Hydrogen sulfide and vascular regulation – An update

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Abstract

Background: Hydrogen sulfide (H$_2$S) is considered to be the third gasotransmitter after carbon monoxide (CO) and nitric oxide (NO). It plays an important role in the regulation of vascular homeostasis. Vascular remodeling has proved to be related to the impaired H$_2$S generation.

Aim of Review: This study aimed to summarize and discuss current data about the function of H$_2$S in vascular physiology and pathophysiology as well as the underlying mechanisms.

Key Scientific Concepts of Review: Endogenous hydrogen sulfide (H$_2$S) as a third gasotransmitter is primarily generated by the enzymatic pathways and regulated by several metabolic pathways. H$_2$S as a physiologic vascular regulator, inhibits proliferation, regulates its apoptosis and autophagy of vascular cells and controls the vascular tone. Accumulating evidence shows that the downregulation of H$_2$S pathway is involved in the pathogenesis of a variety of vascular diseases, such as hypertension, atherosclerosis and pulmonary hypertension. Alternatively, H$_2$S supplementation may greatly help to prevent the progression of the vascular diseases by regulating vascular tone, inhibiting vascular inflammation, protecting against oxidative stress and proliferation, and modulating vascular cell apoptosis, which has been verified in animal and cell experiments and even in the clinical investigation. Besides, H$_2$S system and angiotensin-converting enzyme (ACE) inhibitors play a vital role in alleviating ischemic heart disease and left ventricular dysfunction. Notably, sulfhydryl-containing ACEI inhibitor zofenopril is superior to other ACE inhibitors due to its capability of H$_2$S releasing, in addition to ACE inhibition. The design and application of novel H$_2$S donors have significant clinical implications in the treatment of vascular-related diseases. However, further research regarding the role of H$_2$S in vascular physiology and pathophysiology is required.

Keywords: Hydrogen sulfide, Blood vessels, Hypertension, Atherosclerosis, Pulmonary hypertension

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Introduction

Hydrogen sulfide (H$_2$S) was discovered to be the third gaseous molecule after nitric oxide (NO) and carbon monoxide (CO). This novel gaseous molecule has been proved to be widely involved in the regulation of various systems in human body [1]. Moreover, H$_2$S has attracted great attention in regulating the structure and function of blood vessels. Many researchers have shown that H$_2$S exerts vital effects on vascular cellular processes, such as inflammation, apoptosis, cell cycle, cytoprotection, and mitochondrial metabolic function and biogenesis [2].

In the vasculature, H$_2$S modulates vascular tension, suppresses the proliferation, and exerts a bidirectional effect on apoptosis and autophagy of vascular smooth muscle cells (VSMCs). Furthermore, the development of many vascular remodeling-associated diseases, including hypertension, atherosclerosis and pulmonary hypertension has been proved to be related to the impaired H$_2$S generation. In addition, H$_2$S and the use of zofenopril, one of the ACE inhibitors that can promote the release of H$_2$S, in cardiovascular diseases, including hypertension, atherosclerosis and pulmonary diseases, have been reported to be 5.8 ± 1.7 pmol s$^{-1}$ mg wet tissue$^{-1}$, respectively [14].

After synthesis by transsulfuration from L-cysteine, various metabolic pathways participate in the regulation of H$_2$S concentration in the cell. Significant pathways for H$_2$S metabolism include oxidation by sulfide quinone oxidoreductase (SQR) and persulfide dioxygenase (ETHE1) in the mitochondrion and methylation by cysteine dioxygenase (CDO) in the cytoplasm [15]. Sulfide is oxidized in the mitochondrion by SQR to generate persulfide. Persulfide is further oxidized to sulfite by ETHE1, and sulfite is finally oxidized by rhodanese or sulfite oxidase. After ubiquinone captures electrons released in the SQR reaction, the electrons are transferred to complex III in the electron transport chain [16]. In addition to the above oxidation pathway metabolism, Olson et al [17] proved that superoxide dismutase (SOD) also oxidizes H$_2$S to produce polysulfides. Methemoglobin and molecules containing metallo or disulfides such as oxidized glutathione may also eliminate H$_2$S [3,18].

Physiological regulation of blood vessels by H$_2$S

H$_2$S on vascular tone

H$_2$S has a bidirectional regulatory effect on vascular tone. H$_2$S can not only relax blood vessels, but also contract blood vessels [19]. A study published in Science [20] showed that the activation of CSE by calcium-calmodulin (CaM) under physiological conditions is the main mechanism of H$_2$S production in the vascular system. Mutant mice lacking CSE displayed lower levels of H$_2$S, with abnormally elevated blood pressure and loss of endothelium-dependent vasodilatory function. These findings directly prove the significance of H$_2$S for the maintenance of vascular function. Intriguingly, the vasodilation of H$_2$S on the portal vein and the ileum was notable stronger than that on the thoracic aorta [21]. In addition, compared with H$_2$S, hydrogen polysulfides (H$_2$S$_n$) tended to contain more sulfane sulfur atoms which have a relaxing effect and ultimately lowered blood pressure [22,23].

H$_2$S also has vasconstrictive effects under certain conditions. NaHS contracts VSMCs at concentrations between 5 × 10$^{-6}$ M and 10$^{-4}$ M [24]. A study by Ping reported similar results [25]. NaHS at concentrations ranging from 10 to 300 μM induced coronary artery constriction in rats. Therefore, the regulation of H$_2$S on vascular tone is bidirectional.

The mechanisms underlying H$_2$S-induced vasodilatation are not fully understood. The effects of vasodilatation have been attributed to iron channels that are activated by H$_2$S according to previous studies [26]. It is suggested that H$_2$S exerts a vasorelaxant effect via opening ATP-sensitive potassium channels (K$_{ATP}$ channels) in VSMCs [27]. H$_2$S mediates a new type of protein post-translational modification that is sensitive to redox, namely sulfhydration. More specifically, H$_2$S causes sulfhydration of cysteine-43 (C43) in Kir6.1 (a subunit of K$_{ATP}$ channel), resulting in a decrease in the capacity of Kir 6.1 binding to ATP, while the capacity of Kir 6.1 binding to PiP$_2$ is enhanced. This event eventually causes K$_{ATP}$ channels to open and VSMCs to relax [29]. Excepting the K$_{ATP}$ channel, growing evidence demonstrates that calcium-activated potassium channels (K$_{Ca}$ channels) are also activated by H$_2$S [30,31]. H$_2$S increases smooth muscle Ca$^{2+}$ spark activity to activate endothelial large-conductance calcium-activated potassium channels (BK$_{	ext{Ca}}$ channels) [32]. Transient receptor potential cation channel V4 (TRPV4) is also modified by H$_2$S through sulfhydration. This is followed by the activation and the opening of...
TRPV4-dependent Ca$^{2+}$ internal flow and the endothelial BKCa channel and results in vasodilation [33]. In addition, the SK3 channel which acts as an α-subunit isomorph of the SKCa channel is activated by H$_2$S through S-sulphydrilation [34]. Moreover, the activation of voltage sensitive potassium channels (Kv channels) and Kv7.4 voltage-gated potassium channels which are predominantly expressed in VSMCs are seen as targets for H$_2$S action on vascular tone [35,36]. Recent reports have also demonstrated that H$_2$S caused S-sulphydrilation of L-type Ca$^{2+}$ channels, leading to a decrease in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) [37].

Whether H$_2$S participates in the regulation of the cyclic guanosine monophosphate (cGMP) pathway remains controversial. A compelling amount of evidence indicates that H$_2$S exerts a vasodilative effect through the activation of endothelial nitric oxide synthase (eNOS) and the inhibition of cGMP degradation [38–40]. There are several primary mechanisms thought to participate: (1) H$_2$S directly reacts with NO to produce nitroxyl (HNO), thereby activating the HNO–transient receptor potential ankyrin 1 (TRPA1)–calcitonin gene-related peptide (CGRP) pathway to regulate vascular tone [41]. (2) H$_2$S inhibits the activity of phosphodiesterase 5 (PDE5) by reducing cGMP degradation and promoting cGMP signaling, followed by the activation of cGMP-dependent protein kinase (PKG) to phosphorylate the vasodilator-stimulated phosphoprotein (VASP), eventually resulting in vasodilation [42]. In addition, Sun et al. [43] believed that H$_2$S sulfhydrated associated PDE5A dimerization to exert the vasorelaxant function. (3) H$_2$S may alleviate oxidative stress, resulting in increased eNOS coupling by phosphorylation of eNOS$^{3177}$ [44,45]. (4) The reaction of soluble guanylyl cyclases (sGCs) to NO can be enhanced by H$_2$S [40,46]. It might be related to the reduction of sGC heme Fe by H$_2$S, so as to facilitate NO-regulated cellular signaling processes [47]. However, there is disagreement over the role of H$_2$S. For instance, Wang et al. [48] et al. suggested that H$_2$S did not rely on cGMP pathway to exert vasodilation, although vasodilation was strengthened by specific sGC inhibitors (ODQ and NS-2028). Similarly, NaHS-induced relaxation was unaffected by ODQ in rat coronary arteries [49]. Taken together, the vasorelaxation of H$_2$S varied very widely in different species and cell types. This might explain the conflicting results [46].

The vasodilation of H$_2$S was also related to the suppression of mitochondrial complexes I and III. It was shown that NaHS (100–1000 μM) suppressed mitochondrial electron transport to exert a vasodilation effect in rat mesenteric arterioles. This effect was inhibited by complex I and complex III inhibitors [30].

Accumulating evidence from H$_2$S studies demonstrates that H$_2$S derived from perivascular adipose tissue (PVAT) also exerts a critical effect in the regulation of vascular tension [33,50]. PVAT exerts predominantly anti-contractile effects, which is induced by adipocyte-derived relaxing factor (ADRF) [51,52]. Schleifenbaum et al. [53] suggested that H$_2$S could be an ADRF to regulate vascular tone. The mechanism of H$_2$S as ADRF could relate to activate K$_{ATP}$ and (or) voltage-sensitive K$_{CNQ}$ potassium channels [54,55]. Importantly, the findings from Kohn et al. [55] suggest that with technical progress, future studies on the vascular H$_2$S/K$_{CNQ}$ pathways make it possible to relieve vascular dysfunction.

In summary, H$_2$S-induced vasorelaxation takes place via the activation of iron channels, the interactions with NO–cGMP signaling, the inhibition of mitochondrial complexes I and III, and H$_2$S as an ADRF. However, under certain conditions, H$_2$S has vasoconstrictive effects which appear to involve the activation of Na$^+$–K$^+$–2Cl$^–$–co-transporters and voltage-gated calcium ion channels by H$_2$S [24]. Additionally, Ping et al. [25] suggested that the activation of the Rho kinase signaling pathway by H$_2$S may participate in the contraction of rat coronary arteries.

**Effects of H$_2$S on proliferation and apoptosis of vascular smooth muscle cells**

Accumulating evidence implicates H$_2$S as an inhibitor of VSMC proliferation. It was shown that the VSMC proliferation rate in CSE knockout mice was dramatically increased. However, endogenous H$_2$S significantly inhibited the proliferation of smooth muscle cell (SMC) in CSE knockout mice [56]. Similarly, NaHS, a commonly used H$_2$S donor, dose-dependently suppressed the proliferation of VSMCs [57]. The potential mechanisms for H$_2$S-induced proliferation are as follows: Du et al. [57] demonstrated that H$_2$S suppressed the activity of mitogen-activated protein kinase (MAPK), which might be responsible for H$_2$S-inhibited VSMC proliferation. Furthermore, endogenous CSE/H$_2$S pathway can inhibit the cascade conduction of MAPK/thioredoxin interacting protein (TXNIP) signals [58], thereby protecting endothelial function. In addition, H$_2$S dramatically inhibited the transcription and expression of Brg1 gene, reduced the recruitment of Brg1 in the promoter region of proliferating genes (pcna, nf3 and Pdgfra) and consequently inhibited the proliferation of VSMCs [59]. On the other hand, H$_2$S not only decreased the expression of insulin-like growth factor-1 receptor (IGF-1R), but also modified IGF-1R through sulfhydration to prevent IGF-1 binding, ultimately inhibiting VSMC proliferation [60]. Recently, Wang et al. [61] demonstrated that calcium-sensing receptor (CaSR) increased endogenous generation of H$_2$S via calcium-CaM signal pathways, ultimately inhibiting the proliferation of VSMCs. Therefore, several genes, molecules, and signaling pathways (such as MAPK/TXNIP signals, Brg1, ERK1/2, IGF-1R and CaSR) have been identified in the regulation by H$_2$S, and contribute to the suppression of VSMC proliferation.

H$_2$S can promote or inhibit vascular cell apoptosis. Several studies agree with the view that H$_2$S promotes apoptosis. Studies [62,63] have demonstrated that H$_2$S can activate the ERK/caspase 3 pathway and promote the apoptosis of human aorta smooth muscle cell (HASMC). CSE overexpression or exogenous H$_2$S supplementation promotes apoptosis via stimulating extracellular regulated protein kinases (ERK) 1/2, p38 MAPK, and p21$^{Cip1/WAF1}$ but suppressing cyclin D1 [56,62]. In contrast, several studies suggest that H$_2$S inhibits apoptosis. H$_2$S decreased the elevated ratio of Bcl2-associated x (Bax)/B-cell lymphoma-2 (Bcl-2) and the activity of caspase-3, thus inhibiting apoptosis caused by high glucose [64]. It was also shown that NaHS suppressed apoptosis by reducing the expression of caspase-12, C/EBP homologous protein (CHOP), and glucose-regulated protein 78 (GRP78) which are related to endoplasmic reticulum stress (ERS), thus protecting vascular endothelial function [65]. Therefore, the regulation of apoptosis by H$_2$S is bidirectional. It can promote and inhibit apoptosis under different pathological conditions.

**Effect of H$_2$S on vascular autophagy**

Autophagy is essential for homeostasis in processes including cell development and differentiation, regulation of cell longevity and programmed cell death, degradation of invading pathogens, and provision of antigens to the immune system [66]. Pathogens, abnormal proteins and organelles are engulfed by autophagosomes and undergo lysosomal degradation [67,68]. H$_2$S is reported to either promote or inhibit autophagy depending on the different pathological process [69,70]. NaHS was shown to activate mitophagy in rat aortic endothelial cells (RAEAS) [71]. Mechanistically, NaHS facilitates Parkin recruited by PTEN induced putative kinase 1 (PINK1), and then ubiquitylates mitofusin 2 (Mfn2), leading to the upregulation of mitophagy [71]. However, several studies showed that both supplementation of H$_2$S and the overexpression of its synthetases mitigated mitophagy [72]. H$_2$S inhibited adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK)-
mammalian target of rapamycin (mTOR) pathway, which is closely associated with autophagy [73]. On the other hand, the ratio of microtubule-associated protein 1A/1B-light chain 3 (LC3)-II to LC3-1 is commonly used as an indicator of autophagy. Expression of LC3A I/II was significantly decreased with supplementation of H2S (30 μM) [72]. NaHS could also inhibit the excessive autophagy of vascular endothelial cells by suppressing nuclear factor erythroid-2-related factor 2 (Nrf2)-reactive oxygen species (ROS) -AMPK signaling pathway [74]. Taken together, there are still different opinions of vascular autophagy regulation by H2S. A variety of pathological conditions likely contribute to the differences in the effect that have been observed.

**Pathophysiological regulation of H2S on blood vessels**

**H2S and hypertension**

Treating hypertension which is defined as ≥ 140/90 mmHg with chronically increased blood pressure remains a great challenge. Several clinical studies showed a close correlation between hypertension and reduction of H2S. The reduction of endogenous H2S synthesis and H2S-dependent vasodilation led to a microvascular dysfunction in hypertensive patients [75]. Notably, CBS, CSE and 3-MST as the H2S generating enzymes, were markedly decreased in humans with hypertension [76], suggesting that H2S generation pathway may be involved in the pathogenesis of hypertension. Similar results have also been shown in animal research. For instance, a decreased endogenous H2S content in the aorta was observed in the development and progression of spontaneously hypertensive rats (SHRs) [77]. The use of DL-propargylglycine (PPG), a CSE inhibitor, dramatically elevated the level of basal blood pressure in WKY rats and promoted vascular remodeling, demonstrating that a sufficient H2S level is necessary for the maintenance of basal blood pressure [78]. Similar to that of SHRs, it was shown that CBS/H2S pathway was down-regulated in salt-sensitive Dahl rats [79].

Extensive evidence shows that H2S exerts a crucial effect on blood pressure regulation in pathological cases. For instance, studies by Sun et al. [80] suggested that NaHS lowered tail artery pressure in SHRs. Similarly, it was shown that H2S delayed the shift from prehypertensive to hypertensive status in SHRs [81]. Notably, H2S improved endothelial function in renovascular hypertensive rats and ameliorated the damaged endothelium-dependent contraction (EDC) and endothelium-dependent relaxation (EDR) [82,83]. Furthermore, the H2S donor alleviated hypertension, reversed aortic remodeling, and inhibited the renin–angiotensin–aldosterone (RAS) system in renal tissue of Dahl rats [79]. These experimental results demonstrate that H2S dramatically suppressed the elevation of blood pressure in two animal models.

Many scholars have discussed the protective effect of H2S on hypertension and its potential mechanisms. Previous studies [82,83] showed that the H2S donor NaHS significantly suppressed the activation of NOD-like receptors (NLRP3), inflammasomes, and oxidative stress in SHRs. Moreover, the amelioration of excessive EDC of H2S was associated with the inhibition of the bone morphogenetic protein 4 (BMP4) and its downstream signal molecules [84]. NaHS can also protect renal artery endothelial cells and improve endothelial function through the activation of the peroxisome proliferator-activated receptor α (PPARα) signaling [85]. In addition to improvements in the vascular endothelium, NaHS also regulated immune function by reducing the expression of connexin 40 (Cx40)/connexin 43 (Cx43) T lymphocytes in SHRs, and reversed changes in multiple T lymphocyte subtypes in SHRs [86], which may explain the anti-inflammatory effect of H2S. Ion channels are considered as key targets for H2S depressurization. A report from Sun et al. [80] suggested that the KATP channel is activated by H2S and causes vasodilation. Furthermore, H2S may activate the KATP channel by inhibiting Forkhead box O1 (FOXO1) and Forkhead box O3a (FOXO3a) phosphorylation, subsequently inducing their nuclear binding to SUR2B and Kir6.1. In addition to the regulation of the KATP channel, H2S can also activate the TRP vanilloid 1 (TRPV1) ion channel through S-sulfhydration, increasing the sensitivity of carotid sinus pressure receptors in SHRs [87]. TRPA1 channels were also activated by H2S, inducing the release of CGRP and promoting vasodilation [88,89]. On the other hand, H2S also inhibited the pathological state of SHRs by regulating the RAS system. H2S reduced the expression of RAS-related mRNA (Ren, Atp6ap2, Agt, Ace, and Agrtr1α) in the kidneys of SHRs, which blocked the RAS system and exerted a vasomotor effect [81]. Finally, an underlying H2S mechanism may be related to the inhibition of collagen deposition. H2S dose-dependently inhibited MAPK activation induced by angiotensin II in SHRs and down-regulated the affinity of angiotensin II type 1 (AT1), ultimately inhibiting vascular remodeling and collagen deposition in SHRs [90]. Furthermore, reduced collagen deposition by H2S may be related to the suppression of transforming growth factor-β /Smad signaling pathway [91].

The mechanism by which H2S regulates blood pressure in high-salt Dahl rats may be as follows. Liang et al. [92] showed that H2S reduced the oxidative stress response in the paraventricular nucleus of high-salt Dahl rats, attenuated sympathetic activity, and promoted the secretion of anti-inflammatory factors, thus inhibiting the inflammatory response. H2S may also regulate blood pressure by the inactivation of epithelial sodium channels (ENaC). Reabsorption of sodium by the ENaC promotes the progress of salt-sensitive hypertension. It was shown that H2S completely blocked abnormal activation of ENaC caused by excessive H2O2. H2O2 increased sodium reabsorption by up-regulating phosphatidylinositol 3, 4, 5-trisphosphate. H2S can significantly inhibit PTEN inactivation caused by H2O2, thereby reducing oxidative stress [93].

To summarize, the mechanisms by which H2S inhibits hypertension are complicated, including the reduction of oxidative stress and inflammation, the modulation of immune function and ion channels, and the inhibition of collagen deposition and vascular remodeling.

**H2S and atherosclerosis**

Atherosclerosis (AS) is a chronic, complicated and progressive pathological process of large and medium-sized arteries. Several studies have shown that H2S deficiency is related to the pathogenesis of AS. For example, Gao et al. [94] suggested that H2S deficiency may predispose stable coronary artery disease (CAD) patients to vulnerable plaque rupture. As reported in many clinical studies, Wang et al. [95] found disorders of the vascular CSE/H2S pathway in apolipoprotein E (ApoE)-knockout mice. Another study from Meng et al. [96] also demonstrated that decreased endogenous H2S generation accelerated AS in CSE-knockout mice. Accumulating evidence [97,98] has shown that endogenous H2S produced by CSE in blood vessels has an anti-AS effect. Unstable plaques generated by AS are prone to rupture and have the risk of infarction [99]. In ApoE-knockout mice, H2S stabilizes atherosclerotic plaques and suppresses lipid deposition [100,101].

Key mechanisms for the anti-AS effect of H2S include anti-oxidative stress, anti-inflammatory effect, and regulation of ion channels [102] to protect the vascular endothelium. Intriguingly, it was reported that vascular CSE/H2S, as the target of estrogen, was involved in the mechanism by which estrogen protected against AS [103]. The detailed mechanism is as follows.
First, H$_2$S attenuates oxidative stress to protect against AS. It induces S-sulfhydration of glutathione peroxidase 1 (GPX1) to prompt glutathione synthesis, resulting in alleviating lipid peroxidation and improving antioxidant capacities [104]. Several studies [105,106] further found that H$_2$S may induce Nrf2 to dissociate from kelch-like ec-associated protein 1 (Keap1) by sulfhydration of Cys151 in Keap1, enhancing nuclear translocation of Nrf2 and thereby exerting antioxidant stress and cardiovascular protection. Moreover, translocation of Nrf2 further stimulated its downstream molecules, including the NADPH quinoneoxidoreductase 1 (NQO1), thus preventing the release of inflammatory cytokines [107]. H$_2$S was found to attenuate atherosclerotic lesions by blocking oxidative modification of low density lipoprotein (LDL) and elevating antioxidant activity [108]. A recent study shows that H$_2$S-induced antioxidant stress is also related to its elimination of oxidized hemoglobin (Hb) and inhibition of the interaction between Hb and lipid in AS [109]. Through the regulation of above molecules, H$_2$S exerts a critical role in prevention of collagen deposition and protection of vascular function.

Secondly, H$_2$S attenuates inflammation to protect against AS. Inactivation of nuclear factor kappa-B (NF-kB) caused by H$_2$S reduces the expression of inflammatory factor intercellular cell adhesion molecule-1 (ICAM-1), which may be an important reason for H$_2$S to maintain the stability of AS plaques [95]. Moreover, Du et al. [110] found that H$_2$S modified cysteine 38 in p65 via sulfhydrylation, which was responsible for NF-κB inactivation. Recent studies also showed that the anti-inflammatory effect of H$_2$S might suppress TXNIP, an activator of NLRP3, which inhibited excessive production of interleukin 18 (IL-18) and interleukin 1β (IL-1β) [111]. Additionally, H$_2$S was identified as an agonist of histone deacetylase Sirtuin-1 (SIRT-1). H$_2$S directly induced deacetylation of SIRT-1 and its target proteins (P53, P65, and sterol response element-binding protein), alleviating inflammation in the endothelium and macrophages, inhibiting macrophage cholesterol uptake in ApoE knockout mice, and eventually reducing the formation of AS plaques [112]. Furthermore, it is worth noting that the activation of matrix metalloproteinases (MMPs) was involved in AS. As a member of MMPs, MMP9 is considered to be a critical factor in the transformation from pro-atherosclerosis to anti-atherosclerosis [116].

Thirdly, the interactions between NO and H$_2$S may also be one of the anti-AS mechanisms. Specifically, H$_2$S upregulates the expression of inducible nitric oxide synthase (iNOS) protein and promotes NO production. [114].

Fourthly, H$_2$S has an anti-apoptotic effect. Studies showed that H$_2$S increased the stability of plaques in ApoE knockout mice by inhibiting caspase-3/9 activity and lipoprotein receptor-1 (Lox-1) [100].

Additionally, there are other mechanisms that mediate the anti-AS effect of H$_2$S. H$_2$S donors can reduce the level of adrenomedullin (ADM) and increase the level of atrial natriuretic peptide (ANP) in AS rats, thus antagonizing the formation of AS [115]. Mani et al. [96] proposed that H$_2$S plays an anti-AS effect, which may inhibit intimal proliferation and adhesion molecule expression. Recently, a study also showed that NaHS notably activated angiotensin converting enzyme 2 (ACE2)-related pathways, so as to promote the transformation from pro-atherosclerosis to anti-atherosclerosis [116].

In conclusion, H$_2$S retarded the development of AS by a variety of molecular mechanisms that include anti-oxidative stress, anti-inflammation, anti-apoptosis, and interactions with NO.

H$_2$S and pulmonary hypertension

Abnormal vascular remodeling and increased pulmonary artery pressure that results in right ventricular (RV) hypertrophy and heart failure are characteristic pathological features of pulmonary hypertension (PH). PH consists of hypoxic pulmonary hypertension (HPH) and PH caused by high pulmonary blood flow and so on. Acute or chronic hypoxic stimulation leads to the progression of HPH, which is typically characterized by PH and increased pulmonary vascular resistance. It was shown that both the expression of CSE and its activity were inhibited in lung tissues during HPH [117]. In another model of PH, endogenous H$_2$S pathway was also downregulated in rat PH models caused by high pulmonary blood flow [118]. In addition, Feng et al. [119] suggested that the contents of H$_2$S in lung tissues and serum of rats in the monocrotaline (MCT)-induced PH group were obviously inhibited, and CSE expression was dramatically co-downregulated.

However, a clinical study demonstrated that H$_2$S at 500 μM induced an average dilation of 42.3% from the pre-constricted tension in dissected human arterial rings. In addition, H$_2$S at 500 μM also induced an average reduction of 17.7% in pulmonary artery pressure [120]. This effect was also seen in animal models. For instance, H$_2$S donors reduced pulmonary artery pressure and alleviated structural remodeling of pulmonary vessels during HPH [117]. In addition, exogenous H$_2$S restored H$_2$S contents in plasma, alleviating pulmonary artery remodeling caused by HPH.

The mechanisms by which H$_2$S protects against PH include but are not restricted to anti-inflammation [121], anti-endoplasmic reticulum stress (ERS) [122], induction of apoptosis [123], anti-proliferation [124,125] and upregulation of the CO/HO pathway [126]. The detailed mechanisms are as follows.

First, H$_2$S antagonizes pulmonary vascular inflammation. Inflammation exerts a central effect on the pathogenesis of PH. Previous studies [122,127] demonstrated that H$_2$S inhibited pro-inflammatory and oxidative stress. It was shown that H$_2$S alleviates pulmonary artery endothelial inflammation by inhibiting NF-κB signaling pathway [127]. Moreover, H$_2$S not only inhibits the NF-κB signaling pathway, but also alleviates ERS by inhibiting the expression of NADPH oxidase 4 (Nox4), as well as GRP78 and CHOP the ERS-related molecule markers [122,125].

Secondly, H$_2$S induces PASMC apoptosis. The effect of H$_2$S on apoptosis is bidirectional, which can promote and inhibit apoptosis. However, Li et al. [123] suggested that H$_2$S induces apoptosis through inhibiting Bcl-2 and activating Fas signaling pathway of PASMCs in PH rats.

Thirdly, H$_2$S significantly inhibited the expression of proliferative cell nuclear antigen (PCNA) and urotensin II (U-II), which are critical molecules related to cell proliferation [128]. This anti-proliferative effect may be related to the up-regulation of cyclooxygenase-2 (COX-2)/prostaglandin 12 (PGI$_2$) signaling pathway [124,125].

Fourthly, H$_2$S exerts the anti-oxidative stress effect in PH model. Oxidative stress is another important cause of elevated pulmonary arterial systolic pressure in humans. H$_2$S enhances the ratio of GSH/oxidized glutathione (GSSG), which represents antioxidant capacity, by scavenging GSSG, thus exerting antioxidant capacity in HPH [129]. Moreover, the expression of collagen-promoting molecules connective tissue growth factor (CTGF) and MMP-13 were increased after the application of D, L-propargylglycine (PPG), whereas the expression of tissue inhibitor of metalloproteinase 1 (TIMP-1) was significantly decreased. All of the above results indicate that H$_2$S alleviates oxidative stress injuries, thus inhibiting pulmonary vascular remodeling [130,131].

Lastly, H$_2$S upregulates the CO/heme oxygenase (HO-1) pathway and is regulated by NO simultaneously in PH [132,133].
interaction between CO and H$_2$S potentially contributes to the pathogenesis of HPH. Zhang et al. demonstrated that H$_2$S might modulate the pathogenesis of HPH by activating HO-1 [126]. However, the mechanisms underlying H$_2$S through regulation of the CO/HO pathway in PASMCs remain unknown. Accumulating evidence [134] also demonstrates that defects of NO signaling possibly contribute to the progression of PH. The NO substrate, L-arginine, is known to upregulate CSE/H$_2$S signaling in PH caused by high blood flow [135]. Therefore, H$_2$S protects pulmonary vascular structure through the interaction with the other two gas molecules—NO and CO.

In summary, H$_2$S attenuates PH through several mechanisms, including anti-inflammation, induction of apoptosis, anti-proliferation, anti-oxidative stress, and regulating CO and NO signaling pathways.

H$_2$S and other cardiovascular diseases

Previous studies have confirmed that the abnormality of endogenous H$_2$S pathway may participate in the pathogenesis of ischemic heart disease (IHD) and left ventricular dysfunction [136]. Overexpression of CSE or supplementation of H$_2$S donors significantly improved cardiac function and structural lesions [137,138,139]. The following mechanisms might be involved in the protective effect of H$_2$S on the IHD and left ventricular dysfunction: 1) suppression of oxidative stress: H$_2$S increases the activity of antioxidant enzymes SOD, CAT and GSH in the cardiac tissues of mice with ischemia/reperfusion (IR) injury [140]. Furthermore, a 7-day treatment of H$_2$S donor Na$_2$S promoted the nuclear translocation of Nrf2, an important transcription factor that regulates antioxidant genes as an adaptive response to oxidative stress, in the hearts of mice with left coronary artery occlusion and reperfusion, which might contribute to the increase in the antioxidant enzymes [137]. Moreover, the upregulation of the rhythm gene Bmal1 expression was also involved in the antioxidant effects of H$_2$S in the ischemic cardiomyocyte H9c2 cells [141]. 2) inhibition of apoptosis and autophagy: H$_2$S reduced the proportion of apoptotic cells in the myocardium of mice with heart failure (HF) by increasing the expression of Bcl-2 and inhibiting the expression of Bax and caspase 3 [138]. In another study, H$_2$S alleviates autophagy of myocardial ischemia in SOD1 KO mice through the inhibition of S6 kinase (S6K) phosphorylation and AMPK phosphorylation [142]. 3) regulation of macrophage-related cardiac inflammatory response: H$_2$S promoted the infiltration of macrophages into the infarcted myocardium in both wild type and CSE-KO mice targeting on the macrophage integrin $\beta_1$ and its downstream Src-FAK/Pyk2-Rac pathway [143]. Moreover, the polarization of infiltrated macrophage in the heart of mice with MI was also governed by H$_2$S. The results showed that H$_2$S donor NaHS promoted the number and the proportion of anti-inflammatory M2 macrophages in the hearts of mice with MI by increasing mitochondrial biosynthesis and fatty acid oxidation [144]. 4) interaction with other bioactive molecules: In the previous studies, the interaction between H$_2$S and NO was involved in the vascular regulation [145]. Similarly, it is reported that H$_2$S enhanced endogenous NO generation by increasing the mRNA level of eNOS and nNOS and decreasing the mRNA level of iNOS in the...
heart tissues of myocardial IR rats [146]. Mitochondrial protec-

ation: H2S maintains mitochondrial homeostasis by restoring the balance of Bcl-2/Bax and reducing mitochondrial-dependent apoptosis in HF rats [138], and improving mitochondrial respiration and ATP synthesis in isolated cardiac mitochondria from HF mice [137]. In addition, a blocker of mitoKATP channel 5-HD completely blocked the protective effect of H2S donor on the isolated I/R rat heart, suggesting that the opening of mitoKATP channel might be

![Fig. 2. Regulation of H2S on hypertension. \(\rightarrow\) means stimulating effect, whereas \(\downarrow\) means inhibiting effect. \(P\) means phosphorylation.](image)

![Fig. 3. Regulation of H2S on atherosclerosis. \(\rightarrow\) means stimulating effect, whereas \(\downarrow\) means inhibiting effect. \(–SSH\) means S-sulphydrylation. Ace means acetylation. oxLDL, oxidized low-density lipoprotein; Ang II, angiotensin II; Ang (1–7), angiotensin (1–7).](image)
involved in the regulatory effect of H2S on the cardiac mitochondria [147].

**Application of sulfhydryl group-containing angiotensin-converting enzyme (ACE) inhibitor in cardiovascular diseases**

Angiotensin-converting enzyme (ACE) inhibitors are widely used as therapeutic agents in the treatment of cardiovascular diseases such as hypertension, IHD and left ventricular dysfunction in experimental studies and clinical trials [148–150]. The protective mechanisms of ACE inhibitors were mainly mediated by the inhibition of angiotension II generation and bradykinin degradation. For example, the mechanisms of cardioprotection in patients treated with ACE inhibitors might include the reduction in LV preload and afterload, suppression of sympathetic stimulation, restoration of the balance of myocardial oxygen supply and demand, improvement in endogenous fibrinolysis, and alleviation of diastolic dysfunction, etc [151]. Compared with other ACE inhibitors, a sulfhydryl-group-containing ACE inhibitor zofenopril has been demonstrated to have a better clinical efficacy and safety in patients with hypertension, acute myocardial infarction (AMI) or CAD, particularly in high risk patients such as diabetes mellitus, in many clinical and preclinical studies such as SMILE series studies [152–154]. Borghi et al compared the difference in the efficacy between zofenopril and other ACE inhibitors in patients with AMI. The results showed that early administration of zofenopril in the patients ≥ 1 cardiovascular risk factor had a better prognosis and less risk of cardiovascular events than the administration of lisinopril and ramipril [153]. It has been reported that the peculiar protective effects of zofenopril including the capability of scavenging

![Fig. 4. Regulation of H2S on pulmonary hypertension. → means stimulating effect, whereas ↓ means inhibiting effect, \| means scavenging.](image)

| Action | Mechanisms | Models | H2S gas/donor application (concentration) | Refs. |
|--------|------------|--------|------------------------------------------|-------|
| Relaxation | Activation of KATP channel | Mesenteric artery VSMCs of rats | NaHS (100–300 μM) | [27,29] |
| | Activation of KCa channel | Rat cerebral arteries | NaHS (10 and 100 μM) | [31] |
| | Activation of Ca2+ spark activity | Rat mesenteric small arteries | NaHS (10 μM) | [32] |
| | Activation of TRPV4 channel | Rat mesenteric small arteries | NaHS (1–1000 μM) | [33] |
| | Activation of BK channels | Rat mesenteric small arteries | NaHS (1–1000 μM) | [33] |
| | Activation of KCa and SKCa channels | Mouse mesenteric arteries and aortas | NaHS (100–1000 μM) | [34] |
| | Activation of Kv7 channels | Rat mesenteric small arteries | NaHS (100–3000 μM) | [30] |
| | Activation of Kv7,4 channels (subtype of Kv7) | Rat aortic rings | NaHS (1000 μM) | [35] |
| | Activation of Kv7,4-type K+ channels | Rat and mouse aortas | NaHS (10–3000 μM) | [55] |
| | Activation of HNO-TRPA1-CGRP pathway | Rat mesenteric arteries | NaHS (10 μM) | [41] |
| | Activation of cGMP-PKG-VASP pathway | Mouse aortic rings | NaHS (30 μM) | [42] |
| | Inhibition of sGC heme Fe | Mouse thoracic aorta | NaHS (50 μM) | [24] |
| Constriction | Activation of Na+(K+–2Cl–) co-transporters and voltage-gated calcium ion channels | Rat thoracic aorta | NaHS (5–100 μM) | [25] |
| | Activation of Ca2+ influx | Rat coronary arteries | NaHS (10–300 μM) | [25] |
Table 2
Effects of H2S on proliferation and apoptosis of vascular smooth muscle cells.

| Action                      | Mechanisms                                                                 | Cells/Models | H2S gas/donor application (concentration) | Refs. |
|-----------------------------|-----------------------------------------------------------------------------|--------------|------------------------------------------|-------|
| Anti-proliferation           | Inhibition of Brg1 transcription and expression by reducing the recruitment of Brg1 to the PcnA, Nf1, and Pdgfa promoter regions | VSMCs        | NaHS (1000 μM)                           | [59]  |
| Anti-proliferation           | Inhibition of the MAPK pathway                                             | VSMCs isolated from rat thoracic aorta | NaHS (50–500 μM) | [57]  |
| Anti-proliferation           | Inhibition of the MAPK/TKNIP cascade                                        | HUVECs/CSE-KO mice | NaHS (56 μM/kg/d) | [58]  |
| Anti-proliferation           | Inhibition of the expression of IGF-1 and the binding of IGF-1 with IGF-1 via S-sulfhydration | SMCs isolated from mouse mesenteric arteries | NaHS (10–100 μM) | [60]  |
| Inducing apoptosis           | Increasing ERK1/2, p38\(^{\text{MAPK}}\), and decreasing cyclin D1 in SMCs-KO mice. | SMCS-KO mice/CSE-KO mouse/HASMCs | H2S (100 μM) | [5662]|
| Inducing apoptosis           | Activation of MAPKs and caspase-3                                           | HASMCs       | H2S (50–100 μM)                          | [63]  |
| Inhibiting apoptosis         | Activation of SOD activity                                                  | HUVECs       | NaHS (50 μM)                             | [64]  |
| Inhibiting apoptosis         | Inhibition of ROS generation and MDA levels                                 | PAECs        | NaHS (56 μM/kg/d)                        | [65]  |

Table 3
Effect of H2S on vascular autophagy.

| Action                      | Mechanisms                                                                 | Cells/Models | H2S gas/donor application (concentration) | Refs. |
|-----------------------------|-----------------------------------------------------------------------------|--------------|------------------------------------------|-------|
| Promoting mitophagy         | Activation of Parkin recruited by PINK1 and then ubiquitination of Mfn2      | RAECs        | NaHS (100 μM)                           | [71]  |
| Inhibiting mitophagy        | Phosphorylation of Akt and dephosphorylation of FoxO3a                      | MAECs        | NaHS (30 μM)                             | [72]  |
| Inhibiting autophagy        | Dethosphorylation of AMPK and phosphorylation of mTOR                      | VSMCs isolated from rat thoracic aorta | NaHS (100 μM) | [73]  |
| Inhibiting autophagy        | Dethosphorylation of AMPK and activation of Nrf2                           | RAECs/db/db mice | NaHS (100 μM) | [74]  |

ROS, preventing of endothelial dysfunction, suppressing inflammatory response, promoting of NO generation and bioactivity, and regulating of cell apoptosis might be related to its sulfhydryl groups [151]. However, Bucci et al found that H2S could be released from S-zofenoprilat, an active metabolite of S-zofenopril, in a cell-free assay and directly play a vasorelaxant effect in vitro. Also, the key H2S-producing enzyme CSE expression in the vessel and the endothelial-dependent vasodilatation in SHRs treated with S-zofenopril was recovered to normal level [155]. As well as the regulation of vessel function, H2S was found to mediate the proangiogenic effect of zofenopril, supported by the fact that CSE inhibitor or CSE siRNA blocked the zofenopril-induced angiogenesis in vivo and in vitro [156]. In addition, CSE-dependent H2S was also involved in the anti-inflammatory effect of zofenopril in IL-1β-induced endothelial inflammation model [157]. Interestingly, an increase in the H2S and NO level in the myocardial tissue and plasma was found to be associated with the cardioprotective effect of zofenopril pretreated before I/R injury in mouse and pig I/R [158]. Therefore, although further studies are needed, the above-mentioned studies suggest that the property of H2S donor/generator might contribute to the superior clinical application of sulfhydral ACE inhibitor zofenopril compared with other ACE inhibitors, which would open a new avenue for the treatment of cardiovascular diseases.

Conclusions

H2S participates in the physiological and pathological regulation of vasculature. The mechanisms underlying H2S-induced vasodilation are complex. H2S induced vasorelaxation predominantly by activating iron channels, interacting with NO-cGMP signaling, inhibiting mitochondrial complex I and III, and acting as an ADRF. In addition, H2S inhibits the proliferation of VSMCs in association with MAPK/ TXNIP, Brg1, ERK1/2, IGF-1R and CaSR signals. The regulation of H2S on vascular cell apoptosis and autophagy is bidirectional. It can either promote or inhibit autophagy and apoptosis depending on the different pathological process (see Figs. 1–4 and Tables 1–3).

Recent experimental data provide evidence that H2S can prevent vascular-related diseases, such as hypertension, atherosclerosis and PH. The underlying mechanisms may include the regulation of vascular tone, anti-inflammation, anti-oxidative stress, the inhibition of VSMC proliferation, and the modulation of VSMC apoptosis. Regulating H2S level provides a novel therapeutic method against these vascular diseases. In addition, the application of H2S system and ACE inhibitors in the treatment of cardiovascular diseases has gradually been paid attention. Notably, the effectiveness of zofenopril in clinical trials is significantly better than other ACE inhibitors due to its capability of H2S releasing. Therefore, H2S has important clinical implications. Further understanding of its protective role in cardiovascular system is needed.

Future studies should investigate the interaction amongst H2S and other gaseous signaling molecules including NO and sulfur dioxide (SO2). There remain many opportunities to explore its role in atherosclerosis, PH and hypertension. Of note, drugs targeting H2S producing enzymes (CBS, CSE and 3-MST) merits further clinical research.

Conflict of Interest

The authors declare no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.
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