Endogenous hydrogen sulfide is involved in the pathogenesis of atherosclerosis

Wang Qiao a, Tang Chaoshu b,c, Jin Hongfang a,**, Du Junbao a,**

a Department of Pediatrics, Peking University First Hospital, Beijing 100034, China
b Department of Physiology and Pathophysiology, Health Sciences Center, Peking University, Beijing 100034, China
c Key Laboratory of Molecular Cardiovascular Medicine, Ministry of Education, China

Abstract

Atherosclerosis is a chronic, complex, and progressive pathological process in large and medium sized arteries. The exact mechanism of this process remains unclear. Hydrogen sulfide (H₂S), a novel gasotransmitter, was confirmed as playing a major role in the pathogenesis of many cardiovascular diseases. It plays a role in vascular smooth muscle cell (VSMC) proliferation and apoptosis, participates in the progress of hyperhomocysteinemia (HHcy), inhibits atherogenic modification of LDL, interferes with vascular calcification, intervenes with platelet function, and there are interactions between H₂S and inflammatory processes. The role of H₂S in atherosclerotic pathogenesis highlights the mysteries of atherosclerosis and inspires the search for innovative therapeutic strategies. Here, we review the studies to date that have considered the role of H₂S in atherosclerosis.

1. Introduction

Atherosclerosis is a chronic, complex, and progressive pathological process in large and medium sized arteries. The exact mechanism of this process remains unclear. According to previous studies, vascular inflammation and abnormal immune response, proliferation of smooth muscle cells, endothelial damage, foam cell accumulation, intraplaque hemorrhage, lipid deposition, proliferation of neovessels, and plaque regression contribute to atherosclerotic plaque formation (Fig. 1) [1–8].

Currently, the cardiovascular regulatory characteristics of endogenous H₂S are becoming increasingly important. Wang et al. [16] first reported a direct correlation between endogenous H₂S and atherosclerosis. Their study indicated that there was a disturbance of the CSE/H₂S pathway in apoE⁻/⁻ mice, and they demonstrated an inhibitory role of H₂S on intercellular adhesion molecules (ICAM-1), which resulted in a protective effect against atherosclerosis. The investigation of H₂S and atherosclerosis has continued, and in-depth study on the role of H₂S in atherosclerosis has demonstrated the important theoretical and clinical value of hydrogen sulfide in the pathologic of atherosclerosis and new insights on the biological features of H₂S have resulted.

2. The regulatory role of H₂S on vascular structural remodeling

According to our studies, the basic processes of many vascular diseases include vasoconstriction and structural remodeling of vessels. The most important process of vascular remodeling is the proliferation of VSMCs, which plays a crucial role in the development of atherosclerosis. It was confirmed that H₂S suppressed VSMC proliferation in different ways (Fig. 2). Du et al. [17] previously reported that H₂S was shown to be an inhibitor of VSMC proliferation through the MAPK pathway, and the suppression effect was dose-dependent. NaHS in the concentrations of 5 × 10⁻⁵, 1 × 10⁻⁴, and 5 × 10⁻³ mol/L, as a source of H₂S, attenuated VSMC proliferation which was induced by endothelin by 16.8%, 26.6%, and 37.40%, respectively, via the MAPK pathway. Apoptosis of VSMCs also plays a dominant role in vascular structural remodeling. Yang et al. [18] found that H₂S induced apoptosis of human aorta smooth muscle.
cells (HASMCs) at concentrations ≥ 200 μM, was modulated by the level of endogenous H₂S. In addition, the pro-apoptotic effect of H₂S was confirmed to be mediated by activation of ERK, which caused stimulation of caspase-3 in HASMCs. After inhibiting ERK and caspase-3, the apoptosis of HASMCs induced by H₂S was significantly attenuated. It was hypothesized that H₂S might activate ERK first, then the activated ERK would catalyze the downstream factors as caspase-3, and finally, all activated factors induced cell apoptosis cooperatively. Another study by Yang et al. [19] reported that overexpressed CSE in HASMCs showed growth inhibition and apoptosis induction that was due to the overproduction of H₂S. This study suggested a novel therapeutic approach; i.e., CSE gene transferring, for treating vascular diseases involved in cell proliferation. A report by Qing et al. [20] first showed that altered CSE/H₂S levels were involved in the pathogenesis of balloon injury-induced atherosclerosis in rat carotid arteries. After balloon injury, CSE mRNA, CSE activity, and endogenous H₂S were reduced. More importantly, they found that treatment with NaHS significantly attenuated balloon-injury-induced neointimal hyperplasia using the method of morphometric analysis (0.15 ± 0.01 versus 0.21 ± 0.01 mm²; P < 0.001). These researchers hypothesized that anti-proliferation and pro-apoptotic effects were related to the inhibitory effect of H₂S on balloon injury-induced neointimal hyperplasia. In addition, S-diclofenac, a novel H₂S releasing derivative, was demonstrated to inhibit the proliferation of rat VSMCs dose-dependently (cell survival: 10 μM – 94.62%, 30 μM – 91.88%, 50 μM – 79.93%, and 100 μM – 5.34%; p < 0.05). More importantly, they considered the evidence that the pro-apoptotic effect of H₂S was involved in the cell cycle and was associated with activating some cyclin-dependent kinases. S-diclofenac treatment
decreased the percentage of cells in the G1 phase of the cell cycle significantly. S-diclofenac treatment increased p53, p21, p53AIP1, and Bax in cells. On the other hand, S-diclofenac did not increase the anti-apoptotic factor Bcl-2 [21]. These advances in investigating the modulating effect of H2S on VSMC proliferation shed light on the potential use of H2S to treat atherosclerosis.

### 3. H2S is involved in the pathogenesis of hyperhomocysteinemia

Homocysteine (Hcy) is a metabolite of methionine. Hcy accumulation in plasma is termed as hyperhomocysteinemia (HHCY). In 1969, McCully found early stages of atherosclerosis and thrombotic lesions in patients with HHCY [22]. A research showed that HCY induces the cholesterol metabolism of LDL in atherogenesis. Ox-LDL is deposited in the endothelium and causes the atherogenic modification of LDL. Currently, there is emphasis on the oxidation of LDL. S-diclofenac treatment increased the anti-oxidative stress effect. It was shown that low concentrations of H2S (30 and 50 μM) increased Hcy (100 μM)-incubated cell survival by 7% and 6.1%, respectively. Moreover, it was demonstrated that H2S (30 μM) attenuated the Hcy (100 μM)-induced overproduction of oxidized DCF (reflecting the levels of H2O2 and ONOO- and O2- by 13.8% and 55.8%, respectively [35]. This study showed the important role of H2S in HCY-induced cellular cytotoxicity via redox cell pathways. Subsequently, researchers also found injection of H2S reduced the concentration of Hcy in plasma, and decreased lipid peroxidation formation [36]. These results not only suggest that there is a protective function of H2S in HCY-induced anti-oxidative stress, which could interfere with the promoting effect of HHCY on cardiovascular diseases, but also provide insight for new areas of research on the influence of H2S on oxidative stress.

### 4. Inhibiting atherogenic modification of LDL

The third effect of H2S on atherosclerosis is inhibiting atherogenic modification of LDL. Currently, there is emphasis on the oxidation of LDL in atherogenesis. Ox-LDL is deposited in the endothelium and initiates the scavenging effects of macrophages and the proliferation of VSMCs. According to Hilde et al. [37], H2S (50 and 100 μM) reduced the atherogenic modification of LDL induced by HOCl (the product of the activated myeloperoxidase/H2O2/chloride system) significantly. In the presence of HOCl/NaHS (1/0.75 mmol/L), REM (relative electrophoretic mobility) was decreased by 50%. The report also showed an ability of H2S to inhibit the activity of the myeloperoxidase/H2O2/chloride system. Furthermore, H2S exhibited the property of interfering with the myeloperoxidase/H2O2/chloride system substrates (MPO and H2O2) and scavenging HOCl.

### 5. Attenuating calcification

As an important risk factor of atherosclerosis, calcification increases the risk of myocardial infarction and aggravates plaque instability [38]. Wu and coworkers [39] showed that vascular calcification was successfully treated by administering vitamin D3 plus nicotine. Compared with controls, calcium content; i.e., 45Ca2+ accumulation and ALP activity, in calcified arteries were increased by 6.77-, 1.42-, and 1.87-fold, respectively (P < 0.01). Also, the osteopontin mRNA concentration was elevated in calcified arteries. The CSE/H2S pathway was downregulated significantly by calcification. The H2S content in the aorta and plasma were decreased by 57% and 39%, and CSE activity and the amount of CSE mRNA were downregulated by 53% and 76%, respectively. Administration of NaHS in VDN-treated rats resulted in a decrease of the vascular calcium content, 45Ca2+ accumulation, ALP activity, and aortic OPN mRNA by 34.8%, 40.75%, 63.5%, and 74%, respectively, in the low-dose group, and by 84%, 38%, 46%, and 86% in the high-dose group, respectively. These results indicate that H2S interferes with vascular calcification dose-dependently. This report speculated that the effect on vascular calcification of H2S might be induced by regulating oxidative stress [39]. The exact pathogenic mechanisms, however, by which H2S regulates vascular calcification needs more investigation. A new target for the prevention and treatment of vascular calcification and atherosclerosis may result from this line of investigation.

### 6. Intervening in platelet function

In the formation of atherosclerotic plaque, the activation, adhesion, and aggregation of platelets can induce vessel occlusion and further ischemia. We found H2S could intervene in platelet function through different mechanisms. The disulfide bond arrangement has been considered in platelet function as well as the extracellular platelet protein function [40]. Giovanni et al. [41] reported that H2S had the effect of inhibiting platelet aggregation in vitro without toxic effects (up to 10 mM). This effect depended on the strength of the stimulus, such as ADP, collagen, epinephrine, arachidonic acid, thromboxane mimetic, U46619, and thrombin. Moreover, they found NaHS in the concentration of 30 μM, which is within the physiologic H2S dose in plasma, plus ADP (0.8 μM) showed a significant inhibition of platelet aggregation. It could be hypothesized that H2S in the physiologic state has the function of inhibiting platelet aggregation. Though the exact mechanism is still unclear, researchers speculate that interference with disulfide metabolism of some activators of platelets is involved in mechanisms responsible for the inhibitory effect of H2S.

### 7. The interaction between H2S and inflammation

Atherosclerosis is well-known as a chronic inflammatory process. Inflammation induces the atheromatous process, promotes atherosclerotic plaque complications, triggers neovascularization, and enhances microvessel permeability. Studies have confirmed that H2S was associated with the development of inflammation [42]. The research by Jia Song Bian et al. evidenced H2S attenuated LPS-induced inflammation in cultured microglia [43]. They found both exogenous application of NaHS and stimulating endogenous H2S production displayed a strong inhibitory effect on LPS-stimulated NO production and TNF-α secretion. Moreover, researchers indicated the mechanism for the anti-inflammatory effect of H2S was associated with inhibition of LPS-induced iNOS and p38 MAPK signaling pathways. In addition, Zanardo et al. [44] reported H2S inhibited leukocyte infiltration and adhesion, which is associated with activation of KATP channels. The report by Wang et al. [16] brought the direct correlation of H2S and atherosclerosis to light, and explored the role of H2S on regulation of ICAM-1. ICAMs are responsible for the migration of inflammatory cells, whereas inflammatory cells from the circulation crossed the endothelium
The decreased production of H2S resulted in a compensatory increase in H2S/CSE pathway. This study demonstrated the role of H2S in the procession of atherosclerosis in apoE−/− mice. H2S levels were decreased in both plasma (44.6 ± 4.52 versus 57.69 ± 7.03 μmol/L) and the aorta (1.98 ± 0.82 versus 4.36 ± 2.46 nmol/min mg protein). However, aortic expression of CSE mRNA was significantly increased (3.09 ± 1.31 versus 1.05 ± 0.30, P < 0.05). This indicates an abnormal CSE/H2S system may be another causative factor for atherosclerosis. ICAM-1 levels in apoE−/− mice were significantly increased (plasma: 0.37 ± 0.02 versus 0.21 ± 0.07 μg/L, P < 0.01; aorta: 2.07 ± 1.04 versus 1.08 ± 0.44, P < 0.05). After apo E−/− mice were treated with NaHS, the atherosclerotic plaque dwindled, whereas the level of ICAM-1 in plasma, ICAM-1 mRNA levels and expression in atherosclerotic aorta were significantly depressed, and the activation of NF-KB, a promotor of ICAM-1, was suppressed. This study demonstrated the role of H2S in the progression of atherosclerosis by regulating ICAM-1 and showed the effect of H2S in maintaining the stability of atherosclerotic plaque. In this research, H2S showed an effectively therapeutic value for atherosclerosis in apoE−/− mice, and prompted us to further investigate the causative role of endogenous H2S in the pathogenesis of atherosclerosis.

8. Conclusions and perspectives

It is widely recognized that H2S is not only a toxic gas with a strong odor of rotten eggs, but it is also an endogenous gas produced by the catalyzation of CSE in the cardiovascular system at a concentration range of 10–100 μM in the blood [45]. It has been shown that endogenous H2S participates in the regulation of many cardiovascular diseases. Here we summarized important pathways in which H2S regulates atherosclerosis. Such investigations have stimulated the development of research on the therapeutic potential of H2S for atherosclerosis by scientists and clinicians worldwide. H2S, as a gasotransmitter, is involved in the occurrence, development, and outcome of atherosclerosis. But these studies also suggest challenges and questions. Positive feedback in the H2S/CSE pathway was first demonstrated by Wang et al. [16]. The decreased production of H2S resulted in a compensatory increase of CSE gene expression. The exact mechanism of this “positive feedback” has not been explained to date. The causative role of the abnormality of the H2S/CSE pathway in atherosclerosis remains controversial. Though NO and H2S were both reported as being involved in the pathogenesis of atherosclerosis (Fig. 3), the interaction between NO and H2S in the pathogenesis of atherosclerosis is not clear. This provides a new area of research for a new treatment for atherosclerosis. Clinical studies of H2S on the treatment of cardiovascular diseases are very rare and many opportunities to study H2S in atherosclerotic patients remain. The relation between H2S and atherosclerosis requires further investigation.

Acknowledgments

This work is supported by grants from National Natural Science Foundation of China (30630301, 30821001, and 30801251) and the Major Basic Research Development Program of People’s Republic of China (2006CB503807).

References