REVIEW

Hydrogen sulfide-mediated cardioprotection: mechanisms and therapeutic potential

Madhav LAVU, Shashi BHUSHAN and David J. LEFER
Department of Surgery, Division of Cardiothoracic Surgery, The Carlyle Fraser Heart Center, Emory University School of Medicine, Atlanta, GA 30308, U.S.A.

ABSTRACT

H_2S (hydrogen sulfide), viewed with dread for more than 300 years, is rapidly becoming a ubiquitously present and physiologically relevant signalling molecule. Knowledge of the production and metabolism of H_2S has spurred interest in delineating its functions both in physiology and pathophysiology of disease. Although its role in blood pressure regulation and interaction with NO is controversial, H_2S, through its anti-apoptotic, anti-inflammatory and antioxidant effects, has demonstrated significant cardioprotection. As a result, a number of sulfide-donor drugs, including garlic-derived polysulfides, are currently being designed and investigated for the treatment of cardiovascular conditions, specifically myocardial ischaemic disease. However, huge gaps remain in our knowledge about this gasotransmitter. Only by additional studies will we understand more about the role of this intriguing molecule in the treatment of cardiovascular disease.

INTRODUCTION

Despite the recent discovery that H_2S (hydrogen sulfide) is a biologically important signalling molecule with a myriad of physiological actions, this gas has long been considered a deadly toxic pollutant. H_2S is believed to be the cause of at least one mass extinction, the Permian–Triassic extinction event that occurred 251.4 million years ago [1,2]. It is believed that vast amounts of H_2S were generated in the oceans and atmosphere during these periods, resulting in the death of a majority of species [3]. Only species with the ability to use H_2S in a process known as chemosynthesis were able to survive these hostile environments and this led to the continuation of life. As the environment was slowly depleted of H_2S, primitive organisms known as autotrophs slowly evolved to use water instead of H_2S and switched to photosynthesis [4]. Amazingly, the primitive process of chemosynthesis is still active in remote regions of the earth. Chemosynthesis sustains whole ecosystems discovered at deep-sea hydrothermal vents such as those in proximity to the Galapagos Islands [5]. Here, 2438 m (8000 feet) below the surface, in the absence of sunlight, bacteria thrive on noxious chemicals including H_2S to sustain a diverse range of living organisms [6]. In fact, some marine biologists believe that life originated in deep waters near these hydrothermal vents where H_2S is abundantly present. Interestingly the concentrations of H_2S present in these harsh habitats are comparable with those present in the human colon, which is home to gut flora [7].

Whether H_2S sustains life or harms it depends on the concentration at which it is present in the environment and how it is metabolized by the organisms that encounter it. Effects also depend upon the species in question. For instance, H_2S in controlled doses has

Key words: cardiovascular disease, cardioprotection, drug therapy, hydrogen sulfide (H_2S), nitric oxide (NO).

Abbreviations: BP, blood pressure; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; DADS, diallyl disulfide; I/R, ischaemia/reperfusion; IPC, ischaemic preconditioning; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; 3-MST, 3-mercaptopyruvate sulfurtransferase; NOS, NO synthase; eNOS, endothelial NOS; Nrf, nuclear factor-erythroid 2-related factor; NSAID, non-steroidal anti-inflammatory drug; PKC, protein kinase C; SAC, *S*-allylcysteine; SNP, sodium nitroprusside; SPRC, *S*-propargylcysteine.

Correspondence: Professor David J. Lefer (email dlefer@emory.edu).

been shown to induce a state of suspended animation in some mammals [2,8,9]. At high doses, however, H_2S is lethal. The harmful nature of H_2S was discovered in 1713 by Bernardino Ramazzini, an Italian physician, who observed that workers involved in cleaning the then cesspits and privies were exposed to a 'volatile acid', which led to inflammation of eyes. Ramazzini noted further that copper and silver coins in the pockets of these workers turned black. Since this astute observation, most studies have focused on the toxic effects of H_2S. It was only during the last decade or so that H_2S has been shown not only to be present widely in mammalian tissues, but that it also plays a critical role in many pathophysiological processes leading to cell preservation. In the present review, we will summarize experimental studies that have led us to begin to understand the role of H_2S in cardiovascular physiology and pathophysiology. We will also point out the potential for H_2S-based therapeutics in the treatment of cardiovascular disease.

PRODUCTION AND METABOLISM OF H_2S

Endogenously, H_2S is produced from the degradation of L-cysteine by the action of the enzymes CBS (cystathionine β-synthase) and CSE (cystathionine γ-lyase) [10]. The kinetics of H_2S synthesis and release are currently not understood. [11,12]. CBS and CSE are cytosolic enzymes that synthesize H_2S in many tissues, including the cardiovascular system, nervous system and gastrointestinal system [13]. H_2S is also generated from 3-MST (3-mercaptopyruvate sulfurtransferase) derived from sulfane sulfur in the presence of appropriate levels of cellular reductants. [14]. Although 3-MST is both a mitochondrial and cytosolic enzyme with approximately two-thirds of 3-MST existing in the mitochondria [15–17], its contribution to *in vivo* generation of H_2S is currently unknown [14].

At physiological pH, four-fifths of H_2S exists as HS^- and only one-fifth exists as undissociated H_2S [18,19]. A trace amount of S^{2-} may also exist in solution [20]. Generally in the scientific literature, the term 'H_2S' refers to the sum of all three species [18,19]. After synthesis, excess H_2S can be stored in two different forms: sulfane sulfur [21,22] and acid-labile sulfur [23].

Individual organs have been reported to differ in their ability to synthesize H_2S. In adult rats, H_2S generation is greatest in the liver and brain, followed by kidney, heart, aorta and small intestines [10,24–26]. Liver produces H_2S in amounts greater than vascular tissue and has been proposed to be the chief source of circulating H_2S [24]. Additionally, gut flora and intestinal enzymes produce H_2S [27].

The activity of H_2S-synthesizing enzymes may be regulated by multiple factors such as circulating glucocorticoid hormones and cAMP [28]. Additionally, CBS is inhibited by endogenously produced gasotransmitters such as NO (nitric oxide) and CO (carbon monoxide) [29]. Although dietary aspartate reduces H_2S synthesis, several dietary proteins and amino acids [such as methionine, cysteine, arginine, SAM (*S*-adenosylmethionine), glycine, *N*-acetylcysteine and NMDA (*N*-methyl-D-aspartate)] augment the production of H_2S [30].

Once released, H_2S can be metabolized by a variety of metabolic pathways. Mitochondrial oxidative pathways convert H_2S into thiosulfate, which is converted further into sulfite and finally into sulfate (the major end product of H_2S metabolism) [31]. Since oxidation of cysteine also contributes to urinary sulfate content [32], urinary thiosulfate is a specific marker of whole-body H_2S production [33]. The second metabolic pathway is methylation by thiol S-methyltransferase in the cytosol [34]. Additionally, H_2S binds to methaemoglobin and other disulfide-containing molecules such as GSSG (oxidized glutathione) [35]. Finally, H_2S is oxidized by NADPH oxidase to produce SO_2 (sulfur dioxide) [36]. Both SO_2 and H_2S are exhaled through the lungs. These metabolic pathways have been established only recently and more pathways may be uncovered in the near future.

The exact physiological concentration of H_2S in various tissues is still unknown and there is a wide variation in the level of sulfide reported in circulating blood since methods used to measure bound as well as free sulfide levels are different [37,38]. Therefore with the current knowledge a comprehensive view of H_2S metabolism is lacking [12]. However, this limitation has not deterred a substantial number of workers from describing the actions of both endogenously produced and exogenously administered H_2S.

ROLE OF H_2S IN PHYSIOLOGY AND PATHOPHYSIOLOGY

Role in vascular tissues

Although H_2S is being projected as chiefly a vasodilator in vascular tissues, a number of conflicting actions have been reported in the literature [12,20] (Table 1). On one hand, H_2S has been reported to relax several isolated non-coronary blood vessels [39–48], but, on the other, H_2S failed to relax coronary vessels *ex vivo* [49]. In contrast, H_2S is reported to cause vasoconstriction [50]. Furthermore, a few *in vitro* studies have reported that H_2S causes both contraction and relaxation of isolated vessels depending on the concentration of H_2S administered [19,51]. The variable effects also depend on the oxygen concentration at which the study is conducted, with vasoconstriction at high oxygen concentration, but vasodilation at low oxygen concentration [52]. Perplexingly, in several studies, H_2S caused a 'triple-phase response' with initial relaxation, followed by constriction

Table 1 Actions of H_2S on blood vessels

Effect	Reference
Vessel wall relaxation	Hosoki et al. [39]
	Zhao et al. [40]
	Ali et al. [41]
	Cheng et al. [42]
	Fiorucci et al. [43]
	Kiss et al. [44]
	Lee et al. [45]
	Wang et al. [46]
	Srilatha et al. [47]
	Zhao et al. [48]
Vessel wall constriction	Lim et al. [50]
Both vessel wall relaxation and constriction	Dombkowski et al. [18]
	Webb et al. [19]
	Kubo et al. [51]
	Koenitzer et al. [52]
	Predmore et al. [53]
	Dombkowski et al. [54]
	Olson et al. [55]
No action on vessel wall	Johansen et al. [49]

and then ending again with relaxation [18,53,54]. It also appears that H_2S administration amounts to different effects on different vascular tissues from the same animal, with some tissues showing vasoconstriction, while others show no change in vessel diameter [55].

In vivo, H_2S treatment revealed similar conflicting responses, increasing BP (blood pressure) in some studies [41,56] while decreasing BP in others [40,57,58]. Infusions directly into the CNS (central nervous system) also disparately showed both elevated MAP (mean arterial pressure) [56] and decreased MAP [57]. The effects of H_2S on heart rate are also ambiguous. H_2S administration did not alter heart rate in some studies [49,56], whereas it decreased heart rate in others [57].

Thus far a majority of studies seemingly favour the hypothesis that H_2S predominantly causes vasodilation. In additional support of this view, suppression of H_2S production either pharmacologically [24] or genetically [59] leads to increase in BP. In addition, H_2S appears to be elevated in most shock states where concurrently low BPs are encountered. As such, CSE inhibitors given in haemorrhagic shock led to decreased levels of H_2S, increased BP and decreased heart rate [60]. Coincidentally, in human patients with shock, H_2S levels were observed to be higher [61].

Reports of the interactions between H_2S and NO in the regulation of BP are also discrepant. Some studies report that H_2S may inactivate NO forming a nitrosothiol [41,62], and this has been proposed to explain how H_2S causes vasoconstriction in the presence of NO. H_2S has been shown to decrease the level of NO, an effect that was dependent on the presence of bicarbonate in the buffer solution [63]. Additionally, H_2S specifically inhibited eNOS [endothelial NOS (NO synthase)] and the resultant NO production [64]. Other instances where H_2S inhibited all three isoforms of NOS have also been reported [65]. H_2S preconditioning has also been reported to inhibit the actions of NO, since pre-treatment with H_2S reduced the vasorelaxant effect of the NO donor SNP (sodium nitroprusside) [48]. Furthermore, H_2S, in the absence of NO, fails to cause vasoconstriction [51]. Other studies show that blockade of NOS [46] or absence of NO [66] resulted in vasodilation by H_2S, suggesting that H_2S may be a vasoconstrictor in the presence of NO. However, questioning the dependency of H_2S on NO to cause vasoconstriction, in an animal incapable of NO production, H_2S still caused vasoconstriction [55], indicating that H_2S may not require NO to cause vasoconstriction.

An entirely different point of view is that H_2S and NO are believed to act synergistically to mediate vasodilation [67]. This is because blockade of NOS curtailed the vasorelaxant effect of H_2S [48], suggesting that NO may be needed for vasorelaxation by H_2S. Additionally, NO-induced vasodilation was enhanced by exogenously administered NaHS [39], and SNP, an NO donor, increased the synthesis of H_2S in both vascular tissues and organs [24]. Thus it is currently unclear whether H_2S in the presence of NO mediates vasoconstriction or vasodilation, or if NO is required for H_2S to exert physiologically relevant actions on blood vessels.

Numerous mechanisms have been proposed by which H_2S is perceived to moderate vascular tone. Some authors have proposed cAMP activation as one of the possible mechanisms of vasodilation by H_2S. However, as observed by Whiteman and Moore [20], H_2S fails to do this consistently, as H_2S activated the cAMP/PKG (protein kinase G) pathway in some studies [68], whereas it did not activate cAMP in cardiomyocytes [69] or endothelial cells [70]. Consistently, however, H_2S is reported to mediate vasorelaxation through the opening of K_{ATP} channels in the smooth muscle [19,40,42,43,57,71]. Another mechanism proposed by which H_2S relaxes vascular smooth muscle cells is by lowering intracellular pH [45]. Others have proposed metabolic inhibition, where H_2S is observed to reduce cellular ATP levels and thereby mediate smooth muscle relaxation [44]. In addition, H_2S has been shown to inhibit ACE (angiotensin-converting enzyme) activity of endothelial cells and hence is deemed to have a potential for decreasing BP [72].

The role of H_2S in the pathophysiology of cardiovascular diseases is somewhat variable since endogenous H_2S levels vacillate in different disease states. In haemorrhagic shock, plasma levels of H_2S are increased [60]. Coincidentally, mRNA levels of CBS and CSE are elevated in endotoxic, septic and haemorrhagic shock [60,61,73–76]. Rats subjected to haemorrhagic

shock when given PAG (D,L-propargylglycine) or BCA (β-cyanoalanine), inhibitors of H_2S biosynthesis, had improved MAP [60]. Hence H_2S may mediate immunological and inflammatory responses during shock. In contrast, H_2S levels are decreased in coronary heart disease [77] and in hypertensive rats [78]. Levels correlate with the severity of disease, as patients with two-vessel and three-vessel disease have lower H_2S levels compared with those with single-vessel disease [77]. H_2S levels are lower in diabetic mice as well [79,80]. CSE expression and activity are also reduced during hypoxic pulmonary hypertension [81], and administering H_2S exogenously or up-regulating CSE leads to improved pulmonary pressure [82,83]. In addition, exogenous H_2S reversed the increase in pulmonary artery pressure in left-to-right shunts [84]. Hence, although the levels of H_2S are higher in shock, they are lower in coronary heart disease, hypertension and diabetes, suggesting a potential for sulfide-based therapies in these disease states.

In summary, although a unifying mechanism responsible for vasoactivity of H_2S remains elusive, H_2S appears to modulate smooth muscle relaxation in vascular tissues. However, modulation of vascular tone by H_2S may depend on multiple factors, such as the species in question, the concentration at which the effects are studied, the experimental conditions and also the type of vascular tissue under investigation [54]. Furthermore, its interactions with other physiological regulators of BP (such as NO) are unclear at this time and warrant further investigation.

Role in inflammation, oxidative stress and atherosclerosis

H_2S has been shown to mediate pro-inflammatory effects by potentiating sulfite production in neutrophils [85] and mediating leucocyte activation [86]. However, numerous studies characterize H_2S as being anti-inflammatory. H_2S donors inhibit leucocyte adherence to the endothelium, thereby suppressing inflammation [87]. H_2S promotes short-term survival of neutrophils via inhibition of p38 MAPK (mitogen-activated protein kinase) activation and of caspase 3 cleavage, accelerating the resolution of inflammation [88]. Additionally, pre-treatment with NaHS inhibits LPS (lipopolysaccharide)-induced iNOS (inducible NOS) expression and derogatory NO production [68,89]. In vascular smooth muscle cells subjected to homocysteine-induced toxicity, treatment with NaHS decreases formation of ROS (reactive oxygen species) [90]. H_2S also protects against cytotoxicity induced by peroxynitrite [91], β-amyloid [92] and hypochlorous acid [72,93]. Thus there is currently strong evidence supporting H_2S as an anti-inflammatory agent.

Apart from anti-inflammatory effects and cytoprotection against oxidative stress, H_2S is reported to have beneficial anti-platelet and anti-atherosclerotic effects. NaHS inhibits platelet aggregation induced by a wide range of pro-thrombotic agents such as ADP, collagen, adrenaline (epinephrine), arachidonic acid, the thromboxane mimetic U46619 and thrombin [94]. H_2S has also been shown to inhibit proliferation of vascular smooth muscle cells by reducing MAPK activity [95]. In addition, H_2S attenuated remodelling of vascular tissue in hypertension [58]. Furthermore, treatment with NaHS inhibits neointima formation of balloon-injured carotid arteries and reduces the intima/media ratio [96], and long-term treatment reduces thickness of coronary arteriolar vasculature [97]. Furthermore, exogenous H_2S decreases modification of LDL (low-density lipoprotein) and retards the development of atherosclerosis [72,98]. Taken together, these studies point to a potential for use of sulfide-based therapeutics in conditions associated with abnormal smooth muscle proliferation, derogatory platelet activation and plaque formation.

Table 2 Mechanisms of cardioprotection by H_2S during I/R

Mechanism	Reference
Opening of K_{ATP} channels	Zhang et al. [102]
	Sivarajah et al. [103]
	Ji et al. [104]
Anti-apoptotic signalling	Sivarajah et al. [103]
	Jha et al. [105]
	Osipov et al. [106]
	Sodha et al. [107]
Inhibition of cellular respiration	Forgan et al. [111]
	Cooper et al. [112]
	Volpato et al. [113]
Inhibition of myocardial contractility	Sun et al. [69]
	Yong et al. [115]
Nrf-1 and Nrf-2 activation	Calvert et al. [121]
eNOS-mediated Akt phosphorylation	Yong et al. [109]
Angiogenesis	Cai et al. [70]
Mitochondrial preservation	Elrod et al. [110]

CARDIOPROTECTION MEDIATED BY H_2S

H_2S has been reported to be cytoprotective during reperfusion injury in multiple organ systems [99]. In the heart, reducing the level of H_2S by inhibiting CSE increases myocardial infarct size, pointing to a role for endogenous H_2S production in combating myocardial ischaemia [100]. Similarly, exogenous H_2S therapy has been reported to reduce the amount of myocardial ischaemic injury, reduce mortality rate, improve LV (left ventricular) pressures, suppress leucocyte infiltration and attenuate fibroblast hyperplasia [101]. A number of additional mechanisms have been proposed by which H_2S mediates cardioprotection during I/R (ischaemia/reperfusion) injury (Table 2).

In myocardial ischaemic studies, H_2S is believed to protect the heart against I/R injury by opening K_{ATP} channels [102–104]. However, Sun et al. [69] have shown that H_2S failed to open K_{ATP} channels in cardiomyocytes. More convincingly, H_2S has been shown to activate anti-apoptotic signalling. H_2S modulates Bcl-2 expression [105], alters phosphorylation of stress-activated protein kinases [103] and reduces expression of Beclin-1 [106]. H_2S administration reduces level of cleaved caspase 3 and decreases cleaved PARP [poly(ADP-ribose) polymerase] expression [107]. Additionally, H_2S leads to Nrf (nuclear factor-erythroid 2-related factor)-1 and Nrf-2 mediated Akt phosphorylation with a resultant decrease in oxidative stress [108]. eNOS-related Akt activation has also been shown to be involved in H_2S-mediated cardioprotection [109]. Furthermore, Akt phosphorylation following H_2S administration has been shown to induce angiogenesis in the heart [70].

Apart from the activation of the above survival pathways, H_2S has been shown to preserve both the structure and function of mitochondria and thus protect against myocardial ischaemic injury [110]. Evidence continues to evolve regarding inhibition of cellular respiration by inhibiting cytochrome *c* oxidase [111,112] with one study even reporting a reversible inhibition of cardiometabolic function [113]. In constraining cellular respiration, H_2S is somewhat similar to NO [114].

The potential for H_2S in decreasing myocardial contractility has been investigated. H_2S has been shown to inhibit contractility of cardiomyocytes both by inhibition of L-type calcium channels [69] and by inhibition of the cAMP/PKA (protein kinase A) pathway (coupled to β-adrenergic receptors) [115]. By contrast, H_2S is reported to activate L-type calcium channels in non-cardiac tissues [116]. Furthermore, administration of NaHS under normal conditions does not alter *in vivo* contractility in rats [101]. Hence the role of H_2S in modulating contractility specifically in disease states needs further investigation.

In contrast with the majority of the I/R studies mentioned above, where H_2S was administered during reperfusion, additional studies have assessed the role of raising tissue H_2S levels prior to the occurrence of ischaemia. Pharmacological preconditioning with NaHS reduced the severity of arrhythmias and increased cell viability following ischaemia and reperfusion [117,118]. Mechanistically, preconditioning with NaHS leads to a decreased expression of c-Fos protein [119], improved clearance of cytosolic calcium in a PKC (protein kinase C)-dependent manner [120], activated Nrf-2 signalling [121] and activated both ERK1/2 (extracellular-signal-regulated kinase 1/2) and PI3K (phosphoinositide 3-kinase)/Akt pathways [122].

Additionally, H_2S mediates IPC (ischaemic preconditioning) [117]. During IPC, the presence of H_2S results in activation of PKC and K_{ATP} channels [117]. Similarly, ischaemic postconditioning leads to cardioprotection, and H_2S has been shown to be involved in this strategy as well [109]. During postconditioning, H_2S stimulates PKC-α, PKC-ε and eNOS pathways thereby reducing infarct size and improving cardiac function following myocardial ischaemia [109].

H_2S treatment was also examined in other cardiovascular disease conditions. H_2S has been shown to be protective in heart failure [108] and adriamycin-induced cardiomyopathy [123]. Additionally, H_2S demonstrated beneficial effects during cardioplegia associated with cardiopulmonary bypass [124] and improved post-cardiac arrest survival in mice [125]. H_2S balneotherapy (a spa-based treatment) in patients with coronary heart disease improved exercise tolerance, controlled symptomatology and reduced the required dose of nitrates [126].

H_2S therapy may be beneficial in hyperhomocysteinaemia. This disorder, which is the result of mutated and/or dysfunctional CBS, rapidly leads to atherosclerosis [127]. Homocysteine is a substrate for CBS and hence the presence of functional CBS would lead to generation of endogenous H_2S. Although CBS is absent in the peripheral tissues, it can theoretically be up-regulated. Hence in hyperhomocysteinaemia, it is speculated that gene therapy with CBS may be therapeutic since the H_2S produced acts as an antioxidant [128]. Alternatively, administering H_2S by itself is protective in hyperhomocysteinaemia [129]. Certain cardiovascular conditions may benefit from high concentration of sulfide locally. CSE gene therapy in smooth muscle cells of the aorta resulted in the facilitation of apoptosis and inhibition cell growth [130]. When corroborated by further studies, this therapy may be useful in treating vascular conditions involving abnormal smooth muscle proliferation such as in-stent stenosis.

The studies described above suggest that sulfide-based therapeutics may prove efficacious in situations such as myocardial I/R injury, heart failure, cardiomyopathy, cardioplegia, homocysteinaemia and in-stent stenosis. This list is by no means exhaustive as it might accrue more conditions as research progresses.

DEVELOPMENT OF H_2S-BASED THERAPEUTICS FOR CARDIOVASCULAR DISEASE

A significant amount of effort is currently being channelled into developing novel therapeutics based on delivering H_2S [131]. Administration of an H_2S-releasing drug, *S*-diclofenac, during LPS-induced inflammation led to release of H_2S and further reduced lung and liver myeloperoxidase activity with significantly less gastric toxicity than diclofenac [133]. Administration

of this drug also protected against the development of myocardial I/R injury [134]. *S*-Diclofenac may in actuality be more potent that diclofenac with regard to an anti-inflammatory action [135]. Additionally, *S*-diclofenac inhibits smooth muscle cell growth and may inhibit progression of vascular injury [136]. This effect can potentially be directed at preventing arterial thrombosis or for plaque stabilization. GYY4137 is a water-soluble compound capable of releasing H_2S slowly, administration of which led to decreased BP in hypertensive rats [137]. NSAIDs (non-steroidal anti-inflammatory drugs) are some of the most commonly used medications, but their use is hampered by occurrence of side effects, mainly gastric ulceration. H_2S has been shown to have an ulcer-healing effect [138] and hence numerous H_2S-releasing analogues of existing NSAIDs are being developed [139–143]. These have potential for use in inflammatory cardiovascular conditions such as pericarditis.

Garlic (rich in polysulfides with proven cardioprotective benefits) is an avidly researched topic. Garlic, due to its inherent H_2S-releasing capability, mediates vasoactivity in an oxygen-dependent manner [52,144]. Additionally, organic sulfide donors derived from garlic, such as DADS (diallyl disulfide) and DATS (diallyl trisulfide), attenuate the deleterious effects of oxidized LDL on NO production [145] and attenuate myocardial I/R in mice [146]. DADS is also known to inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-CoA), and thus is a potential anti-hyperlipidaemic agent [147]. SAC (*S*-allylcysteine), another derivative of garlic, significantly lowers mortality and reduces infarct size following myocardial infarction [148]. SPRC (*S*-propargylcysteine), a structural analogue of SAC, was found to protect against myocardial infarction in rats both in *in vivo* and *in vitro* studies with an accompanying increase in CSE activity and plasma H_2S concentration [149]. SPC (*S*-propyl-L-cysteine), SAC and SPRC are all cardioprotective in myocardial infarction by reducing the deleterious effects of oxidative stress by modulating the endogenous levels of H_2S and preserving the activities of antioxidant-defensive enzymes like SOD (superoxide dismutase) [150]. Apart from these amino acid derivatives, an H_2S-donating analogue of sildenafil has also been shown to decrease oxidative stress induced by buthionine sulfoximine [151].

Clinical studies on sulfide-based therapies have been dominated by interest in garlic and garlic extracts as a whole in treating a variety of conditions. A very limited number have attempted to establish a link between the effects of garlic and its direct capability to release polysulfides. Currently only one such trial comes to mind: the bioavailability of garlic-derived allicin (which is a precursor form of allyl methyl sulfide) is being examined in healthy volunteers in a Phase 0 clinical trial (ClinicalTrials.gov identifier, NCT00874666).

CONCLUSIONS AND FUTURE DIRECTIONS

The emerging roles of endogenous H_2S (in the pathophysiology of disease) and the potential for H_2S-based therapeutics assert the importance of a continuation of research in these novel and exciting areas. H_2S has been shown to inhibit smooth muscle cell proliferation and inflammation, and also to counteract oxidative and nitrosative stress. These properties, in conjunction with anti-platelet actions, make sulfide donor drugs promising agents for the treatment of atherosclerosis. H_2S is anti-apoptotic at physiological concentrations and inhibits cellular respiration. These effects, along with potential angiogenic properties, make sulfide therapies useful for the treatment of coronary heart disease.

Despite discordant views on the role and mechanisms of action of H_2S in the body, it is not inappropriate to focus efforts on developing novel H_2S donor drugs simultaneously as we continue to understand its physiology. A number of H_2S-releasing analogues of existing drugs are being designed to harness the beneficial cytoprotective effects of H_2S. Design and actual preparation of novel donor drugs may pose significant challenges due to the instability of many of these compounds.

The evolution of our understanding of the role of novel gaseous transmitters in mediating physiological and pathophysiological processes *in vivo* has been fraught with controversy (especially in the initial stage) and H_2S appears to be no different. Beginning with synthesis and metabolism and ending with endogenous roles in disease, many aspects of H_2S signalling are still unclear. Besides, rapid discovery of novel H_2S metabolic pathways, differences in the levels of H_2S reported, a lack of knowledge about relevant intermediate and storage forms, differences in action depending on local oxygen tension, interactions with other physiologically relevant gases and the possibility of divergent functions of H_2S in various tissues make it extremely difficult to conclusively define the role and functions of H_2S in physiology and disease at this time. This notwithstanding, a significant amount of pre-clinical research has been done on the role of endogenously produced H_2S in physiology and exogenously administered therapy in treating select conditions, leading to an explosion in the field of sulfide-based therapeutics. Only time will tell if these will go on to represent another exciting group in the expanding arsenal of cardiovascular drugs.

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