Role of Hydrogen Sulfide in Acute Lung Injury and Acute Respiratory Distress Syndrome

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Abstract: Accumulating evidence has suggested that hydrogen sulfide (H_2S) naturally occurs during cysteine metabolism in many types of mammalian cells. Since H_2S exhibits vasodilator activity and plays an important role in nervous system and inflammatory diseases, it is currently considered to be the third gaseous mediator. Recently, more and more attention has been paid to the biological functions of H_2S in acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS). In various animal models of lung injury, H_2S has been demonstrated to contribute to the development and progression of lung inflammation and injury. Regulating the endogenous level of H_2S is possible to protect animals against lung injury. H_2S may exert its effect on ALI/ARDS by modulating leukocyte activation. In addition, H_2S may induce lung inflammation and injury *via* activating sensory nerves in lung and eliciting a neurogenic inflammatory response.

INTRODUCTION

Hydrogen sulfide (H_2S) has been recognized as a toxic gas with a characteristic odor of rotten eggs for nearly 300 years [1]. However, it is now evident that H_2S is endogenously generated in many types of mammalian cells and tissues during cysteine metabolism in a reaction catalyzed by cystathionine-γ-lyase (CSE, EC 4.4.1.1) and/or cystathionine-β-synthetase (CBS, EC 4.2.1.22) [2-4]. Although endogenous H_2S was detected in the brainstem at the end of 1980s, its biological role and specific cellular targets in nervous system was first identified a decade ago [5]. Now H_2S is increasingly considered as the third gasotransmitter.

Although much less is known about the biological functions of H_2S than about its two counterparts, nitric oxide (NO) and carbon monoxide (CO), the gas is suggested to fulfill a wide range of physiological and pathological functions. For instance, H_2S opens K^+_{ATP} channels in vascular smooth muscle cells, gastrointestinal smooth muscle cells, cardiomyocytes, neurons, and pancreatic ß-cells, therefore regulates vascular tone, intestinal contractility, myocardial contractility, neurotransmission and insulin secretion [6-8]. In nervous system, H_2S promotes hippocampal long-term potentiation (LTP) by enhancing the sensitivity of NMDA receptors to glutamate and plays a role in neurodegenerative diseases [6-8]. In addition, H_2S may scavenge reactive nitrogen species (RNS), peroxynitrite (OONOO-), oxygen free radicals and lipid peroxidations, resulting in cardiovascular protection and neuron protection [9-11].

Despite the vasodilator and atypical neuromodulator activity of H_2S, it has recently been shown to play an important role in the pathogenesis of inflammatory diseases and associated organ injury, such as acute pancreatitis, sepsis and endotoxemia [12-16]. Overproduction of endogenous H_2S may exacerbate inflammatory response and lung injury in acute pancreatitis, sepsis and endotoxemia and inhibition of H_2S formation may be a potential therapeutic approach in these diseases.

Biosynthesis and Metabolism of H_2S

It is well know that H_2S can be generated by breaking down organic matters in certain bacteria and archea. Interestingly, many types of mammalian cells can produce H_2S. A substantial amount of H_2S is found to be present both in circulation and in various tissues, such as liver, kidney, pancreas, brain, aorta etc [6-8]. For instance, the concentration of H_2S in rat and human serum is reported to be 46 and 43.8 μM, respectively [13, 17]. The physiological level of H_2S in central nervous system is approximately 50-160 μM [5, 18].

The majority of endogenous H_2S generation is catalyzed by cystathionine-γ-lyase (CSE, EC 4.4.1.1) and/or cystathionine-β-synthetase (CBS, EC 4.2.1.22), which use L-cysteine as the main substrate [2-4]. These two enzymes are pyridoxal-5'-phosphate-dependent. Although CBS and CSE are widely expressed in cells and tissues, CSE is the predominant H_2S-forming enzyme in cardiovascular system, liver, kidney and non-vascular smooth muscle cells whereas CBS is mainly distributed in nervous system. As the end product of CSE- and CBS-involved cysteine metabolism, H_2S has a negative feedback on the activity of these two enzymes [19, 20]. In addition, a small proportion of endogenous H_2S may be generated by non-enzymatic steps in erythrocytes [21].

H_2S *in vivo* is metabolized by oxidation in mitochondria or by methylation in cytosol [6]. It can also be scavenged by methemoglobin or by metallo- or disulfide- containing molecules such as oxidized glutathione [6]. In addition, H_2S is

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excreted mainly by the kidney as free or conjugated sulfate [6].

Etiology and Pathogenesis of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS)

ALI and its most severe manifestation, ARDS, is a clinical syndrome characterized by acute hypoxemic respiratory failure, bilateral pulmonary infiltrates on frontal chest radiograph consistent with edema, and normal cardiac filling pressures [22, 23]. ALI/ARDS may occur in patients of all ages from direct or indirect insults. Common direct pulmonary causes of ALI/ARDS include lung viral or bacterial infections, gastric aspiration, blunt thoracic trauma with lung contusion, meconium aspiration (infants), near-drowning, thoracic radiation, hyperoxia, and the inhalation of smoke or other toxicants. Common indirect (systemic) causes of ALI/ARDS include sepsis, closed space burn injury, hypovolemic shock, non-thoracic trauma, multiple transfusions, and pancreatitis. These direct or indirect insults induce pulmonary inflammation, damage the pulmonary capillary permeability and alveolar diffusion capacity, increase intrapulmonary shunt, leading to severe acute respiratory failure [23-28]. Furthermore, complex autocrine and paracrine interrelationships of cytokines and pro-inflammatory mediators initiate and amplify the inflammatory response in ALI/ARDS. Cellular responses including endothelial adhesion molecules expression and migration of PMNs, as well as humoral responses, such as lipid mediators, proteases, oxidants, growth factors, NO, neuropeptides, and nuclear factor-κB (NF-κB) also participate in the pathogenesis of ALI/ARDS [29].

Role of H_2S in ALI/ARDS of Different Etiologies

In the past few years, H_2S has emerged as a novel and increasingly important mediator in inflammatory diseases. Its role in ALI/ARDS has also been investigated in different animal models and clinical cases. Although H_2S may exert different effect in ALI/ARDS induced by different insults, it has suggested that endogenous H_2S may participate in the pathogensis of ALI/ARDS and H_2S related therapy may be a potential therapeutic approach in this condition.

Pro-Inflammatory

Sepsis or Endotoxemia Induced Lung Injury

Sepsis and its sequelae such as septic shock and multiple organ failure are common and serious medical problems in severely ill patients [30]. An overproduction of endogenous H_2S in vascular tissues has been found in cecal ligation and puncture (CLP) induced septic shock or lipopolysaccharide (LPS) induced endotoxic shock. The vascular levels of H_2S may be associated with the hemodynamic collapse during shock [31]. Recently, it has been shown that generation of endogenous H_2S is elevated in plasma, liver and kidney, during CLP induced sepsis as well as LPS induced endotoxemia [13, 15]. Plasma H_2S concentration is also significantly increased in septic patients compared to healthy controls [13]. Furthermore, inhibition of H_2S formation by administration with DL-propargylglycine (PAG), a CSE inhibitor not only suppresses the overproduction of pro-inflammatory cytokines and chemokines in sepsis, which is a key feature of systemic inflammation, but also attenuates acute lung injury caused by sepsis or endotexmia [13-16]. Notably, the protective effect of PAG is independent of the improvement of hemodynamics, since it did not alter blood pressure [14]. In contrast, H_2S donors aggravate inflammation and sepsis associated ALI [15]. The pro-inflammatory effect of H_2S in sepsis may be due to the activation of ERK-NF-κB pathway [16, 32]. In addition, injection of NaHS to normal mice directly results in lung inflammation and inflammatory damage in a dose-dependent manner [33]. Administration with NaHS at a dose of 10 mg/kg but not 1 or 5 mg/kg, caused an obvious lung injury as evidence by lung MPO activity and histological changes in lung sections [33].

Acute Pancreatitis Induced Lung Injury

Since both CBS and CSE, two H_2S forming enzymes, are highly expressed in pancreatic acinar cells, some studies investigated the potential role of H_2S in acute pancreatitis [34]. Induction of acute pancreatitis in mice by caerulein increased plasma H_2S level. In pancreatic acinar cells stimulated by caerulein, the production of H_2S and CSE mRNA were significantly elevated, while the expression of CBS was reduced, suggesting that CSE is the main H_2S forming enzyme in caerulein induced acute pancreatitis [34]. Pretreatment or posttreatment with PAG to inhibit the activity of CSE not only attenuated the inflammatory response (MPO activity and chemokine expression) in pancreas and lung but also mitigated the severity of pancreatitis associated lung injury [35, 36].

Anti-Inflammatory

Burn and Smoke Inhalation Induced Lung Injury

In a murine model of acute lung injury induced by combined burn and smoke inhalation, intraperitoneal administration of NaHS, an H_2S donor alleviated lung injury, as evidenced by a promising improvement in survival rate and lung histological conditions [37]. H_2S may exert the protective effect *via* up-regulating the level of tissue anti-inflammatory cytokines (IL-10) and reducing the generation of pro-inflammatory cytokines (IL-1β). In this model, H_2S can also rescue smoke exposed lung from oxidative stress and therefore improve the outcome of ALI.

Oleic Acid Induced Lung Injury

In oleic acid induced ALI, a significant reduction in H_2S levels in plasma and lung tissues was observed [38]. However, administration of H_2S donors in rats treated with oleic acid down-regulated the production of pro-inflammatory IL-6 and IL-8 in lung but increased the level of anti-inflammatory IL-10 in plasma and lung, thus alleviating lung edema, PMNs infiltration in lung and severity of lung injury.

Application of H_2S - Releasing NSAIDs in ALI/ARDS

S-diclofenac (ACS 15) is a type of H_2S-releasing diclofenac, which comprises a H_2S-releasing dithiol-thione moiety attached by an ester linkage to diclofenac. Prophylactic or therapeutic administration of ACS 15 significantly attenuated lung inflammation and the severity of lung injury induced by acute pancreatitis but had no significant effect on pancreatic damage, suggesting the usefulness of H_2S-releasing nonsteroidal anti-inflammatory drugs as a potential treatment for pancreatitis-associated ALI/ARDS [39]. ACS15 also exhibits enhanced anti-inflammatory and protective effect as compared to the parent drug in a rat model of

endotoxemia and associated lung injury [40]. It is the H_2S released from ACS 15 that may intensify the anti-inflammatory activity of ACS 15 by inhibiting the DNA binding activity of nuclear transcriptional factors (AP-1 and NF-κB) and consequent production of inflammation related genes [40].

Potential Mechanisms of the Role of H_2S in ALI/ARDS

Regulation of Leukocyte Activity

Recent studies have revealed that H_2S may up-regulate the inflammatory response *via* stimulation of immune cells. It has been shown that reactive oxygen species from activated neutrophils converted H_2S to sulfite, which may up-regulate leukocyte adhesion and neutrophil functions [41-43]. The reaction was suppressed by the NADPH oxidase inhibitor and was accelerated by the addition of NaHS. Serum levels of H_2S and sulfite was found to be elevated in LPS injected rats. These data imply that oxidative stress dependent conversion of H_2S to sulfite by activated neutrophils may enhance non-specific host defense in various inflammatory diseases such as pneumonia [43, 44]. Most recently, Zhi *et al*. reported that H_2S dose-dependently activated human monocytes with up-regulating the production of pro-inflammatory cytokines, at least partially *via* the activation of extracellular signal-regulated kinase (ERK)-NF-κB signaling pathway [45]. Pretreatment with NF-κB inhibitor or MEK antagonist significantly inhibited H_2S induced activation of NF-κB and secretion of pro-inflammatory cytokines [45]. In addition, H_2S *in vitro* provoked the short-term survival of granulocyte *via* inhibition of caspase-3 cleavage and p38 mitogen-activated protein kinase (MAPK) activation and therefore contributed to the bactericidal activity of neutrophils [46].

In contrast, some *in vitro* studies performed in macrophages propose the anti-inflammatory role of H_2S. For instance, in cultured murine RAW264.7 macrophages, H_2S down-regulated LPS induced iNOS expression, NO production and NF-κB activation. This anti-inflammatory effect may be mediated by H_2S induced activation of ERK and consequent up-regulation of HO-1 expression and CO production, which exerts inhibitory effect on LPS-induced activation of NF-κB and resulted in a reduction in NO synthesis [47]. In addition, Hu *et al*. observed the effect of H_2S on LPS induced inflammation in both primary and cultured microglia and immortalized murine BV-12 microglia cells [48]. They found that both endogenous and exogenous H_2S significantly reduced LPS-induced NO production and TNF-α secretion in microglia. The effect of H_2S was mediated by inhibition of LPS induced activation of p38 MAPK. However, the anti-inflammatory role of H_2S reported in this study is merely limited to CNS-derived glia cells, in which CBS but not CSE is the primary H_2S-producing enzyme.

Regulation of Leukocyte Trafficking

Recently, one *in vivo* experiment indicates that H_2S may contribute to leukocyte-endothelial interaction, such as leukocyte rolling and adhesion, as well as promote PMN infiltration (MPO activity) into inflamed tissues during polymicrobial sepsis or endotoxemia [13, 15]. H_2S may provoke leukocyte migration by the activation of NF-κB and consequent up-regulation of the expression of adhesion molecules [49]. Furthermore, administration of NaHS in normal mice caused a significant increase in DNA binding activity of NF-κB and production of adhesion molecules [16, 49]. In addition, H_2S has been found to up-regulate the expression of CXCR2 in PMNs and therefore facilitate MIP-2 directed migration of PMNs [49].

On the other hand, some investigators reported that H_2S donors attenuated NSAIDs-induced gastric granulocyte infiltration and leukocyte adherence in mesenteric venules likely *via* opening K_{ATP} channels [50, 51]. Leukocyte infiltration in the air pouch in response to carrageenan was also suppressed by NaHS [50, 51]. This effect was reversed by either inhibition of H_2S formation or pretreatment with K_{ATP} channel blocker. In addition, H_2S also inhibited fMLP-induced leukocyte adherence to the mesenteric microcirculation [51]. The inconsistency in the role of H_2S in regulating leukocyte migration may be due to the different animal models and different doses of H_2S donors used.

Neurogenic Inflammation

Activation of afferent sensory nerve causes the release of bioactive substances, such as substance P, from never terminal, leading to vasodilation, edema and other manifestation of inflammation. This phenomenon is called "neurogenic inflammation" [52-54]. The association between H_2S and substance P in lung inflammation and injury has been investigated both *in vitro* and *in vivo*. Trevisani *et al*. found that NaHS not only provoked the release of substance P and CGRP from the sensory nerve terminals in isolated guinea pig airways but also produced a concentration-dependent contractile response [55]. Recently, one study performed by our group found that i.p. administration of NaHS in normal mice caused a significant rise in the circulatory level of substance P in a dose-and time-dependent manner, coupled with obvious lung inflammation [33]. Genetic deletion of *preprotachykinin-A (PPT-A)* gene, the precursor gene for substance P, or depletion of substance P from sensory neurons by capsaicin or pretreatment with capsazepine, an antagonist of the transient receptor potential vanilloid-1 (TRPV-1), abolished the inflammatory role of H_2S and therefore protected normal mice against H_2S-induced lung inflammation. This study shows for the first time that H_2S may induce neurogenic inflammation and lung injury even without other noxious stimuli.

In sepsis and associated lung injury, H_2S may exert its pro-inflammatory effect *via* up-regulating the production of substance P. Inhibition of the activation of substance P by genetic depletion of *PPTA* gene prevented NaHS from exacerbating inflammatory response and aggravating lung injury in sepsis [56]. Consistent findings were obtained in a murine model of acute pancreatitis. Inhibition of H_2S formation by PAG significantly decreased the expression of substance P in lung and pancreas in caerulein-induced acute pancreatitis and associated lung injury [57]. Taken together, both *in vitro* and *in vivo* studies suggest that neurogenic inflammation may mediate the pro-inflammatory role of H_2S in ALI/ARDS.

CONCLUSION AND PERSPECTIVES FOR THE FUTURE

H_2S is now considered to play a key role in inflammation. However, its role in ALI/ASDS is still far from clear,

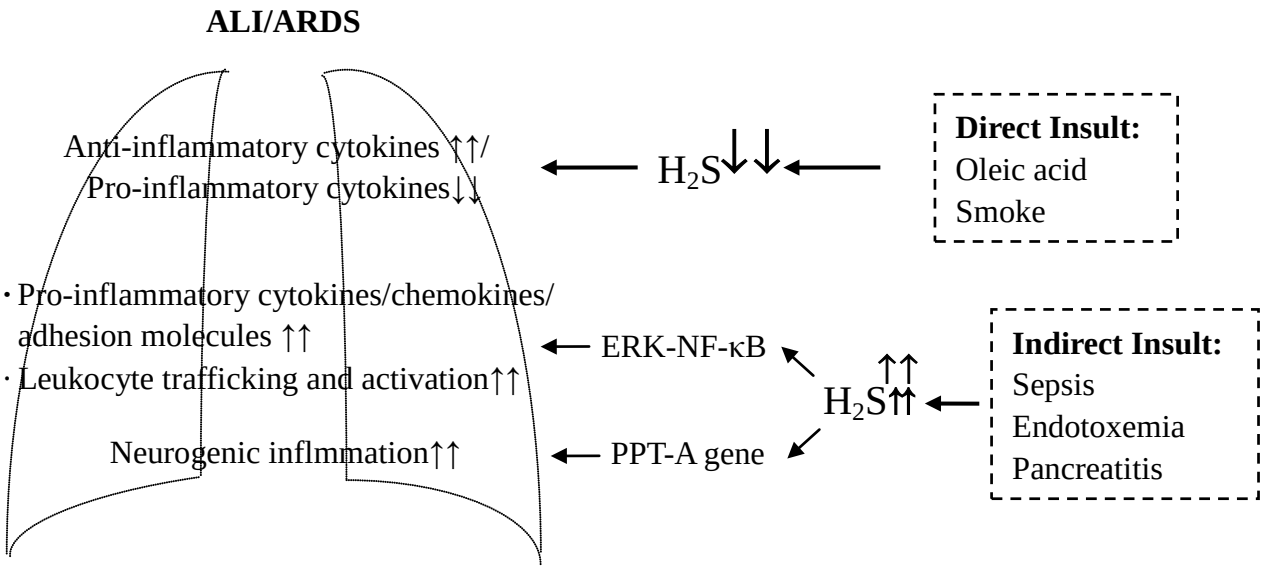

Fig. (1). A schematic summary of the evidence for the potential role of H_2S in ALI/ARDS. ALI/ARDS induced by direct or indirect insults may alter the circulatory level of H_2S. H_2S released from vascular smooth cells, liver, pancreas or lung (?) may regulate the production of inflammatory mediators (cytokines, chemokines and adhesion molecules), and leukocyte activation in lung by activating nuclear NF-κB, therefore leading to ALI/ARDS. H_2S may also facilitate inflammatory manifestations in lung, such as edema and plasma protein extravasation, by promoting neurogenic inflammation *via* upregulating the expression of PPT-A or NK1R gene.

and Fig. (**1**) summarized the relevant findings obtained to date. The apparent discrepancy in the role of H_2S in ALI/ARDS may be due to the different animal models and different doses of H_2S donors used. The protective effect of H_2S in ALI has been reported in some models of ALI caused by direct insults (smoke inhalation) whereas the opposite findings are usually obtained in ALI caused by indirect or systemic insults (e.g. sepsis, pancreatitis). Furthermore, high dose of H_2S donor tends to aggravate lung inflammation and injury while the low dose (or slow release) of H_2S, which approximates the physiological concentration but below that observed following inflammation, has been reported to mitigate ALI.

Further research is needed in the following directions: (1) to further characterize the precise mechanisms by which H_2S contributes to the development and progression of ALT/ARDS, including its interaction with other molecules; (2) to investigate whether H_2S participate in the pathogenesis of ALI/ARDS in humans and whether these results can be translated into the clinic; (3) to explore whether inhibition of H_2S biosynthesis provide a novel and potential forward for the development of treatment for ALT/ARDS. Although research in these directions is still in its infancy, the role of H_2S in ALI/ARDS is likely to be a complex one.

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