola *et al.*, unpublished data).

**Natural sulfur-containing compounds**

Garlic has been shown to contain several sulfur-containing compounds, the most abundant of which is allicin (63). Once extracted from garlic, allicin quickly decomposes into derivatives such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) (64). Similarly to thiol-activated donors, garlic-derived compounds are believed to release H₂S only in the presence of thiols such as GSH. DATS is one of the most studied compounds in various
models of inflammatory diseases, both in vitro and in vivo. In relation to respiratory diseases, DATS administration has been shown to have a beneficial effect in naphthalene-induced lung injury (65). DATS treatment was associated with elevation of GSH levels in lung tissue, inhibition of the production of serum TNF-α and IL-8 and suppression of lung inflammatory cell recruitment, in particular neutrophil infiltration (65).

Very limited data are available regarding the possible antiviral activity of DATS. Fang et al showed the anti-cytomegalovirus (CMV) activity of allitridin (DATS) in vivo using clinical isolates of human and mouse CMV strains (66). Studies from the same group showed the beneficial effect of allitridin on liver pathology after CMV infection and reduction of viral load in affected organs (67;68). The effect of allitridin was attributed to the inhibition of viral gene transcription (69;70) and of immune tolerance to the infection by reducing amplification of T-regulatory helper cells after CMV infection (71;72).

Anethole dithiolethione (ADT)

ADT and its main metabolite (ADT-OH; 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione) have been used extensively as a donor of H₂S, and its ease of esterification with other therapeutics has led to a considerable variety of H₂S “donating” drugs, as it has been done for example with non-steroidal anti-inflammatory drugs (NSAIDs) such as H₂S-aspirin and H₂S-diclofenac [reviewed in (52)]. These novel combinatory drugs have been shown to have similar or higher anti-inflammatory effect with lower adverse effects, compared to their NSAID counterparts. Administration of S-diclofenac (ADT-diclofenac chimera) in a rat model of LPS-induced septic
shock was more effective in reduced lung MPO activity, a sign of lung inflammation, compared to diclofenac alone (73).

Conclusions

H$_2$S is emerging as an important potential therapeutic option for acute and chronic respiratory inflammatory diseases. Significant progress has been made in studying the underlying mechanisms of H$_2$S cellular generation and the potential role of H$_2$S in models of lung diseases in vivo. Future research should focus on continuing to elucidate basic biology of H$_2$S in the respiratory system and its relationship with pathophysiology of lung diseases. As it has been shown that the methods usually employed for measurement of H$_2$S in biological samples are associated with substantial artifacts (74), a better understanding of the chemistry and the problems in the analytical techniques used to measure H$_2$S concentrations is critical to our expanding knowledge on the biology of hydrogen sulfide. From a therapeutic standpoint, there is a need for further development of safe H$_2$S donors with controllable release and possible better water solubility, as well as development of efficient methods for their delivery to patients.

Acknowledgements

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References


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<tr>
<td>Asthma (mouse, rat)</td>
<td>NaHS*</td>
<td>↓ airway hyperresponsiveness, ↓ inflammation, ↓ oxidative stress, ↓ mast cell degranulation, ↓ fibroblast recruitment &amp; proliferation, ↓ pathology, ↑ lung function</td>
<td>Chen et al 2009 (32), Zhang et al 2013 (33), Roviezzo et al 2015 (53), Campos et al 2016 (54)</td>
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<tr>
<td>COPD† (mouse, rat)</td>
<td>NaHS*</td>
<td>↓ pathology, ↓ inflammation, ↓ airway hyperresponsiveness, ↑ lung function</td>
<td>Han et al 2011 (39), Chen 2011 (40)</td>
</tr>
<tr>
<td>Pulmonary Fibrosis (mouse, rat)</td>
<td>NaHS*</td>
<td>↓ pathology, ↓ oxidative stress, ↓ inflammation, ↓ fibrosis</td>
<td>Zhou et al 2014 (56), Fang et al 2009 (57), Wang et al 2016 (58)</td>
</tr>
<tr>
<td>Allergic Rhinitis (guinea pig)</td>
<td>NaHS*</td>
<td>↓ clinical disease, ↓ inflammation</td>
<td>Shaoqing et al 2009 (44)</td>
</tr>
<tr>
<td>Acute Lung injury (mouse, rat)</td>
<td>DATS‡, GYY4137§, DTT</td>
<td></td>
<td>↓ inflammation, ↓ oxidative stress, ↓ clinical disease</td>
</tr>
<tr>
<td>RSV infection (mouse)</td>
<td>GYY4137§</td>
<td>↓ clinical disease, ↓ airway hyperresponsiveness, ↓ viral replication, ↓ inflammation</td>
<td>Ivanciuc et al 2016 (50)</td>
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*NaHS – sodium hydrosulfide; †COPD – chronic obstructive pulmonary disease; ‡DATS – diallyl trisulfide; §GYY4137 – Phosphorodithioate-based donor; ¶DTT - 1,2-Dithiole-3-Thiones; LPS – lipopolysaccharide.
Figure legends

Figure 1. Intracellular synthesis and degradation of H$_2$S. H$_2$S is produced by cytoplasmic and mitochondrial enzymes CSE (cystathionine γ-lyase), CBS (cystathionine β-synthase), 3-MST (3-mercaptopyruvate sulfurtransferase) and CAT (cysteine aminotransferase) using cysteine or homocysteine as substrates. Intracellular non-toxic H$_2$S level is being actively maintained by oxidation in mitochondria by the enzyme sulfide quinone reductase (SQR), or methylation in the cytoplasm using thiol-S-methyltransferase. Free H$_2$S can also be bound by methemoglobin and by molecules with metal- or disulfide bonds and excreted with biological fluids.

Figure 2. Role of H$_2$S in the physiopathology of the airways. Under physiological conditions H$_2$S participates in regulation of respiratory rhythm in central neural system, electrolyte transport, and is necessary for normal development of lung vasculature and alveolarization. In various disease conditions, H$_2$S has been shown to inhibit inflammatory responses, pulmonary fibrosis and oxidative stress, and to possess a broad antiviral activity.
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