Sulfide regulation of cardiovascular function in health and disease

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Abstract | Hydrogen sulfide (H2S) has emerged as a gaseous signalling molecule with crucial implications for cardiovascular health. H2S is involved in many biological functions, including interactions with nitric oxide, activation of molecular signalling cascades, post-translational modifications and redox regulation. Various preclinical and clinical studies have shown that H2S and its synthesizing enzymes — cystathionine γ-lyase, cystathionine β-synthase and 3-mercaptosulfo transferase — can protect against cardiovascular pathologies, including arrhythmias, atherosclerosis, heart failure, myocardial infarction and ischaemia–reperfusion injury. The bioavailability of H2S and its metabolites, such as hydropersulfides and polysulfides, is substantially reduced in cardiovascular disease and has been associated with single-nucleotide polymorphisms in H2S synthesis enzymes. In this Review, we highlight the role of H2S, its synthesizing enzymes and metabolites, their roles in the cardiovascular system, and their involvement in cardiovascular disease and associated pathologies. We also discuss the latest clinical findings from the field and outline areas for future study.

Hydrogen sulfide (H2S) is a naturally occurring, colourless gas that is toxic, corrosive and flammable. H2S is a major component of the sulfur cycle and is present in the environment (such as in decaying organic matter, groundwater and natural gases). With exposure to levels >100 ppm, H2S typically causes asphyxiation, with shock and convulsions that can be fatal1. However, H2S is also an important biological molecule that was crucial in the evolution of life2,3 and is synthesized in nanomolar to micromolar concentrations in vivo. In the past few decades, the essential role of H2S in cellular signalling and protection and in regulating numerous biological functions has been recognized1.

H2S is one of three known gaseous signalling molecules or ‘gasmotransmitters’ with crucial pathophysiological roles in cardiovascular function4–6. Carbon monoxide (CO) and nitric oxide (NO) are the other two gaseous neurotransmitters in this class. Before the identification in the 1940s of the biological role of H2S in vertebrates4, NO had long been considered the major vascular gaseous signalling molecule1. The current literature clearly demonstrates that H2S is an important independent effector4–11, as well as an enhancer of NO-mediated signalling events affecting the cardiovascular system12–14. A cardioprotective role for H2S has been suggested in cardiac arrhythmias, cardiac fibrosis, heart failure, cardiac hypertrophy, ischaemia–reperfusion injury (IRI) and myocardial infarction (MI)15. Although the role of H2S and its metabolites as biomarkers of human cardiovascular disease (CVD) is not yet well established16, improved detection techniques have identified novel sulfide metabolites, including hydropersulfides and polysulfides, and have begun to reveal previously unknown molecular mechanisms and their biological relevance in cardiovascular pathology. In this Review, we discuss the involvement of H2S, hydropersulfides and polysulfides in cardiovascular function and CVD and provide timely insights into potential clinical applications and interventions.

Chemical biology of sulfides

The oxidation state of sulfur has a broad range, from −2 in H2S, 0 in elemental sulfur (S8), +2 in sulfur monoxide (SO), and a maximum oxidation state of +6 in sulfate (SO42−). Owing to its lower oxidation state, H2S acts as a reductant. Although H2S does not react readily with oxygen in the air, it easily undergoes oxidation in aqueous solutions. Sulfide can be present as other oxidation products, including polythionates, thiosulfate, sulfite (SO32−), sulfate and small oxoacids of sulfur (H2SO3, H2SO4). Sulfate and small oxoacids of sulfur (H2SO3, H2SO4) can contribute to other metabolites, such as acid-labile sulfide (such as iron–sulfur clusters) and bound sulfane sulfur (−2). H2S predominantly exists (~80%) as the anionic form HS− under physiological conditions (pH 7.4).
Hydrogen sulfide (H₂S) has a crucial role in regulating cardiovascular function; reduced H₂S regulates various pathophysiological functions via interaction with nitric oxide, atherosclerosis and cardiac ischaemia–reperfusion injury. Findings from clinical studies demonstrate that H₂S and its metabolites, including activation of molecular signalling cascades, post-translational modification of H₂S and its synthesizing enzymes, affects cellular function. These findings are important because they demonstrate that hydropersulfides and polysulfides can be synthesized independently of H₂S. However, further studies are required to understand how these various pathways participate in cardiovascular pathophysiological responses.

**Production of sulfides**

Endogenous H₂S is produced in mammalian tissues by both enzymatic and non-enzymatic pathways. The basal level of production is determined by the activity of three main enzymes — cystathionine γ-lyase (CTh), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (MPST) — as well as by cysteine aminotransferase. Homocysteine, l-cysteine and their derivatives are common substrates of these H₂S-generating enzymes. Cysteine can also produce H₂S in the blood, catalysed by iron and vitamin B₆. Additionally, l-cysteine can be metabolized by n-amino acid oxidase to 3-mercaptopyruvate, which is subsequently converted to H₂S via MPST in mammalian cells. This pathway is functional only in the kidneys and the brain, particularly the cerebellum.

The synthesis of H₂S and its metabolites can be promiscuous with respect to substrate utilization and reactivity. The transsulfuration pathway of H₂S production via CBS and CTh uses homocysteine and l-cysteine, but these enzymes can also produce other biochemical forms of sulfide. CBS and CTh can use substrates such as cysteine or glutathione disulfide, resulting in the formation of cysteine hydrosulfide or glutathione hydrosulfide as well as polysulfides that are biologically important forms of bound sulfane sulfur. Hydroperoxides or polysulfides can be carried by proteins, such as plasma albumin, which can transport sulfane sulfur equivalents functioning as signalling mediators for various biological activities.

**Localization of H₂S-producing enzymes**

CBS and CTh are pyridoxal 5'-phosphate-dependent enzymes localized in the cytosol, with CBS being predominantly found in the brain and central nervous system and CTh primarily expressed in the cardiovascular system, although both enzymes are also found in the kidneys, liver, lymphocytes, placenta and pancreatic islets. MPST is localized in mitochondria and has been found in the heart, kidneys, liver and retina. Importantly, all three of these H₂S-synthesizing enzymes are expressed in cardiovascular cells. Translocation of CTh to the mitochondria under hypoxic conditions has been reported, and this enzyme can metabolize cysteine to produce H₂S and increase ATP production in the mitochondria when MPST activity is concomitantly reduced. Interestingly, this process has been attributed to CBS, which accumulates in mitochondria under hypoxic conditions because the degradation of CBS by Lon protease in the mitochondrial matrix is greatly reduced in the absence of oxygen. However, H₂S production in the brain is possible via MPST as an alternative to CBS. Likewise, upregulation of CBS can replenish H₂S levels in the cerebral cortex of CTh-deficient mice. Together, these findings show that translocation or expression of any of these enzymes can change to maintain H₂S synthesis when one of the other enzymes is genetically removed. Further studies are required to investigate the compensatory mechanisms of H₂S production under various pathophysiological conditions, including the tissue-specific roles of these enzymes.

**Sulfide catabolism**

The metabolic clearance of H₂S via detoxification pathways is crucial to maintaining an appropriate physiological balance of H₂S and its metabolites. The bioavailability of H₂S is influenced by both the direct catabolism and cysteine metabolism of endogenous H₂S in biological systems. Several enzymes catalyze H₂S — mitochondrial sulfide–quinone oxidoreductase (SQOR), which oxidizes H₂S to a hydropersulfide; mitochondrial persulfide dioxygenase ETHE1 (also known as ethylmalonic encephalopathy protein 1), which oxidizes the sulfide downstream of SQOR; and cysteine dioxygenase, which catalyzes cysteine to cysteine sulfinic acid. Additionally, cytosolic methylation, glutathione disulfide, or other metallo-containing or disulfide-containing molecules can scavenge H₂S and regulate its levels. Sulfates, such as thiosulfate, are major end products of
H₂S metabolism under physiological conditions⁴[FIG. 1a]. Sulfates can be further converted into sulfite and sulfate by thiosulfate–cyanide sulfurtransferase and sulfite oxidase, respectively. Lastly, H₂S and methaemoglobin form sulfhaemoglobin, resulting in H₂S depletion⁴⁸.

Detection of sulfide metabolites
Improved technology and novel analytical methods to identify H₂S in its various chemical forms have allowed the intricacies of this molecule’s bioavailability and biological function to be studied. However, the
Reviews those using analytical methods for detecting sulfide, hydropersulfides and polysulfides, such as accuracy of this approach for detecting important metabolic responses. Other analytical methods for detecting sulfide, hydropersulfides and polysulfides, such as those using β-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM) and N-iodoacetyl l-tyrosine methyl ester (TME-IAM), have also been reported. Polargraphic H2S sensors can also detect H2S levels in the nanomolar range and provide real-time measurement of H2S from biological samples. Although this method is reliable, some reports suggest that it might not detect sulfide. A gas chromatography–chemiluminescence sulfur detection method using an alkylation technique to extract H2S has also been reported to accurately measure H2S in biological samples at the nanomolar level. Numerous H2S and sulfane sulfur-sensitive fluorescence probes (including Washington State probe-1, synchronous fluorescence-1/2, dansyl azide, sulfide-selective fluorescent probe-1/2/7-azido-4-methylcoumarin, sulfane sulfur probe 4 and PSP-3) have been identified, and their use has rapidly expanded in the field of H2S pathobiology.

measurement of H2S can still be challenging owing to its complex chemical signature and the various biological forms of sulfide. Detection methods for free and acid-labile H2S and pools of sulfur have included hydroperoxides, polysulfides and oxoacids of sulfur — have been reviewed previously. In contrast to H2S, the biological effects of sulfur metabolites, including hydroperoxides and polysulfides, are largely unknown. Also, the functions of the H2S-producing enzymes in vascular disease remain unexplored and are a major knowledge gap. Fortunately, new analytical and biochemical methods have been developed to study hydroperoxides sulfane and polysulfide species. The pitfalls associated with sulfide quantification analysis have been reviewed previously.

Sulfides in the cardiovascular system

The three gasotransmitters are involved in regulating an array of vital biological functions in the cardiovascular, neurological and immune systems at the cellular and organ levels. NO and H2S have similar and inter-relating physiological and pathological functions in the cardiovascular system, and the signalling pathways of these molecules often work in tandem. H2S was initially identified as an endogenous neuromodulator and vasorelaxant, with subsequent studies revealing broader functions. The literature clearly demonstrates the protective effects and regulatory functions of H2S in animal models of cardiovascular pathophysiology, but the role of H2S and its metabolites in clinical CVD is less well studied.

H2S-synthesizing enzyme polymorphisms. H2S-synthesizing enzymes have a significant association with CVD. A correlation was found between H2S and NO bioavailability in patients with CVD, and H2S metabolite levels were predictive of CVD in a sex-specific and ethnicity-specific manner. Decreased levels of bound sulfane sulfur and total sulfide found in patients with coronary artery disease or peripheral artery disease were a statistically indicative biomarker for CVD. Moreover, a specific single-nucleotide polymorphism (SNP) in CTH (1364G>T) was also identified as a potential risk factor in a substudy cohort, with a greater allelic mutation frequency across all forms of CVD than the previously identified 894G>T SNP in NOS3 (encoding endothelial NO synthase (eNOS)), which was associated only with coronary artery disease. Similarly, a CTH 1364G>T polymorphism was identified in 178 white Greek patients undergoing coronary artery bypass graft surgery. Interestingly, the frequency
of the CTH 1364TT genotype was numerically higher (but not significantly different) in female patients than in healthy female control individuals, whereas there was no difference in the frequency of this SNP between male patients and controls. These studies suggest an association between CTH polymorphisms and CVD; however, molecular studies of these SNPs in other, larger populations is needed.

**Cardioprotective effects in IRI.** MI occurs when the heart muscle is deprived of blood carrying oxygen and nutrients, leading to acute tissue ischaemia and cell death. Although reperfusion relieves ischaemia, it also results in complex reactions leading to inflammation and oxidative damage, which contribute to infarct development. Growing evidence demonstrates that exogenous delivery of H₃S or modulation of endogenous H₃S improves cardiac function and reduces cardiac complications in IRI and various other cardiac conditions, including arrhythmias, heart failure, cardiac hypertrophy, myocardial fibrosis and MI. Exogenous H₃S therapy was shown to be cardioprotective in a mouse model of IRI. H₃S delivery reduced infarct size and preserved left ventricular function. Additionally, endogenous H₃S production by cardiac-specific CTH overexpression significantly limited myocardial injury. This study established that CTH−/−H₃S-mediated cryoprotection and inhibition of myocardial inflammation preserves myocardial and mitochondrial structure and function. Subsequent research from the same group identified the underlying protective mechanisms of CTH−/−H₃S therapy in a mouse model of heart failure. H₃S-induced cardiac protection was mediated via increased phosphorylation of RACa serine–threonine-protein kinase (AKT1; also known as protein kinase B), and nuclear localization of nuclear respiratory factor 1 and nuclear factor erythroid 2-related factor 2, which significantly increased antioxidative signalling, inhibited apoptosis and increased mitochondrial biogenesis. Treatment with the H₃S donor diallyl trisulfide for 12 weeks preserved left ventricular function, reduced left ventricular remodelling and improved angiogenesis mediated via vascular endothelial growth factor (VEGF)–NO signalling in a mouse model of transverse aortic constriction. These findings clearly imply the involvement of endogenous H₃S in maintaining basal physiological cardiac function.

H₃S therapy can protect against IRI via activation of the tyrosine–protein kinase JAK2–signal transducer and activator of transcription 3 (STAT3) signalling pathway. In a pig model of IRI, H₃S treatment markedly reduced MI-related damage, improving left ventricular function while concomitantly reducing apoptosis and increasing autophagy. Sodium hydrosulfide pretreatment protected rat isolated hearts against IRI by inhibiting opening of the mitochondrial permeability transition pore. Pharmacological inhibition of CTH increased infarct size in a rat model of IRI, which was rectified by H₃S therapy, leading to myocardial protection. Additionally, cardiac-specific CTH overexpression in transgenic mice significantly reduced infarct size and improved cardiac function compared with wild-type mice after IRI. These findings indicate that both exogenous H₃S donors and endogenously elevated H₃S levels protect the heart against IRI, revealing potentially important therapeutic targets.

Studies have suggested that the cardioprotective effects of H₃S are mediated through various pathways. H₃S has an important role in promoting angiogenesis and ameliorating type 2 diabetes mellitus that also protect against IRI. Endogenous H₃S production also augments antioxidant signalling via nuclear factor erythroid 2-related factor 2, reduces nuclear factor-κB (NF-κB)-mediated inflammatory signalling and facilitates NO signalling. Studies in animal models of MI, IRI and heart failure have revealed significant reductions in endogenous H₃S production that contribute to disease progression. H₃S also protects against MI and IRI by opening K⁺ATP channels. Furthermore, H₃S interacts with NO in a Cth−/− mouse model of heart failure. Cardiac remodelling and dysfunction were found to be worse in CTH-deficient mice than in wild-type mice. Reduced circulating H₃S levels in Cth−/− mice directly led to cardiac dilatation and dysfunction, whereas exogenous H₃S therapy had cardioprotective effects via upregulation of the VEGF–AKT1–eNOS–NO–cGMP pathway, resulting in preserved mitochondrial function and increased myocardial vascular density. Therapy with the sulfur-donating drug SG1002 in Cth−/− mice increased myocardial vascular density and improved cardiac remodelling and function via the same pathway. In a later study, SG1002 was found to effectively increase circulating H₃S and circulating NO bioavailability, while attenuating B-type natriuretic peptide levels (a marker of cardiomyocyte stress and left ventricular dysfunction) in patients with heart failure with reduced ejection fraction (NYHA class II–III).

**Cardiac dysfunction and hypertrophy.** Cardiac hypertrophy is a crucial compensatory mechanism in the failing heart. It increases cardiac output and can occur in response to chronic pressure or volume overload and after MI. However, persistent hypertrophy is deleterious, resulting in cardiac dilatation, loss of contractile function and decreased ejection fraction, subsequently leading to heart failure. The protective role of H₃S in pathogenic cardiac hypertrophy is being increasingly demonstrated. In a model of cardiac hypertrophy, endogenous H₃S reduced the production of reactive oxygen species (ROS) in the mitochondria and preserved cardiac mitochondrial membrane potential, thereby inhibiting hypertrophy and cardiomyocyte apoptosis and improving cardiac function. Furthermore, reduced levels of endogenous CTH and H₃S increased oxidative stress and induced cardiomyocyte apoptosis. Hypertrophic signalling pathways activated in response to MI are defective in Cth−/− mice, but treatment with the exogenous H₃S donor GYY4137 from 2 h after the onset of MI reduced infarct size, cardiac hypertrophy and adverse remodelling and preserved cardiac function in both Cth−/− and wild-type mice. An age-dependent association was found between MPST and cardiac hypertrophy in mice. In young adult animals (aged 2–3 months), knocking out Mpst had a cardioprotective effect;
however, in older mice (aged >18 months), the Mpo knockout resulted in reduced antioxidant signalling and subsequent hypertension and cardiac hypertrophy108.

**Endothelial function and vasodilatation.** The vascular endothelium is the active component lining the entire circulatory system and controls numerous responses, such as vascular tone, vessel remodelling, oxidative stress defences, thrombosis and inflammation107,108. Endothelial dysfunction is a crucial predictor of CVD108–110. NO is one of the most important substances produced by the vascular endothelium and, as discussed above, the association and interaction between the H₂S and NO signalling pathways have substantial implications for cardiovascular protection107–109. Our group and others have demonstrated that H₂S can preserve endothelial function through various mechanisms, including the post-translational stabilization of eNOS, leading to an increase in NO bioavailability, and the augmentation of nitrite–NO signalling6,11,111,112.

H₂S can exert vasodilator effects via regulation of the soluble guanylate cyclase (sGC)–phosphodiesterase–cGMP–protein kinase G (PKG; also known as cGMP-dependent protein kinase) vascular relaxation signalling pathway11 or via K⁺ ATP-channel and cGMP pathways117. In a rat renal hypertension model, treatment with the fast-releasing H₂S donor sodium hydro-sulfide (NaHS) dilated isolated aortic rings by relaxing vascular smooth muscle cells, mediated by increased cGMP–PKG activity, in a dose-dependent manner111. Similarly, the use of an H₂S and NO conjugated donor, ZYZ-803, induced time-dependent and dose-dependent vasodilatation of rat aortic rings by stimulating the cGMP pathway111. This vasorelaxant effect was suppressed with H₂S and NO inhibition. Inhibitors of PKG or the K⁺ ATP-channel had similar effects, demonstrating that the protective effects of H₂S and NO are mediated via K⁺ ATP-channel and cGMP pathways117. Another study, using human mesenteric arteries obtained from patients undergoing abdominal surgery, demonstrated NaHS-mediated K⁺ ATP-channel-dependent vasorelaxation118. This response was completely inhibited after endothelium denudation or inhibition of eNOS or cGMP, indicating a role for these signalling pathways in NaHS-mediated vasorelaxation118. Researchers demonstrated dose-dependent H₂S-induced vasoregulation in isolated blood vessels (including aortic, carotid, renal and iliac arteries) from rabbits119. As with NO donors, vasodilation occurred with low doses of H₂S, but vasoconstriction occurred with high doses of H₂S119. These studies clearly indicate that H₂S has a prominent role in regulating endothelium-dependent signalling activities (Fig. 2a).

Interestingly, in addition to the effects of H₂S, prolonged NO and cGMP signalling might be sustained by sulfide metabolite modifications of eNOS, cGMP or PKG120–122 (Fig. 2b). H₂S-mediated sulfhydration of eNOS Cys443 facilitates its catalytic activity, maximizing NO generation120. The HS⁻ anion can also mediate the electrophilic sulfhydration of 8-nitro-cGMP to form 8-SH-cGMP, which stabilizes cGMP release and modulates cellular redox signalling122. H₂S can also stabilize cGMP release by catalysing the formation of a protein disulfide within PKG1α, thereby stimulating the activity of PKG122. This modification has been shown to have substantial physiological effects that can reduce blood pressure. H₂S significantly lowers blood pressure in wild-type mice, but not in PKG1α Cys42Ser knock-in mice122, revealing the functional implications of this modification.

H₂S can induce sGC activation and decrease cGMP degradation by blunting phosphodiesterase activity. The involvement of CTH and H₂S in mediating the vasodilatation of aortic rings via cGMP was demonstrated through inhibition of cGMP-specific 3’,5’-cyclic phosphodiesterase (PDE5; also known as phosphodiesterase type 5)113. H₂S can increase sGC levels via sulfhydration of sGCβ1 and reducing sGCαβ1 dimers in vascular tissues124. Furthermore, H₂S markedly decreased PDE5A homodimer formation via sulfhydration of PDE5, thereby reducing PDE5 activity, facilitating cGMP stabilization and significantly decreasing levels of 5’-GMP124. Other studies have also demonstrated endothelium-dependent vasodilatation in response to H₂S donors via a NO–cGMP-dependent pathway113,125,126.

H₂S enzymatic pathways are important in the regulation of endothelial vascular function124. As discussed above, CTH-generated H₂S mediates smooth muscle relaxation and subsequent vasodilatation113,124. However, regulation of CTH in the vascular endothelium remains poorly characterized. Studies have shown that genetic deletion of H₂S-producing enzymes, and the subsequent reduction in H₂S levels, results in impaired vasodilatation124,114. In a global Cth−/− mouse model, reduced H₂S levels lead to hypertension124. Additionally, mesenteric arteries were markedly impaired in Cth−/− mice, and removal of the endothelium prevented methacholine-induced relaxation in both wild-type and mutant arteries125. Our group has extended this observation using a non-invasive, flow-mediated dilatation model in global Cth−/− mice126. Femoral artery dilatation was defective, and distal tissue blood flow was compromised. Both these effects were mediated by sulfide-dependent reduction of nitrite back to NO by xanthine oxidase and were reversed with dialyl trisulfide treatment124 (Fig. 2c). Another study demonstrated that deletion of Cth decreased H₂S and cardiac NO production, impairing endothelial-dependent vasorelaxation. Transgenic overexpression of endothelial CTH restored H₂S and NO levels in the cardiovascular system and vasorelaxation in thoracic aorta127. These studies reveal interactions between H₂S and NO signalling in the regulation of vascular tone. However, further research is needed to understand the mechanisms mediated by cell-specific functions of CTH, H₂S and its metabolites.

**Inflammation and atherosclerosis**

Evidence suggests that H₂S protects against the development and progression of atherosclerosis129,130, which involves endothelial dysfunction and vascular inflammation and is a major mediator of clinical CVD. Exogenous H₂S supplementation has salutary effects on atherogenesis, and the reduction in endogenous CTH or H₂S levels accelerates atherosclerosis129,131,132. Atherosclerotic
lesion formation was inhibited by NaHS in ApoE−/− mice, whereas the CTH inhibitor D,L-proparglyglycine significantly reduced H₂S levels and resulted in accelerated plaque formation. Genetic CTH deficiency significantly increases atherosclerosis development in ApoE−/− mice. Disruption of the vascular redox status was observed, as well as increased intimal proliferation and inflammatory adhesion molecule expression. Exogenous H₂S treatment inhibits the expression of endothelial cell adhesion molecules, including intercellular adhesion molecule 1, vascular cell adhesion protein 1 and E-selectin, by suppressing NF-κB activity and attenuating atherosclerotic pathogenesis. Exogenous H₂S therapy protects the endothelium, inhibits the development of vascular lesions and reduces blood pressure in ApoE−/− mice fed a high-fat diet. In this study, H₂S donors such as diallyl disulfide and diallyl trisulfide protected against oxidized LDL-induced atherosclerotic plaque formation by inhibiting the activation of eNOS.

Homocysteine metabolizes in the body to produce H₂S. However, increased homocysteine synthesis (hyperhomocysteinaemia) inactivates CTH. Hyperhomocysteinaemia has a strong correlation with premature coronary artery disease secondary to atherosclerosis via decreased H₂S production, which leads to sustained endothelial cell injury and the induction of vascular smooth muscle cell proliferation. H₂S can induce anti-inflammatory signalling via peroxisome proliferator-activated receptor-δ (PPARδ) and suppressor of cytokine signalling 3 (SOCS3), which mediates vascular remodelling. Therefore, endogenous H₂S deficiency could be a risk factor for vascular smooth muscle cell dysfunction. Endogenous H₂S deficiency generated vascular remodelling with aggregated active and passive contraction, thickened aortic walls, collagen deposition, increased STAT3 phosphorylation and decreased aortic production of PPARδ and SOCS3, which were all reversed by treatment with NaHS. Importantly, SOCS3 mediates anti-inflammatory effects in hypertension and obesity via inhibition of tyrosine-protein kinase JAK1–STAT signalling, preserving endothelial function in experimental hypertension, suppressing inflammation in macrophages after treatment with lipopolysaccharides and inhibiting vascular smooth muscle cell proliferation. These studies strongly establish anti-atherogenic and anti-inflammatory roles for CTH and H₂S in animal models of atherosclerosis.
Angiogenesis and vascular remodelling. Angiogenesis is a regulated process of microvascular growth that can revascularize ischaemic tissue. H₂S induces angiogenesis by increasing endothelial cell proliferation and migration. Exogenous H₂S (NaHS) increases cell growth, migration and the formation of tube-like structures in cultured endothelial cells. These effects were concentration-dependent and mediated via phosphatidylinositol 3-kinase (PI3K)–AKT1 signalling. The researchers confirmed their observations in vivo using a Matrigel plug assay to assess neovascularization in mice.
Studies from our group and others have established that H₂S promotes arteriogenesis and angiogenesis, and improves regional blood flow in ischaemic limbs, indicating prominent vascular growth and remodelling in ischaemic tissues. Chronic ischaemia of the limb during peripheral vascular disease remains largely resistant to medical therapy, and translational approaches to restore perfusion to the distal limb and improve outcomes are limited. Therefore, H₂S is an attractive therapeutic target for limb ischaemia. A study showed the pro-angiogenic effects of H₂S in a rat model of chronic limb ischaemia. H₂S upregulated collateral vessel growth and capillary density mediated by upregulation of the VEGF–VEGFR2 pathway. Similarly, an H₂S donor restored vascular density and remodelling and, subsequently, blood flow and tissue perfusion in mice with hindlimb ischaemia. These effects were mediated via upregulation of the hypoxia-inducible factor 1α–VEGF–VEGFR2 pathway that induces the eNOS–sGC–cGMP–PKG system downstream. Our group has demonstrated a unique interaction between H₂S and NO, in which H₂S significantly increases NO levels in plasma and ischaemic limb tissue, followed by downregulation of H₂S when NO levels are elevated, suggesting a hierarchical order of gasotransmitter production. These beneficial effects of H₂S on NO levels in ischaemic tissue did not depend exclusively on NO activity, because nitrite anion reduction back to NO was also involved and was blunted by febuxostat-dependent inhibition of xanthine oxidase.

In Cth−/− mice, chronic tissue ischaemia was associated with impaired ischaemic vascular remodelling and reductions in endogenous H₂S production, monocyte recruitment and expression of VEGF and fibroblast growth factor 2 (FGF2; also known as basic fibroblast growth factor). Exogenous treatment with diallyl trisulfide restored plasma and tissue NO levels, monocyte recruitment, arteriogenesis, ischaemic vascular remodelling and an angiogenic cytokine expression pattern. VEGF receptor 2 (VEGFR2) can also act as a receptor target for H₂S during angiogenesis. Downregulation of VEGFR2 during ischaemia can be reversed by H₂S via phosphorylation at Tyr996 of the receptor. Exogenous H₂S can also increase AKT1 phosphorylation and upregulate angiogenic signalling including mitogen-activated protein kinase 1 (MAPK1), MAPK3 and MAPK11 (also known as ERK2, ERK1 and p38, respectively), which can be attenuated by MAPK inhibition, indicating a role for this pathway in H₂S-mediated angiogenesis.

Shear stress. Shear stress has major effects on vascular function and stimulates adaptive changes in blood vessel structure and size. Vascular endothelial cells are exposed to haemodynamic forces, which modulate their functions in health and disease. Low, or oscillatory, shear stress can promote vascular dysfunction and atherosclerosis, whereas physiological high shear stress is protective. Changes in blood flow can trigger a cascade of biochemical signalling that mediates changes in biological events. Endothelial cells are crucial sensors of shear stress, but the mechanisms by which they decode complex shear stress environments to regulate physiological and pathophysiological responses are incompletely understood.

Shear stress-induced collateral vessel formation can be inhibited by blocking NO–VEGF–Rho GTPase signalling pathways and by upregulation of signaling mechanisms facilitating monocyte recruitment and attachment to the endothelium via adhesion molecules. Our group has revealed the role of CTH and H₂S in shear stress. In a Cth−/− mouse model of partial carotid ligation, reduced medial thickening and a dilated arterial phenotype was identified, indicating a defective inward vascular remodelling response (Fig. 5a). Oscillatory shear stress upregulated CTH expression and subsequent sulfane sulfur levels, which induced monocyte and macrophage recruitment into regions of disturbed flow. Importantly, a reduction in inward vascular remodelling in Cth−/− mice was associated with increased NO bioavailability that was reversed by the NO scavenger cPTIO. These findings reveal that CTH expression is important in shear stress-dependent responses in athroprone vascular regions and involves both endothelial activation and flow-dependent vascular remodelling through altered NO bioavailability. In accordance with our observations, other groups have demonstrated the role of CTH and sulfane sulfur in atherosclerosis under varied shear stress. Endothelial-specific Cth deletion accelerated the development of endothelial dysfunction and atherosclerosis. CTH expression was upregulated in a mouse model of partial carotid artery ligation and in atheromas from human patients. However, circulating and intraplaque H₂S levels were reduced owing to Ser377 phosphorylation of CTH, which inhibits the enzyme (Fig. 5b). Consistent with the loss of H₂S, human atheroma R (HuR) sulfhydration was blunted in atherosclerosis, resulting in stabilization of the HuR target mRNAs encoding E-selectin and chemokine S, both of which are linked to endothelial cell activation and atherosclerosis. CTH-derived H₂S can sulfhydrate HuR Cys13 and prevent its homodimerization and activity, thereby attenuating the expression of E-selectin and chemokine S. As such, increased E-selectin expression facilitates
monocyte adherence and recruitment under atherogenic conditions. The endothelial dysfunction and atherosclerosis associated with Cth deletion in endothelial cells were reversed with administration of the polysulfide donor SG1002, indicating its potential use in modulating inflammatory vascular responses.10

Another study by the same group demonstrated the molecular mechanisms of shear stress-mediated reduction of CTH expression in human and mouse endothelial cells.58 An inverse relationship was observed between CTH and Krüppel-like factor 2 (KLF2), which is involved in shear-stress mediated atheroprotective pathways.59 CTH was identified as a direct target of the KLF2-regulated microRNA-27b. Increased CTH expression in human plaque-derived endothelial cells also negatively correlated with KLF2 and microRNA-27b levels.64 However, decreased CTH expression led to the loss of peroxiredoxin 6 (PRX6) Cys47 sulfhydration causing PRX6 hyperoxidation and inhibition, which subsequently increased endothelial ROS and lipid membrane peroxidation. These effects were reversed by polysulfide supplementation.60 Additionally, statin therapy, which can activate KLF2, decreased CTH expression and increased CTH activity, thereby preventing phosphorylation of CTH at Ser377 and partially restoring PRX6 sulfhydration in plaque specimens from arteries of statin-treated patients.60

In 2021, the same group of researchers reported mechanotransduction signalling changes via proteome S-sulfhydration in the setting of atherosclerotic vascular dysfunction.61 In this study, 3,446 cysteine residues from 1,591 proteins in endothelial cells that can influence vascular reactivity were analysed. S-sulfhydration of β3 integrin was required for mechanotransduction in native endothelial cells isolated from mouse and human vessels. Exogenous sulfide treatment with SG1002 resulfhydrated endothelial cell proteins and β3 integrin, partially restoring endothelial cell function and vascular blood flow.62 These observations indicate a potential role for polysulfide therapeutics in rectifying vascular function in human vascular disease.

Vascular barrier function. Vascular permeability and endothelial selective molecular sieving are crucial for several physiological functions, including tissue–fluid homeostasis, angiogenesis and vessel tone.63 Regulated passage of macromolecules between the blood and interstitial space is important for physiological homeostasis. Vascular hyperpermeability is associated with numerous physiological and pathophysiological processes, such as inflammation, tumorigenesis, ischaemic injury, wound healing, and vascular growth and remodelling.64 As discussed above, CTH and H2S have important regulatory roles in vessel remodelling and maintenance of cellular homeostasis, and cytotoxic effects.65,66

Vascular permeability can be increased via upregulation of VEGF and extracellular matrix signalling pathways, which causes endothelial contraction and junction protein disruption, resulting in intercellular gaps with greater permeability.67 H2S therapy inhibits vascular hyperpermeability and endothelial blood–brain barrier disruption in mice undergoing cardiac arrest. Treatment with exogenous H2S was shown to decrease matrix metalloproteinase 9 (MMP9) activity and VEGF expression, and increase the expression of angiogenin I, preserving the normal function of the blood–brain barrier.68 A study of ethanol-induced toxicity in mouse brain endothelial cells demonstrated the protective effects of H2S on endothelial hyperpermeability.69 In a subarachnoid haemorrhage model, NaHS therapy attenuated brain oedema, blood–brain barrier disruption and cerebral vasospasm.68 In addition, exogenous H2S was shown to reduce vascular protein leakage and leukocyte infiltration in a mouse model of particulate matter-induced lung inflammation.68

Our group has shown that H2S and polysulfides regulate permeability and barrier function in mouse aortic endothelial cells.64 Reduction of CTH expression in either Cth−/− cells or via small interfering RNA inhibition resulted in tighter endothelial barrier function. Genetic loss of CTH expression and reduced bound sulfane sulfur levels prevented VEGF-mediated permeability in vivo. Importantly, the reduction in CTH and sulfide metabolite levels augmented claudin 5 expression and enhanced tight junction arrangement, contributing to improved endothelial barrier function (Fig. 3c). Although permeability is crucial in regulating both cardiovascular and cerebrovascular homeostasis, most of the literature is currently focused on the blood–brain barrier.65,67 Further studies investigating CTH regulation of sulfide and its metabolites on changes in endothelial solute permeability are needed to increase our understanding of the endothelial barrier dysfunction during pathophysiological conditions.

Cardiac arrhythmias. H2S is postulated to be antiarrhythmic but, although some molecular pathways have been explored, cell studies, animal models and translational research on this hypothesis are limited. The clearest evidence so far linking H2S and arrhythmias is the capacity of this molecule to regulate the electrical properties of cardiac tissues. H2S modulates ion channels both directly and indirectly, leading to electrical remodelling (Fig. 3c,d). Ca2+ and Ca2+-binding proteins are intrinsically involved in cardiac arrhythmias. Variants in L-type Ca2+ channels are linked to a variety of arrhythmias, and sulfide donors are known to inhibit L-type Ca2+ currents and reduce intracellular Ca2+ concentrations.67,68 A decrease in action potential duration (APD) was reported with sodium hydrosulfide, facilitated by the reduction in peak L-type Ca2+ current and Ca2+ transients.69 Although sulfide donors are also known to modulate T-type Ca2+ channels in the nervous system and gastrointestinal tract, no studies have been reported on the effects of H2S on T-type Ca2+ currents in cardiomyocytes.14,68

In addition to regulating voltage-gated ion channels, H2S also affects Ca2+-binding proteins. Ca2+/calmodulin-dependent protein kinase II (CaMKII), a ubiquitous and abundant serine–threonine kinase, has emerged as an important signalling molecule in cardiac arrhythmias. CaMKII has been implicated in the mechanisms of sinus node dysfunction, atrial tachyarrhythmias and ventricular arrhythmias.15,16 H2S inhibits
CaMKII, thereby potentially acting as an antiarrhythmic molecule. Sulfide donors, such as sodium NaHS, inhibit CaMKII phosphorylation through its sulfhydration. Moreover, reduced levels of H$_2$S in Cth$^{-/-}$ mice have been associated with increased CaMKII activity$^{97}$.

Treatment of rat atrial myocytes with NaHS has been shown to reduce APD and decelerate the sinus rhythm$^{98}$. Decreases in APD at 50% and 90% repolarization by NaHS were blocked by the K$_{ATP}$ channel blocker glibenclamide, suggesting that sulfide-induced APD shortening is mediated by K$_{ATP}$ channels$^{99}$. This effect of NaHS on APD shortening has been replicated in rat ventricular myocytes$^{100}$. Although the mechanism behind the effects of sulfide donors in opening the K$_{ATP}$ channels is not well understood, on the basis of findings in vascular smooth muscle cells, sulfide donors are thought to cause sulfhydration of the K$_{6,1}$ subunit of the K$_{ATP}$ channel$^{99}$.

In addition to modulating K$_{ATP}$ channels, blockade of H$_2$S biosynthesis with DL-propargylglycine has been shown to increase angiotensin II-induced K$_{ATP}$ expression in cultured atrial myocytes from neonatal rats$^{101}$. Moreover, in the same study, 24-h rapid atrial pacing in a beagle model of atrial fibrillation (AF) increased atrial angiotensin II and K$_{ATP}$ expression, which was inhibited by NaHS supplementation$^{101}$. Although the effects of H$_2$S on ion channels might be the primary antiarrhythmic mechanism, H$_2$S can also reduce adverse structural remodelling$^{102}$. Electrical anisotropy increases with age-related fibrosis by aiding electrotonic coupling between cardiomyocytes and fibroblasts or myofibroblasts, and can lead to electrical dissociation in the atrium and AF$^{103}$. In cell proliferation assays with human cultured fibroblasts, NaHS reduced atrial fibroblast proliferation induced by transforming growth factor-β1, mothers against decapentaplegic homologue 3 (SMAD3) and angiotensin II$^{104}$. Furthermore, H$_2$S also inhibits the differentiation of fibroblasts into myofibroblasts$^{105}$.

Diabetes increases atrial fibrosis, decreases atrial expression of the PI3K–AKT–eNOS pathway, and increases the inducibility and duration of AF in rats$^{106}$. These effects were inhibited by intraperitoneal injection of NaHS$^{107}$. Our group found that Cth$^{-/-}$ mice with reduced levels of endogenous H$_2$S had increased AF inducibility and duration compared with wild-type mice, which was reversed by extrinsic supplementation with the H$_2$S donor diallyl trisulfide$^{108}$. Low sulfide levels in the atria of Cth$^{-/-}$ mice were related to increased superoxide levels, increased frequency of atrial cell Ca$^{2+}$ sparks, prolonged APD and atrial effective refractory period, and slower atrial conduction velocity [FIG. 5d]. In a case–control analysis performed in parallel to this study, we found that patients with AF had reduced levels of acid-labile sulfide (the storage form of H$_2$S) compared with control individuals who had other cardiovascular conditions. We also showed a novel association between endothelial dysfunction and atrial remodelling mediated by H$_2$S in the pathogenesis of AF$^{109}$. Uniquely, H$_2$S can also act as a paracrine signalling molecule. In the global Cth$^{-/-}$ mouse model of AF discussed above, transgenic reconstitution of CTH in endothelial cells reduced the atrial effective refractory period and APD, normalized the frequency of Ca$^{2+}$ sparks, and decreased the inducibility and duration of AF$^{109}$.

H$_2$S has been shown to be antiarrhythmic not only in the atria; emerging research indicates that sulfide donors might also have a role in preventing life-threatening ventricular arrhythmias. NaHS was first shown to reduce the arrhythmia burden in an ex vivo model of IRI$^{110}$. In another rat model of IRI, α-lipoic acid increased H$_2$S and sulfane sulfur levels, thereby reducing ventricular ectopy and sustained ventricular arrhythmias$^{112–114}$. CTH was reported to be upregulated in the heart of rats with IRI and, interestingly, plasma H$_2$S levels were inversely related to the arrhythmia scores$^{115}$. In a later study, mitochondrial sulfide donor compounds, but not global sulfide donors, reduced the incidence of ventricular arrhythmias in a rat in vivo model of ischaemia–reperfusion$^{116}$. These studies show that intracellular and paracrine H$_2$S signalling can regulate electrical and structural remodelling in the heart, reducing the risk of arrhythmias mediated by various risk factors.

**Sulfide therapies for CVD**

As discussed in this Review, many cardiovascular conditions — including hypertension, stroke, IRI, cardiac hypertrophy and fibrosis, atherosclerosis, arrhythmias and vascular pathologies related to diabetes — can potentially be treated with H$_2$S$^{146–149}$. Clinical studies have shown that plasma H$_2$S levels inversely correlate with the severity of CVD, particularly hypertension and stroke, and children with hypertension have reduced plasma H$_2$S levels compared with healthy children$^{150,151}$. TABLE 1 lists interventional and observational clinical trials related to sulfide treatment for CVD.

**Administration of sulfides**

Many natural products and drugs in current use carry sulfur-derived functional groups. Garlic has been used for centuries in traditional medicine and contains allicin that rapidly degrades into diallyl polysulfides, which can act as H$_2$S donors in the presence of thiols$^{152}$. Preclinical and clinical trials have shown that garlic consumption reduces the risk of CVD$^{152–154}$. Pharmacologically, H$_2$S can be administered in several ways, including by direct inhalation of the gas and orally or intravenously as inorganic sulfides or natural and synthetic H$_2$S donors$^{155,156}$. Each method has advantages and disadvantages. Inhalation of H$_2$S can provide targeted treatment for conditions involving pulmonary defects, but carries a risk of toxicity and flammability. Oral or intravenous administration of inorganic sulfides can be site-directed, but these compounds have short half-lives and oxidize rapidly, which limits their use. Many natural and synthetic H$_2$S donors have poorly understood pharmacological mechanistic effects and possible toxicities$^{156}$.

**Synthetic H$_2$S donors**

Many currently available sulfide salts, natural H$_2$S compounds and synthetic H$_2$S donors have unsuitable pharmacokinetic profiles and undergo rapid hydrolysis, releasing H$_2$S in an uncontrollable manner that limits their clinical utility$^{157}$. Therefore, various novel, chemically stable and efficacious H$_2$S donors are being
| Trial name | Study type | Number of patients | Status | Study population | Main findings | Intervention | Study period (year) | Ref. |
|-----------------|-------------|-------------------|--------|------------------|---------------|--------------|---------------------|------|
| **Interventional trials using sulfide donors** | | | | | | | | |
| Assessing the safety and ability of SG1002 to overcome deficits in hydrogen sulfide in heart failure patients | Randomized controlled trial | 16 | Completed | Patients with heart failure and healthy individuals | SG1002 increases H₂S and NO bioavailability | SG1002 versus placebo | 2014–2015 | 102 |
| Assessing the safety and bioactivity of SG1002 in heart failure patients | Randomized, double-blind, placebo-controlled trial | 50 | NA | Patients with heart failure | N/A | Sodium polysulphionate versus placebo | 2016–2018 | 204 |
| Sodium thiosulfate to preserve cardiac function in STEMI | Multicentre, double-blind, randomized controlled trial | 38 | Active, not recruiting | Patients with MI and/or heart failure | N/A | Sodium thiosulfate versus placebo | 2018–2021 | 205 |
| Taurine supplementation on lower extremity vasculopathy in patients with diabetes | Randomized, double-blind, placebo-controlled trial | 20 | NA | Patients with diabetes mellitus and/or lower-extremity artery disease | N/A | Taurine versus placebo | 2017–2018 | 209 |
| Effects and safety of taurine granule on blood pressure in prehypertensive (ESTAB) | Randomized, double-blind, placebo-controlled trial | 12 | NA | Patients with prehypertension | N/A | Taurine granules versus placebo | 2012–2015 | 207 |
| Short-term endogenous hydrogen sulfide upregulation | Randomized clinical trial | Planned 40; actual 9 | Completed | Patients with carotid stenosis and undergoing carotid endarterectomy | Dietary intervention increased abundance of sulfide-producing bacteria and was protective in patients undergoing carotid endarterectomy | Protein calorie restriction versus controlled regular diet | 2017–2018 | 208 |
| Effect of garlic (Allium sativum) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease | Placebo-controlled trial (randomization unclear) | 60 | Completed | CAD | Polysulfides (diallyl disulfide and diallyl trisulfide) in garlic oil showed antiplatelet activity | Garlic oil versus placebo | 1997 | 209 |
| A randomized trial of the effects of garlic oil upon coronary heart disease risk factors in trained male runners | Randomized, double-blind, placebo-controlled trial | 27 | Completed | Healthy male runners aged 17–45 years | Garlic oil supplementation reduced total cholesterol and triglyceride levels, thereby lowering the risk of chronic heart disease | Garlic oil versus placebo | 2000 (publication date) | 210 |
| Clinical study on effect of garlicin in stabilizing the carotid artery atherosclerotic plaque in patients with primary hypertension and coronary artery disease | Randomized controlled trial | 79 | Completed | Patients with primary hypertension and CAD | Garlicin is vasoprotective in patients with primary hypertension and carotid artery atherosclerotic plaque | Garlicin and fosinopril versus fosinopril alone | 2006 (publication date) | 211 |
| Effect of combined supplementation of fish oil with garlic pears on the serum lipid profile in hypercholesterolemic subjects | Controlled clinical trial (no randomization, no placebo) | 32 | Completed | Patients with hypercholesterolaemia | Co-administration of garlic pears with fish oil can be effective in managing dyslipidaemia | Fish oil with garlic versus placebo | 2005 (publication date) | 212 |
Sodium thiosulfate is stable relative to other H₂S donors and is used for the treatment of cyanide intoxication, calcific uremic arteriolopathy and renal toxicity induced by chemotherapy. This compound could also have value in treating CVD. For example, in mice with arteriovenous fistula-induced heart failure, treatment with sodium thiosulfate-supplemented drinking water attenuated cardiac decline and reduced the expression of MMP1, MMP9 and adenylate cyclase type 6, suggesting that this H₂S donor restores cardiac function partly by increasing endogenous ventricular H₂S synthesis. In rats with angiotensin II-induced hypertensive heart disease, intraperitoneal injection of sodium thiosulfate attenuated hypertension, increased mRNA expression of natriuretic peptides, and reduced cardiac hypertrophy, oxidative stress, fibrosis and fibrosis-associated gene expression. Similarly, in rats with chronic deficiency of NO induced by the administration of Nω-nitro-L-arginine, sodium thiosulfate-supplemented drinking water improved systolic function and reduced hypertension, left ventricular hypertrophy, cardiac fibrosis and oxidative stress. Interestingly, sodium thiosulfate was also cardioprotective in a rat model of cardiac ischaemia–reperfusion. SG1002 is a novel, α-sulfur derivative markedly suppresses gastric prostaglandin synthesis without causing the gastric mucosal damage associated with chronic administration of non-steroidal anti-inflammatory drugs.

**Novel targets**

An exciting aspect of H₂S donors and CVD lies in the many novel targets yet to be examined. For example, the mitochondrial protein mitofusin 2 is regulated by H₂S, and its dysfunction contributes to several cardiovascular pathologies, including dilated cardiomyopathy, heart failure and IRI. Currently, no data exist on the...
Conclusions

Sulfides are crucially involved in cardiovascular health and disease. Although much has been learned about the various roles of sulfides, their synthesis and their catabolism, the field is still striving to understand specific mechanisms, mediators and conditions in which therapeutic sulfides could affect cardiovascular pathophysiology. Many important questions remain in the field of sulfide-based therapeutics for CVD. For example, how do sulfide metabolites affect cardiovascular cell function and disease? How do sulfide-synthesizing enzymes function in specific cardiovascular cell types and under various pathological conditions? What are the key molecular targets for sulfide-dependent cytotoxic protection against CVD? Are these molecules robust biomarkers for measuring the clinical efficacy of sulfide therapies? Which sulfide-based therapies are most effective in the treatment of CVD? We hope that future studies will help to provide the data needed to support the clinical use of sulfides in the treatment of CVD.
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Competing interests

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