Review Article

The Cardioprotective Effects of Hydrogen Sulfide in Heart Diseases: From Molecular Mechanisms to Therapeutic Potential

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Hydrogen sulfide (H₂S) is now recognized as a third gaseous mediator along with nitric oxide (NO) and carbon monoxide (CO), though it was originally considered as a malodorous and toxic gas. H₂S is produced endogenously from cysteine by three enzymes in mammalian tissues. An increasing body of evidence suggests the involvement of H₂S in different physiological and pathological processes. Recent studies have shown that H₂S has the potential to protect the heart against myocardial infarction, arrhythmia, hypertrophy, fibrosis, ischemia-reperfusion injury, and heart failure. Some mechanisms, such as antioxidative action, preservation of mitochondrial function, reduction of apoptosis, anti-inflammatory responses, angiogenic actions, regulation of ion channel, and interaction with NO, could be responsible for the cardioprotective effect of H₂S. Although several mechanisms have been identified, there is a need for further research to identify the specific molecular mechanism of cardioprotection in different cardiac diseases. Therefore, insight into the molecular mechanisms underlying H₂S action in the heart may promote the understanding of pathophysiology of cardiac diseases and lead to new therapeutic targets based on modulation of H₂S production.

1. Introduction

Hydrogen sulfide (H₂S) has been thought of to be just a toxic gas with a strong odor of rotten eggs for hundreds of years. However, with the advancement of scientific technology over the years, researchers have discovered that H₂S takes part in a series of physiological and pathological processes in mammals. A pioneering study reported by Abe and Kimura [1] in 1996 determined that H₂S facilitated the induction of hippocampal long-term potentiation by enhancing the activity of N-methyl-D-aspartate (NMDA) receptors. From then on, scientific interest has grown in the investigation of the function of H₂S as a gasotransmitter.

Now H₂S has been regarded as a novel gaseous signaling molecule, similarly to nitric oxide (NO) and carbon monoxide (CO) [2, 3]. H₂S is endogenously produced by several enzymes, including cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopuruvate sulfurtransferase (3-MST) along with cysteine aminotransferase (CAT) [4–7]. The distributions of these enzymes’ expressions are tissue specific. CBS is the critical enzyme for H₂S production in the nervous system and CSE is the major H₂S-producing enzyme in the cardiovascular system [8]. A number of studies have demonstrated that H₂S may be involved in a multitude of pathophysiologic processes, such as oxidative stress, inflammation, apoptosis, and angiogenesis [3]. In recent years, growing evidence has shown that H₂S is a critical regulator of heart functions and plays a protective role in the pathogenesis and development of heart diseases.

In this review, we summarize the biosynthesis and physiological functions of H₂S and explore its emerging pathogenic significance in several heart diseases including myocardial ischemia/reperfusion (I/R) injury, myocardial infarction, arrhythmias, cardiac hypertrophy, cardiac fibrosis,
and heart failure. Furthermore, we also discuss the molecular mechanisms involved in the cardioprotective effects of $H_2S$ and how these might be used therapeutically to overcome some of the heart diseases.

2. Biosynthesis and Metabolism of $H_2S$

$H_2S$ is a small molecule which can pass through cell membranes freely. The basal level of its production in mammalian tissues is determined by the activity of three key enzymes: CBS, CSE, and 3-MST together with CAT (Figure 1). Recent studies have provided a broader picture of enzyme distribution; for example, CBS is expressed in brain, liver, kidney, ileum, uterus, placenta, and pancreatic islets, and it is the predominant producer of $H_2S$ in the central nervous system [9–11]. CSE is the main $H_2S$-generating enzyme in the cardiovascular system and is also found in the liver, kidney, ileum, thoracic aorta, portal vein, uterus, and placenta and is weakly detected in the brain [9, 10, 12, 13]. 3-MST, along with CAT, is a third $H_2S$-producing enzyme in neurons, vascular endothelium, and the retina [14–17]. Both CBS and CSE are pyridoxal-5-phosphate- (PLP-) dependent enzymes and located in cytosol; they use L-cysteine as their principal substrate to produce $H_2S$ [18]. Unlike CBS and CSE, 3-MST and CAT have been found in both mitochondria and cytosol, although approximately two-thirds of 3-MST exists in the mitochondria [19]. 3-MST produces $H_2S$ from 3-mercaptopyruvate (3MP), which is produced by CAT from L-cysteine and $\alpha$-ketoglutarate ($\alpha$-KG); on the other hand, it is also produced by D-amino acid oxidase (DAO) from D-cysteine.

![Figure 1: Biosynthesis pathways of endogenous $H_2S$. Cystathionine-$\beta$-synthase (CBS) and cystathionine-$\gamma$-lyase (CSE) use L-cysteine as a substrate to produce $H_2S$. However, 3-mercaptopyruvate sulfur-transferase (3-MST) uses 3-mercaptopyruvate (3-MP) as a substrate to form $H_2S$. 3-MP is produced by cysteine aminotransferase (CAT) from L-cysteine in the presence of $\alpha$-ketoglutarate ($\alpha$-KG); on the other hand, it is also produced by D-amino acid oxidase (DAO) from D-cysteine.](image)

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3-MST and CAT have been found in both mitochondria and cytosol, although approximately two-thirds of 3-MST exists in the mitochondria [19]. 3-MST produces $H_2S$ from 3-mercaptopyruvate (3MP), which is produced by CAT from L-cysteine and $\alpha$-ketoglutarate ($\alpha$-KG). In addition to the above pathway, Kimura group discovered a novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells [20]. D-Cysteine is metabolized by d-amino acid oxidase (DAO) to 3MP, which is a substrate for 3-MST to produce $H_2S$. This pathway is functional only in the kidney and the brain, particularly in the cerebellum.

$H_2S$ can undergo several catabolic pathways in order to maintain a proper physiological balance of its metabolism under physiological conditions. Firstly, once deprotonated, $H_2S$ is rapidly oxidized in the mitochondria to form thiosulfate (nonenzymatic conversion), followed by further conversion into sulfite and finally into sulfate, the major end product of $H_2S$ metabolism [21]. Secondly, $H_2S$ can also be methylated by thiol S-methyltransferase to form dimethylsulfide and methanethiol. Lastly, $H_2S$ can react with methemoglobin to form sulhemoglobin [22]. Metabolic labeling studies with Na$_{35}$S have indicated tissue specific differences in sulfide catabolism rates and in product distribution [23]. Rat liver converts sulfide primarily to sulfate, kidney to a mixture of thiosulfate and sulfate, and lung predominantly to thiosulfate. These biosynthetic and degradative pathways for $H_2S$ will likely prompt more interest into the translational cardioprotective potential of this gasotransmitter in the future.

3. Disturbance of Endogenous $H_2S$ Generation in Heart Diseases

The discovery of CSE in the rat heart as well as identification of $H_2S$ as an important modulator is a breakthrough in the investigation of the role of $H_2S$ in heart function. Increasing evidence has demonstrated that disturbed $H_2S$ production is relevant to heart disease. In clinical patients, Jiang et al. [24] found plasma $H_2S$ levels were significantly lowered in coronary heart disease (CHD) patients compared with that in angiographically normal control subjects. Moreover, in CHD patients, plasma $H_2S$ levels in unstable angina patients and acute myocardial infarction patients were significantly lower than that in stable angina patients. In addition, Polhemus et al. [25] found that heart failure (HF) patients had marked reductions in circulating $H_2S$ levels compared to age matched controls. In experimental animal model, studies also show that the endogenous production of $H_2S$ is significantly reduced in many heart diseases, including myocardial ischemia, myocardial infarction- (MI-) induced or arteriovenous fistula-induced HF, and spontaneous, pulmonary, or hyperhomocysteinemia-induced hypertension [26]. These findings imply that cardiac disease may impair the endogenous synthesis of $H_2S$, which may further exacerbate the disease state. Meanwhile, these findings are clear evidence which support the involvement of endogenous $H_2S$ in maintaining basal physiological functions of the heart.

4. Role of $H_2S$ in Heart Diseases

4.1. Myocardial I/R Injury. I/R injury is one critical cause of tissue destruction and often leads to heart failure. Although reperfusion relieves ischemia, it also results in a complex...
reaction that leads to cell injury caused by inflammation and oxidative damage [27]. A growing body of evidence indicates that H$_2$S is involved in myocardial I/R injury. H$_2$S postconditioning effectively protects isolated rat hearts against I/R injury via activation of the JAK2/STAT3 signaling pathway, an important component of the survivor activating factor enhancement (SAFE) pathway [28]. In another study, sulfur dioxide (SO$_2$) preconditioning can significantly reduce I/R-induced myocardial injury in vivo, which is associated with increased myocardial antioxidative capacity and upregulated H$_2$S/CSE pathway [29]. H$_2$S infusion but not bolus administration markedly reduced myocardial infarct size and improved regional left ventricular function in a porcine I/R model by suppressing cardiomyocyte apoptosis and autophagy [30]. Furthermore, NaHS pretreatment protects isolated rat hearts against I/R injury by inhibition of mitochondria permeability transition pore (MPTP) opening [31]. Our group also found pharmacologic inhibition of CSE results in an increase in infarct size in a rat I/R model; conversely, H$_2$S replacement displayed myocardial protection [32]. Additionally, cardiac specific CSE overexpressed in transgene mice significantly reduced infarct size and improved cardiac function compared to the wild-type group after 45 minutes of ischemia and 72 hours of reperfusion [33]. These findings reveal that both exogenous donors and endogenously elevated H$_2$S serve to protect heart against I/R injury and may serve as an important therapeutic target.

4.2. Myocardial Infarction. Myocardial infarction (MI) is the leading cause of death worldwide. It occurs when a coronary artery is occluded, leading to insufficient oxygen supply to the myocardium and resulting in death of cardiomyocytes and nonmyocyte cells [34, 35]. More and more evidence indicates that H$_2$S has direct benefits for myocardial infarction. Our group demonstrated for the first time that decreased H$_2$S levels in the plasma were associated with an increased infarct size and mortality. NaHS significantly decreased the infarct size of the left ventricle and mortality after acute MI in rats [36]. We also found S-propargyl-cysteine (SPRC), a novel modulator of endogenous hydrogen sulfide, could protect against MI by reducing the deleterious effects of oxidative stress through increased CSE activity and plasma H$_2$S concentration [37]. Moreover, we found that increased CSE and H$_2$S levels in vivo by miR-30 family inhibitor can reduce infarct size, decrease apoptotic cell number in the peri-infarct region, and improve cardiac function in response to MI [38]. Qipshidze et al. [39] also found that administration of H$_2$S remarkably ameliorated infarct size and preserved left ventricular function during development of MI in mice. This cardioprotective effect was associated with the improvement of angiogenesis due to inhibition of antiangiogenic proteins and stimulation of angiogenic factors such as vascular endothelial growth factor (VEGF). In another study, Xie et al. [40] found that H$_2$S preconditioning effectively promoted mesenchymal stem cells (MSCs) survival under ischemic injury and helped cardiac repair after myocardial infarction in rats.

4.3. Cardiac Arrhythmias. Cardiac arrhythmias are an important problem in coronary I/R therapy and constitute a major risk for sudden death after coronary artery occlusion [41]. The primary causes for I/R-induced arrhythmias are considered to be the endogenous metabolites, such as reactive oxygen species (ROS), calcium, thrombin, and platelet activating factor, produced and accumulated in the myocardium during reperfusion.

Zhang et al. [42] found that reperfusion with NaHS after ischemia attenuated arrhythmias in the isolated Langendorff-perfused heart and improved cardiac function during I/R. These effects could be blocked by the ATP-sensitive potassium (K$_{ATP}$) channel blocker glibenclamide, indicating that the cardioprotective effect of H$_2$S against arrhythmias during reperfusion at least partially depends on the opening of K$_{ATP}$ channel. Bian et al. [43] also found that blockade of endogenous H$_2$S synthesis increased both the duration of I/R-induced arrhythmias and the severity of the arrhythmias. However, preconditioning with 100 μM NaHS attenuated arrhythmias in the isolated heart, increased cell viability, and improved cell function in cardiac myocytes during I/R, and these effects may be mediated by protein kinase C (PKC) and sarcolemmal K$_{ATP}$ channels. Connexin 43 (Cx43) is the principal connexin in the mammalian ventricle and has been proven to have a close association with arrhythmia [44]. Huang et al. [45] found that H$_2$S ameliorated the expression of Cx43 in cardiac tissue, which indicated that endogenous H$_2$S may play an important role in regulating heart function and arrhythmia. Furthermore, Yong et al. [46] found that lowered H$_2$S production during ischemia may cause overstimulation of the β-adrenergic function which was closely linked with the incidence of ventricular arrhythmias. Exogenous application of H$_2$S negatively modulated β-adrenergic function by inhibiting adenyl cyclase activity and finally protected heart against cardiac arrhythmias.

Based on these findings, H$_2$S replacement therapy may be a significant cardioprotective and antiarrhythmic intervention for those patients with chronic ischemic heart disease whose plasma H$_2$S level is reduced.

4.4. Myocardial Fibrosis. Cardiac fibrosis is characterized by net accumulation of extracellular matrix proteins in the
cardiac interstitium and contributes to both systolic and diastolic dysfunction in many processes of cardiac disorders [47]. Although the fibroblast activation and proliferation are important for maintaining cardiac integrity and function early after cardiac injury, the development of fibrous scar tissue in the infarct zone often leads to chronic complications and functional insufficiencies [48].

Mishra et al. [49] found cardiac fibrosis and apoptosis in chronic heart failure (CHF) were reversed by administration of H$_2$S, which was associated with a decrease in oxidative and proteolytic stresses. In addition, Huang et al. [45] revealed that H$_2$S markedly prevented the development of cardiac fibrosis and decreased the collagen content in the cardiac tissue by inhibiting the activity of intracardiac Ang-II. It is well known that multiple potassium channels are expressed in cardiac ventricular fibroblasts [50], whereby their modulations may have major significance in cardiac fibrosis. Sheng et al. [51] found that H$_2$S potentially modulate cardiac fibrosis by inhibiting large conductance Ca$^{2+}$-activated K$^+$ current (BK$_{Ca}$), transient outward K$^+$ current (Ito), and Ba$^{2+}$-sensitive inward rectifier K$^+$ current (IK,$\beta$), independent of K$_{ATP}$ channels, leading to decreased proliferation and suppression of transforming growth factor-$\beta$1- (TGF-$\beta$1-) induced myofibroblast transformation of atrial fibroblasts. Our previous finding has demonstrated that H$_2$S therapy significantly attenuated ischemia-induced cardiac fibrosis in chronic heart failure rats [52]. We also found that treatment with H$_2$S substantially inhibited AngII-stimulated cardiac fibroblasts, as evidenced by the reduction in $\alpha$-SMA and type I collagen expression as well as effective suppression of the fibrotic marker CTGF. In addition, we proved that the pharmacologic supplementation of exogenous H$_2$S attenuated fibrotic and inflammatory responses induced by MI. The beneficial effects of H$_2$S, at least in part, were associated with a decrease of Nox4-ROS-ERK1/2 signaling axis and an increase in heme oxygenase-1 (HO-1) expression [53].

4.5. Cardiac Hypertrophy. Cardiac hypertrophy, usually considered as an effective compensation mechanism, can maintain or even increase cardiac output. However, in the long term, persistent hypertrophy will ultimately result in cardiac dilatation, decreased ejection fraction, and subsequent heart failure [54]. Pathological hypertrophy usually occurs in response to chronically increased pressure overload or volume overload, or following MI.

A large number of experiments confirm that H$_2$S play a positive role in protecting heart against cardiac hypertrophy. Lu et al. [55] demonstrated that H$_2$S could improve cardiac function and reduce myocardial apoptosis in the isoproterenol- (ISO-) induced hypertrophy rat model by reducing Nox4 expression and ROS production in the mitochondria. Treatment of mice with sodium sulfide (Na$_2$S) leads to less cardiac hypertrophy and left ventricular dilatation as well as improved left ventricular function after the induction of heart failure in a thioredoxin 1- (Trx1-) dependent manner [56]. In addition, pharmacologic H$_2$S therapy during heart failure serves to mitigate pathological left ventricular remodeling and reduce myocardial hypertrophy, oxidative stress, and apoptosis [49]. In an endothelin-induced cardiac hypertrophy rat model, Yang et al. [57] found that H$_2$S treatment could decrease left ventricular mass index, volume fraction of myocardial interstitial collagen, and myocardial collagen content and improve cardiac hypertrophy. In another hypertrophy model induced by abdominal aorta coarctation, Huang et al. [58] revealed that exogenous administration of H$_2$S significantly suppressed the development of cardiac hypertrophy and also greatly downregulated the Ang-II levels in cardiac tissue, suggesting that H$_2$S plays a pivotal role in the development of pressure overload-induced cardiac hypertrophy. Interestingly, Padiya et al. [59] showed that administration of freshly prepared homogenate of garlic, which have been shown to generate H$_2$S after interaction within cellular proteins, can activate myocardial nuclear-factor-E2-related factor-2 (Nrf2) through PI3K/AKT pathway and attenuate cardiac hypertrophy and oxidative stress through augmentation of antioxidant defense system in fructose-fed insulin resistant rats.

5. Heart Failure

Heart failure (HF) is a heterogeneous syndrome that can result from a number of common disease stimuli, including long-standing hypertension, myocardial infarction, or ischemia associated with coronary artery disease. The pathogenesis of HF has not been fully elucidated and the current treatments for HF are woefully inadequate. H$_2$S therapy has recently been shown to ameliorate ischemic-induced heart failure in a murine model. Cardiac-restricted overexpression of CSE in mice resulted in increased endogenous H$_2$S production and a profound protection against ischemia-induced heart failure and decreased mortality [60]. In contrast, knockout of CSE in murine models of heart failure showed worsened myocardial function and greater infarct size [61].

In a hypertension-induced heart failure model, it has been demonstrated clearly that H$_2$S decelerated progression to adverse remodeling of the left ventricle and induced angiogenesis in the myocardium [62]. Polhemus et al. [63] also found H$_2$S therapy attenuated left ventricular remodeling and dysfunction in the setting of heart failure by creating a proangiogenic environment for the growth of new vessels. In another model of pressure overload-induced heart failure, mice administered Na$_2$S exhibited enhanced proangiogenesis factors, such as matrix metalloproteinase- (MMP-) 2, and suppressed antiangiogenesis factors, including MMP-9 [64]. H$_2$S also play a protective role in volume overload-induced CHF by upregulating protein and mRNA expression of HO-1 [65].

Local cardiac renin-angiotensin system (RAS) is required for the development of heart failure and left ventricular remodeling. Liu and coworkers [66] have demonstrated that treatment with NaHS could protect against isoproterenol-induced heart failure by suppression of local renin levels through inhibition of both mast cell infiltration and renin degranulation in rats, suggesting a novel mechanism for H$_2$S-mediated cardioprotection against heart failure. Our group found NaHS markedly inhibited cardiac apoptosis and improved mitochondrial derangements, both of which led to cardioprotection in a rat model of heart failure [52].
In addition, we also showed that NaHS decreased the leakage of cytochrome c protein from the mitochondria to the cytoplasm, improved mitochondrial derangements, and increased CSE mRNA and protein levels in heart failure rats [52]. SPRC, reported also as ZYZ-802, could reduce infarct size and improve cardiac function in a rat model of MI-induced heart failure via antiapoptosis and antioxidant effects as well as angiogenesis promotion [67, 68]. All these illustrate that the CSE/H$_2$S pathway plays a critical role in the preservation of cardiac function in heart failure.

6. Molecular Mechanisms of H$_2$S-Induced Cardioprotection

Similar to NO and CO, the effects of H$_2$S on the heart are mediated via a diverse array of cellular and molecular signals. The mechanisms by which H$_2$S protects against cardiac diseases are through antioxidative action, preservation of mitochondrial function, reduction of cardiomyocyte apoptosis, anti-inflammatory responses, angiomyocyte action, regulation of ion channel, and increasing the production of NO (Figure 3).

6.1. Antioxidative Action. Oxidative stress is a process due to an imbalance between oxidant and antioxidant systems. Oxidative stress-induced cellular injury is often caused by excessive formation of ROS, such as superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), peroxynitrite (ONOO$^-$), and hydrogen peroxide (H$_2$O$_2$). The occurrence of the majority heart diseases is associated with ROS generation, including myocardial I/R injury, cardiac hypertrophy, myocardial fibrosis, and arrhythmias. H$_2$S has been reported as a strong antioxidant and widely proposed to protect the cardiac system through its antioxidant role. The robust antioxidant actions of H$_2$S are associated with direct scavenging of ROS and/or increased expressions and functions of antioxidant enzymes.

Sun et al. [76] found that H$_2$S inhibited mitochondrial complex IV activity and increased the activities of Mn-SOD and CuZn-SOD and decreased the levels of ROS in cardiomyocytes during I/R. H$_2$S decreased lipid peroxidation by scavenging hydrogen peroxide and superoxide in a model of isoproterenol-induced myocardial injury [77]. The activation of Nrf2 dependent pathway mediated by H$_2$S results in upregulated gene expression of specific factors, such as HO-1, glutathione reductase, glutathione S-transferase, thioredoxin, and catalase, which play role in endogenous antioxidant defense. Furthermore, H$_2$S has an inhibitory effect on phosphodiesterase-5 (PDE-5), which results in decreased NADPH oxidase formation, and the level of antioxidant enzymes increases [78]. Besides these mechanisms, H$_2$S also acts as a direct scavenger to neutralize cytotoxic reactive species like peroxynitrite [79] and directly destroys organic hydroperoxides of pathobiological importance, like fatty acid hydroperoxides (LOOHs) [80]. Collectively, these findings suggest that H$_2$S is capable of preventing the generation of ROS, scavenging ROS, and strengthening the endogenous antioxidant system.

6.2. Preservation of Mitochondrial Function.Mitochondrial function is compromised under hypoxic conditions or in the presence of increased ROS [81]. Growing evidence has shown that H$_2$S has the ability to protect mitochondria and ultimately improve respiration and promote biogenesis. Elrod and colleagues [33] found a dose dependent reduction of oxygen consumption in isolated murine cardiac mitochondria after hypoxia, and the administration of H$_2$S was shown to improve the recovery of posthypoxic respiration rate significantly. Moreover, electron microscopy showed a notable reduction in mitochondrial swelling and increased matrix density in mice after treatment with H$_2$S, further suggesting a prominent role of H$_2$S in the preservation of mitochondrial function in the cytoprotection. In addition, H$_2$S can affect mitochondria of cardiac cells by inhibition of cytochrome c oxidase in a potent and reversible way, which leads to preservation of mitochondrial structure and function [52]. H$_2$S may protect mitochondrial function by
6.3. Antiapoptosis. There is increasing proof that H\(_2\)S has antiapoptotic actions. Most data indicate the antiapoptotic effects of H\(_2\)S are mainly due to the preservation of mitochondrial function, and many of the cytoprotective actions of H\(_2\)S during ischemic states may be a result of potent actions on mitochondria [85]. It is reported that H\(_2\)S significantly protected against high glucose-induced cardiomyocyte apoptosis by altering Bax and Bcl-2 gene expression [86]. Moreover, it is found that NaHS treatment suppressed the activation of caspase-3 and reduced apoptotic cell numbers in both mice [33] and swine [87], suggesting that H\(_2\)S was capable of inhibiting the progression of apoptosis after I/R injury.

Survivin is an antiapoptotic gene implicated in the initiation of mitochondrial-dependent apoptosis. In an in vivo I/R rat model, our group found administration of NaHS for 6 days before surgery significantly upregulated survivin mRNA and protein expressions by 3.4-fold and 1.7-fold, respectively [32], suggesting another way of action for H\(_2\)S-induced cardioprotection.

The activity of glycogen synthase kinase-3 (GSK-3\(\beta\)), which has been proposed as a viable target in the ischemic heart injury, is associated with both apoptosis and cell survival. Osipov et al. [30] found that H\(_2\)S infusion increased the expression of the phosphorylated form of GSK-3\(\beta\) significantly. Similarly, Yao et al. [88] also demonstrated that NaHS upregulated the phosphorylation of GSK-3\(\beta\) (Ser9) expression and subsequently resulted in inhibiting the opening of MPTP, preventing apoptosis and protecting the heart against ischemic damage.

6.4. Anti-Inflammation. Inflammation is involved in the main pathological processes of ischemic heart disease. For example, cytokines mediate the development of ischemic injury in the heart and depress myocardial function [89]. IL-6 and IL-8 are released on myocardial I/R damage and then...
increase neutrophil adhesion and inflammatory responses \[90\]. TNF-\(\alpha\) plays multiple roles in the pathogenesis of myocardial I/R injury by inducing endothelium adhesion molecules, allowing for neutrophil infiltration, increasing the production of ROS, amplifying the inflammatory response, and having direct myocardial depression and apoptotic actions \[91\].

Studies have shown that \(\text{H}_{2}\text{S}\) may play dual roles in inflammatory process. Whiteman and Winyard \[92\] reviewed 14 studies showing an anti-inflammatory effect of \(\text{H}_{2}\text{S}\) and 15 studies showing a proinflammatory effect of \(\text{H}_{2}\text{S}\). However, the anti-inflammatory effect of \(\text{H}_{2}\text{S}\) plays a dominant role in heart disease. In myocardial I/R experiments, Elrod et al. \[33\] have demonstrated that, at the time of heart reperfusion, \(\text{H}_{2}\text{S}\) decreased the number of leukocytes within the ischemic zone as well as neutrophils within the myocardial tissue. The evaluation of inflammatory cytokines revealed myocardial levels of IL-1\(\beta\) to be markedly reduced after administration of \(\text{H}_{2}\text{S}\). Additionally, \(\text{H}_{2}\text{S}\) was found to potently reduce in vivo leukocyte-endothelial cell interactions. Using the ischemic porcine heart, Sodha et al. \[93\] found that NaHS treatment decreased the level of TNF-\(\alpha\), IL-6, and IL-8 as well as the activity of myeloperoxidase. Therefore, \(\text{H}_{2}\text{S}\) restrained the extent of inflammation and limited the extent of MI by preventing leukocyte transmigration and cytokine release. In another study, the \(\text{H}_{2}\text{S}\) donor, \(\text{Na}_{2}\text{S}\) and NaHS were both able to inhibit leukocyte adherence and the resultant inflammatory pathology via activation of \(\text{K}_{\text{ATP}}\) channels \[94\].

In the lipopolysaccharide-induced inflammatory response of rat embryonic ventricular myocardial cells (H\(_9\)C\(_2\) cells), our group also found \[95\] that SPRC prevented nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) activation and suppressed LPS-induced extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation and intracellular reactive oxygen species (ROS) production. In addition, SPRC induced phosphorylation of Akt, attenuated LPS-induced mRNA and protein expression of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), and inhibited mRNA expression of intercellular adhesion molecule-1 (ICAM-1) and inducible nitric oxide synthase (iNOS). Therefore, SPRC produced an anti-inflammatory effect in LPS-stimulated H\(_9\)C\(_2\) cells through the CSE/H\(_2\)S pathway by impairing \(\text{IkB}\)/NF-\(\kappa\)B signaling and by activating PI3K/Akt signaling pathway. These studies provide strong evidence of the function of \(\text{H}_{2}\text{S}\) as anti-inflammatory agent.

6.5. Angiogenesis. The cardioprotective role of \(\text{H}_{2}\text{S}\) could also be due to its angiogenic action on the ischemic area in the heart. Angiogenesis plays a pivotal role in the early stage of wound healing. In \textit{in vitro} studies, incubation with low micromolar concentrations of \(\text{H}_{2}\text{S}\) increased endothelial cell number, cell migration, and capillary morphogenesis on matrigel \[96\]. Chicken chorioallantoic membranes, an \textit{in vivo} model of angiogenesis, displayed increased branching and lengthening of blood vessels in response to 48 h treatment with \(\text{H}_{2}\text{S}\) \[97\]. Aortic rings isolated from CSE knockout mice exhibited markedly reduced microvessel formation. Additionally, in a wound healing model, topically applied \(\text{H}_{2}\text{S}\) accelerated wound closure and healing \[97\].

Angiogenesis is very important in chronic ischemia as poorly vascularized tissue will result in loss of function. Therefore, increasing myocardial vascularity and perfusion in concert with cardiac myocyte growth are critical to prevent the progression of heart failure. In a hypertension-induced heart failure model, administration of \(\text{H}_{2}\text{S}\) induced angiogenesis in the myocardium and decelerated the progression of left ventricle remodeling \[63\]. In a similar heart failure model, NaHS treatment improved cardiac function and mitigated transition from compensatory hypertrophy to heart failure, which was associated with a significant increase in capillary density \[98\]. In another MI model, \(\text{H}_{2}\text{S}\) supplementation showed improvement of heart function and mitigation of cardiac remodeling by increasing angiogenic vessels and blood flow in MI mice \[39\].

Multiple signaling mechanisms are involved in the angiogenic action of \(\text{H}_{2}\text{S}\), including activation of \(\text{K}_{\text{ATP}}\) channels \[99\]. By using the \(\text{K}_{\text{ATP}}\) channel inhibitor glibenclamide, Papapetropoulos et al. \[97\] found that \(\text{K}_{\text{ATP}}\) channel was involved in \(\text{H}_{2}\text{S}\)-stimulated angiogenesis. Additionally, \(\text{H}_{2}\text{S}\) can stimulate angiogenesis through phosphatidylinositol 3-kinase (PI3K) and Akt activation \[96\]. \(\text{H}_{2}\text{S}\) can also activate hypoxia inducible factor-1a (HIF-1a) and thus increase expression of VEGF \[100\]. VEGF is a key growth factor in physiological angiogenesis and induces angiogenesis in myocardial ischemia and MI. \(\text{H}_{2}\text{S}\) is reported to promote angiogenesis in a MI model by increasing the expression of VEGF and its specific receptors such as the tyrosine kinase receptor-flk-1 and the fms-like tyrosine kinase-flt-1 \[39\]. It is also reported that \(\text{H}_{2}\text{S}\) can regulate the matrix metalloproteinase/tissue inhibitor of metalloproteinase (MMP/TIMP) axis to promote VEGF synthesis and angiogenesis \[98\]. Furthermore, Zhu group identified VEGFR2 as a receptor for \(\text{H}_{2}\text{S}\) for inducing angiogenesis in vascular endothelial cells and found that an intrinsic inhibitory Cys1045–Cys1024 disulfide bond acted as a molecular switch for \(\text{H}_{2}\text{S}\)-stimulated angio genesis and VEGF synthesis \[98\].

6.6. Regulation of Ion Channel. The effects of \(\text{H}_{2}\text{S}\) on heart electrophysiology have been reported. There are two different types of Ca\(^{2+}\) channels (L-type and T-type) in the myocardial membrane. L-type Ca\(^{2+}\) channels are absolutely essential for maintaining the electrophysiological basis for the plateau phase of action potentials and for excitation-contraction (EC) coupling \[102\]. Whole patch clamp experiments in rat cardiomyocytes revealed that NaHS negatively modulates L-type Ca\(^{2+}\) channels composed by the CaV1.2 subunits in rat cardiomyocytes \[103–105\]. T-type Ca\(^{2+}\) channels can be reexpressed in atrial and ventricular myocytes in a variety of pathological conditions such as cardiac hypertrophy and heart failure and participate in abnormal electrical activity and EC coupling \[106\]. A recent report has showed that NaHS (10 \(\mu\)M–1 mM) selectively inhibits Cav3.2 T-type Ca\(^{2+}\) channels which are heterologously expressed in HEK293 cells \[107\].
K\textsubscript{ATP} channels are located on the surface of cell membranes and mitochondria and are widely distributed in the myocardium. The opening of K\textsubscript{ATP} channels is an important endogenous cardioprotective mechanism involved in cardiac ischemia preconditioning. The K\textsubscript{ATP} channel opening generates outward currents and causes hyperpolarization, which reduces calcium influx via L-type Ca\textsuperscript{2+} channels and prevents Ca\textsuperscript{2+} overload. Tang and coworkers [108] found evidence that NaHS (100 \mu M) opened the K\textsubscript{ATP} channels in vascular smooth muscle cells. Furthermore, H\textsubscript{2}S may also indirectly activate the K\textsubscript{ATP} channels by inducing intracellular acidosis [109]. By activation of the K\textsubscript{ATP} channels, H\textsubscript{2}S shortens action potential duration (APD) and produces cardioprotective effects [110, 111], though H\textsubscript{2}S has no significant effect on the amplitude of action potential and resting potential [104].

Study has demonstrated that voltage-dependent Na\textsuperscript{+} channels (Nav) can be regulated by H\textsubscript{2}S. In Native Nav from jejunum smooth muscle and recombinant Nav (Nav1.5) heterologously expressed in HEK293, Streve et al. [112] found NaHS increased peak sodium currents and also right-shifted the voltage dependence of Na\textsuperscript{+} current inactivation and activation. This effect could extend beyond the jejunum, since Nav1.5 is also expressed in other tissues. In the heart, Nav1.5 gives rise to the upstroke of the cardiac action potential; thus, it is possible that H\textsubscript{2}S may have the same effect on the Nav expressed in the heart.

Growing studies show that chloride channels play an important role in normal physiological function in myocardial cells, but abnormal changes can be found in pathological conditions such as myocardial ischemia and arrhythmias. Malekova et al. [113] investigated the effect of H\textsubscript{2}S on single-channel currents of chloride channels using the patch clamp technique and found that NaHS inhibited the chloride channels by decreasing the channel open probability in a concentration-dependent manner. The inhibitory effect of H\textsubscript{2}S on the chloride channels may be involved in the biological actions of H\textsubscript{2}S in the heart.

6.7. Interaction with NO. H\textsubscript{2}S protects cardiac muscles from I/R injury by increasing the production of NO [114]. H\textsubscript{2}S is known to interact with the other biological mediators and signal transduction components to produce its effects in the cardiovascular system. H\textsubscript{2}S can activate endothelial nitric oxide synthase (eNOS) through phosphorylation at the S1177 active site and augment NO bioavailability [61], highlighting that there is an interaction between NO and H\textsubscript{2}S of physiological significance. There is evidence that NO and peroxynitrite react with H\textsubscript{2}S to form a novel nitrosothiol, which has been proposed to regulate the physiological effects of both NO and H\textsubscript{2}S [115]. Moreover, mice treated with the H\textsubscript{2}S donor, diallyl trisulfide (DATS), showed marked increases in plasma nitrite, nitrate, and nitrosylated protein (RXNO) levels 30 minutes after injection [116].

In CSE knockout mice, the levels of H\textsubscript{2}S and bound sulfane sulfur in tissues and blood as well as the levels of NO metabolites were decreased significantly. However, administration of H\textsubscript{2}S rescued the heart form I/R injury by activating eNOS and increasing NO availability. In addition to these observations in CSE knockout mice, the administration of H\textsubscript{2}S failed to protect the cardiac muscle from I/R injury in eNOS defective mutant mice [114]. Similar results were also obtained by Kondo et al. [61] in a mouse model of pressure overload-induced heart failure, which suggests that H\textsubscript{2}S protects the heart by upregulating eNOS phosphorylation accompanied by increasing NO production. Interestingly, plasma H\textsubscript{2}S levels, CSE gene enzymatic activity, and expression in the cardiovascular system were reduced in rats after treated with a NOS inhibitor chronically, indicating the physiological significance of NO in the regulation of H\textsubscript{2}S production in the cardiovascular system [117].

6.8. Regulation of miRNA Expression. MicroRNAs (miRNAs) are evolutionarily conserved molecules that modulate the expression of their target genes by mRNA degradation or translational repression, and they may participate in various physiological and pathological processes of heart diseases [118]. An increasing body of evidence shows that H\textsubscript{2}S exerts its role by regulating the expression of miRNA. Shen et al. [119] found H\textsubscript{2}S was involved in regulating the expression of drought associated miRNAs such as miR-167, miR-393, miR-396, and miR-398 and their target genes, and therefore improved the tolerance of Arabidopsis to drought. A recent study [120] demonstrated that H\textsubscript{2}S played a role in the protection of hepatic I/R injury in the young rats by downregulating the expression of miR-34a, which resulted in the promotion of Nrf-2 signaling pathway. More importantly, Liu et al. [121] found H\textsubscript{2}S inhibited cardiomyocyte hypertrophy by upregulating miR-133a. In addition, H\textsubscript{2}S donor, Na\textsubscript{2}S, would attenuate myocardial injury through upregulation of protective miR-21 and suppression of the inflammasome, a macromolecular structure that amplifies inflammation and mediates further injury [122]. These data suggest a new mechanism for the role of H\textsubscript{2}S and indicate that miRNA could be a new target of H\textsubscript{2}S in cardiac disorders.

7. H\textsubscript{2}S-Based Therapeutic Potential for Heart Diseases

More and more H\textsubscript{2}S donors with varying chemical and pharmacological properties have been reported as potential therapeutics. Among them, Na\textsubscript{2}S and NaHS were the first H\textsubscript{2}S-releasing agents studied in the cardiac system [33, 123]. As inorganic salts, Na\textsubscript{2}S and NaHS have the advantage of rapidly increasing H\textsubscript{2}S concentration within seconds, but they also rapidly decline within tissue and could exert adverse side effects because of rapid increases in H\textsubscript{2}S at high concentrations [124]. This somewhat limits their therapeutic potential. Thus, it is important to develop novel H\textsubscript{2}S-releasing drugs used to treat heart diseases.

Synthetic H\textsubscript{2}S-releasing compounds have been developed. GYY4137, a water-soluble compound capable of releasing H\textsubscript{2}S slowly, has been reported to protect against high glucose-induced cytotoxicity by activation of the AMPK/mTOR signal pathway in H\textsubscript{9}C\textsubscript{2} cells [73]. SG-1002 [61] and penicillamine based donors [125] are examples of synthesized H\textsubscript{2}S donors whose release is more precisely controlled.
H₂S therapy with SG-1002 resulted in cardioprotection in the setting of pressure overload-induced heart failure via upregulation of the VEGF-Akt-eNOS-NO-cyclic guanosine monophosphate (cGMP) pathway with preserved mitochondrial function, attenuated oxidative stress, and increased myocardial vascular density. Penicillamine based donors showed potent protective effects in an in vivo murine model of myocardial I/R injury.

In recent years, some natural plant-derived compounds, such as garlic, have been found to produce H₂S. Naturally occurring H₂S donors such as DATS, a polysulfide derived from garlic, is known to protect against myocardial I/R injury in mice through preservation of endogenous H₂S [126]. It also has been shown to protect against hyperglycemia-induced ROS-mediated apoptosis by upregulating the PI3 K/Akt/Nrf2 pathway, which further activates Nrf2-regulated antioxidant enzymes in cardiomyocytes exposed to high glucose [127]. Additionally, organic sulfide donors derived from garlic, such as diallyl disulfide (DADS), attenuate the deleterious effects of oxidized LDL on NO production [128] and protect the ischemic myocardium. SAC (S-allylcysteine), another derivative of garlic, significantly lowers mortality and reduces infarct size following MI [129]. SPRC, a structural analogue of SAC which was synthesized by our group, was found to protect against myocardial ischemic injury both in in vivo and in vitro studies through the increase in CSE activity and plasma H₂S concentration [130]. SAC and SPRC are both cardioprotective in MI by modulating the endogenous levels of H₂S, reducing the deleterious effects of oxidative stress and preserving the activities of antioxidant-defensive enzymes like SOD [37]. As novel H₂S releasing agents or H₂S donors develop, these novel agents should ultimately address the clinically relevant issues such as sustained release or half-life, route of administration, tissue specificity, and low toxicity.

8. Conclusion and Perspectives

Following in the footsteps of NO and CO, H₂S is rapidly emerging as a critical cardiovascular signaling molecule. We have summarized the current knowledge on the function of H₂S in heart disease and discussed the possible molecular mechanisms involved in its cardioprotective effect. Although the complete actions of this gas remain under investigation and the underlying mechanisms should be further elucidated, the therapeutic options relating to heart disease are extremely promising. We also reviewed the current H₂S donors which have been verified to have the therapeutic potential for heart disorders. Most of the current H₂S donors have the drawback of rapid degradation and difficult to control. Furthermore, whether the therapeutic effects of these donors in animal studies can be transferable to clinical studies needs to be determined. However, we believe a long-acting donor with controlled H₂S release will be developed. In short, a better understanding of the function of the H₂S in heart disease as well as development of novel H₂S-based therapeutic agents may be helpful to reduce the risks of heart disease in the future.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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