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Review

Targeting hydrogen sulfide as a promising therapeutic strategy for atherosclerosis

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ABSTRACT

Physiological concentrations of nitric oxide (NO) and carbon monoxide (CO) have multiple protective effects in the cardiovascular system. Recent studies have implicated hydrogen sulfide (H_2S) as a new member of vasculoprotective gasotransmitter family, behaving similarly to NO and CO. H_2S has been demonstrated to inhibit multiple key aspects of atherosclerosis, including atherogenic modification of LDL, monocytes adhesion to the endothelial cells, macrophage-derived foam cell formation and inflammation, smooth muscle cell proliferation, neointimal hyperplasia, vascular calcification, and thrombogenesis. H_2S also decreases plasma homocysteine levels in experimental animal models. In the human body, H_2S production is predominantly catalyzed by cystathionine-β-synthase (CBS) and cystathionine γ-lyase (CSE). CSE is the primary H_2S-producing enzyme in the vasculature. Growing evidence suggests that atherosclerosis is associated with vascular CSE/H_2S deficiency and that H_2S supplementation by exogenous H_2S donors (such as NaHS and GYY4137) attenuates, and H_2S synthesis suppression by inhibitors (such as D, L-propargylglycine) aggravates the development of atherosclerotic plaques. However, it remains elusive whether CSE deficiency plays a causative role in atherosclerosis. A recent study (Circulation. 2013; 127: 2523–2534) demonstrates that decreased endogenous H_2S production by CSE genetic deletion accelerates atherosclerosis in athero-prone ApoE$^{-/-}$ mice, pinpointing that endogenously produced H_2S by CSE activation may be of benefit in the prevention and treatment of atherosclerosis. This study will facilitate the development of H_2S-based pharmaceuticals with therapeutic applications in atherosclerosis-related cardiovascular diseases.

1. Introduction

Atherosclerosis, the leading cause of cardiovascular morbidity and mortality worldwide, is considered to be a chronic inflammatory, progressive and multifactorial disease, in which immunity disturbance also plays an important role [1,2]. Lipid-lowering statins are the mainstream therapeutic strategy in slowing or reversing atherosclerotic plaque progression in clinic, but the therapeutic benefits of statins in hypercholesterolemic patients without previous cardiovascular diseases are very limited [3]. Therefore, searching for other anti-atherosclerotic agents has been the focus of cardiovascular medicine. In 1998, the Nobel Prize in Physiology or Medicine was awarded to Robert F Furchgott, Louis J Ignarro and Ferid Murad for their discoveries concerning "the nitric oxide (NO) as a signaling molecule in the cardiovascular system" (www.nobelprize.org). Since then, burgeoning evidence has indicated that NO is an endogenous signaling molecule that functions as endothelium-derived relaxing factor. Later on, other gaseous molecules, such as carbon monoxide (CO) [4], hydrogen (H_2) [5,6] and hydrogen sulfide (H_2S) [7,8] have also demonstrated some/all of the vasculoprotective effects of NO.

2. H_2S as a member of the family of gasses similar to NO

It is well recognized that NO, CO and H_2 are vasculoprotective gaseous molecules. Like NO and CO, H_2S emerged as the third member of the gasotransmitter family [8,9]. Due to the fact that H_2S is a small molecule that is freely permeable to the cell membrane, H_2S can directly exert its biological functions via interacting with multiple signaling molecules, as occurs with the production of NO from endothelial NO synthase (eNOS) or CO from heme oxygenases (HO) [10,11]. Mounting studies have begun to unveil the vasculoprotective functions of this promising gas. However, whether H_2S attenuates the development of atherosclerosis remained unexplored until Wang et al. [12] first reported that H_2S supplementation (by sodium hydrosulfide (NaHS)) protects against atherosclerosis in ApoE$^{-/-}$ mice. Intriguingly, H_2S possesses almost all of the protective effects of NO without reacting with superoxide to form the toxic metabolite peroxynitrite [13,14]. Under some disease conditions, these three members of gasotransmitter

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family (NO, CO, and H_2S) may not work independently, but cooperatively to modulate disease pathogenesis [15], especially given the recent appreciation of the mutual cooperation between H_2S and NO in regulating angiogenesis and endothelium-dependent vasorelaxation [16]. Therefore, these three gasotransmitters may cooperate with each other in the regulation of atherogenesis. To retrospectively analyze the studies of H_2S, we searched the PubMed for research articles and reviews. From 2002 to 2012, there was a trend of substantial increase in the publications covering H_2S biology and medicine year by year (Fig. 1). It is clear that the H_2S will continue to create exciting research opportunities for the scientific community, especially for cardiovascular medicine.

3. Natural biogenesis and metabolic pathways of H_2S

In mammalian cells, endogenous H_2S is predominantly synthesized by cystathionine-β-synthase (CBS, EC4.2.1.22), cystathionine γ-lyase (CSE, EC 4.4.1.1), cysteine aminotransferase (CAT, EC 2.6.1.3), and 3-mercaptopyruvate sulfurtransferase (MST, EC 2.8.1.2). All the four enzymes are involved in the transsulfuration and reverse transsulfuration pathways as described elsewhere [11]. Among them, CBS and CSE are the most extensively studied isoforms, using amino acids L-cysteine, L-homocysteine and L-cystathionine to produce H_2S with pyridoxal 5′-phosphate (vitamin B6) as a cofactor [11]. CBS is the main H_2S-generating enzyme in the central nervous system, while CSE is primarily expressed in the vasculature, liver and kidney [13,17]. CSE catalyzes the main reaction that produces the H_2S by the following reaction: L-cysteine + H_2O → Ammonia + pyruvate + H_2S. In addition to CBS and CSE, CAT and MST also catalyze the production of H_2S, and have been localized both in cytosol and mitochondria. In addition, a small portion of H_2S can be generated non-enzymatically by glucose-reducing equivalents and GSH. In order to maintain a proper physiological balance of H_2S metabolism, the mammalian system has developed four major routes of elimination, including mitochondrial oxidation, cytosolic methylation, scavenging (by methemoglobin, hemoglobin, metallo- or disulfide-containing molecules such as horseradish peroxidise, catalase, and oxidized glutathione), as well as expiration and excretion [11,17].

4. Vasculoprotective effects of H_2S

Evidence gathered from the last decade has implicated CSE/H_2S pathway as a pleiotropic vasculoprotective pathway in cardiovascular diseases. It has been reported that CSE expression and activity were reduced in balloon-injury induced neointimal hyperplasia [18] and diet-induced atherosclerosis [19], suggesting that H_2S may be beneficial for preventing coronary artery restenosis after percutaneous coronary intervention or coronary artery bypass grafting. Emerging evidence converged to demonstrate that H_2S possesses multiple vasculoprotective effects, which include: (1) inhibiting atherogenic modification of LDL induced by hypochlorous acid (HOCl) [20]; (2) inhibiting monocytes adhesion to activated endothelium [12,21] and promoting angiogenesis [22]; (3) improving vasorelaxation [23]; (4) inhibiting VSMC proliferation and migration [18,24] and stimulating VSMC apoptosis [25] (thereby decreasing intimal hyperplasia); (5) inhibiting macrophage foam cell formation [26], chemotaxis [19], and inflammation [27,28]; and (6) decreasing vascular calcification [29], platelet aggregation and thrombogenesis [30,31].

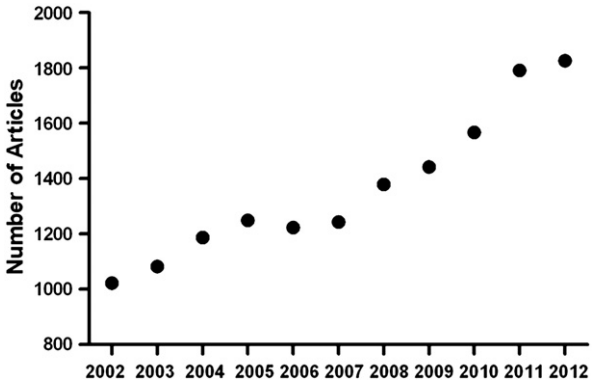

Fig. 1. Rising number of publications in H_2S biology and medicine. Review criteria: A search for original and review articles published in 2002–2012 was performed in the PubMed database (www.ncbi.nlm.nih.gov/pubmed) using key terms "hydrogen sulfide" or "H_2S".

Hypertension and hyperhomocysteinemia represent two major risk factors of atherosclerotic cardiovascular diseases; in this aspect, H_2S significantly lowers blood pressure as well as reduces plasma homocysteine levels in experimental animals. However, the role of endogenous H_2S in modulating lipid metabolism remains unclear. Despite previous studies [12,19] have shown that plasma lipid levels were not significantly altered after treatment with NaHS or DL-propargylglycine (PAG) in ApoE$^{-/-}$ mice, the role of endogenous H_2S in atherosclerosis development is inconclusive as the pharmaceutical manipulation of H_2S by donors or inhibitors does not truly reflect the status of endogenous H_2S metabolism [11]. H_2S (donated by NaHS, 56 μmol/kg/d, intraperitoneal injection (i.p.)) has also been found to inhibit atherogenesis by regulating endoplasmic reticulum stress [32]. The protective effects of H_2S-releasing compounds against the pathogenesis of atherosclerosis are summarized in Table 1. For detailed information, we refer the readers to recent elegant reviews [7,11,22,33–35].

5. In vivo evidence that links H_2S with atherosclerosis

5.1. CBS/CSE knock-out and pharmacological inhibitors accelerate atherosclerosis

Despite the report that CBS$^{-/-}$ApoE$^{-/-}$ mice developed larger lesion size compared with ApoE$^{-/-}$ mice after 6 months of age without dietary manipulation, the pathophysiological relevance of CBS$^{-/-}$ to H_2S metabolism in atherosclerosis was not clear since CBS is not expressed in the vasculature and only 2% of the double knockout mice can survive to 6 months of age [36]. In vitro, a recent study has suggested that oxidized LDL (oxLDL) stimulation downregulated CSE/H_2S pathway and increased inflammatory responses in cultured macrophages [27]. This effect can be prevented by exogenous supplementation of NaHS and Na_2S, whereas, exacerbated by CSE inhibitor PAG [27]. Therefore, CBS and CSE inhibitors are useful tools in elucidating the effect of endogenous H_2S on atherosclerosis. More recently, Zhang et al. [19] observed that treatment with PAG (10 mg/kg, i.p., daily) increased aortic CX3CR1 and CX3CL1 expression and exacerbated the extent of atherosclerosis in male ApoE$^{-/-}$ mice fed with a high-fat diet (HFD), suggesting that endogenous H_2S limits atherogenesis by inhibiting chemokine-mediated chemotaxis in macrophages. However, we should bear in mind that the data obtained with these inhibitors should be interpreted with caution due to the issue of non-specificity of these inhibitors (which also inhibits other vitamin B6-dependent enzymes with pyridoxal phosphate binding site) [17]. Previous studies have indicated that CSE$^{-/-}$ mice developed spontaneous hypertension [8], hyperhomocysteinemia [37], and neointima formation [24]. The only evidence linking decreased endogenous synthesis of H_2S (by CSE deficiency) with atherosclerosis is not completely conclusive.

In the study by Mani et al. [38], the authors directly addressed whether CSE deficiency impacts the development of atherosclerosis, by knocking out CSE gene in wild type (WT) and ApoE$^{-/-}$ mice, fed with a normal diet (ND) or HFD. The authors first analyzed the effect of endogenous H_2S on lipid metabolism. The phenotypic differences between CSE$^{-/-}$ vs WT and CSE$^{-/-}$ApoE$^{-/-}$ vs ApoE$^{-/-}$ were summarized in Table 2. These data expand previous findings showing