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MINIREVIEW

Hydrogen Sulfide, the Next Potent Preventive and Therapeutic Agent in Aging and Age-Associated Diseases

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Hydrogen sulfide (H_2S) is the third endogenous signaling gasotransmitter, following nitric oxide and carbon monoxide. It is physiologically generated by cystathionine-γ-lyase, cystathionine-β-synthase, and 3-mercaptopyruvate sulfurtransferase. H_2S has been gaining increasing attention as an important endogenous signaling molecule because of its significant effects on the cardiovascular and nervous systems. Substantial evidence shows that H_2S is involved in aging by inhibiting free-radical reactions, activating SIRT1, and probably interacting with the age-related gene *Klotho*. Moreover, H_2S has been shown to have therapeutic potential in age-associated diseases. This article provides an overview of the physiological functions and effects of H_2S in aging and age-associated diseases, and proposes the potential health and therapeutic benefits of H_2S.

Aging has been a topic of great interest to the scientific community for a long time. This biological process is characterized by time-dependent progressive decline of physiological functions accompanied by increased incidence of age-associated diseases. The basic cellular and biochemical features of aging are very complex to elucidate clearly. Although various theories have been proposed to explain the process of aging, there is none that has been generally accepted by gerontologists. Denham Harman proposed that aging and age-associated diseases are basically caused by the deleterious side attacks of free radicals on cell constituents and connective tissues (1). This proposal, which implies that limiting or inhibiting the formation of free radicals could reduce the rate of aging and prevent age-associated diseases, is receiving growing acceptance as a possible explanation of the aging theory. Studies showing that nutritional antioxidants (vitamin C, vitamin E, and resveratrol) and antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], and glutathione [GSH] peroxidase) can promote better health and longevity by suppressing free-radical reactions are still limited and equivocal (2). However, antioxidants are receiving considerable attention and their use is increasingly being adopted in Western countries. Hydrogen sulfide (H_2S), which was previously regarded as a poisonous gas, is generated endogenously in mammals. With its antioxidant and other physiological functions, it has the potential to provide many health benefits. Several studies have shown that H_2S prevents free radical-induced impairment and is beneficial in treating age-associated diseases. A recent study reported that the plasma H_2S level in humans over 50 to 80 years of age declines with age (3). However, the precise relationship between H_2S and physiological changes that constitute aging is largely unknown. This review aims to provide detailed information on the various physiological effects of H_2S on aging and age-associated diseases known to date.

GENERATION OF ENDOGENOUS H_2S

H_2S is a colorless, flammable, water-soluble gas with the characteristic smell of rotten eggs. In the past several centuries, H_2S had been known only for its toxicity and environmental hazards. However, as a member of a family of gasotransmitters that includes nitric oxide and carbon monoxide, it is produced endogenously in mammals, including humans, and has important physiological roles in the human body (4, 5). Previous studies reported that H_2S is physiologically generated by cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS) (6, 7). These two pyridoxal-5′-phosphate-dependent enzymes, which are expressed in the liver, kidney, brain, thoracic aorta, ileum, pancreatic islets, uterus, and placenta, among other locations, are crucial in the synthesis of H_2S (8–13). CBS is predominantly expressed in the brain and the nervous system (6, 14, 15). However, expression of CSE proteins has been mainly observed in vascular smooth muscle cells and in the heart (16, 17). In addition, 3-mercaptopyruvate sulfurtransferase and cysteine aminotransferase localized to the endothelium of the thoracic aorta have also been reported to produce H_2S from cysteine and alpha-ketoglutarate (18) (Fig. 1).

PHYSIOLOGICAL EFFECTS OF ENDOGENOUS H_2S

H_2S has various physiological effects on the human body. First, H_2S is a major endothelium-derived hyperpolarizing factor (EDHF) that causes hyperpolarization and vasorelaxation of vascular endothelium and smooth muscle cells by activating ATP-sensitive, intermediate-conductance and small-conductance potassium channels through cysteine S-sulfhydration (19, 20). Second, H_2S can prevent cytokine- or oxidant-induced oxidative damage through its antioxidative effects (21–23). Additionally, H_2S can inhibit the expression of proinflammatory factors by downregulating NF-κB activation or by upregulating heme oxygenase 1 expression (24–28). Moreover, H_2S may help regulate various functions of the human body with its cytoprotective, antifibrotic, antiapoptotic, and angiogenic properties (29–32). Taken together, these known functions of H_2S imply an important role in aging and age-associated diseases (Table 1).

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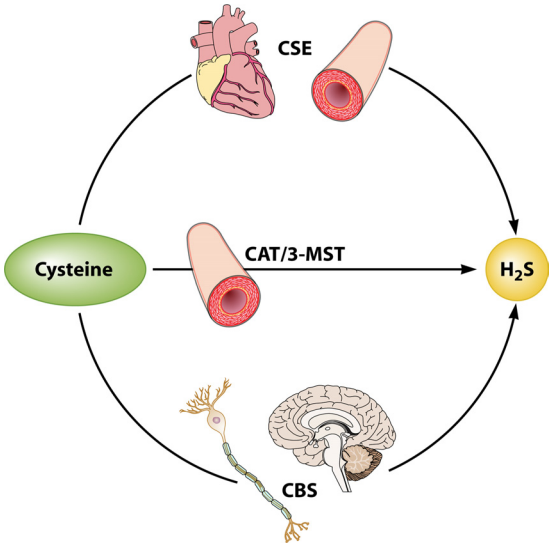

FIG 1 Generation of endogenous H_2S. H_2S is synthesized by two cytosolic pyridoxal-5′-phosphate-dependent enzymes responsible for the metabolism of L-cysteine, namely, cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), as well as by the combined action of 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT).

ROLE OF H_2S IN REGULATION OF AGING

H_2S inhibits free-radical reactions and oxidative stress. Continuous accumulation of deleterious free-radical reactions in cells and tissues is a major contributor to aging. Hence, the aging process may be caused by free-radical reactions. Free radicals and oxidative stress are believed to be important risk factors in aging and various age-associated diseases. A judicious selection of diet and antioxidant supplements is expected to promote longevity (34, 35). Consistent with this idea, many healthy volunteers with improved body redox status benefit from sulfurous water consumption, which can reduce biomolecule oxidation and provide valid protection against oxidative damage commonly associated with aging and age-related degenerative diseases. However, endogenously produced H_2S can be hydrolyzed to hydrosulfide and sulfide ions. Interestingly, it has been reported that levels of both lipid and protein oxidation products, namely, malondialdehyde (the lipid peroxidation product), advanced oxidation protein products, and carbonyls decrease significantly in plasma samples of healthy volunteers subjected to a cycle of hydroponic therapy with H_2S-rich water (500 ml day^{-1} for 2 weeks) (36). Although the underlying mechanisms of this effect are not completely understood, it implies that H_2S can protect against oxidative stress. Oxidative damage to biological macromolecules is caused by increased levels of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) which can disrupt the cellular reduction-oxidation (redox) balance. The primary ROS/RNS species generated in a cell are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and nitric oxide ($NO^{\bullet}$) (37). H_2S can protect mouse brain endothelial cells (bEnd3) from methionine (Met)-induced oxidative stress and cytotoxicity. Pretreatment of bEnd3 cells with NaHS (0.05 mM) can decrease H_2O_2, peroxynitrite ($ONOO^-$), and $O_2^{\bullet -}$ generation induced by Met. In the ROS scavenging pathway, SOD converts $O_2^{\bullet -}$ to H_2O_2, which is subsequently reduced to H_2O and O_2 by CAT. However, low concentrations of H_2S can synergistically increase inhibitory effects of apocynin, *N*-acetyl-L-cysteine (NAC), reduced GSH, CAT, SOD, and Nω-nitro-L-arginine methyl ester (L-NAME) on ROS production and redox enzyme levels induced by Met (38). $ONOO^-$, a powerful oxidant and cytotoxic species which can induce nitration of tyrosine and tyrosine residues in proteins, is formed from rapid interaction of $NO^{\bullet}$ and $O_2^{\bullet -}$ (39). H_2S has also been considered a potent scavenger of $ONOO^-$. H_2S at low concentrations (30 μM) can significantly inhibit tyrosine nitration mediated by $ONOO^-$ to an extent similar to that seen with reduced GSH, a major antioxidant in the cellular defense against oxidative stress. Additionally, H_2S can also significantly inhibit cytotoxicity mediated by $ONOO^-$ in human neuroblastoma SH-SY5Y cells at physiological levels (40). In addition, Whiteman et al. have shown that H_2S can similarly quench $NO^{\bullet}$ to form a novel nitrosothiol compound *in vitro*. This suggests that H_2S has direct chemical interactions with $NO^{\bullet}$ free radicals to suppress oxidative stress (41). Moreover, Kimura et al. demonstrated that H_2S can protect mouse brain neuroblastoma Neuro2a cells from oxidative stress caused by H_2O_2 and recover GSH levels suppressed by H_2O_2 in those cells (42). Furthermore, modulation of oxidative stress by calorie restriction (CR) is recommended as one mechanism to slow the aging process and decline of body functions in animals (43). Thus, H_2S may also slow the aging process by inhibiting free-radical reactions and oxidative stress.

The free-radical theory was revised when mitochondria were found to be responsible for the initiation of most free-radical reactions occurring in cells. The life span is also postulated to be determined by the rate of free-radical damage to mitochondria. In fact, owing to continuous generation of free radicals throughout the life of a cell, mitochondria, especially mitochondrial DNA, are the key targets of free-radical attack (44). Kimura and coworkers showed that H_2S reduces cystine to cysteine in the extracellular space and increases intracellular concentrations of cysteine to increase the production of intracellular GSH and enhances the redistribution of GSH into mitochondria in Neuro2a cells. It enables H_2S to suppress oxidative stress in the mitochondria (42). In rat models of ischemia and reperfusion (I/R) injury, it has been observed that preconditioning with H_2S can prevent mitochondrial dysfunction in rat intestinal mucosa by a calcium-activated, large-conductance potassium channel-dependent mechanism (45). Under I/R conditions, production of ROS increases, which has a pivotal role in the pathogenesis of myocardial I/R injury. Inhibition of oxidative stress has been reported to underlie the

TABLE 1 Physiological effects of hydrogen sulfide on aging

Parameter	Effect[a]		References
	Aging	H_2S	
Blood pressure	↑	⊢	19, 20
Oxidative damage	↑	⊢	21, 22, 23
Inflammation			
Inducible NO synthase	↑	⊢	24, 25, 26, 27, 28
NF-κB activation	↑	⊢	
Heme oxygenase 1	↓	↑	
Apoptosis	↑	⊢	30, 33

[a] ↑, increase; ↓, decrease; ⊢, blunting of the increase.

cardioprotective effects of H_2S under I/R conditions. Sun et al. showed that treating cardiomyocytes with 50 μM NaHS, which is close to the level found in rat plasma, decreases the levels of ROS by inhibiting mitochondrial complex IV3 and increasing SOD activities in cardiomyocytes under I/R conditions. H_2S has also been found to inhibit electron transport to reduce harmful ROS generation under cardiac I/R conditions (46). Thus, protection against oxidative stress-induced mitochondrial damage may be a novel mechanism through which H_2S could furnish valid protection and possibly increase life span.

H_2S activates SIRT1 to resist aging. The silent information regulator 2 gene (*SIR2*) family, which is conserved from prokaryotes to eukaryotes (*Staphylococcus aureus*, fission yeast, *Arabidopsis*, *Caenorhabditis elegans*, mouse, rat, human), has functions in silencing, cell cycle progression, and chromosome stability (47). *SIR2* is a determinant of life span in yeast mother cells. It can promote longevity in *Saccharomyces cerevisiae* by suppressing the generation and accumulation of extrachromosomal ribosomal DNA (rDNA) circles, which are considered a cause of aging in yeast (48). Similarly, it has been demonstrated that *SIR2*, which encodes a histone deacetylase, extends the life span of *Caenorhabditis elegans* through signaling pathways involving *DAF-2*/insulin receptor and *DAF-16*/FOXO (49, 50). Mammals have seven Sir2 homologues (SIRT1 to -7), of which SIRT1 is most closely related to Sir2. These proteins have a highly conserved NAD-dependent sirtuin core domain, first identified in the yeast Sir2 protein, making them good candidates for life span regulators (51). Interestingly, it has been reported that exogenous H_2S has a protective effect on maintaining the circadian rhythm of clock genes in isolated hepatocytes by changing the NAD^+/NADH ratio and enhancing the activity of SIRT1 protein (52). SIRT1 has thus emerged as a major life span regulator, and H_2S has been hypothesized to delay aging, in part by activating SIRT1.

Calorie restriction is the only established antiaging experimental paradigm in a variety of species, including mammals, flies, nematodes, and yeast, that has been accepted by many researchers. It has been reported that CR can extend life span by increasing Sir2 activity and activating Sir2 deacetylase in yeast and *Drosophila*. Additionally, the expression of mammalian Sir2 (SIRT1) is induced in calorie-restricted rats and also in human cells cultured in the presence of serum from such rats. SIRT1 can deacetylate the DNA repair factor Ku70, causing it to sequester the proapoptotic factor Bax away from the mitochondria, thereby inhibiting stress-induced apoptotic cell death. A major cause of aging is perceived to arise from the cumulative effects of cell loss over time. Moreover, it has also been reported that CR can inhibit the insulin/insulin-like growth factor 1 (IGF-1) pathway to increase SIRT1 expression. Thus, CR can promote longevity by inducing SIRT1 expression and promoting long-term survival of irreplaceable cells (33). Correspondingly, when exposed to H_2S, nematodes are apparently healthy and do not exhibit phenotypes consistent with metabolic inhibition. Instead, animals exposed to H_2S are long-lived, and this phenotype requires Sir2 activity, which may translate environmental changes into physiological alterations to improve survival (53). CR also has potential benefits for CSE and CBS in both the aorta and liver by reducing oxidative stress and ameliorating the negative effect of age on H_2S concentration. Consistent with this, Predmore et al. inferred from their study that CR may help maintain the H_2S signaling system during aging (54). CR and H_2S apparently have a synergistic antiaging effect by regulating SIRT1 activity. Our laboratory recently demonstrated that H_2S has protective effects against human umbilical vein endothelial cell senescence, which may involve the regulation of SIRT1 activity. Similar to resveratrol, H_2S is likely a CR mimetic with potential antiaging functions mediated via SIRT1 activation. It has been reported that SIRT1 could deacetylase the FOXO transcription factors *in vitro* and within mammalian cells to increase cellular stress resistance, which is correlated with extended organismal longevity (55). However, the hypothesis that the antiaging effects of H_2S are mediated via the SIRT1/FOXO pathway needs to be validated with further investigations.

Resveratrol, a polyphenol found in red wine, is a CR mimetic with potential antiaging and antidiabetogenic properties. Resveratrol ameliorates age-related metabolic phenotypes by inhibiting cyclic AMP (cAMP) phosphodiesterases (PDE). Inhibiting PDE4 with rolipram reproduces all the metabolic benefits of resveratrol, including prevention of diet-induced obesity and promotion of mitochondrial function, physical stamina, and glucose tolerance in mice. Therefore, PDE4 inhibitors may also prevent and ameliorate the symptoms of age-related metabolic diseases (56). H_2S also acts as an endogenous inhibitor of phosphodiesterase activity (57). However, whether H_2S uses the same mechanism for inhibiting phosphodiesterases to prevent age-related diseases and for exerting antiaging effects via SIRT1 activation remains unknown. Figure 2 gives an overview of the pathways that underlie the antiaging effects of H_2S.

Effect of H_2S on the age-related gene *Klotho*. The age-related gene *Klotho* suppresses the expression of multiple age-associated phenotypes to extend life span. Klotho increases resistance to oxidative stress by activating the FOXO forkhead transcription factors that induce expression of manganese SOD (58). Klotho also represses DAF-2 (insulin/IGF-like) receptors under physiological conditions, thereby inducing derepression of DAF-16 (FOXO) and subsequent overexpression of factors such as antioxidant enzymes that improve longevity and stress resistance (59).

Klotho is predominantly expressed in the kidney. Downregulation of *Klotho* in the kidneys aggravates the development of renal damage induced by angiotensin II via increased oxidative stress, whereas induction of *Klotho* expression has therapeutic effects against angiotensin II-induced end organ damage (60). Yoon et al. confirmed that angiotensin II plays a pivotal role in regulating expression of *Klotho* in cyclosporine (CsA)-induced renal injury and that an angiotensin II type 1 (AT1) receptor blocker inhibits aging by decreasing CsA-induced oxidative stress (61). Interestingly, H_2S has a direct inhibitory effect on angiotensin-converting enzyme (ACE) activity, which catalyzes the conversion of angiotensin I into angiotensin II (62). Moreover, H_2S can decrease angiotensin II-induced mitogen-activated protein kinase (MAPK) activation, AT1 receptor binding, and the binding affinity of AT1 receptor in a dose-dependent manner (63). In addition, Lu et al. demonstrated that H_2S inhibits renin activity, which participates in the renin-angiotensin system (64). Thus, H_2S may enhance *Klotho* expression via negative regulation of angiotensin II activity. Given these findings, H_2S may be a potent preventive and therapeutic agent in aging and age-associated diseases. Figure 3 summarizes the indirect effects of H_2S on *Klotho* expression to show that H_2S may be involved in regulation of aging through the *Klotho* pathway.

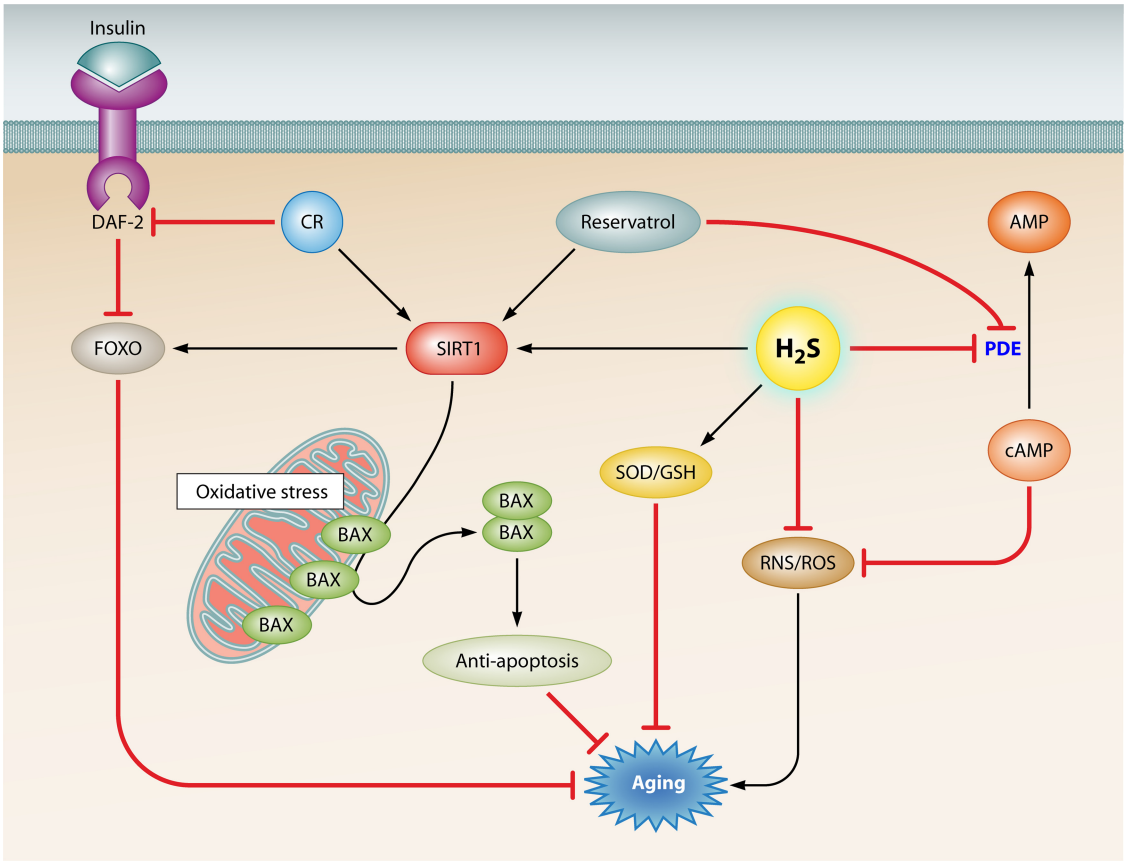

FIG 2 H_2S exerts its antiaging effects by directly inhibiting free radicals and oxidative stress and by upregulating SIRT1 activity. H_2S can directly inhibit the free radicals ROS/RNS and increase inhibitory effects of GSH and SOD on ROS production and redox enzyme levels to increase cellular stress resistance, which is correlated with extended organismal longevity. CR and resveratrol have been reported to have antiaging properties. CR and resveratrol share similar antiaging mechanisms: they both increase SIRT1 expression to inhibit oxidative stress or to play antiapoptotic roles. Resveratrol can also ameliorate age-related metabolic phenotypes by inhibiting cAMP phosphodiesterases (PDE). Similarly, H_2S has been reported to enhance the activity of SIRT1 and to inhibit PDE activity. As shown in the figure, H_2S may have antiaging functions through several shared pathways related to CR and resveratrol.

H_2S: A NOVEL THERAPEUTIC AGENT IN AGE-ASSOCIATED DISEASES?

H_2S has been generally recognized as an important signaling molecule in cardiovascular and nervous systems. Enhancement of the physiological effects of H_2S has been confirmed by understanding the therapeutic implications of H_2S in age-associated diseases, such as cardiovascular system diseases, central nervous system degenerative diseases, diabetes, and cancer, among others (Table 2).

Cardiovascular system diseases. Aging is considered a principal risk factor in the progression of hypertension (81). The plasma concentration of H_2S in spontaneously hypertensive rats is substantially lower than that in normotensive control animals (65). Similarly, the plasma H_2S level and H_2S production are greatly reduced and transcription and activity of CSE in vascular tissues are also obviously inhibited in rats with hypertension induced by N^G-nitro-L-arginine methyl ester (L-NAME). However, H_2S can prevent the development of hypertension induced by L-NAME. This suggests that the H_2S synthase/H_2S pathway participates in the pathogenesis of hypertension (82). The fundamental hemodynamic abnormality in hypertension is increased peripheral vascular resistance (83). It has been reported that H_2S can relax rat aortic tissues *in vitro* in an ATP-sensitive K^+ (K_{ATP}) channel-dependent manner and that H_2S at physiologically relevant concentrations induces vasorelaxation via activation of K_{ATP} channels in isolated mesenteric artery smooth muscle cells (17, 84). K_{ATP} channels are activated by binding of its Kir subunits to phosphatidylinositol-4,5-bisphosphate (PIP_2) along with a reduction in binding to the inhibitor ATP (85). H_2S causes vascular endothelial and smooth muscle cell hyperpolarization and vasorelaxation by activating K_{ATP} channels through cysteine S-sulfhydration, which can enhance Kir6.1-PIP2 binding and reduce Kir6.1-ATP binding (19). Additionally, considering that human hypertensive pulmonary vascular disease represents a disease of excessive smooth muscle cell proliferation (86), H_2S could also suppress the proliferation of cultured aortic vascular smooth muscle cells of rats *in vitro* through the MAPK pathway (87). Because H_2S is a major EDHF and a primary determinant of vasorelaxation in numerous vascular beds (20), drugs that alter CSE activity or H_2S-mediated channel sulfhydration may be effective therapeutic agents for treating hypertension.

Atherosclerosis, characterized by lesions in large and medium arteries, is the primary cause of coronary artery disease. Not only is this condition a chronic inflammatory disease that can lead to an acute clinical event by plaque rupture and thrombosis, but it is also an inevitable degenerative consequence of aging (88). Athero-

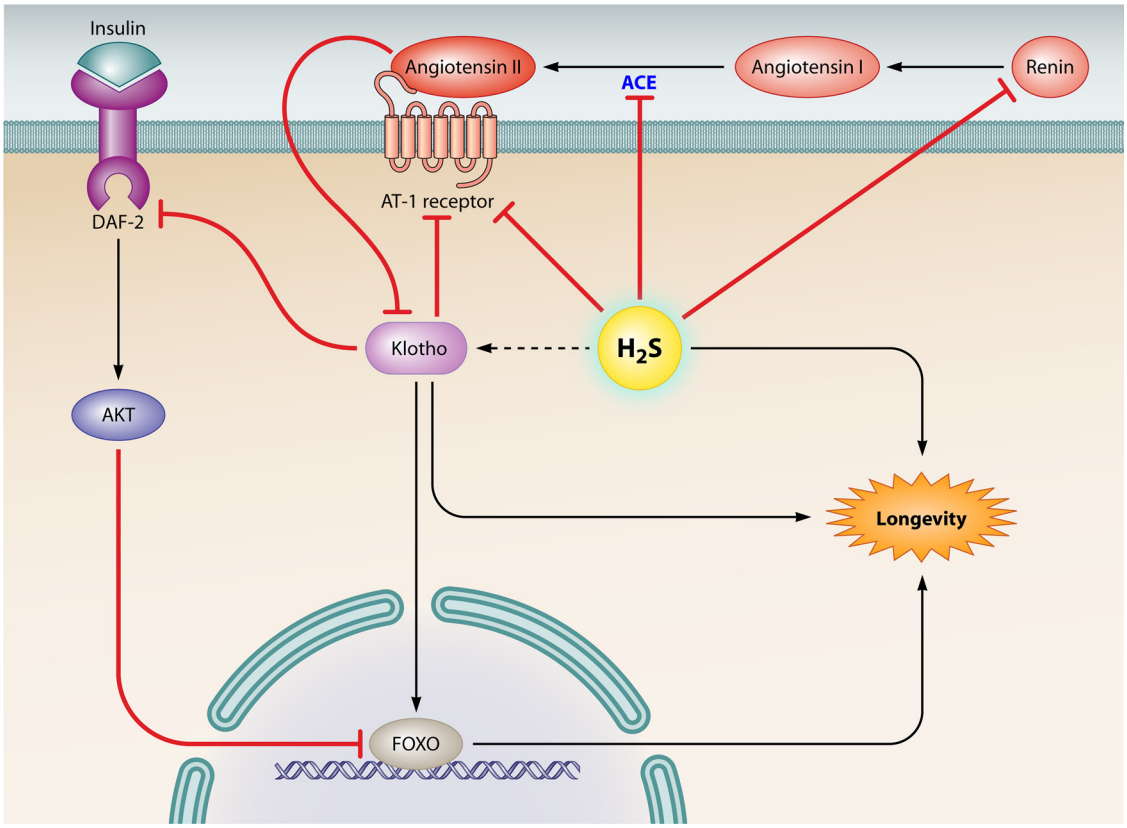

FIG 3 H_2S may have an effect on the age-related gene *Klotho* to promote longevity. *Klotho* can improve longevity by inhibiting IIS signaling, inducing FOXO derepression, and decreasing angiotensin II-induced oxidative stress, among other effects. However, angiotensin II may downregulate the expression of *Klotho*. Interestingly, H_2S exhibits direct inhibitory action on ACE activity, which catalyzes the conversion of angiotensin I to angiotensin II. Moreover, H_2S can decrease the binding affinity between angiotensin II and AT1 receptor and can inhibit renin activity, which participates in the renin-angiotensin system. Hence, H_2S may improve *Klotho* expression to promote longevity via negative regulation of angiotensin II production.

sclerosis is characterized by multiple key events, including endothelial dysfunction, infiltration of monocytes and their differentiation into macrophages, conversion of lesion-resident macrophages into foam cells, and smooth muscle cell proliferation. Fortunately, atherosclerosis can be interrupted by H_2S. Increasing evidence has proven that H_2S plays a significant role in all these biological processes and that disruption of H_2S homeostasis may contribute to the pathogenesis of atherosclerosis (89). For instance, H_2S plays an antiatherogenic role by suppressing the formation of human macrophage foam cells. H_2S inhibits the formation of macrophage-derived foam cells, which is a crucial event in the development of atherosclerosis, by downregulating CD36, SR-A, and ACAT-1 expression and inhibiting oxidized low-density lipoprotein (oxLDL) binding and uptake of macrophages (66, 67). In addition to its important role in preventing atherosclerosis, H_2S has also direct benefits for coronary heart disease (CHD) patients. Plasma H_2S levels in patients with CHD show a significant inverse correlation with the severity of CHD and changes in the coronary artery (90). Moreover, providing H_2S balneotherapy to CHD patients has been shown to result in significant prolongation of stress bicycle exercise. In the hot climate of arid zones, use of moderate H_2S baths raises the tolerance of CHD patients to exercise, attenuates clinical manifestations of CHD, and consequently reduces daily nitrate need (91). Additionally, stem cell transplantation has become a promising therapeutic approach for treatment of myocardial infarction (MI). However, poor survival of the donor cells after transplantation has restricted its therapeutic efficacy. H_2S has been applied to inhibit cell apoptosis and promote cell survival in such cases. It has been demonstrated that H_2S preconditioning effectively promotes stem cell survival under conditions of ischemic injury and helps cardiac repair after MI (92).

Degenerative diseases of the central nervous system. Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive loss of dopaminergic neurons in the substantia nigra (SN). In a 6-hydroxydopamine (6-OHDA)-induced PD rat model, the endogenous H_2S level has been found to be markedly reduced in the SN. However, H_2S treatment can specifically inhibit 6-OHDA-evoked NADPH oxidase activation, oxygen consumption, and microglial activation in the SN, and accumulation of proinflammatory factors in the striatum (68). PD is one of the most common neurodegenerative diseases with various manifestations, among which cognitive deficiency, namely, dementia, has a prominent role. Dementia is usually ascribed to changes in the nucleus basalis of Meynert and the cerebral cortex (93). H_2S has also been suggested to attenuate vascular dementia injury via inhibition of apoptosis by markedly improving the ratio of Bcl-2 to Bax, upregulating Bcl-2 expression, and downregulating Bax expression (69).

Alzheimer's disease (AD) is the most common form of demen-

TABLE 2 Effects of H_2S or its donors on age-associated diseases[a]

Age-associated disease	Exptl model	H_2S level	Cellular response elicited by H_2S or its donors	Reference(s)
Hypertension	Spontaneously hypertensive rats	↓	Relaxing vascular muscle cell by involving different ion channels	65
Atherosclerotic disease	Human monocyte-derived macrophages	↓	Inhibiting foam cell formation by downregulating CD36, SR-A, and ACAT1 expression via the K_{ATP}-ERK1/2 pathway	66, 67
PD	6-OHDA-induced PD rat	↓	Inhibiting NADPH oxidase activation and oxygen consumption; inhibiting accumulation proinflammatory factors via NF-κB pathway; inhibiting neuronal apoptosis by improving Bcl-2/Bax	68, 69
AD	Amyloid β-induced cell toxicity in murine BV-2 microglial cells	↓	Inhibition of inflammation; promotion of cell growth and preservation of mitochondrial function in a p38- and JNK-MAPK-dependent manner	70, 71, 72
Diabetes	Streptozotocin-induced diabetes in mice	In pancreas, ↑	Stimulating β-cell apoptosis and inducing K_{ATP} channel activity	73, 74, 75, 76
		In plasma, ↓	Protecting diabetic vascular endothelial cells	77
Prostate cancer	PC-3 cells (a human prostate cancer cell line)	?	Inhibiting PC-3 cells viability by activating p38 MAPK and JNK	78
ER(−) breast cancer	Mice with human ER(−) breast cancer xenografts	?	Suppressing the growth of cancer cells by induction of G_0/G_1 arrest and apoptosis; downregulation of NF-κB; reduction of thioredoxin reductase activity; increased levels of reactive oxygen species	79
Colon cancer	HT-29 human colon cancer cells	?	Inhibiting cell proliferation by reducing PCNA expression; inducing G_0/G_1 cell cycle arrest and apoptosis	80

[a] PD, Parkinson's disease; AD, Alzheimer's disease; ER(−), estrogen receptor negative; 6-OHDA, 6-hydroxydopamine; SR-A, scavenger receptor A; ACAT1, acyl coenzyme A-cholesterol acyltransferase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinases; PCNA, proliferating cell nuclear antigen.

tia. AD initially targets memory and progressively destroys the mind (94). This disease is pathologically characterized by the accumulation of senile plaques containing activated microglia and amyloid beta peptides (Abeta) (95). As mentioned previously, endogenous H_2S is predominantly produced in the brain from cysteine by CBS (5, 96). Endogenous levels of H_2S of between 50 and 160 μM are detected in the brains of humans (40). In the brains of AD patients, lower levels of H_2S as well as accumulation of homocysteine (Hcy), a strong risk factor for the development of Alzheimer's disease (AD), are observed (71, 72). Neurotoxicity of elevated Hcy is associated with inhibition of endogenous H_2S generation and downregulation of expression and activity of CBS in PC12 cells, which is mediated by extracellular signal-regulated kinase 1 and 2 (ERK1/2) activation. It has been suggested that H_2S could reduce neurotoxicity induced by Hcy and that enhancement of H_2S synthesis may be a useful therapeutic strategy against Hcy-induced AD (97). Accordingly, Liu and Bian have demonstrated that H_2S demonstrates protective effects against Abeta-induced cell injury by inhibiting inflammation, promoting cell growth, and preserving mitochondrial function in a p38- and Jun N-terminal protein kinase (JNK)–mitogen-activated protein kinase (MAPK)-dependent manner (70). Moreover, H_2S can protect neurons from oxidative stress, which is responsible for neuronal damage and degeneration in AD. H_2S protects neurons against glutamate-mediated oxidative stress by enhancing the activities of γ-GCS and cystine transport, which results in incremental changes of glutathione levels (98). These findings suggest that H_2S is a promising therapeutic target for treating neurodegenerative diseases.

Diabetes. Diabetes is a chronic metabolic disease with influence on the metabolism of carbohydrates and other nutrients. The pathogenesis of diabetes is associated with decreased functional beta cell mass and increased activities of K_{ATP} channels in pancreatic beta cells (99, 100). It has been reported that in streptozotocin-induced diabetes in mice, pancreatic H_2S production and CSE activity are significantly upregulated (73, 76). Similarly, it has also been found that pancreatic CSE expression and H_2S production are higher in Zucker diabetic fatty (ZDF) rats than in Zucker fatty and Zucker lean rats (101). Enhanced endogenous production of H_2S in diabetes can induce apoptosis of insulin-secreting beta cells by enhancing endoplasmic reticulum (ER) stress via p38 MAPK activation and also stimulate K_{ATP} channels in insulin-secreting cells (73–75). These results imply that H_2S at high concentrations contributes to the pathogenesis of diabetes mellitus.

Diabetes mellitus is also characterized by the resistance of peripheral tissues to insulin (102, 103). L-Cysteine which is synthesized from methionine is a main precursor of H_2S. However, adipose tissue is an important organ of methionine metabolism (104) and is also an insulin-sensitive organ that mediates glucose uptake and metabolism. With age, the endogenous CSE/H_2S system is upregulated in adipose tissues. Increased H_2S in adipose tissues inhibit basal or insulin-stimulated glucose metabolism and regulate insulin sensitivity. This suggests that H_2S may be a novel insulin resistance regulator (105). Additionally, Kaneko et al. suggested that elevation of the H_2S levels as a result of L-cysteine metabolism may inhibit insulin release from isolated mouse islets and the mouse β-cell line MIN6 under diabetic conditions (11). In ZDF rats, abnormally high pancreatic production of H_2S has been reported to suppress insulin release (101). These findings indicate that high concentrations of H_2S in pancreas could have deleterious effects on the development of diabetes and that new therapeutic measures for diabetes may involve inhibiting endogenous H_2S production from pancreas.

In contrast, Jain et al. have reported that blood H_2S levels are significantly lower in type 2 diabetes patients than in age-matched healthy subjects and in streptozotocin-treated diabetic rats than in

control Sprague-Dawley rats. Low blood H_2S levels may contribute to the vascular inflammation observed in diabetes because supplementation with H_2S can prevent inflammatory factor interleukin-8 (IL-8) and monocyte chemoattractant protein 1 (MCP-1) secretion by monocytes cultured in high-glucose medium (77). It has also been observed that H_2S levels in plasma and aortic tissue are progressively reduced in nonobese diabetic (NOD) mice. This suggests that reduced endogenous H_2S production is associated with diabetes-related endothelial dysfunction in NOD mice (106). Endothelial dysfunction plays a pathogenic role in the development of diabetic vascular complications. However, protective effects of H_2S on the endothelial cells under high-glucose conditions have also been observed. Pretreatment with NaHS (50 μmol/liter) protects against high-glucose-induced apoptosis in endothelial cells by increasing SOD activity, decreasing ROS generation and malondialdehyde levels, and downregulating the Bax/Bcl-2 ratio (107). These findings are consistent with those of Suzuki et al. showing that *in vitro* hyperglycemia is associated with increased H_2S degradation caused by mitochondrial ROS overproduction and that H_2S replacement has cytoprotective effects on endothelial cells in hyperglycemia *in vitro*. H_2S protects against the development of hyperglycemia-induced endothelial dysfunction by attenuating the hyperglycemia-induced enhancement of ROS formation, attenuating nuclear DNA injury, reducing the activation of the nuclear enzyme poly(ADP-ribose) polymerase, and improving cellular viability (108).

Furthermore, inhibiting ACE, a Zn-dependent enzyme, has been recommended to prevent diabetic nephropathy (109). The H_2S level and CSE expression in the renal cortex of diabetic rats have been shown to be significantly decreased (110). As indicated earlier, H_2S at a physiological concentration exhibits direct inhibitory action on ACE activity in human umbilical vein endothelial cells by interfering with the Zn^{2+} in the active center of the enzyme (62). Thus, it appears that H_2S could also protect against diabetes nephropathy by functioning as an ACE inhibitor. These findings imply that H_2S at normal physiological concentrations may have beneficial effects on the control of diabetes owing to its anti-inflammatory and antioxidative roles. Therefore, inhibiting pancreatic H_2S biosynthesis may be a potential approach to protect β cells from death during the induction phase of diabetes, whereas supplementation with H_2S could be considered a potential approach to maintain diabetic blood vessel potency (111).

Cancer. The slow-releasing H_2S donor GYY4137 can kill seven different human cancer cell lines (HeLa, HCT-116, Hep G2, HL-60, MCF-7, MV4-11, and U2OS) in a concentration-dependent manner. This indicates that H_2S could be a potential anticancer agent (112). Moreover, H_2S-releasing nonsteroidal anti-inflammatory drugs (HS-NSAID) composed of a traditional NSAID to which an H_2S-releasing moiety is covalently attached have significant anti-inflammatory properties. It has been shown that HS-NSAID can inhibit the growth of all cancer cells, such as human colon, breast, pancreatic, prostate, lung, and leukemia cancer cells, by inhibiting cell proliferation, inducing apoptosis, and causing G_0/G_1 cell cycle block. These data indicate that H_2S may have potential antigrowth activity against various human cancer cells (113).

Sulforaphane, a sulfur-containing compound with properties against prostate cancer, can release a large amount of H_2S. Pei et al. showed that H_2S can decrease the viability of PC-3 cells (a human prostate cancer cell line) in a dose-dependent manner (78). Importantly, both CSE and CBS are expressed in the prostate. Thus, H_2S-releasing diets or drugs may be beneficial in the treatment of prostate cancer. Similarly, H_2S-releasing aspirin, a novel and safer derivative of aspirin, has shown promise as an anticancer agent against hormone-independent estrogen receptor-negative breast cancers by inhibiting cell proliferation, inducing apoptosis, and decreasing NF-κB levels (79). Moreover, NOSH-aspirin, a NO- and H_2S-releasing agent, has been found to inhibit colon cancer growth by inhibiting cell proliferation, inducing apoptosis, and causing G_0/G_1 cell cycle block (80). Overall, these results indirectly demonstrate that H_2S has strong anticancer potential that merits further evaluation.

PERSPECTIVES

The findings discussed in this review strongly argue in favor of a role for H_2S in aging and age-associated diseases. However, the mechanisms of action of this role of H_2S have not yet been sufficiently characterized and await elucidation by further studies. A better understanding of the roles of H_2S in aging can provide insights into potential therapeutic interventions against aging and reduce age-associated diseases. More specifically, data available so far strongly suggest that H_2S may become the next potent preventive and therapeutic agent for preventing and ameliorating the symptoms of aging and age-associated diseases, and this should be addressed in future studies.

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