H_2S and Inflammation: An Overview

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Contents

Abstract

Inflammation is a response to traumatic, infectious, post-ischemic, toxic, or autoimmune injury. However, uncontrolled inflammation can lead to disease, and inflammation is now believed to be responsible for several disease conditions. Research in our laboratory has shown that hydrogen sulfide (H_2S) acts as a novel mediator of inflammation. At present, work in several research

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groups worldwide is focused on determining the role of H_2S in inflammation. H_2S has been implicated in different inflammatory conditions. Most of this research involved working with animal models of disease and in vitro systems. Recent research, however, points to a role of H_2S in clinical inflammatory disease as well. This chapter describes our current understanding of the role of H_2S in inflammation.

Keywords
Hydrogen sulfide • Inflammation • Systemic inflammatory response syndrome • Leukocytes

1 Introduction

Inflammation is a response to traumatic, infectious, post-ischemic, toxic, or autoimmune injury. It is a highly orchestrated, tissue-based process, characterized by "rubor" (redness), "calor" (heat), "dolor" (pain), and "tumor" (swelling). Inflammation is a normal response to injury and a useful physiological event. However, uncontrolled inflammation can lead to disease. Recent research has shown a key role of hydrogen sulfide (H_2S) in inflammation. This has been possible with the use of experimental approaches, such as cell culture systems and in vivo disease models. At the time of writing this chapter, evidence has started emerging on the role of H_2S in clinical inflammatory disease.

In this chapter, our current understanding of the role of H_2S in inflammation is discussed.

2 Inflammation

Currently, inflammation is an important research question in systems biology in the academia, as well as a multibillion dollar market for the pharmaceutical industry. In a disease in which primary pathogenic events are unknown, control of inflammation is the next best option.

In response to the initial insult/injury, leukocytes are activated. A key component of the inflammatory process is the trafficking of inflammatory cells to the site of injury/infection. Cytokine/receptor interactions on the surface of these cells lead to the expression of gene products that bring about the inflammatory response. However, uncontrolled production of inflammatory products is injurious to host cells and, in some cases, can lead to cancer. Therefore, endogenous mechanisms have evolved to limit the production of inflammatory molecules and permit the resolution of the inflammatory response. An understanding of these mechanisms is important because defects in the pathway may contribute to inflammatory disorders, and the pathway itself may present targets for novel anti-inflammatory therapeutic strategies (Bhatia and Moochhala 2004; Bhatia 2010, 2012; Hegde and Bhatia 2011; Nathan 2002; Mazumder et al. 2010).

3 H_2S and Inflammation

Over the years, various studies have indicated a role of H_2S in the inflammatory process. In mammals, including humans, H_2S is produced by the action of the enzymes, cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), cysteine aminotransferase (CAT, EC 2.6.1.3), (followed by 3-mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2), and cysteine lyase (EC 4.4.1.10), (Bhatia 2012; Li et al. 2011; Moore et al. 2003; Wang 2012). As the end product of CBS- and CSE-catalyzed cysteine metabolism, H_2S exerts a negative feedback effect on the activity of these enzymes (Bhatia 2012; Moore et al. 2003). Endogenous H_2S synthesized by CSE has been shown to be primarily responsible for its inflammatory action.

Recent work in our laboratory and others has shown a key role of H_2S as a mediator of inflammation in different clinical conditions. Current understanding of the role of H_2S in different inflammatory conditions is summarized in Table 1.

4 Disease Conditions with Role of H_2S in Inflammation

4.1 Acute Pancreatitis

Acute pancreatitis is a common clinical condition, the incidence of which has been increasing worldwide over recent years (Bhatia et al. 2000, 2005c; Bhatia 2012). For example, in the United States alone, acute pancreatitis is the most common reason for hospitalization (274,119 discharges in the year 2009, a 30 % increase over 2000) amongst all gastrointestinal diseases (Peery et al. 2012). It also inflicts a heavy economic burden; the direct cost in the United States alone in the year 2009 was US$2,599,686,000 (Peery et al. 2012). Acute pancreatitis was the cause of death in 3065 cases and a contributing cause in an additional 5500 deaths in the year 2009 (Peery et al. 2012).

Most cases of acute pancreatitis are secondary to biliary disease or excess alcohol consumption. The exact mechanisms by which diverse etiological factors induce an attack are still unclear, but once the disease process is initiated, common inflammatory and repair pathways are invoked. There is a local inflammatory reaction at the site of injury, which if marked leads to systemic inflammatory response syndrome (SIRS), and it is this systemic response that is believed to be ultimately responsible for the majority of the morbidity and mortality (Bhatia et al. 2000, 2005c; Bhatia 2012). Lung injury, which is clinically manifested as acute respiratory distress syndrome (ARDS), is a major component of the multiple organ dysfunction syndrome (MODS) that results from SIRS in acute pancreatitis.

CBS and CSE, the two major H_2S synthesizing enzymes, are highly expressed in the pancreas. Endogenously produced H_2S has been shown as a mediator of inflammation in acute pancreatitis (Bhatia et al. 2005a). mRNA for CSE is expressed in mouse pancreas and that pancreas homogenates convert L-cysteine to H_2S ex vivo. Also, circulating levels of H_2S are increased in mice upon induction

Table 1 Role of H_2S in inflammatory disease

Disease model	Effect on inflammation	Reference
Cerulein-induced acute Pancreatitis in the mouse	Plasma H_2S is increased in inflammation. Treatment with the cystathione-gamma-lyase (CSE) inhibitor propargylglycine (PAG) protects against acute pancreatitis and associated lung injury	Bhatia et al. (2005a)
	Treatment with slow H_2S-releasing diclofenac protects mice against acute pancreatitis-associated lung injury	Bhatia et al. (2008a)
	Treatment with s-propargyl-cysteine (SPRC), a slow H_2S-releasing donor, protects against acute pancreatitis and associated lung injury	Sidhapuriwala et al. (2012)
	Knockout mice genetically deficient in CSE ($CSE^{-/-}$) and protected against acute pancreatitis and associated lung injury	Ang et al. (2013)
Lipopolysaccharide (LPS)-induced endotoxemia in the mouse	Plasma H_2S levels, H_2S-synthesizing activity, and CSE expression are increased in inflammation. PAG treatment protects against inflammation	Li et al. (2005)
	Treatment with slow H_2S-releasing diclofenac protects mice against LPS-induced endotoxemia	Li et al. (2007)
	Treatment with morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate (GYY4137), a slow H_2S-releasing donor, protects mice against LPS-induced endotoxemia	Li et al. (2009)
Cecal ligation and puncture (CLP)-induced sepsis	Plasma H_2S levels, H_2S-synthesizing activity, and CSE expression are increased in inflammation. PAG treatment protects against sepsis. The H_2S donor sodium hydrogen sulfide (NaHS) further aggravates inflammation in sepsis	Zhang et al. (2006)
Carrageenan-induced hindpaw edema in the mouse	H_2S-synthesizing activity increased in inflammation. PAG treatment protects against inflammation in this model	Bhatia et al. (2005b)
	Treatment with slow H_2S-releasing diclofenac protects mice against carrageenan-induced hindpaw edema	Sidhapuriwala et al. (2007)
Burn injury-induced inflammation	Plasma H_2S levels, H_2S-synthesizing activity, and CSE expression are increased in inflammation. PAG treatment protects against burn injury-induced inflammation	Zhang et al. (2010)
Trinitrobenzene sulfonic acid-induced colitis	Slow H_2S-releasing mesalamine protects against inflammation in colitis	Fiorucci et al. (2007)
Direct administration of H_2S as NaHS to mouse	Direct administration of H_2S by intraperitoneal (i.p) administration of NaHS causes lung inflammation characterized by an increase in lung myeloperoxidase (MPO) activity and histological evidence of lung injury	Bhatia et al. (2006)

of acute pancreatitis. The conversion of L-cysteine to H_2S in pancreas homogenates is significantly reduced in mice pretreated with DL-propargylglycine (PAG) (Bhatia et al. 2005a). Furthermore, treatment of animals with PAG (either prophylactic or therapeutic) reduces the severity of pancreatitis as evidenced by a significant attenuation of hyperamylasemia, acinar cell injury/necrosis, and pancreatic myeloperoxidase (MPO) activity and by histological evidence of diminished pancreatic injury. Lung injury in severe acute pancreatitis is characterized by sequestration of neutrophils within the lung (i.e., increased lung MPO activity) and histological evidence of lung injury. Prophylactic or therapeutic administration of PAG additionally protected mice against acute pancreatitis-associated lung injury as evidenced by a significant attenuation of lung MPO activity and by histological evidence of diminished lung injury (alveolar thickening and leukocyte infiltration) (Bhatia et al. 2005a). The pro-inflammatory role of H_2S synthesized by CSE has been confirmed recently using knockout mice deficient in CSE (Ang et al. 2013). CSE knockout mice are protected against acute pancreatitis and associated lung injury, when compared to wild-type controls (Ang et al. 2013). These effects of CSE blockade/gene deletion suggested an important pro-inflammatory role of H_2S in regulating the severity of pancreatitis and associated lung injury and raised the possibility that H_2S may exert similar activity in other forms of inflammation. Following this study, an important role of CBS in the pathogenesis of acute pancreatitis and associated lung injury has been shown (Shanmugam et al. 2009). Acute pancreatitis was associated with increased plasma and tissue H_2S and ammonia (NH_3). Prophylactic or therapeutic administration of aminooxyacetate (AOA), a reversible inhibitor of CBS, directly inhibited CBS in the pancreas thereby reducing H_2S and NH_3 production, and protected against acute pancreatitis, showing a role of H_2S synthesized by CBS in inflammation in acute pancreatitis (Shanmugam et al. 2009).

4.2 Sepsis

Sepsis is defined as the presence of infectious organisms, such as bacteria, viruses, protozoa, or fungi, and/or their toxins in blood or tissue and the systemic response that follows. Sepsis leading to organ failure characterizes severe sepsis, and septic shock is defined by severe sepsis accompanied by hypotension unresponsive to fluid resuscitation. Severe sepsis and septic shock are one of the leading causes of mortality among intensive care unit and postoperative care patients (Bhatia 2012; Bhatia et al. 2009; Levy et al. 2010; Martin et al. 2003; Ramnath et al. 2006). Sepsis is a major health problem worldwide. The incidence of sepsis in North America, for example, is 3.0 per 1000 population, which transforms into an annual number of 750,000 cases, with 210,000 of them being fatal and a large socioeconomic burden (Bhatia 2012; Bhatia et al. 2009; Levy et al. 2010; Martin et al. 2003; Ramnath et al. 2006). The incidence of mortality due to sepsis is increasing, and the most likely causes are the increased incidence of resistant pathogens and the advances of medical and surgical procedures that save the lives of many patients but at the cost

of leaving them immunocompromized and in a state susceptible to death from severe sepsis and septic shock (Bhatia 2012; Bhatia et al. 2009; Levy et al. 2010; Martin et al. 2003; Ramnath et al. 2006).

H_2S has been shown to act as a mediator of inflammation in sepsis. In a clinically relevant mouse model of cecal ligation and puncture (CLP)-induced polymicrobial sepsis, liver CSE expression, H_2S synthesis, and plasma H_2S levels are significantly elevated (Zhang et al. 2006). Prophylactic, as well as therapeutic, administration of PAG significantly reduced sepsis-associated systemic inflammation, as evidenced by decreased MPO activity and histological changes in lung and liver and attenuated the mortality in CLP-induced sepsis (Zhang et al. 2006). On the other hand, administration of sodium hydrogen sulfide (NaHS), an H_2S donor, significantly aggravated sepsis-associated systemic inflammation (Zhang et al. 2006). Similar to CLP-induced sepsis, a pro-inflammatory action of H_2S has also been shown in lipopolysaccharide (LPS)-induced endotoxemia (Li et al. 2005; Collin et al. 2005). These studies show that H_2S plays a pro-inflammatory role in regulating the severity of sepsis and associated organ injury. H_2S has also been shown to modulate sinusoidal constriction in the liver and contribute to hepatic micorcirculatory dysfunction during endotoxemia (Norris et al. 2013), and endogenous H_2S formation mediates the liver damage in endotoxemia in the rat (Yan et al. 2013).

4.3 Burn Injuries

Burn injuries are ranked among the leading causes of morbidity and mortality worldwide. In severe cases, burn injuries lead to SIRS, and associated MODS, which is a major contributor to death following burns (Bhatia 2012; Church et al. 2006; Endorf and Ahrenholz 2011). H_2S has been shown to act as a critical mediator of severe burn injury (to 25 % total body surface area full thickness burn)-induced inflammation in mice (Zhang et al. 2010). The result of this study shows that burn injury in mice resulted in a significant increase in plasma H_2S levels, liver and lung CSE mRNA expression, and liver H_2S-synthesizing activity. The enhanced H_2S/CSE pathway correlates with increased burn-associated systemic inflammation, as evidenced by increased MPO activity and histological evidence of lung and liver injury. There was protection against systemic inflammation and multiple organ damage by prophylactic or therapeutic treatment of PAG. Administration of NaHS at the same time of burn injury resulted in a further increase in MPO activity and more severe tissue injury in the lung and liver. These findings show that H_2S plays a key pro-inflammatory role in burn injury (Zhang et al. 2010).

4.4 Joint Inflammation/Arthritis

Joint inflammation/arthritis is a major health problem worldwide. In the United States, for example, back pain and arthritis (osteoarthritis, rheumatoid arthritis) are

amongst the most common and costly conditions, affecting more than 100 million individuals and costing more than US$200 billion per year (Ma et al. 2014). The possible role of endogenous H_2S in the development of joint inflammation induced by intraplantar administration of carrageenan to the rat hindpaw has been investigated. An increase in H_2S synthesis in inflamed hindpaws was seen, suggesting a localized overproduction of H_2S during inflammation (Bhatia et al. 2005c). Pretreatment with PAG resulted in a dose-dependent inhibition of hindpaw edema as well as hindpaw MPO activity. These findings suggest that H_2S is an endogenous mediator of the development of hindpaw local inflammation. Recent reports in the literature in human patients point to a role of H_2S in rheumatoid arthritis, but there is a difference of opinion as to the pro- versus anti-inflammatory action of H_2S (Fox et al. 2012; Kloesch et al. 2012).

4.5 Colitis

Colitis, or inflammation of the colon, presents itself in several clinical forms, such as inflammatory bowel disease (IBD), ulcerative colitis (UC), and Crohn's disease (CD). Mucosal changes in colitis are characterized by ulcerative lesions accompanied by prominent inflammatory infiltrates in the bowel wall (Polytarchou et al. 2014). Mesalamine (5-aminosalicylic acid) is the first-line therapy for colitis. An H_2S-releasing derivative of mesalamine has been reported to protect against inflammation in trinitrobenzene sulfonic-induced colitis (Fiorucci et al. 2007) and against nociception in hapten-induced colitis (Coletta et al. 2012).

4.6 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a common clinical condition characterized by progressive airflow obstruction that is only partly reversible, inflammation in the airways, and systemic effects or comorbities. The main cause is tobacco smoking (Decramer et al. 2012). In patients with stable COPD, serum H_2S levels have been found to be significantly increased as compared to age-matched control subjects or those with acute exacerbation of COPD (AECOPD) (Chen et al. 2005). In this study, serum H_2S levels were negatively correlated with the severity of airway obstruction in patients with stable COPD, whereas they were positively correlated with the lung function in all patients with COPD and healthy controls. This study also reported that serum H_2S levels were lower in smokers than nonsmokers regardless of their health status (COPD or healthy controls). In addition, patients with AECOPD, whose serum H_2S levels were decreased, had greater neutrophil proportion but lower lymphocyte proportion in sputum than patients with stable COPD, suggesting a potential role of H_2S in regulating inflammatory response at different types or stages of COPD. This study (Chen et al. 2005) demonstrated that endogenous H_2S may participate in the development of airway obstruction in COPD and that the level of endogenous

H_2S may be correlated with the progression and severity of COPD. Also, there is a correlation between exhaled H_2S and exhaled nitric oxide (NO) in COPD (Sun et al. 2013).

5 Mechanisms of Action of H_2S in Inflammation

H_2S has been shown to contribute to inflammation via cytokines, chemokines, adhesion molecules (and leukocyte recruitment), and transient receptor potential vanilloid type 1 (TRPV1) and substance P.

5.1 Cytokines and Chemokines

In isolated pancreatic acinar cells stimulated by cerulein (an in vitro system that resembles acinar changes in acute pancreatitis), inhibition of H_2S formation by PAG has been shown to decrease mRNA expression and production of the chemokines monocyte chemoatractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and MIP-2 (Tamizhselvi et al. 2007, 2008). Cerulein-induced acute pancreatitis is associated with a significant increase in mRNA for MCP-1, MIP-1α, and MIP-2 in both the pancreas and lungs, suggesting that they are important early mediators in both local as well as distant inflammatory response (Tamizhselvi et al. 2008). Blockade of H_2S biosynthesis with PAG ameliorates the development of inflammatory process in cerulein-induced acute pancreatitis, acting through downregulation of chemokine expression (Tamizhselvi et al. 2008). Also, results indicate a key role of the phosphatidylinositol 3-kinase-protein kinase B pathway in relation to the action of H_2S on cerulein-induced cytokine production in isolated mouse pancreatic acinar cells (Tamizhselvi et al. 2009).

Both prophylactic and therapeutic administration of PAG significantly reduces the mRNA and protein levels of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, MCP-1, and MIP-2 in lungs and liver, coupled with decreased nuclear translocation and activation of NF-κB in lungs and liver following sepsis (Zhang et al. 2007a). In contrast, injection of NaHS significantly aggravates sepsis-associated systemic inflammation and increases nuclear factor (NF)-κB activation. In addition, H_2S-induced lung inflammation is blocked by the NF-κB inhibitor BAY 11-7082. Therefore, H_2S upregulates the production of pro-inflammatory mediators and exacerbates the systemic inflammation in sepsis through a mechanism involving NF-κB activation (Zhang et al. 2007a).

In human monocyte cell line U937, treatment with H_2S donor NaHS results in significant increase in the mRNA expression and protein production of TNF-α, IL-1β, and IL-6. This effect is mediated via NF-κB and extracellular signal-related kinase (ERK) pathway (Zhi et al. 2007). Recent research, using mouse macrophage RAW264.7 cells, has shown that the activation of macrophages by lipopolysaccharide (LPS) results in higher levels of CSE mRNA and protein as well as the increased production of pro-inflammatory cytokines and chemokines IL-1 β,

IL-6, TNF-α, and MCP-1. Silencing of the CSE gene by small interference ribonucleic acid (siRNA) results in decreased levels of pro-inflammatory cytokines (Badiei et al. 2013).

H_2S regulates inflammatory response by activating the extracellular signal-related kinase ERK pathway in polymicrobial sepsis (Zhang et al. 2008). Maximum phosphorylation of ERK1/2 and degradation of IκBα in lung and liver is observed 4 h after CLP. Inhibition of H_2S formation by PAG significantly reduces the phosphorylation of ERK1/2 in lung and liver, coupled with decreased degradation of IκBα and activation of NF-κB. In contrast, injection of NaHS significantly enhances the activation of ERK1/2 in lung and liver, therefore leading to a further rise in tissue NF-κB activity. In sepsis, pretreatment with PAG significantly reduces the production of cytokines and chemokines via NF-κB and ERK1/2, whereas exogenous H_2S greatly increased it. In addition, pretreatment with PD98059, an inhibitor of MEK-1, significantly prevented NaHS from aggravating systemic inflammation in sepsis (Zhang et al. 2008). Binding site for NF-κB has been shown on the CSE gene promoter and is critical for LPS-induced CSE expression (Wang et al. 2014). Therefore, H_2S may regulate systemic inflammatory response in sepsis via the NF-κB-ERK pathway.

5.2 Adhesion Molecules and Leukocyte Recruitment

H_2S induces intercellular adhesion molecule (ICAM)-1 expression and neutrophil adhesion to cerulein-treated pancreatic acinar cells through nuclear factor (NF)-κB and Src-family kinases (SFK) pathway (Tamizhselvi et al. 2010). H_2S activates SFKs in acinar cells, and inhibition of SFKs impairs H_2S-induced ICAM-1 expression secondary to the inhibition of NF-κB activation. The effect of SFK inhibition on NF-κB activation occurs together with IκBα degradation. The results further demonstrate that neutrophil attachment onto H_2S-treated acinar cells is increased and that inhibition of SFK function inhibits H_2S-induced neutrophil attachment onto acinar cells. Taken together, these data indicate that H_2S engages SFKs in order to signal ICAM-1 expression by a mechanism involving induction of NF-κB (Tamizhselvi et al. 2010).

Using intravital microscopy, it has been shown that in sepsis, prophylactic and therapeutic administration of PAG reduces leukocyte rolling and adherence significantly in mesenteric venules coupled with decreased mRNA and protein levels of adhesion molecules (ICAM-1, P-selectin, and E-selectin) in lung and liver. In contrast, administration of NaHS upregulates leukocyte rolling and attachment significantly, as well as tissue levels of adhesion molecules in sepsis. Conversely, in normal mice given NaHS to induce lung inflammation, NaHS treatment enhanced the level of adhesion molecules and neutrophil infiltration in lung. These alterations can be reversed by pretreatment with BAY 11-7082 (Zhang et al. 2007b). Therefore, H_2S acts as an important endogenous regulator of leukocyte activation and trafficking during an inflammatory response.

5.3 Transient Receptor Potential Vanilloid Type 1 and Substance P

Substance P is a mediator of inflammation and plays an important role in the pathogenesis of several inflammatory conditions. Intraperitoneal administration of the H_2S donor NaHS to mice causes an increase in circulating levels of substance P (Bhatia et al. 2006). NaHS, by itself, causes lung inflammation, as evidenced by a significant increase in lung MPO activity and histological evidence of lung injury. This is associated with a significant increase in lung levels of tumor necrosis factor (TNF)-α and interleukin (IL)-1β. In preprotachykinin (PPT)-$A^{-/-}$ mice, genetically deficient in substance P, H_2S does not cause any lung inflammation. Also, pretreatment of mice with CP-96345, an antagonist of the neurokinin-1 receptor (NK-1R), the receptor for substance P, protects mice against lung inflammation caused by H_2S. Depleting neuropeptide from sensory neurons by capsaicin significantly reduces the lung inflammation caused by H_2S. In addition, pretreatment of mice with capsazepine, an antagonist of the transient receptor potential vanilloid-1 (TRPV-1), protects mice against H_2S-induced lung inflammation. These results demonstrate an important role of substance P and neurogenic inflammation in H_2S-induced lung injury in mice (Bhatia et al. 2006).

In acute pancreatitis, PAG treatment significantly attenuates the increases in substance P concentrations in plasma, pancreas, and lung (Bhatia et al. 2008b). Moreover, administration of PAG significantly reduces PPT-A mRNA expression and NK-1R mRNA expression in both pancreas and lung when compared with cerulein-induced acute pancreatitis. This reduction in PPT-A mRNA expression and NK-1R mRNA expression is associated with a protection against acute pancreatitis and associated lung injury (Bhatia et al. 2008b). These results suggested that the pro-inflammatory effects of H_2S may be mediated by SP-NK-1R pathway in acute pancreatitis (Bhatia et al. 2008b). Furthermore, PPT-A deficiency and blockage of H_2S synthesis can regulate the toll-like receptor 4 (TLR4) pathway and subsequent innate immune response in acute pancreatitis, implying an interaction between SP/H_2S occurs via TLR4 and NF-kB pathway. PPT-A gene deletion regulates H_2S-induced TLR4 signaling pathway in cerulein-treated pancreatic acinar cells, suggesting that in acute pancreatitis, H_2S may upregulate the TLR4 pathway and NF-κB via substance P (Tamizhselvi et al. 2011).

In sepsis, PAG treatment significantly decreases the PPT-A gene expression and the production of substance P in lung, whereas administration of NaHS results in a further rise in the pulmonary level of substance P (Zhang et al. 2007c). PPT-A gene deletion and pretreatment with the NK-1R antagonist L703606 prevent H_2S from aggravating lung inflammation. In addition, septic mice genetically deficient in PPT-A gene or pretreated with L703606 do not exhibit further increase in lung permeability after injection of NaHS (Zhang et al. 2007c). These findings show that in sepsis, H_2S upregulates the generation of substance P that contributes to lung inflammation and lung injury mainly via activation of the NK-1R. Also, H_2S induces systemic inflammation and multiple organ damage characteristic of sepsis via transient receptor potential vanilloid type 1 (TRPV1)-mediated neurogenic

inflammation (Ang et al. 2010). Pretreatment with capsazepine, a TRPV1 antagonist, significantly attenuates systemic inflammation and multiple organ damage caused by sepsis. Moreover, capsazepine delays the onset of lethality and protects against sepsis-associated mortality. As expected, administration of NaHS exacerbates sepsis, but capsazepine reverses these deleterious effects. In the presence of PAG, capsazepine causes no significant changes to the PAG-mediated attenuation of systemic inflammation, multiple organ damage, and mortality. More importantly, capsazepine has no effect on endogenous generation of H_2S, suggesting that H_2S is located upstream of TRPV1 activation, and may play a critical role in regulating the production and release of sensory neuropeptides in sepsis. This study showed for the first time that H_2S induces systemic inflammation and multiple organ damage in sepsis via TRPV1-mediated neurogenic inflammation (Ang et al. 2010). Capsazepine treatment results in an attenuation of circulating and pulmonary levels of substance P in septic mice. Capsazepine also inhibits NaHS-augmented substance P production but has no effect on PAG-mediated abrogation of substance P levels in both plasma and lung. Capsazepine significantly reduces H_2S-induced inflammatory cytokines, chemokines, and adhesion molecules expression and protects against lung and liver dysfunction in sepsis. In the absence of H_2S, capsazepine caused no significant changes to the PAG-mediated attenuation of sepsis-associated systemic inflammatory response and multiple organ dysfunction. Capsazepine inhibits phosphorylation of ERK1/2 and IκBα, concurrent with suppression of NF-κB activation. Results in this study showed that H_2S regulates TRPV1-mediated neurogenic inflammation in polymicrobial sepsis through enhancement of SP production and activation of the ERK-NF-κB pathway (Ang et al. 2011a). Also, a recent study has shown that H_2S upregulates cyclooxygenase-2 (COX-2) and prostaglandin E metabolite (PGEM) in sepsis-evoked acute lung injury via TRPV-1 channel activation (Ang et al. 2011b).

6 H_2S Donors

NaHS and sodium sulfide (Na_2S) are the two "classical" H_2S donors that release the gas rapidly in solution. In most of the studies, they have been substantiated a pro-inflammatory action of H_2S. In recent years, slow/controlled release donors have emerged, and results using these compounds have been quite interesting. For example, S-diclofenac (ACS 15) is H_2S-releasing diclofenac, which comprises an H_2S-releasing dithiol-thione moiety attached by an ester linkage to diclofenac (a nonsteroidal anti-inflammatory drug—NSAID). The effect of treatment with the NSAID diclofenac and its H_2S-releasing derivative ACS15 on cerulein-induced acute pancreatitis and the associated lung injury has been investigated (Bhatia et al. 2008a). Although these two drugs do not have any significant effect on the local pancreatic injury, ACS15 affords significant protection against acute pancreatitis-associated lung injury (Bhatia et al. 2008a). ACS 15 also more effectively inhibits hindpaw swelling and neutrophil infiltration after carrageenan injection as compared to its parent NSAID (Sidhapuriwala et al. 2007). Furthermore, in a

rat model of LPS-induced endotoxemia, ACS 15 exhibits enhanced anti-inflammatory effect as compared to the parent drug (Li et al. 2007). Although these results suggest the potential for the use of controlled release H_2S donor compounds against inflammation, the protective action of ACS 15 in LPS-induced endotoxemia was associated with an inhibition of endogenous H_2S synthesis (Li et al. 2007). Therefore, protective actions of H_2S-releasing compounds may be caused by an inhibition of endogenous H_2S formation, possibly by a negative feedback mechanism caused by very low local levels of H_2S. Another slow H_2S-releasing drug is S-propargyl-cysteine (SPRC), a structural analog of S-allyl cysteine (SAC) with a common cysteine-containing structure. Pretreatment with SPRC has been shown to protect mice against acute pancreatitis and associated lung injury (Sidhapuriwala et al. 2012). Another slow H_2S-releasing compound is GYY4137 (morpholin-4-ium-4-methoxyphenyl(morpholino) phosphinodithioate), which has been reported to have an anti-inflammatory action (Li et al. 2009, 2013).

7 Conclusion

Figure 1 summarizes our current understanding of the mechanism of action of H_2S in inflammation. Several gaps in knowledge still remain on this subject. H_2S synthesis inhibitors, such as PAG, have proved useful in the proof of principle

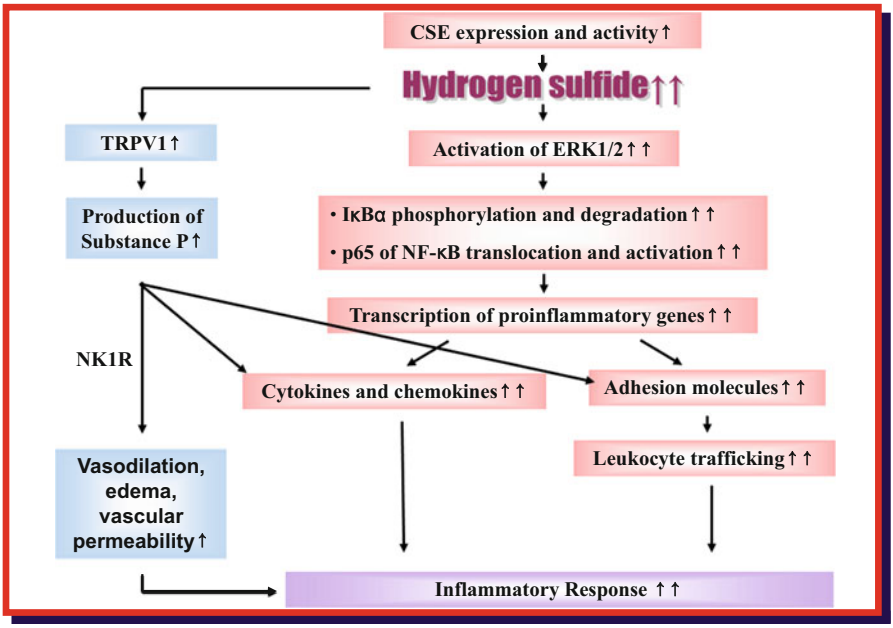

Fig. 1 A summary of our current understanding of the mechanisms of action of H_2S in inflammation

studies, but they are not entirely specific. Studies using CSE knockout mice and siRNA have contributed greatly to research in this area, but there is need to develop novel synthesis inhibitors that are more selective and have a better safety profile than the ones currently available. Also, following up on the progress made in basic/preclinical research, now is the time to investigate the role of H_2S in inflammation in clinical disease. As described in this article, some groups have already started working on this, and early results have been promising.

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