

Cardioprotective effects of hydrogen sulfide in attenuating myocardial ischemia-reperfusion injury (Review)

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Abstract. Ischemic heart disease is one of the major causes of cardiovascular-related mortality worldwide. Myocardial ischemia can be attenuated by reperfusion that restores the blood supply. However, injuries occur during blood flow restoration that induce cardiac dysfunction, which is known as myocardial ischemia-reperfusion injury (MIRI). Hydrogen sulfide (H_2S), the third discovered endogenous gasotransmitter in mammals (after NO and CO), participates in various pathophysiological processes. Previous *in vitro* and *in vivo* research have revealed the protective role of H_2S in the cardiovascular system that render it useful in the protection of the myocardium against MIRI. The cardioprotective effects of H_2S in attenuating MIRI are summarized in the present review.

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1. Introduction

Cardiovascular diseases (CVD) contribute to a high morbidity and mortality burden globally (1). In 2019, the number of patients with CVD was ~523 and ~18.6 million cases succumbed to CVD (2). Myocardial ischemia is a common clinical symptom resulting from atherosclerosis and myocardial infarction (3). Reperfusion is often used to repair

myocardial structure damage and improve cardiac function following ischemia. However, reperfusion may also result in myocardial ischemia-reperfusion injury (MIRI), which aggravates cardiac dysfunction. Therapeutic strategies, such as preconditioning, postconditioning and administration of antiplatelet or antithrombotic agents, have been utilized to alleviate MIRI (4).

Hydrogen sulfide (H_2S), the third discovered gaseous signaling molecule (after NO and CO), has been extensively studied in recent years (5). H_2S was traditionally acknowledged as an environmental toxicant, however, it has recently gained significance as an endogenous-generated biological transmitter in mammal tissues (6). Multiple studies have revealed the physiological and pathological roles of H_2S in the onset and progression of cardiac diseases (7,8). Thus, H_2S is considered to be a potential treatment for MIRI. The present review has summarized the protective effects of H_2S against MIRI.

2. Pathophysiological mechanism of MIRI

Oxidative stress. Oxygen homeostasis plays a vital role in the maintenance of physiological functions. Reactive oxygen species (ROS) are generated during the normal metabolism of oxygen and participate in signal transduction. ROS are then scavenged by various endogenous free radical scavenging enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase and thioredoxin (9). However, overproduction of ROS or insufficient enzyme activity may impair the equilibrium between ROS and antioxidants, resulting in damage to proteins, DNA and lipids (10). SOD1 knockout mice were shown to have excessive oxidative stress and aggravated myocardial injuries following acute myocardial ischemia (11). Moreover, excessive ROS impairs heart contraction by modifying excitation-contraction coupling proteins. Excessive ROS also activates various signaling kinases and transcription factors associated with myocardial hypertrophy. In addition, the proliferation of cardiac fibroblast and the activity of MMP are promoted by ROS (12,13).

Mitochondrial function. The mitochondria are the main source of ROS production. ROS are generated in the electron transport chain (ETC) located on the mitochondrial membrane during the process of ATP production, namely oxidative phosphorylation. Electrons are then transported by a train of proteins known as the mitochondrial complex

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via oxidation-reduction reactions and combine with oxygen molecules to produce water. During this process, some oxygen molecules are reduced to form ROS (14).

Mitochondria may also act as a target of ROS damage. During the early process of reperfusion, the excessive ROS generated may induce oxidative stress, leading to the abnormal opening of the mitochondrial permeability transition pore (mPTP). Opening of the mPTP leads to mitochondrial Ca^{2+} overload, usually accompanied by oxidative or nitrosative stress and ATP depletion. Abnormal opening of mPTP also causes loss of mitochondrial membrane potential (15), respiratory chain uncoupling and impaired ATP synthesis. The impaired mitochondrial function results in mitochondrial swelling, rupture and cell apoptosis or necrosis (16,17). Mitochondria morphological changes observed during a MIRI in rat myocardial tissues mainly manifest as mitochondrial cristae and membrane damage, disordered fiber arrangement and larger perinuclear space (18). Furthermore, inhibition of mPTP opening using pharmaceutical agents, such as cyclosporine A, has been shown to reduce myocardial infarct size in acute ischemia-reperfusion injury (IRI) animals (19).

Autophagy. Autophagy plays a key role in cell survival by transferring damaged proteins and organelles to lysosomes for degradation. However, the autophagy process is controversial in MIRI. Autophagy is activated via the AMP-activated protein kinase pathway during ischemia to promote cell survival. However, during reperfusion, autophagy exerts a harmful role via Beclin activation (20). Loos *et al* (21) observed the activation of autophagy in mild ischemia. However, severe ischemia did not activate autophagy. This demonstrates that autophagy induction is closely associated with the degree of MIRI.

Reperfusion injury salvage kinase (RISK) pathway. Ischemic-induced apoptosis (cell death) is accelerated by reperfusion (3). Thus, anti-apoptotic mechanisms may be exploited as potential methods to decrease reperfusion-induced cell death. Reperfusion can activate several anti-apoptotic pathways in the RISK pathway, including PI3K/Akt and ERK1/2 pathways, that regulate cell survival (22). Protein kinase C, protein kinase G and GSK-3 β are also regarded as members of the RISK pathway (23). Type 2 diabetes has been shown to impair nuclear factor-erythroid factor 2-related factor 2 (Nrf2) signaling via BTB domain and CNC homolog 1 (Bach1), thereby blocking the binding of Nrf2 to the heme oxygenase-1 promoter. Moreover, db/db diabetic mice treated with Na_2S for 7 days was shown to overcome this impairment by removing Bach1 from the nucleus in an ERK1/2-dependent manner (24).

3. Characteristics of H_2S

Generation and metabolism of endogenous H_2S in mammals. Endogenous H_2S is produced via enzymatic or nonenzymatic pathways in mammalian tissues. Cystathionine β -synthase (CBS) and cystathionine γ -lyase (CSE) are pyridoxal-5'-phosphate-dependent enzymes expressed in the cytosol that synthesize H_2S using L-cysteine or homocysteine as substrates (25). H_2S may also be synthesized in a catalytic reaction by 3-mercaptopyruvate sulfurtransferase (3-MST), involving α -ketoglutarate (25). These three

enzymes are tissue-specific. CSE is mainly located in the kidney, liver, heart and vessels (26). CBS is found in neurons and astrocytes of the central nervous system, while 3-MST is mainly expressed in the liver, kidney, brain and heart (Fig. 1) (27). The concentration of H_2S varies in tissues, with the highest concentration observed in the heart (28-31). Fig. 2 shows the concentration of H_2S in tissues and plasma in mice.

In mammals, there are three main catabolic pathways of H_2S : i) H_2S is oxidized to thiosulfate catalyzed by mitochondrial thioquinone oxidoreductase, S-dioxygenase and S-transferase. The thiosulfate is then catalyzed by cyanide thioltransferase to sulfite, which is then oxidized by sulfite oxidase to sulfate; ii) H_2S generates methyl mercaptan and dimethyl sulfide in a reaction catalyzed by cytoplasmic thiol S-methyltransferase; and iii) H_2S interacts with methemoglobin to produce thiolhemoglobin (Fig. 1) (25).

H_2S donors and inhibitors of H_2S synthetic pathways. Various H_2S donors have been employed for elucidating the physiological and pathological role of H_2S . These donors are divided into the following categories: Inorganic salts, sulfur-containing organic compounds and derivatives of *Allium sativum* extracts (32). The H_2S releasing mechanisms and protective effects of typical donors are summarized in Table I.

The most widely-used H_2S donors are sulfur-containing inorganic salts that release H_2S rapidly in large amounts. The utilization of sulfur-containing inorganic salts in research may be limited by the superphysiological concentration of H_2S (32). Morpholin-4-ium 4-methoxyphenyl-morpholino-phosphinodithioate (GYY4137) was synthesized to overcome this challenge (39). GYY4137 achieves lower concentrations of H_2S , which can be maintained for longer period with improved efficacy and reduced cytotoxicity.

Researchers have also synthesized derivatives of naturally occurring sulfur-containing organic compounds, such as S-propargyl-cysteine, S-allylcysteine and diallyl sulfide, to improve the effectiveness of the H_2S donors. In contrast to conventional H_2S donors that release H_2S directly, *Allium sativum* extract derivatives increase the levels of H_2S by increasing the expression and activity of CSE and CBS. This is advantageous as the levels of H_2S are controlled and, thus, have a lower risk of toxicity.

Szczesny *et al* (46) reported a novel H_2S donor, AP39, [(10-oxo-10-(4-(3-thioxo-3H-1,2-dithiol-5yl)phenoxy)decyl) triphenylphosphonium bromide] that had a preferential response in the mitochondrial regions, as triphenylphosphonium tends to accumulate in mitochondria. Exposure of cells to different concentrations of AP39 (30-300 nmol/l) revealed that the effect of AP39 on mitochondrial activity was dependent on the concentration of H_2S . It was shown that lower concentrations (30-100 nmol/l) promoted mitochondrial electron transport and cellular bioenergetic functions. By contrast, higher concentrations (300 nmol/l) had an inhibitory role. Thus, the antioxidant and cytoprotective effects of AP39 against oxidative mitochondrial DNA damage have been reported.

Inhibitors blocking H_2S synthesis enzymes have also been examined. In colon cancer cells, CBS inhibitor aminooxyacetic acid (AOAA) can reduce tumor growth dose-dependently (47). However, the effect of CSE inhibitor D,

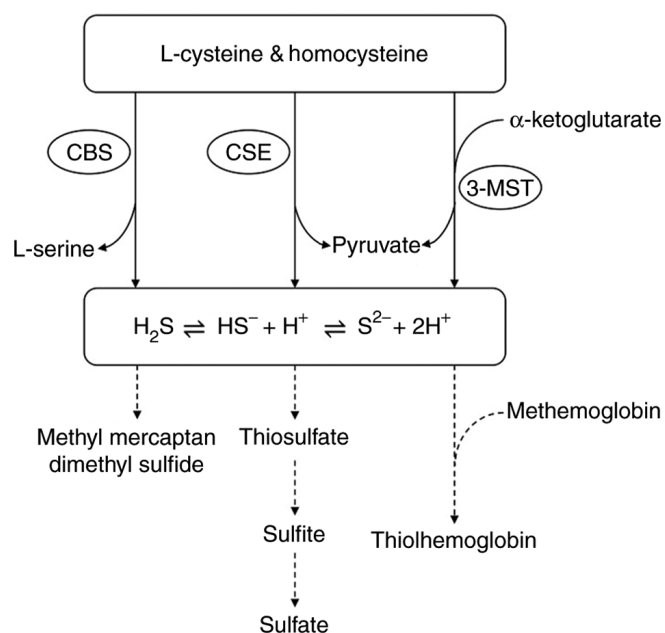


Figure 1. Biogenesis and metabolism of H₂S in mammals. L-cysteine or homocysteine can be catalyzed by CBS, CSE and 3-MST to produce H₂S. Sulfide is oxidized into methyl mercaptan, dimethyl sulfide, thiosulfate and thiolhemoglobin. CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; 3-MST, 3-mercaptopyruvate sulfurtransferase; H₂S, hydrogen sulfide.

L-propynylglycine (PAG) on myocardial injury remains controversial. In acute myocardial infarction and heart failure animal models, PAG could upregulated oxidative stress and apoptosis by suppressing H₂S generation (8,48). Nevertheless, PAG administration can exacerbated acute lung inflammation in a rat model (49).

4. Role of H₂S in the cardiovascular system

Physiological role of H₂S in the cardiovascular system. H₂S has a dual biological effect in mammals. High concentrations of H₂S exert pathological and toxicological effects, such as inhibition of cellular bioenergetics, pro-oxidant effects, genotoxicity, proinflammatory effects and promotion of cell death. By contrast, low H₂S concentrations stimulate mitochondrial electron transport, suppress inflammation, promote physiological vasodilatation, stimulate angiogenesis and inhibit oxidative stress, which are beneficial to cell survival (50).

Therapeutic role of H₂S in the cardiovascular system. In recent years, the protective role of H₂S in the cardiovascular system has been confirmed. The cardioprotective effects of H₂S and the possible mechanism are summarized in Table II. These studies have revealed that multiple signaling pathways are involved in the therapeutic effects of H₂S in cardiovascular system (6,51-67). Notably, S-sulfhydration may be the core mechanism of H₂S in mediating protein function and regulating pathophysiological processes of the cardiovascular system.

5. Protective effects of H₂S in MIRI

During MIRI, the plasma level of H₂S and activity of CSE in the myocardium are decreased, leading to a further reduction

in H₂S synthesis. However, the mRNA expression level of CSE is enhanced following reperfusion, which contributes to positive feedback following the depressed H₂S level (68). CSE knockout mice were observed to have lower levels of H₂S in the blood and heart, followed by exacerbated oxidative stress and severe MIRI (69). Furthermore, acute H₂S therapy significantly reduces myocardial infarct size per area-at-risk and lowers the plasma level of troponin-I in myocardial I/R mice (69). A meta-analysis reported that preconditioning with H₂S *in vivo* significantly decreases the infarct size by 20.25% (95% CI 25.02; 15.47), while postconditioning with H₂S notably reduced the infarct size by 21.61% (95% CI 24.17; 19.05) (70). *In vivo* results have shown that pretreatment with H₂S before MIRI resulted in improved myocardial function, ameliorated coronary microvascular reactivity and reduced infarct size (67). Apolipoprotein E knockout mice were also revealed to have enhanced plaque stability and blood lipid levels and reduced plaque formation when treated with NaHS compared with vehicle-treated controls (71).

H₂S inhibits oxidative stress. Administration of H₂S restores cardiac function and enhances antioxidant function. Sun *et al* (72) compared the effects of diallyl trisulfide-mesoporous silica nanoparticles (DATS-MSN), a long-term and slow-releasing H₂S donor, with two classical donors NaHS and GYY4137. The results of this study demonstrated that these three donors preserved the levels of glutathione and the activities of SOD and catalase, while DATS-MSN had the highest antioxidant effects. This result may be attributed to the slow-release and long-term H₂S effects of DATS-MSN, which mimic the generation and function of endogenous H₂S. It was shown that treatment with GYY4137 for 7 days before ischemia and reperfusion decreased the serum levels of malondialdehyde and myeloperoxidase, as well as suppressed superoxide anion levels and phosphorylation of MAPKs in the myocardium (68). In a Yorkshire swine model of mid-left anterior descending coronary artery, sulfide treatment before and throughout reperfusion decreased myeloperoxidase and inflammation, thereby improving myocardial function and conferring protection against MIRI (67).

NaHS (10 μmol/l) postconditioning was revealed to decrease the myocardial infarct size of isolated rat hearts and inhibit oxidative stress by stimulating SOD activity and reducing malondialdehyde levels via the activation of the sirtuin1/peroxisome proliferator-activated receptor-γ coactivator-1α pathway in an *ex vivo* study (73).

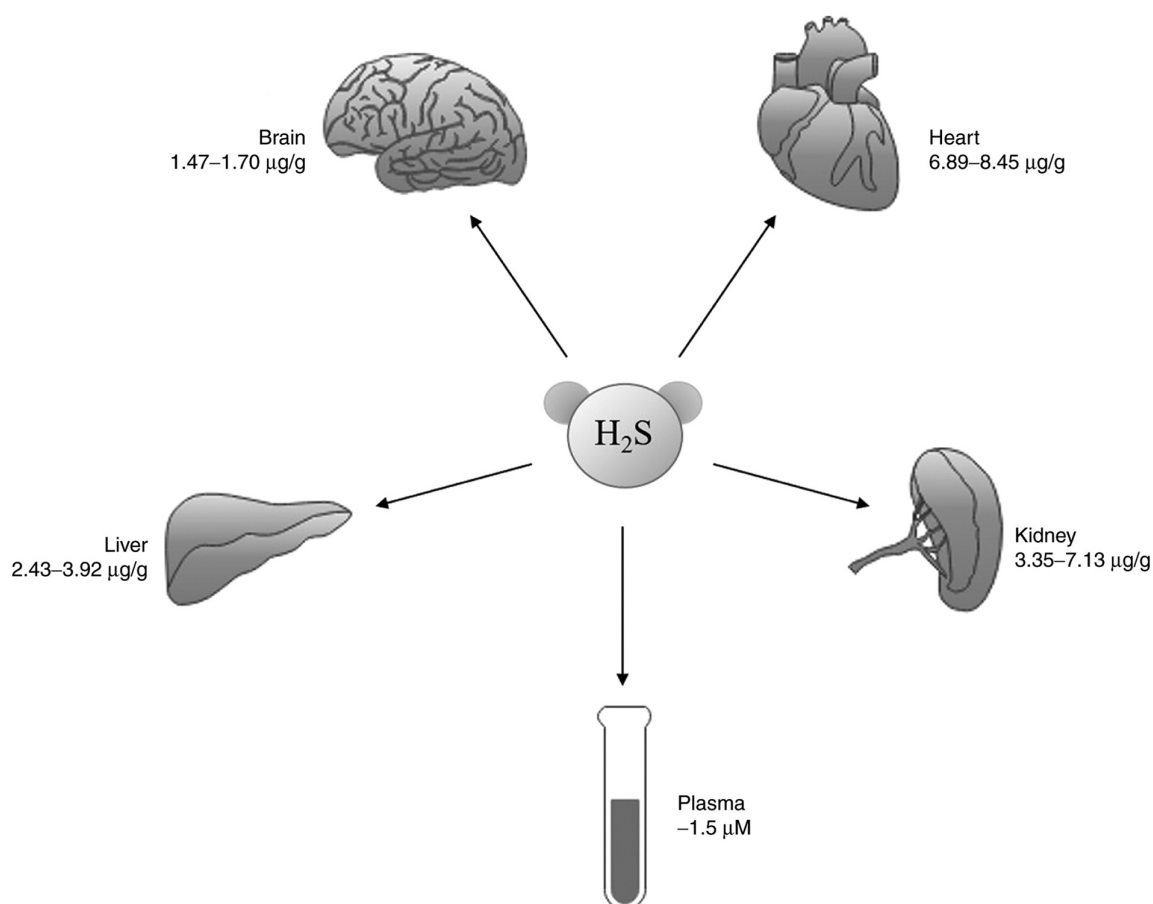
On the contrary, AP39 exhibited antioxidative effects via ROS generation rather than scavenging. The alleviation of myocardial infarction induced by AP39 during MIRI partly arose from reduced production of ROS in interfibrillar and subsarcolemmal mitochondria of cardiomyocytes, which were dose-dependent (Fig. 3) (74).

H₂S improves mitochondrial function. The cardioprotective effects mediated by exogenous NaHS depend on mitochondrial ETC enzymes. Hemodynamic parameters and mitochondrial ETC functional assessment revealed that the cardioprotective effects of H₂S require active mitochondria (75). Following MIRI, mouse hearts showed mitochondrial swelling, disorganized cristae and lower matrix density. However, treatment

Table I. Mechanism and protective effects of H₂S-releasing compounds.

Donor type	Typical donor name	H ₂ S-releasing mechanism	Protective effects
Inorganic salts	NaHS Na ₂ S CaS	Hydrolysis	Stimulating ROS scavenging (33) Inhibiting ROS production (34) Anti-inflammation (35) Vasodilation (36) Promoting angiogenesis (37)
Sulfur-containing organic compound	GY4137	Hydrolysis	Protecting mitochondria (38) Vasodilation (39) Anti-inflammation (40) Anti-oxidative stress (41)
Derivative of allium sativum extract	SPRC S-allylcysteine Diallyl sulfide	H ₂ S generation enzyme Glutathione	Anti-apoptosis (42) Inhibiting ROS production (42) Anti-inflammation (43) Promoting angiogenesis (44)
Mitochondria-targeting compound	AP39	Hydrolysis	Protection against mitochondrial DNA oxidative damage (45)

ROS, reactive oxygen species; SPRC, S-propargyl-cysteine; H₂S, hydrogen sulfide.

Figure 2. Concentrations of H₂S in plasma and tissues in mice. H₂S, hydrogen sulfide.

with H₂S during reperfusion resulted in significantly improved mitochondrial structure, stimulated mitochondrial respiration

and oxygen consumption (76). Karwi *et al* (74) reported that AP39 inhibited ROS generation and mPTP opening during

Table II. Therapeutic effects of H₂S and possible mechanism.

Animal	Dosage	H ₂ S concentration	Possible mechanism	Therapeutic effects
Mouse	GY4137 (133 μ mol/kg/d, IP)	86.36 \pm 17.78 μ M in plasma	Inducing Keap1 S-sulphydration	Inhibiting atherosclerosis (51)
Mouse	NaHS (50 μ mol/kg/d, IP)	~7 μ M in plasma	Increasing SIRT3 promoter activity and expression	Inhibiting oxidative stress in myocardial hypertrophy (52)
Mouse	SPRC (10, 25 mg/kg/d, IP)	~1.5, 2.5 μ M in plasma	Inducing CaMKII S-sulphydration	Anti-oxidative stress and anti-apoptosis (6)
Mouse	NaHS (80 μ mol/kg/2d, IP)	N/A	Inducing USP8 S-sulphydration	Cardioprotection (53)
Mouse	NaHS (80 μ mol/kg/2d, IP)	N/A	Inducing Hrd1 Cys ¹¹⁵ S-sulphydration	Cardioprotection (54)
Mouse	N/A	~1.1 nmol/mg in left ventricle	Alleviating pyroptosis	Cardioprotection (55)
Mouse	SG-1002 (20 mg/kg/d, PO)	N/A	Activating AMPK/PGC-1 α pathway	Promoting cardiac mitochondrial biogenesis (56)
Mouse	NaHS (1 mg/kg, IP)	N/A	Decreasing CD11b ⁺ Gr-1 ⁺ cell numbers in blood and myocardium after MI.	Anti-inflammation in chronic myocardial ischemia (57)
Mouse	NaHS (14 μ mol/kg/d, IP)	N/A	Inducing FoxO1 phosphorylation and nuclear exclusion	Cardioprotection (58)
Mouse	GY4137 (50 μ mol/kg/d, IP)	N/A	Downregulating cardiac hypertrophy, fibrosis, and apoptosis-related gene expression	Cardioprotection (59)
Rat	NaHS (39 μ mol/kg/d, IP)	N/A	Inducing muscle RING finger-1 Cys ⁴⁴ S-sulphydration	Alleviating cardiac muscle degradation (60)
Rat	GY4137 (10, 25, 50 mg/kg/d, IP)	N/A	Inducing specificity protein 1 Cys ⁶⁶⁴ S-sulphydration	Inhibiting myocardial hypertrophy (61)
Rat	NaHS (90 μ mol/kg/d, IP)	~30, 42 μ M in plasma	Inducing TRPV1 S-sulphydration	Enhancing carotid sinus baroreceptor sensitivity (62)
Rat	NaHS (56 μ mol/kg/d, IP)	~1.5 μ M in plasma	Activating Akt/eNOS/NO pathway	Cardioprotection and anti-hypertension (63)
Rat	GY4137 (10, 25, 50 mg/kg/d, IP)	N/A	Inhibiting TGF- β 1/Smad2 pathway, inhibiting oxidative stress and downregulating α -SMA in cardiac fibroblasts	Inhibiting myocardial fibrosis (64)
Rat	Controlled release formulation of SPRC (30 mg/kg/d, PO)	6-fold compared with model	Anti-apoptosis and anti-oxidative stress	Cardioprotection (65)
Rat	NaHS (100 μ M/d, IP)	N/A	Anti-apoptosis	Cardioprotection (66)
Swine	Na ₂ S (100 μ g/kg bolus + 1 mg/kg/h infusion)	N/A	Anti-oxidative stress and anti-inflammation	Protecting against MIRI (67)

IP, intraperitoneal injection; PO, Oral ingestion; d, day; Keap-1, Kelch-like ECH-associated protein-1; MIRI, myocardial ischemia-reperfusion injury; SPRC, S-propargyl-cysteine; H₂S, hydrogen sulfide; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; TRPV1, transient receptor potential cation channel subfamily V member; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator-1; AMPK, AMP-activated protein kinase; Hrd1, HMG-coA reductase degradation 1 homolog (*S. cerevisiae*); USP8, ubiquitin specific peptidase 8; SIRT3, sirtuin3; CaMKII, calcium/calmodulin-dependent protein kinase II.

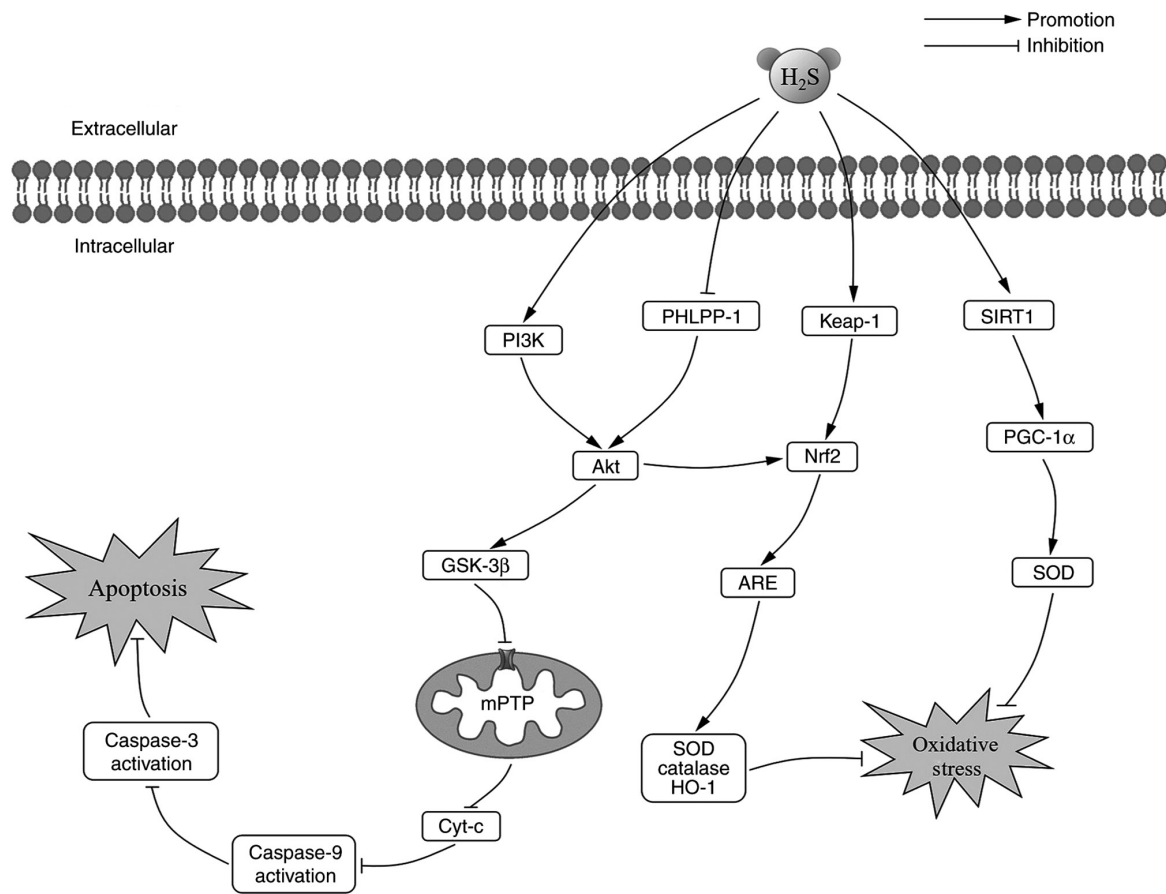


Figure 3. Cardioprotective effect of H₂S during MIRI are exerted by inhibiting oxidative stress and improving mitochondrial function. H₂S promotes PI3K, Keap-1 and SIRT1 activity, inhibits PHLPP-1 activity respectively. Increase of PI3K and decrease of PHLPP-1 can activate Akt and then promote GSK-3β to inhibit mPTP opening, which can decrease Cyt-c release from mitochondria to cytosol and inhibit apoptosis. Keap-1 can activate Nrf2, ARE and SOD, to suppress oxidative stress. Increase of SIRT1 can promote PGC-1α and SOD, and further inhibit oxidative stress. ARE, antioxidant response elements; Cyt-c, cytochrome c; HO-1, heme oxygenase-1; Keap-1, Kelch-like ECH-associated protein-1; Nrf2, nuclear factor erythroid 2-related factor 2; mPTP, mitochondrial permeability transition pore; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1; PHLPP-1, PH domain leucine-rich repeat protein phosphatase-1; SIRT1, sirtuin1; SOD, superoxide dismutase; H₂S, hydrogen sulfide.

MIRI. However, inhibition of the PI3K/Akt pathway, endothelial nitric oxide (NO) synthase (eNOS) or soluble guanylyl cyclase did not reverse the protective effects of AP39. Further research is required to investigate the association of these effects to post-translation modifications mediated by H₂S and the interaction with NO in mitochondria.

H₂S also leads to mitochondrial ATP-sensitive K⁺ (K_{ATP}) channel opening. Ji *et al* (77) reported that treatment with NaHS before reperfusion resulted in the reduction of infarct size and inhibited creatine kinase release in isolated rat hearts. However, these observations were shown to be reversed by K_{ATP} channel blockers (glibenclamide or 5-hydroxydecanoate). Moreover, novel H₂S-donor 4-carboxyphenyl isothiocyanate was reported to activate the mitochondrial K_{ATP} channel and partially depolarize the mitochondrial membrane potential (Fig. 3) (78).

H₂S regulates the RISK pathway. The RISK pathway, activated at the onset of reperfusion, can be regulated by H₂S, thereby protecting against MIRI. In primary cultures of neonatal cardiomyocyte damage induced by hypoxia/reoxygenation (H/R), NaHS was shown to reduce apoptosis in a dose-dependent manner. Furthermore, H₂S inhibits mPTP

opening at a concentration of 30 μ mol/l by increasing the phosphorylation of GSK-3β at Ser9 (78). H₂S administration was not shown to inhibit mPTP opening in isolated mitochondria owing to the lack of intracellular signaling elements, such as GSK-3β (79). In db/db diabetic mice, which are at an increased risk of MIRI, Na₂S therapy administered at the time of reperfusion activated the ERK1/2 pathway, thereby increasing anti-apoptotic proteins and inhibiting the activation of GSK3β (79). Na₂S also significantly reduced the infarct size and circulating troponin-I levels in an ERK1/2-dependent manner (80).

Kelch-like ECH-associated protein-1 (Keap-1)/Nrf2/antioxidant response elements (ARE) pathway is a primary pathway involved in the cellular defense against oxidative stress. In response to oxidative stress, H₂S dissociates Nrf2 from Keap1 (81). During early preconditioning, H₂S promotes the nuclear translocation of Nrf2 and increases the phosphorylation of protein kinase C epsilon and STAT-3. Moreover, H₂S increases the expression of heme oxygenase-1 and thioredoxin 1 during late preconditioning (82). As a result of Nrf2 nuclear translocation, ARE is activated and enhances the transcription of SOD, catalase and heme oxygenase-1 (83). PH domain leucine-rich repeat protein

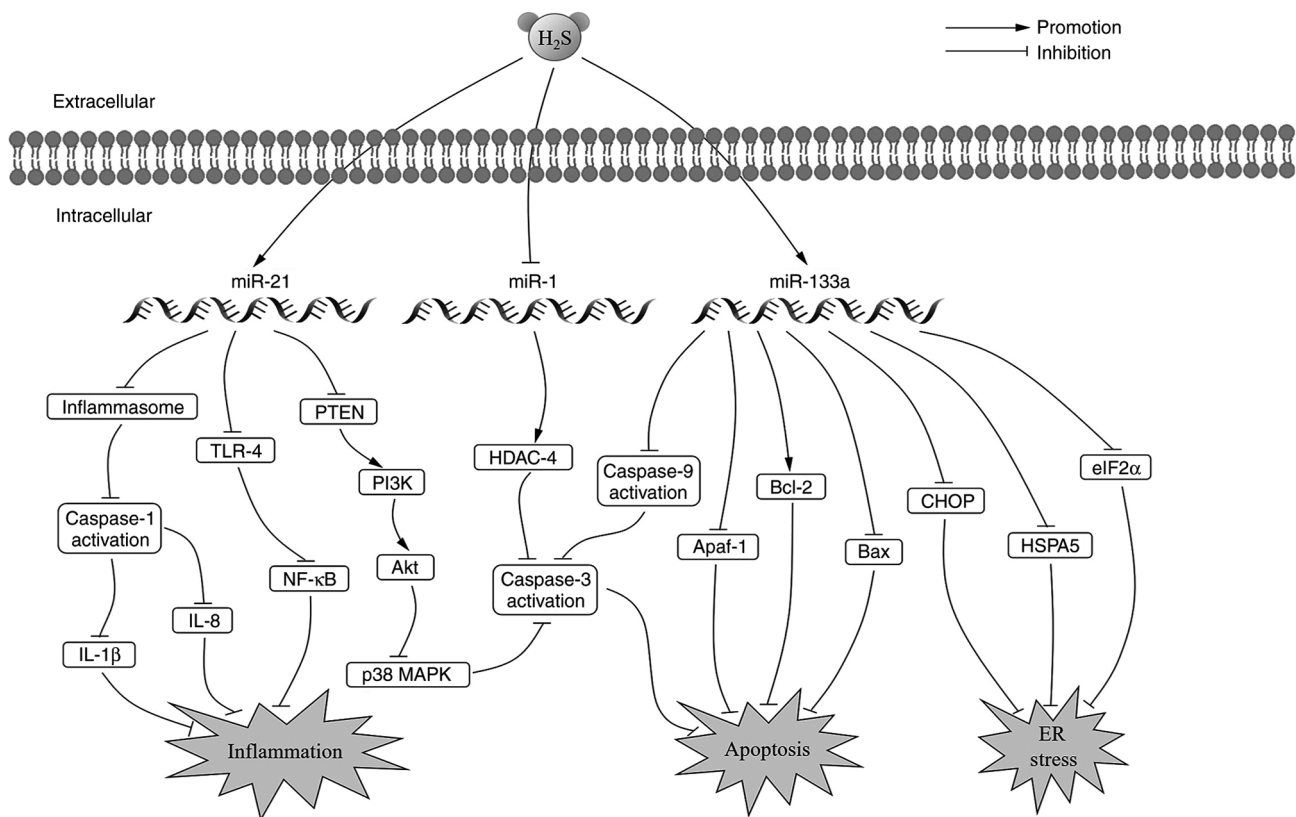


Figure 4. Cardioprotective effects of H_2S during MIRI are exerted by regulating miRNA. H_2S can increase miR-21 expression to inhibit inflammasome production and suppress inflammation through TLR-4/NF- κB pathway. Moreover, increase of miR-21 can decrease apoptosis level through PTEN/PI3K/Akt pathway. As well as miR-21, miR-133a expression can be increased by H_2S . miR-133a increases Bcl-2 level and decreases Apaf-1 and Bax level, resulting in the suppression of apoptosis. miR-133a can also decrease CHOP, HSPA5 and eIF2 α level to inhibit ER stress. H_2S can also inhibit miR-1 expression to enhance HDAC-4 level and suppress apoptosis. Apaf-1, apoptotic peptidase activating factor-1; ER stress, endoplasmic reticulum stress; HDAC4, histone deacetylase 4; TLR-4, Toll-like receptor-4; H_2S , hydrogen sulfide; miRNA/miR, microRNA; eIF2 α , eukaryotic initiation factor-2 α ; HSPA5, heat shock protein family A (Hsp70) member 5.

phosphatase-1 (PHLPP-1) has recently been shown to dephosphorylate Akt at Ser473, which increases infarct size and aggravates MIRI (84,85). During MIRI, the levels of cardiac malondialdehyde are increased, while the expression levels of SOD and heme oxygenase-1 are downregulated. Pretreatment with GYY4137 was shown to reverse the oxidative stress induced by MIRI. GYY4137 also increased the protein expression levels of Akt and Nrf2 by downregulating the level of PHLPP-1. Thus, the antioxidant effect of H_2S in MIRI partly depended on the PHLPP-1/Akt/Nrf2 pathway (41). PI3K, an upstream factor of Akt, is considered an important molecule in the underlying mechanism of H_2S protection against ischemia-reperfusion. The PI3K/Akt/Nrf2 pathway has been reported to play a major role in alleviating cerebral ischemia-reperfusion injury (86). However, to the best of our knowledge, this mechanism has not been reported in the cardiovascular system.

H_2S regulates microRNA (miRNA/miR). Several studies have reported that the expression of miRNA is influenced by H_2S in MIRI (Fig. 4). In cardiomyocytes of neonatal rats, H/R injury was shown to promote the expression of miR-1. The expression of histone deacetylase 4 (HDAC4) was also observed to be decreased (at mRNA and protein levels) during H/R. Preconditioning with H_2S treatment downregulated miR-1,

increased HDAC4 expression and reduced caspase-3 cleavage and release of lactate dehydrogenase. However, a study showing that the protective effects of H_2S could be partially reversed by transfection of cardiomyocytes with miR-1 mimic, demonstrates that H_2S protected neonatal rat cardiomyocytes from apoptosis and enhanced cell viability via the miR-1/HDAC4 signaling pathway (87).

H_2S reduced the activity of caspase-1, as well as the formation and activity of inflammasome in a miR-21-dependent manner. Caspase-1 is an effector enzyme of the inflammasome that is mainly responsible for the processing and release of IL-1 β and IL-18 (88). Na_2S administration was demonstrated to inhibit apoptosis or necrosis in cardiomyocytes in *in vitro* studies and reduce infarct size following MIRI *in vivo* by activating miR-21 (89). A potential target of interaction between miR-21 and Toll-like receptor-4 exists. For instance, in lipopolysaccharide-induced acute lung injury, miR-21 was shown to negatively regulate inflammatory responses via the Toll-like receptor-4 and NF- κB signaling pathway (90). In addition, miR-21 activates the PI3K/Akt signaling pathway to participate in rheumatoid arthritis by inhibiting PTEN expression (91). miR-21 was also shown to reduce p38 MAPK protein expression, which inhibits activation of caspase-3 via PTEN/Akt (92). However, the involvement of these mechanisms in the protection against MIRI by H_2S requires further study.

Endoplasmic reticulum (ER) stress is activated to protect cells when they are exposed to hypoxia. However, sustained activation of ER stress causes apoptosis (93). Ren *et al* (94) reported that the expression levels of ER stress biomarkers, heat shock protein family A (Hsp70) member 5, CHOP and eukaryotic initiation factor-2 α , were significantly increased during ischemia/reperfusion. However, *in vitro* and *in vivo* results revealed that pretreatment with H₂S alleviated ER stress and subsequent apoptosis via the miR-133a signaling pathway by reversing the cardiomyocyte trauma induced by MIRI. The combination of H₂S intervention and miR-133a overexpression notably increased the proliferation, migration and invasion of cardiomyocytes. miR-133a was also observed to promote anti-apoptotic protein Bcl-2 expression and inhibit pro-apoptotic protein Bax, caspase-3, caspase-9 and apoptotic peptidase activating factor-1 expression. Consequently, decreasing apoptosis in the cardiomyocytes (95,96).

Crosstalk between H₂S and NO. Accumulating evidence has revealed that there is a crosstalk between H₂S and NO. CSE knockout mice showed a reduction in NO levels due to decreased eNOS expression. Acute treatment with H₂S in CSE knockout mice was found to increase NO bioavailability and restore eNOS protein expression, which consequently attenuated oxidative stress and MIRI (69). In another *in vivo* study, H₂S, donated by diallyl trisulfide, activated eNOS protein expression and NO metabolites, reduced infarct size and restored myocardial contractile function (97). H₂S was also confirmed to attenuate cardiac arrest-induced mitochondrial injury and cell death in cardiopulmonary resuscitation in mice (98). These protective effects are conferred by increasing phosphorylation of eNOS in the left ventricle and increasing serum nitrite/nitrate levels (98). However, further research is required to confirm the protective role of H₂S in MIRI.

S-sulphydration. In recent years, increased attention has been paid to S-sulphydration, a post-translational modification between H₂S and cysteine residues of proteins that modifies the structure and biological activities of protein targets (99). Pharmacological postconditioning performed at the onset of reperfusion with NaHS significantly increased S-nitrosylation of cardioprotective proteins, as well as reduced post-ischemic contractile dysfunction and infarct size (100). However, the S-sulphydration of proteins in MIRI has not been fully studied. H₂S was reported to S-sulphydrate Keap1 in response to oxidative stress, thereby mediating the dissociation of Nrf2 from Keap1, and as a result, promoting Nrf2 translocation in sulfur mustard-induced lung injury (81). A similar mechanism was confirmed in diabetic mice, wherein, H₂S attenuated diabetes-accelerated atherosclerosis by S-sulphydrating Keap1 at Cys151, resulting in activation of Nrf2 signaling (51). These mechanisms may contribute to the potential role of H₂S in MIRI.

6. Conclusions

In summary, H₂S plays a vital protective role in attenuating MIRI via mechanisms, such as attenuation of oxidative stress, restoration of mitochondrial function, regulation of miRNA, interaction with NO and S-sulphydration. However, while

these effects have been demonstrated in cellular and animal models, they have not been replicated in humans, to the best of our knowledge. Therefore, the transition of H₂S from bench to bedside is necessary. Off-target effects of H₂S may result in unexpected adverse reactions, including irreversible damage. Therefore, future research should focus on maximizing the potential benefits of H₂S in cardioprotection in MIRI, while minimizing the unwanted side effects.

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Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Authors' contributions

YG wrote the original manuscript and the prepared figures and tables. DW modified the manuscript according to the reviewers and editors' comments. DZ contributed to the revision of the article. All authors have read and approved the final manuscript. Data sharing not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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