The cardioprotective potential of hydrogen sulfide in myocardial ischemia/reperfusion injury (Review)

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Myocardial infarction is responsible for the majority of cardiovascular mortality and the pathogenesis of myocardial damage during and after the infarction involves reactive oxygen species. Serious efforts are under way to modulate the developing ischemia/reperfusion injury and recently the use of hydrogen sulfide (H$_2$S) emerged as a new possibility. H$_2$S has been best known for decades as a pungent toxic gas in contaminated environmental atmosphere, but it has now been recognized as a novel gasotransmitter in the central nervous and cardiovascular systems, similarly to nitric oxide (NO) and carbon monoxide (CO). This finding prompted the investigation of the potential of H$_2$S as a cardioprotective agent and various in vitro and in vivo results demonstrate that H$_2$S may be of value in cytoprotection during the evolution of myocardial infarction. Although several questions remain to be elucidated about the properties of this new gasotransmitter, increased H$_2$S levels may have therapeutic potential in clinical settings in which ischemia/reperfusion injury is encountered. This review article overviews the current understanding of the effects of this exciting molecule in the setting of myocardial ischemia/reperfusion.

**Keywords:** hydrogen sulfide, myocardial ischemia, reperfusion, oxidative stress, cardioprotection, reactive oxygen species

According to the WHO, cardiovascular diseases (CVD) are the leading causes of death worldwide, and as the population becomes increasingly elderly, almost 23.6 million people will die from CVDs by 2030. The majority of these deaths will be due to myocardial infarction (MI). In most cases MI occurs after rupture, erosion or ulceration of an atherosclerotic plaque in a coronary artery, and this event is rapidly followed by acute thrombosis of these vessels, blocking blood flow. The perfusion imbalance between supply and demand leads to ischemia in the affected myocardium around the thrombotic artery. Necrosis of the cells in the ischemic zone completes 2–4 hours after the onset of decreased perfusion. The individual sensitivity of myocytes to ischemia or any preconditioning effect in the past can influence the extension and the rate of cell death (49). In the acute phase of MI, and especially in reperfusion, polymorphonuclear leukocytes migrate to the infarcted area, and they aggravate tissue damage by producing reactive oxygen species (ROS). Importantly, myocardial cells also produce ROS under ischemic conditions by their mitochondrial electron transport chain, enzymes like nitric oxide synthase, NADPH oxidase, xanthine oxidase, CYP 450, lypoxigenase/cyclooxygenase, or by auto-oxidation of catecholamines. Antioxidant defense...
mechanisms of these cardiac cells include non-enzymatic effects (glutathione, thioredoxin, vitamins C, A and E) and enzymatic mechanisms as well (catalase, glutathione peroxidase, superoxide dismutase) (31). In spite of these various antioxidant mechanisms, oxidative stress via reactive oxygen species is still the leading cause of cell death after MI.

**Reactive oxygen species**

Reactive oxygen species are reactive chemical substances which can be grouped into two categories: free radicals (e.g. superoxide $\text{O}_2^{-}$, and hydroxil radical $\cdot \text{OH}$) and non-radical derivatives (e.g. hydrogen peroxide $\text{H}_2\text{O}_2$). Free radicals are distinguished by having one or more unpaired electrons in their outer orbit and are paramagnetic which make them more reactive than the corresponding non-radicals. These substances in low concentrations function as signaling molecules, however if produced in excess they elicit harmful effects (12).

Intracellular sources of ROS are increased electron leakage from the respiratory chain, xanthine oxidase, lipoxygenase / cyclooxygenase, cytochrome P450, uncoupling of the nitric oxide synthase, arachidonic acid metabolism, while the extracellular sources are oxidation of catecholamines (18), secretion of ROS by activated neutrophils (15, 25). ROS levels can also be increased due to the decreased function of the antioxidant defense mechanisms.

**Physiological and pathophysiological effects of ROS**

In physiological conditions ROS takes part in signal transduction and the regulation of gene expression. They are involved in the regulation of calcium induced signaling which in turn can activate calcium dependent protein kinases such as PKC and calcineurin. ROS can also affect the activity of protein kinase pathways by influencing the redox state of the cell. They can alter the gene expression pattern via modulation of transcription factor activity (e.g. nuclear factor-κB, AP-1, peroxisome proliferators-activated receptor family of transcriptional activators) (13).

However, in pathophysiological conditions high levels of ROS evoke deleterious effects. The oxidation and nitration of lipids, proteins and nucleic acids and formation of aggregates of oxidized molecules results in the loss of membrane integrity, structural and functional changes in proteins, and genetic mutations. These changes underlie the loss of cellular function, cellular aging and the inability of cells to withstand physiological stresses. Furthermore ROS alter signal transduction processes and energy metabolism during oxidative stress.

Lipid peroxidation occurs due to the effect of ROS on polyunsaturated fatty acids (16). As a result the lipid bilayer of the cell membrane is disrupted, affecting its functional properties. Furthermore lipid peroxidation generates cytotoxic and mutagenic products such as unsaturated aldehydes and malondialdehyde (MDA). These metabolites can inactivate many cellular proteins by forming protein cross-linkages.

ROS can damage cellular proteins in numerous other ways. Structural oxidation of sulfhydryl groups or methionine residues results in conformational changes, unfolding, fragmentation and polymerization of proteins (11). These reactions can harm structural proteins and inactivate critical enzymes. The modified proteins are known to have altered sensitivity to proteolysis. Proteins which were subjected to modest oxidation are highly
susceptible to proteolysis, while proteins undergoing heavy oxidation show poor proteolytic affinity.

Direct reaction of ROS with DNA can cause nicking, base-pair mutations, rearrangements, deletions, insertions, and sequence amplification. Nitric oxide or the reactive nitrogen species derived from it are mutagenic agents with the potential to cause nitration, nitrosation and deamination reactions on DNA bases. These changes in the DNA may significantly affect gene expression (31). Mitochondrial DNA is even more susceptible to ROS initiated damage since its proximity to the electron transport chain and the lack of protective histones and many of the repair mechanisms of the nuclear genome.

ROS have a broad range of targets in the signal transduction process: they activate Ca\(^{2+}\) channels, mitogen-activated protein kinase enzymes, tyrosine phosphatides, and upregulate transcription factors such as nuclear factor-κB and AP-1. Microarray studies have shown that oxidative stress induces nearly 100 genes (33).

**Myocardial ischemia/reperfusion injury**

Although reperfusion of the ischemic myocardium during early stages of myocardial ischemia is essential to prevent cardiac damage, reperfusion is also known to cause deleterious effects due to the formation of ROS (23). ROS formed after the reoxygenation lead to direct oxidative damage to cellular components and they inflict indirect damage by causing localized inflammation. Involvement of ROS in ischemia/reperfusion injury has been shown directly by spin trap α-phenyl-N-tert-butylnitrone (6, 62), luminal-enhanced tert-butyl-initiated chemiluminescence (38), and electron paramagnetic resonance spectroscopy (62); furthermore indirectly by the beneficial effects of various antioxidants in experimental ischemia-reperfusion models. Moreover exposure of the heart or subcellular organelles to oxyradical generating substances has been reported to produce similar effects to those observed in hearts subjected to ischemia/reperfusion (21).

ROS can interact with important proteins, such as ion channels, sarco-endoplasmic reticulum calcium release channels and myofilament proteins which are associated with the excitation-contraction coupling. The alterations of the proteins can change their activity or their susceptibility to proteolysis. ROS can induce Ca\(^{2+}\)-overload in myocytes either by directly affecting the Ca\(^{2+}\) handling proteins or indirectly by inducing membrane lipid peroxidation and this results in subsequent dysrhythmias, myocardial cell damage and cardiac dysfunction. The combination of high levels of Ca\(^{2+}\) in the mitochondria and ROS can cause mitochondrial dysfunction. ROS are a major factor contributing to the opening of the mitochondrial permeability transition pore, causing the release of cytochrome c and other factors that can lead to hypercontracture and cell death (52).

The cumulative long-term effects of ROS can cause loss of heart contractile function and alterations in the cardiovascular system (9, 31).

**Approaches for cardioprotection**

Many pre- and postconditioning mechanisms are currently under investigation, which may interfere with ROS formation and/or diminish tissue damage induced by myocardial ischemia/reperfusion. In some studies, ischemic preconditioning resulted in reduced ROS formation in the myocardium (39). In other experiments, pretreatment with various pharmaceutical
agents had beneficial effects on the myocardial damage under ischemic conditions and after reperfusion. Treatment of cardiac cells with hydrogen sulfide (H$_2$S) represents a relatively new possibility and it is under intensive research.

Biochemistry of hydrogen sulfide

Up till now, H$_2$S has been regarded as a toxic gas and environmental hazard (7) and its main importance was in inorganic chemistry where it is frequently used (e.g. as a reagent to produce sulfide salts). However, it was recently described in biological systems that H$_2$S is an endogenously produced gasotransmitter and has a role as substrate or product in numerous enzymatic reactions. The main enzymes involving H$_2$S are listed in Table I. Besides the three main H$_2$S producing enzymes from the carbon-sulfur lyases (4.4.1.x) it was recently discovered that H$_2$S is also produced in a coupled reaction with 3-mercaptopyruvate sulfurtransferase (3-MST, E.C. 2.8.1.2) (28) and the thiosulfate byproduct is converted to H$_2$S mainly by two enzymes: thiosulfate reductase (E.C. 2.8.1.3) and interestingly glutathione-dependent thiosulfate reductase (E.C. 2.8.1.5), which could be a link between NO production and H$_2$S metabolism (44).

Table I. The main enzymes involved in endogenous H$_2$S metabolism

<table>
<thead>
<tr>
<th>Enzymes involved in H$_2$S metabolism</th>
<th>E.C.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystathionine gamma-lyase</td>
<td>4.4.1.1</td>
<td>(50)</td>
</tr>
<tr>
<td>Cysteine lyase</td>
<td>4.4.1.10</td>
<td>(32)</td>
</tr>
<tr>
<td>Cystathionine beta-synthase</td>
<td>4.2.1.22</td>
<td>(28)</td>
</tr>
<tr>
<td>3-Mercaptopyruvate sulfurtransferase</td>
<td>2.8.1.2</td>
<td>(53)</td>
</tr>
<tr>
<td>Glutathione-dependent thiosulfate reductase</td>
<td>2.8.1.3</td>
<td>(41)</td>
</tr>
<tr>
<td>Thiosulfate reductase</td>
<td>2.8.1.5</td>
<td>(4)</td>
</tr>
</tbody>
</table>

The most commonly used H$_2$S donors are NaHS and Na$_2$S. The liberation of H$_2$S from these chemicals is due to simple dissociation. However in cases when H$_2$S is liberated from a prodrug (the active component is formed during the metabolism of the used component, i.e. S-allylcysteine (10) or garlic (26)) the mechanism is still unknown. With the exploration of the enzymes involved, the kinetics and mechanism of the prodrug could be more clearly understood, thus a next generation of H$_2$S donor components could be produced or even specific enzyme assay kits could be developed to enable more accurate H$_2$S detection.

H$_2$S can be described diversely, according to the actual environment in which the material is applied. The quantitative analysis of H$_2$S in aqueous systems has many difficulties, mainly because of the dissociation of H$_2$S in water. Three main forms can be present in an aqueous system (Fig. 1) (46). The first acid dissociation constant ($K_{a1}$) is in the 10$^{-7}$ M range, while the second dissociation constant ($K_{a2}$) is in the 10$^{-13}$ M range (24). At atmospheric pressure and at room temperature the saturated H$_2$S solution is 100 mM (45). Based on the equation above, the pH value of the saturated solution at this temperature is around 4, so H$_2$S is a weak acid, therefore the addition of acids or bases changes its solubility. Acids shift the equation to the left, so the presence of H$_2$S increases, while bases shift the equation to the right, so the concentration of S$^{2-}$ increases. These characteristics must be considered to choose the appropriate analytical method for the measurement of endogenous H$_2$S, which is
already problematic. The most widespread techniques are trapping H$_2$S with a metal (usually zinc) followed by acidification and reaction with a dye, N,N-dimethyl-p-phenylenediamine (DMPD), to form methylene blue, which is then measured spectrophotometrically (56). Still, amperometric methods and gas chromatography-mass spectroscopy are also used. Importantly, solubility decreases at higher temperatures and at physiological temperature the concentration of the saturated H$_2$S solution is around 85 mM. Although this concentration would be lethal, some measurements indicate that the H$_2$S concentration in human serum is in the 50–100 µM (28) range, so only 1000 times less than the saturated solution. This serum concentration seems unrealistic as blood samples do not have the characteristic odour of H$_2$S while NaHS solutions at this concentration do have the smell of a rotten egg. Clearly, the development of accurate H$_2$S measurement methods is a must for stepping forward in this field. In most experiments H$_2$S donor concentrations were in the 0.1–40 µM range using perfusion and in the 1–600 µM range using cultured cells (14, 28).

\[
\begin{align*}
H_2S_{(aq)} & \rightleftharpoons K_{a,1} \ H_2O_{(aq)}^+ + HS_{(aq)}^- \\
HS_{(aq)}^- & \rightleftharpoons K_{a,2} \ H_2O_{(aq)}^+ + S_{(aq)}^{2-}
\end{align*}
\]

*Fig. 1. Potential forms of H$_2$S in an aqueous solution*

**Mechanisms of action**

The mechanisms by which H$_2$S affects injured cells are complex (Fig. 2). The anti-inflammatory effect of H$_2$S originates from inhibition of leukocyte rolling, adhesion and diapedesis. Besides these actions, it inhibits the activation of nuclear factor-κB (NF-κB) and reduces the production of inflammatory factors like IL-1β and TNF-α (47). Interestingly, in some studies H$_2$S mediated proinflammatory effects (22), which indicates that the background of these immunomodulatory influences are not clearly understood yet. H$_2$S has several effects on mitochondria of cardiac cells such as the inhibition of cytochrome c oxidase in a potent and reversible way, which leads to preservation of mitochondrial structure and function after ischemia/reperfusion. Inhibition of mitochondrial respiration in the injured myocytes results in attenuated generation of reactive oxygen species and may alter the function of the affected cell as we have shown earlier in relation with the vascular tone (14, 28). A rise in tissue glutathione concentration may occur after treatment (1). Furthermore, H$_2$S has inhibitory effect on phosphodiesterase-5 (PDE-5), which results in decreased NADPH oxidase formation, and the level of antioxidant enzymes increases (7). H$_2$S decreased lipid peroxidation by scavenging hydrogen peroxide and superoxide in a model of isoproterenol-induced myocardial injury (47). The H$_2$S mediated activation of nuclear-factor-E2-related-factor-2 dependent pathway (Nrf2) results in upregulated gene expression of specific factors – such as heme oxygenase-1, thioredoxin, glutathione reductase, glutathione S-transferase and catalase – which play role in endogenous antioxidant defense. Besides these mechanisms, H$_2$S also acts as a direct scavenger neutralizing cytotoxic reactive species like peroxynitrite (37).
H$_2$S also activates three protein-kinase C (PKC) isoforms in cardiac cells: PKC$\alpha$, PKC$\delta$, and PKC$\epsilon$. The $\alpha$ and $\epsilon$ isoforms take part in the cardioprotective signaling after myocardial ischemia/reperfusion, while PKC$\delta$ is associated with ischemia/reperfusion injury. Interestingly, in an in vitro experimental model, activation of PKC$\delta$ did not induce unfavorable effects (7). PKCs play a role in the clearance of Ca$^{2+}$ from the cytosol, by activating the sarco-endoplasmic reticulum Ca-ATPase (SERCA) (4).

Further important players in the cytoprotection are the K$_{ATP}$ channels. These channels are present in the cellular and mitochondrial membrane and open in the presence of H$_2$S, mediating negative inotropic effect in the myocardium. These channels are also responsible – at least in part – for the vasodilatory effect of H$_2$S. Blocking the channels either in the inner mitochondrial membrane (42) or in the sarcolemma (28) abolishes the cardioprotective effects. Besides the effect on K$_{ATP}$ channels, H$_2$S inhibits L-type Ca$^{2+}$ channels in myocytes (7) via an unknown mechanism. It is possible that H$_2$S being a reductant agent directly interacts with the channel proteins, or it can act through PKC, or simply opens the K$_{ATP}$ channels (7).

H$_2$S also has anti-apoptotic properties. In vivo studies have proven that H$_2$S activates pro-survival kinases like PKC/Erk1/2 or PI3K/Akt. Activation of PKC$\epsilon$-STAT3 signaling cascade results in increased expression of Hsp90, Hsp70, Bcl-2 and Bcl-x1 anti-apoptotic molecules, and inactivation of pro-apoptotic factor Bad also occurs. Activation of eNOS enzyme leads to NO formation, and this other gasotransmitter also inhibits apoptosis (28).

In addition, S-sulfhydration of cellular proteins is also generated by H$_2$S. It is a common post-translational modification event in the cells, and it can affect the function of multiple cellular enzymes. The significance of this protein modification needs further examination (47).
Toxicity

As \( \text{H}_2\text{S} \) was long considered a toxic molecule the literature of its toxicological profile is vast. Therefore here we provide a brief outlook on the topic and refer the interested reader to thorough reviews for more details (2, 40, 57). Most of the toxicological studies are concerned about inhaled sulfide as the second most common gas-related fatality is due to such exposure (17). After exposure to 20–100 ppm of \( \text{H}_2\text{S} \), eye irritation, respiratory tract irritation and headache occur. Chronic exposure to moderate concentrations (around 250 ppm) cause pulmonary edema. Above 500 ppm, it causes unconsciousness, collapse, cough or even death. After single inhalation of 1000–2000 ppm, hyperpnoea occurs, which is followed by rapid collapse and respiratory disturbances, and these changes effect coma or death. Above 5000 ppm, the respiratory ganglia are paralyzed by \( \text{H}_2\text{S} \), and this is the cause of death (40).

Information on parenteral and enteral toxicity data is less abundant reflecting the difficulties of measuring the exact concentrations. High doses (10 mg/kg) of intravenous or intraperitoneal \( \text{H}_2\text{S} \) caused inflammation and neutrophil recruitment in the lung (27, 51). However, these doses were well above the doses where therapeutic benefits were already observed in animal models. In the blood, \( \text{H}_2\text{S} \) bonds to methaemoglobin, in the same way as cyanide, and respiratory paralysis, sudden collapse and death are the outcome of the hampered cellular respiration (40). Our own earlier results also stress the potential blockade of cellular respiration (22). Other possible mechanisms of action may include the inhibition of glutamate uptake and inhibition of monoamino oxidase (54), substance P production (3) and perhaps the involvement of the vanilloid receptor 1 (51). Taken together, the various studies reveal that the width of the therapeutic window of \( \text{H}_2\text{S} \) is not entirely clear yet and it is imperative to overcome the difficulties of exact \( \text{H}_2\text{S} \) measurement to clarify the therapeutic/toxic ratio.

Results in cardiovascular disease models

\( \text{H}_2\text{S} \) is widely investigated in models of cardiovascular and other diseases (4, 20). To obtain cardioprotective effects, different \( \text{H}_2\text{S} \)-donor molecules were administered to raise the level of \( \text{H}_2\text{S} \) in the milieu of injured myocardial cells in a pre- or postconditioning fashion. In several in vitro studies, cultured H9C2 rat cardiomyoblasts, or primary adult/neonatal rat cardiomyocytes were used as experimental model (Table II). Other in vitro studies were based on the investigation of isolated rodent hearts using a Langendorff setup (Table III). The \( \text{H}_2\text{S} \) donor molecule NaHS was used in various ways in the studies; as pretreatment (19, 59), as postconditioning (5) or through the whole experiment (60). An experiment with Sprague-Dawley rats used ischemic postconditioning to increase the endogen \( \text{H}_2\text{S} \) level. In the in vivo experiments, rodent models were used (Table IV). In a study, where Sprague-Dawley rats were used, Pan and colleagues found that precondition limited the infarct size more efficiently than postconditioning (35). Other sort of in vivo studies were carried out on large animals, using pig and dog models (Table V).

Besides the \( \text{H}_2\text{S} \) donors, inhibitors of endogenous \( \text{H}_2\text{S} \) formation are also widely used in different investigations. These agents, namely D,L-propargylglycine (PAG) and \( \beta \)-cyanoalanine (BCA) are unfortunately not specific and lead to multiple cellular effects in their effective doses. Some experiments used PAG as a pretreatment in rat myocardial infarction model, in a dose of 50 mg/kg i.p., given once or daily for seven days resulting in an increased infarct size (47).
Table II. In vitro studies on cultured cardiomyocytes and myoblasts

<table>
<thead>
<tr>
<th>Cell type of the experimental model</th>
<th>Experiment</th>
<th>Used H₂S donor and its concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary isolated adult rat cardiomyocytes</td>
<td>metabolic inhibition elicited by sodium cyanide and 2-deoxyglucose</td>
<td>NaHS, 10–100 µM</td>
<td>protected against cell loss</td>
<td>(36)</td>
</tr>
<tr>
<td>Primary isolated adult rat cardiomyocytes</td>
<td>hypoxia-reoxygenation</td>
<td>NaHS, 100 µM</td>
<td>reduced degree of cellular injury and arrhythmias</td>
<td>(58)</td>
</tr>
<tr>
<td>Primary neonatal rat cardiomyocytes</td>
<td>hypoxia-reoxygenation</td>
<td>NaHS, 10–50 µM</td>
<td>protected against apoptosis</td>
<td>(55)</td>
</tr>
<tr>
<td>Cultured H9c2 cardiomyoblasts</td>
<td>H₂O₂ treatment and hypoxia</td>
<td>1–600 µM Na₂S</td>
<td>increased cell viability after H₂O₂ treatment, but not after hypoxia</td>
<td>(5, 20)</td>
</tr>
<tr>
<td>Cultured H9c2 cardiomyoblasts</td>
<td>hyperhomocysteinaemia</td>
<td>100 µM – 1 mM NaHS</td>
<td>reduced nuclear and ultrastructural changes</td>
<td>(59)</td>
</tr>
</tbody>
</table>

Table III. In vitro studies on isolated-perfused rodent hearts

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Used H₂S donor and its concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia-reperfusion in isolated rat hearts</td>
<td>NaHS, 0.1–1 µM</td>
<td>reduced infarct size, duration and severity of arrhythmias protected against the decrease in intracellular Ca-transients</td>
<td>(19)</td>
</tr>
<tr>
<td>Ischemia-reperfusion in isolated rat hearts</td>
<td>NaHS, 40 µM</td>
<td>improved the contractile function, reduced the incidence of arrhythmias</td>
<td>(30)</td>
</tr>
<tr>
<td>Ischemia-reperfusion in isolated rat hearts</td>
<td>NaHS, 1–10 µM</td>
<td>reduced infarct size, decreased biochemical markers of myocyte necrosis</td>
<td>(60)</td>
</tr>
<tr>
<td>Hypoxia-reoxygenation in isolated mouse hearts</td>
<td>Na₂S, 10 µM</td>
<td>improved recovery in the reoxygenation stage</td>
<td>(61)</td>
</tr>
</tbody>
</table>

In summary, both the in vitro and in vivo results indicate that increased amount of H₂S has cyto- and cardioprotective effects in the injured (ischemic or already reperfused) myocardium. In contrast, decreased endogenous H₂S formation (i.e. administering H₂S inhibitors) aggravates tissue damage. Importantly, the dose-dependence of the cardioprotective effect is bell-shaped: administering donors beyond the optimal concentration has less therapeutic efficacy (29). This further strengthens the importance of accurate measurement techniques.
### Table IV. In vivo rodent models

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Used H₂S donor and its concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial ischemia-reperfusion in the rat</td>
<td>NaHS, 0.8 mg/kg/day</td>
<td>reduced infarct size</td>
<td>(14)</td>
</tr>
<tr>
<td>Myocardial ischemia-reperfusion in the rat</td>
<td>NaHS, 1.1 mg/kg/day</td>
<td>reduced infarct size</td>
<td>(55)</td>
</tr>
<tr>
<td>Myocardial ischemia-reperfusion in the mouse</td>
<td>Na₂S, 10–500 µg/kg given at the time of reperfusion</td>
<td>reduced infarct size, improved LV function</td>
<td>(8)</td>
</tr>
<tr>
<td>Hyperhomocysteinaemia-induced myocardial injury in the rat</td>
<td>NaHS, 1.1 mg/kg/day</td>
<td>protected against homocysteine-induced changes in myocardial ultrastructure</td>
<td>(34)</td>
</tr>
<tr>
<td>LAD-ligation induced heart failure in the mouse</td>
<td>Na₂S, 0.1 mg/kg at the start of reperfusion only once, or given daily in the following 7 days</td>
<td>improved left ventricular contractile function and ultrastructure</td>
<td>(48)</td>
</tr>
<tr>
<td>LAD-ligation in the rat</td>
<td>NaHS, 0.1–30 µM/kg for preconditioning (1 day), and/or 0.1–10 µM/kg for postconditioning (3 days)</td>
<td>decreased infarct size, preconditioning is more effective than postconditioning</td>
<td>(35)</td>
</tr>
</tbody>
</table>

### Table V. In vivo large animal models

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Used H₂S donor and its concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial ischemia-reperfusion in the pig</td>
<td>Na₂S, 100 mg/kg bolus + 1 mg/kg/h infusion for 2 h</td>
<td>reduced infarct size, improved contractility, reduced neutrophil infiltration, improved coronary microvascular reactivity</td>
<td>(43)</td>
</tr>
<tr>
<td>Cardiopulmonary bypass induced myocardial and endothelial injury in the dog</td>
<td>Na₂S, 1 mg/kg/h</td>
<td>improved cardiac contractility and vascular function</td>
<td>(48)</td>
</tr>
</tbody>
</table>
Future directions

As the progress on this research field is hampered by the unknown exact H$_2$S concentrations in various samples it is inevitable to put serious effort on finding a suitable, accurate method for gathering such information. Another important aspect is the lack of specific inhibitors of H$_2$S synthetic pathways and stable H$_2$S donors. The current inhibitor molecules have wide-ranging effects and the donor-molecules are unstable and thus varying concentrations of free H$_2$S leading to conflicting results. This may explain the proinflammatory and anti-inflammatory effects. Apart from these methodological considerations it is also important to learn more about the effects of H$_2$S in large animal models and in aged animals. Results from clinical trials will also pave the way to a better understanding of the effectiveness, safety, true blood concentrations and pharmacokinetics of H$_2$S. In one completed trial healthy volunteers and subjects with varying degrees of impaired renal function received known concentrations of sodium sulfide (clinicaltrials.gov, NCT00879645). Results have been partially announced so far, but the treatment can be considered safe as no serious adverse effects were encountered in the 28 patients involved. Still, some caution may be warranted about the potential of H$_2$S as another trial involving coronary artery bypass graft patients was terminated by the company due to undisclosed reasons (NCT00858936) and one has been withdrawn prior to enrollment (NCT01007461). Further results and information from these trials and from others still ongoing (NCT01282905) or recruiting (NCT01088490) will help to elucidate the real physiological and pathophysiological importance of H$_2$S.

Conclusions

In summary, our knowledge on hydrogen sulfide expands continuously and it seems that this molecule may have important physiological roles. Several experimental approaches showed that increased level of H$_2$S exerts beneficial effects in the region of myocardial ischemia. This result was achieved both by increasing endogenous levels of H$_2$S or by adding exogenous H$_2$S. Importantly, decreased amounts of this molecule were associated with increased myocardial damage. Thus, H$_2$S represents a new possibility to modulate the effects of myocardial ischemia/reperfusion and to decrease the amount of damaged myocardium. The ongoing clinical trials will certainly clarify the importance and broaden our knowledge further on the potential of this exciting endogenous gasotransmitter molecule.

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