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Review

Hydrogen sulfide and translational medicine

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Hydrogen sulfide (H_2S) along with carbon monoxide and nitric oxide is an important signaling molecule that has undergone large numbers of fundamental investigations. H_2S is involved in various physiological activities associated with the regulation of homeostasis, vascular contractility, pro- and anti-inflammatory activities, as well as pro- and anti-apoptotic activities *etc*. However, the actions of H_2S are influenced by its concentration, reaction time, and cell/disease types. Therefore, H_2S is a signaling molecule without definite effect. The use of existing H_2S donors is limited because of the instant release and short lifetime of H_2S. Thus, translational medicine involving the sustained and controlled release of H_2S is of great value for both scientific and clinical uses. H_2S donation can be manipulated by different ways, including where H_2S is given, how H_2S is donated, or the specific structures of H_2S-releasing drugs and H_2S donor molecules. This review briefly summarizes recent progress in research on the physiological and pathological functions of H_2S and H_2S-releasing drugs, and suggests hope for future investigations.

Keywords: hydrogen sulfide; K_{ATP} channel; calcium channel; TRPA1; NF-κB; cytochrome oxidase; antioxidant; H_2S donor; H_2S-releasing drug; translational medicine

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Introduction

Hydrogen sulfide (H_2S) is a colorless, gaseous molecule at room temperature. It is widely known and disliked for its pungent, rotten egg-like smell. H_2S is very water-soluble and lipophilic, resulting in quick and convenient transport between cells and tissues[1]. It is also strongly toxic, especially to the central nervous system. In fact, H_2S acts as a more potent inhibitor of mitochondrial respiration than cyanide[2]. The maximum concentration in the air is approximately 10 mg/m^3, and high concentrations of H_2S may cause an instant loss of olfactory sensation or fainting. However, H_2S, resembling CO and NO, is converted from a noxious molecule to a signaling molecule that may have promise in the medical and pharmaceutical fields.

Endogenous H_2S is produced from *L*-cysteine by both CSE and CBS, with the cofactor cysteine amino transferase, through a so-called "trans-sulfuration pathway"[3–5]. Both CSE and CBS enzymes are pyridoxal-5′-phosphate-dependent with different concentrations in various tissues. It was previously reported that CBS is predominantly located in the central nervous system (hippocampus, cerebellum, cerebral cortex, and brain stem)[6], while CSE emerges more in the cardiovascular system (aorta, mesenteric artery, portal vein, and other vascular tissue)[7, 8]. An amount of CBS or CSE is tissue-specific, and coincidence and sole incidence are both allowed. A recent investigation also suggests the presence of another enzyme besides CBS and CSE that can generate endogenous H_2S: 3-MST, which generates H_2S from cysteine with the help of α-ketoglutarate in the brain[9]. 3-MST is located in the liver, kidney, heart, lung, thymus, testis, thoracic aorta, and brain. Three pathways of H_2S degradation exist: (1) desulfurization to thiosulfate by mitochondrial oxidation, then to sulfite or sulfate; (2) cytosolic methylation to dimethylsulfide; and (3) sulfhemoglobin formation by the binding to hemoglobin[9, 10]. A previous investigation shows that after H_2S is synthesized, it is quickly absorbed or stored as a form of bound or acid-labile sulfur, while free H_2S is maintained at a baseline level[11]. Bound sulfur is stored intracellularly in neurons and astrocytes of rats and releases H_2S in the presence of physiologic concentrations of the endogenous reducing substances glutathione and cysteine. Acid-labile sulfur is another form of stored H_2S. It localizes in the mitochondria and the release of H_2S occurs at a pH below 5.4.

Methods to study H_2S conventionally included three aspects. First, changes in substances involved in the down-regulation of the H_2S concentration, such as CBS and CSE antagonists and inhibitors of potent targets are observed. Second, symptoms are compared between a range of H_2S concentrations

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by enhancing the dosage of H_2S, mainly through exogenous H_2S donors, such as NaHS or other H_2S-releasing compounds. Third, the variance in H_2S concentration in certain diseases is measured, and the function of this variability is determined. The effort put into studying the pathological and physiological functions of H_2S has allowed for a general understanding of its behavior in the body, including its synthesis, metabolism, potent targets, and effects. The large amount of evidence gathered through various experiments and observations has shown that the role of H_2S as a vital endogenous gasotransmitter is ubiquitous throughout the human body. The use of H_2S in the central nervous system, cardiovascular system, and gastrointestinal system is promising, and the investigation of its role is important because cardiovascular and cerebrovascular diseases as well as gastrointestinal cancers are among the top causes of human mortality. Hence, a breakthrough in dealing with diseases that severely affect our health in hope of longevity to occur is necessary. Furthermore, H_2S-associated drugs with effectiveness similar to that of nitroglycerin in cases of angina pectoris may be developed in the future.

Recent evidence for the therapeutic application of H_2S

Since 1996, when Ade and Kimura began their investigation of H_2S, the role of H_2S has been gradually revealed by various contributions from scientists worldwide[3]. Recent discoveries regarding H_2S demonstrate its critical importance, much like NO and CO, to the medical field. This small, gaseous molecule appears to be both powerful and ubiquitous.

In the central nervous system, H_2S ameliorates ischemic injuries but leads to the aggravation of stroke. Enhancement of the H_2S concentration in the brain induces brain infarct, while CBS or CSE inhibitors can reverse this effect. The role of H_2S in neuron protection has been shown in glutamate-induced death with increasing concentrations of cysteine and γ-glutamylcysteine, which causes enhanced GSH concentrations[11–14]. In patients with AD, localized increases in H_2S resulted in a delay of aggravation and exacerbation of symptoms[13, 15, 16]. In addition, patients with Down syndrome overproduce H_2S because their urinary excretion of thiosulfate, a specific H_2S end product, was increased, suggesting a positive relationship between H_2S concentration and the aggravation of Down syndrome[16–18].

In the cardiovascular system, H_2S is related to hypertension, atherosclerosis, and myocardial injury. Its utility for treating hypertension may correspond to its vasodilatory effect. H_2S has the ability to relax the rat thoracic aorta, portal vein, and mesenteric artery, which suggests a more fundamental role in the regulation of contractility and blood pressure[19–23]. Conversely, further study has suggested that H_2S is a vasoconstrictor at low concentrations with a possible mechanism for suppressing NO, which is also involved in contractility. As plaque is destabilized and aortic smooth muscle cell proliferation is possibly reduced by H_2S, atherosclerosis is also palliated owing to an enhanced H_2S concentration[24, 25]. Similarly, H_2S is able to help patients recover from myocardial injury, particularly ischemia-reperfusion injury[26–31]. In summary, many cardiovascular diseases demonstrated a relationship with H_2S modulation, prognosticating a comprehensive utility of H_2S in heart diseases.

In the respiratory system, investigation into the link between H_2S and pulmonary hypertension was the first research in the field of pathophysiology and H_2S in the world[32]. In recent years, many investigations examining the role of H_2S in pulmonary hypertension, in which H_2S was administered to models of chronic hypoxia, have been conducted [33, 34].

In the gastrointestinal system, H_2S remains important in the regulation of local homeostasis. H_2S-releasing bacteria in the intestine are a major source of endogenous H_2S. Donation of H_2S contributes to chloride secretion, which aggravates certain types of gastritis[35]. Gastrointestinal contractility shows sensitivity to H_2S, which affects the gastrointestinal smooth muscle cells as well as the neurons in the enteric nervous system[15, 35, 36]. The concentration of H_2S is enhanced when abdominal sepsis or endotoxemia occurs, and administration of H_2S leads to exacerbation of these conditions, mainly because of its pro-inflammatory effect[36, 37]. Apart from these negative effects, H_2S has been shown to have a protective anti-inflammatory effect on the gastrointestinal system in some types of gastritis and colitis[38–40]. An obvious contradiction, thus, emerges, leading to puzzling study results in terms of the actual pro- or anti-inflammatory effects of H_2S. This conflict implies the presence of fundamental unknown mechanisms in addition to those that have been observed. The observed results may be derived from a balance of multiple mechanisms because a balanced result always changes with circumstances.

Furthermore, insulin secretion and diabetes mellitus can be affected by local H_2S concentrations because the pancreas is among the targets of H_2S[41–44]. In the pancreas, CSE is the main enzyme converting cysteine to H_2S. It has also been reported that the concentration of H_2S rose in response to the presence of pancreatitis, which is ascribed to its pro-inflammatory effect. Therefore, it is clear that H_2S-releasing drugs may make a difference in pancreatitis or diabetes. However, the functions of H_2S remain ambiguous and demand further investigation to elucidate its anfractuous signaling network. Current studies suggest that the effects of H_2S are often influenced by its concentration and reaction time as well as the cell and disease type it is acting upon. Hence, the application of H_2S in certain dysfunctions is bound to be difficult considering its potentially low specificity for certain organs and tissues (see Figure 1). On the other hand, various uses of H_2S may be possible because of its power.

New therapeutic targets associated with H_2S

The mechanisms and pathways of H_2S signaling are not yet completely understood. The regulation of H_2S is so complicated that new mechanisms are continuously discovered before the former mechanisms are fully understood. Fortunately, several fundamental mechanisms have been determined and have drawn much attention, and we have gradually begun to fully understand them (shown in Figure 2).

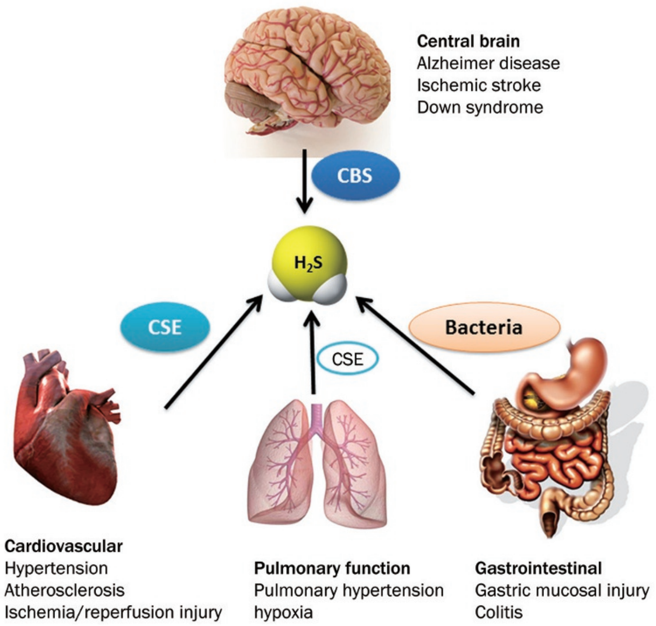

Figure 1. Schematic of the involvement of H_2S in various physiological and pathological functions.

Activation of K_{ATP} channels

A hallmark role of H_2S is the regulation of contractility, which is the most fundamental and valuable use of H_2S. The mechanism directing this effect is a major research topic. Vasodilation has been shown to be caused by the activation of the K_{ATP} channel[8, 26, 45]. Evidence for this hypothesis comes from the observation that the vasodilatory effect of H_2S is attenuated by K_{ATP} channel antagonists, such as glibenclamide, while the K_{ATP}-dependent current is increased by H_2S, as shown by patch-clamp technology. In another study, the non-selective K_{ATP} channel antagonist glibenclamide, the selective K_{ATP} channel antagonist HMR-1098, and the selective mitochondrial K_{ATP} channel antagonist 5-HD were compared to show the effect of H_2S on the K_{ATP} channel[46–48]. The results of this study suggest that H_2S selectively activates the plasma-membrane K_{ATP} channel (only 5-HD treatment lacked mediation of neuroprotection during hypoxia). In addition to having a vasodilatory effect, the activation of the K_{ATP} channel preconditions the body against ischemia-reperfusion injury and promotes myocardial protection, which together provide a theoretical and experimental basis for the application of H_2S in heart disease[26, 27, 45].

Activation of T-type calcium channels

Research has revealed that H_2S is involved in visceral pain-like nociception and somatic hyperalgesia in mice. This observation led to further investigation of the role of indirect activation of T-type calcium channels in facilitation of visceral nociception[49–52]. Because Zn^{2+} acts as an inhibitor of T-type calcium channels, especially the $Cav_{3.2}$ isoform, H_2S, which chelates Zn^{2+}, is reasonably regarded to indirectly activate T-type calcium channels by inhibiting Zn^{2+} [53]. This hypothesis is further proven by the observation that Zn^{2+} chelates similarly to dipicolinic acid, showing the aggravation of colonic pain as well as hyperalgesia.

Activation of TRPA1

The TRP superfamily is comprised of nonselective cation channels that are divided into six main subfamilies: TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA[54]. Among them, both TRPV and TRPA are important targets of pathological research. It is

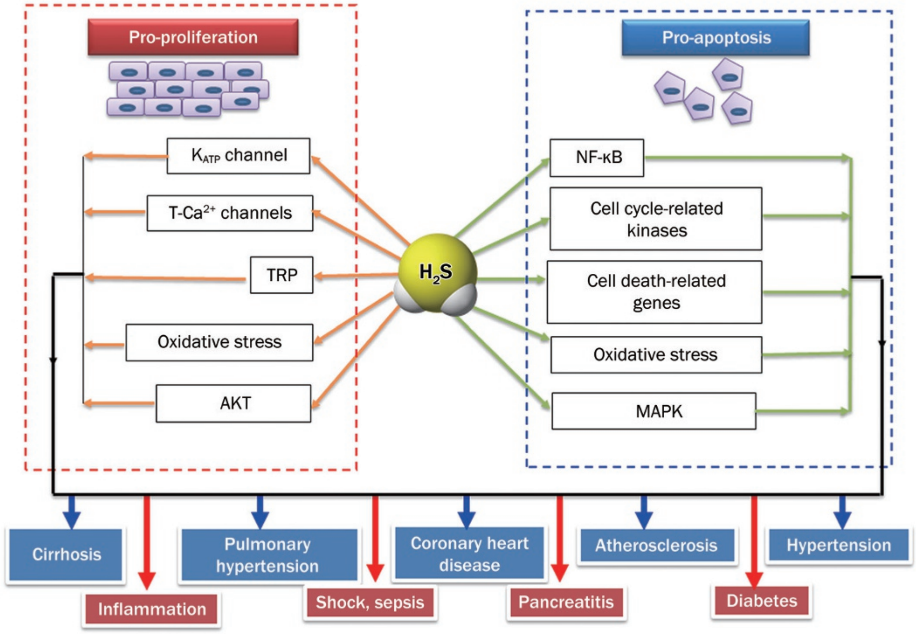

Figure 2. Potential involvement and application of the targets of H_2S, including observed dual effects.

well known that TRPV1 in the bladder wall produces detrusor overactivity. TRPA1 is present on unmyelinated sensory nerve fibers within the urothelium, suburothelial space, and muscle layer as well as around blood vessels throughout the bladder. Because TRPA1 coexists with TRPV1 in major loci, such as sensory neurons in rodent dorsal root and trigeminal ganglia, and because intracellular TRPA1 activators including allyl isothiocyanate and cinnamaldehyde also initiate detrusor overactivity resembling that caused by TRPV1, it is believed that TRPA1 is involved in sensory transduction[55]. A recent investigation demonstrated that H_2S activated TRPA1 in the presence of inflammation by the induction of calcium responses in TRPA1-expressing CHO cells. Moreover, this result is suppressed by ruthenium red, which is a nonselective TRP antagonist, and HC-030031, which is a selective TRPA1 antagonist. This reaction is not observed with the selective TRPV1 antagonist iodoresiniferatoxin. These findings reveal the presence of H_2S-specific activation of TRPA1 channels[56]. Further investigation of H_2S-specific TRPA1 channel activation may provide new solutions to various bladder disorders caused by hyperreflexia, and H_2S may affect nociception, which results from complicated functions of TRPA1, upon activation via the elicitation of pain, protective reflexes, and local release of neurotransmitters in the periphery.

The NF-κB signaling pathway

H_2S-induced inflammation has a relationship with the NF-κB signaling pathway. This pathway is involved in cecal ligation and puncture-induced sepsis, in which H_2S regulates the expression of cytokines, chemokines, and adhesion molecules[57]. There are data indicating that the inhibition of SFKs causes the down-regulation of H_2S-induced NF-κB activity and ICAM-1 expression, suggesting that H_2S is involved in regulating the activity of NF-κB by activating the phosphorylation of SFKs, eventually resulting in mediation of ICAM-1 expression[58]. This pathway may also be an answer to H_2S-induced anti-apoptosis. Mouse embryonic fibroblasts lacking p65, which is a subunit of NF-κB, are sensitive to TNF-α-induced cell apoptosis, indicating that NF-κB is a target molecule involved in apoptosis. Further research has elucidated a possible mechanism of enhanced transcription of CSE, which generates H_2S, during TNF-α-induced apoptosis[6]. H_2S or sulfhydrates then generates the p65 subunit of NF-κB at cysteine-38 and binds to the coactivator ribosomal protein, S3. Finally, the promotion of anti-apoptotic genes occurs, including the cellular inhibitors of apoptosis, caspase-8-c-FLIP, A1, TRAF1, and TRAF2[59].

Cytochrome oxidase

H_2S has long been regarded as a strong toxin that inhibits mitochondrial respiration by combining the cytochrome *c* oxidase copper and/or heme iron site, resembling hydrogen cyanide, a well-known lethal toxin[2]. Studies suggest that H_2S also binds and reduces ferric heme in microsomal cytochrome P450, generating a state of oxidative stress[60]. On the other hand, an interesting phenomenon called "suspended animation," a form of hypometabolism, results from the above mechanism. This phenomenon was first found in rodents that inhaled H_2S gas, and subsequent findings suggest that it is size-dependent or species-related[61–63]. Animals in suspended animation resembled those in hibernation, with reductions in cardiac output, ventilation frequency, and core temperature, finally leading to a decreased demand for energy. Hypometabolism can make great difference, if occurring in the human body, as an emergent protection against ischemia-associated tissue damage and infarction or providing a time delay in patients with stroke.

A scavenger of reactive oxygen and nitrogen species

H_2S is highly predisposed to react with cytochrome oxidase; therefore, the hypothesis that H_2S is also a potent antioxidant has been theorized. Recent studies have suggested that H_2S has the ability to react with ROS and RNS, including the superoxide radical anion, hydrogen peroxide, peroxynitrite, and hypochlorite[9, 64, 65]. Thus, a protective effect against organ damage caused by ROS and/or RNS may be proven. Because the concentration of H_2S in patients with AD is often severely suppressed and because AD has been proven to have a relationship with the increased generation of ROS and RNS, the role of H_2S in the cure of AD merits investigation[6, 66].

H_2S is also reported to have a specific relationship with NO. They both have a synergistic effect on vasodilation as well as an inhibitory effect on the twitching of the ileum stimulated by electricity. While NO may induce CSE activity and then enhance the production of H_2S, H_2S also possess the ability to catalyze release of NO from *S*-nitrosoglutathione[67]. This type of relationship reveals a deeply unexplored mechanism between H_2S and NO. At a low concentration, H_2S displays only a weak relaxation of vessels, mainly due to the scavenging of NO. Thus, the specific concentration of H_2S play a critical role in determining its usability as a biomarker or its therapeutic application[68, 69].

H_2S donor molecules and H_2S-releasing drugs

Ordinarily, H_2S-related drugs are categorized as either administrators of H_2S that enhance local or whole-body H_2S concentrations and cause pathological and physiological changes or as inhibitors of H_2S production enzymes that reduce local or whole-body H_2S concentrations and cause pathological and physiological changes. H_2S donors, including donor molecules and H_2S-releasing drugs, can be divided into three types: inorganic substances, such as NaHS; organic compounds, represented by GYY4137, which is derived from Lawesson's reagent; and agonists of H_2S-synthesized enzymes. Inhibitors of enzymes involved in the synthesis of H_2S are of great importance, especially in the study of the mechanisms of H_2S functions. *DL*-propargylglycine, which easily permeates the cell membrane without obvious damage due to its high lipophilic property, is widely used as a nonspecific inhibitor of CSE and CBS. Disproof is a basic yet practical method for scientific investigation; therefore, the study of the effect of H_2S on certain targets by diminishing its concentration may be more effective than directly administering it. In fact, these inhibitors are effective agents in diseases induced by the overproduction of H_2S.

Inorganic H_2S donors

Simple molecules, such as NaHS and Na_2S, are basic tools used for H_2S research. Their donation of H_2S is rapid upon reaction with water because of high solubility. Gaseous H_2S is sometimes used by respiratory passages through direct absorption by pulmonary alveoli due to high bioavailability. However, there is a fundamental shortage of the use of inorganic H_2S donors in both research and clinical applications because of the need to attain long-term release at a controlled rate[70, 71]. A too rapid release implies increasing the concentration of H_2S instantly, resembling a pulse cure, which is associated with a variety of problems, including concentrations that are too high for the local tissue and a lack of a long-term effect. Both problems led to imprecise experimental parameters, including dose and time, random results, and misleading causes. Thus, this data shortage may be a partial explanation for the emergence of divergent results, even when the same function is investigated.

To obtain a better H_2S donor for sustained and controlled use, organic compounds containing chemically synthesized molecules and natural plant extracts have become prevalent. Some organic compounds have shown ideal therapeutic effects and a large commercial potential.

Precursors for endogenous H_2S synthesis

N-acetylcysteine and *L*-cysteine are precursors for endogenous H_2S synthesis[70]. Enhancing these precursors causes H_2S to increase with the catalysis of CSE and CBS. This precursor increase has minimal side effects associated with their production in the body.

Synthetic H_2S donors

As in other fields, synthetic drugs are predominant among H_2S-releasing drugs. GYY4137, a derivative of Lawesson's reagent, acts as a synthetic H_2S donor. Its sustained release of H_2S in aqueous media with a concentration above baseline has been demonstrated[72]. GYY4137 causes the concentration-dependent suppression of cancer cells, such as MCF-7, by the promotion of apoptosis without an obvious impact in normal cells, such as IMR90. That is to say, our demand for the sustained moderate release of H_2S is met. In a recent study, a series of new H_2S donors based on the *N*-(benzoylthio)benzamide template were synthesized. The authors utilized the theory that the S-N bond is cysteine-activated under certain conditions, which needs to be demonstrated in future studies[73]. The release rate could be manually adjusted by the purposeful modification of the core structure.

H_2S-nonsteroidal anti-inflammatory drug hybrids

NSAIDs, among which aspirin is well known, are widely used for their anti-inflammatory and anti-gout properties. Nevertheless, they are associated with side effects, such as serious gastrointestinal bleeding and heart attack. Aspirin, as a delegate of NSAIDs, is famous for its anti-inflammatory ability as well as its prevention of atherosclerosis by inhibiting COX and platelet aggregation. However, aspirin also stimulates gastric bleeding and, worse, the prevention of platelet aggregation will hinder the cessation of bleeding. H_2S-NSAID hybrids have, thus, been devised based on the protective effect of H_2S on the gastrointestinal and cardiovascular systems[74, 75]. ATB 337, which is derived from diclofenac, shows significantly reduced gastrointestinal toxicity compared with diclofenac and has an enhanced anti-inflammatory effect with no effect on hematocrit or leukocyte adherence. ATB 429, which is derived from mesalamine, also shows an improvement in treating inflammatory bowel disease compared with its parent drug. ATB 346, which is derived from naproxen, remarkably reduces gastrointestinal and cardiovascular toxicity, such as the two aforementioned drugs[70]. The side effects of NSAIDs mainly come from the single inhibition of only COX-1 or COX-2, which generates an imbalance in the expression of COX-1 and COX-2 in different tissues and organs. Therefore, these H_2S-NSAID hybrid compounds can release H_2S when dissolved by enzymes, which will protect organs, such as the stomach as well as the heart, because of the anti-oxidation or other effects of H_2S. It should also be noted that these types of compounds are not a simple addition of both NSAIDs and H_2S but because they can affect each other when in the same place at the same time, they make a more magnified effect than a simple two part addition.

Natural plant-derived compounds

Vegetables, represented by garlic and ginger, are ordinary salubrious foods that have anti-inflammatory functions. In recent years, garlic-derived polysulfide compounds have drawn great attention due to their potential anti-inflammatory and anti-cancer effects. DATS is the first garlic-derived molecule discovered to possess vasoactivity along with the ability to specifically suppress cancer cells[76]. Compounds similar to DATS, such as DAS, DADS, and DATTS, have been evaluated for similar functions, but they are complicated. Different effects have been found. DAS reportedly activates the nuclear receptor CAR which induces various hepatic drug-metabolizing phase I and phase II enzymes in the mouse liver and preventive effects on some types of gastric cancer[77]. DADS has demonstrated vasorelaxation effect in aortic ring preparations via glucose- and thiol-dependent cellular reactions. Interestingly, DADS modified hemoglobin's β-chain at cysteine-93 or cysteine-11_2 in deoxygenated human red blood cells. This was the first instance of a garlic-derived compound that was shown to be able to modify an intracellular protein[77]. Normally, garlic-derived compounds are simply regarded as precursors of H_2S when absorbed and metabolized in blood. Therefore, because DADS itself is able to generate direct protein changes, it is worthy of future study.

S-allylcysteine is believed to have a cardioprotective effect and is a potential source of H_2S. However, whether it plays a role as a H_2S precursor or a modulator of H_2S-related enzymes is controversial[76]. Another novel garlic-derived compound, *S*-propargyl-cysteine, is now under investigation. It was recently shown to attenuate lipopolysaccharide-induced spatial learning and memory impairment through TNF signaling and the NF-κB pathway in rats, which contributes to studies about AD and other diseases related to neuronal damage[6, 79].

Compounds extracted from other plants display similar effects. Sulforaphane, which is derived from isothiocyanates found in cruciferous vegetables, exhibits an anti-cancer property. Sulforaphane suppresses the proliferation of prostate cancer cells and enhances the expression of CBS and CSE. In addition, sulforaphane activates p38 mitogen-activated protein kinase and c-Jun N-terminal kinase, suggesting a potent mechanism[80]. Although most of the significant results have been gained from *in vitro* studies, the study of H_2S-releasing donors *in vivo* is more complex. Therefore, the identification of a feasible drug for clinical use requires further research.

Future developments: challenges for translation of the toxic molecule H_2S

Investigation of the role of H_2S is still in its infancy. H_2S has both scientific and technological value. The latter tends to dominate because of its financial value and direct applicability, which substantiates research efforts. However, the scientific investigation of H_2S is also important for the elucidation of its base fundamental roles. Scientific investigation strives to address disease, enhance quality of life, and disclose the secrets of the human body. Certainly problems and challenges will arise and the limitations of experimental materials will at times constrain further H_2S research. A sustained and controlled H_2S-releasing donor that functions both *in vitro* and *in vivo* has not yet been found. An effective and quick method of measuring the concentration of H_2S is also required, especially a non-invasive method. With an increased understanding of the various H_2S mechanisms in the body, further study of H_2S becomes more difficult and complicated. H_2S is known as a third gaseous signaling molecule, which means that it plays the role of a messenger. The concentration of H_2S has been proven to have relevance in particular diseases; for instance, it is overproduced in sepsis and found at inadequate levels in AD[81]. Therefore, the mechanism controlling the actual concentration of H_2S in certain tissues may become the ultimate problem for H_2S related research. It should be emphasized that a relevant relationship does not mean a relationship of causation. By regulating the H_2S concentration in particular tissues, symptoms of a specific disease can be controlled, which implies that the origin of the disease has not been addressed. That is to say, the regulation of H_2S can only provide transient protection from certain diseases, such as hypertension. The challenges of the sustained and controlled release of H_2S-releasing drugs were mentioned above. Another difficulty in H_2S related research comes from the multiple functions of H_2S, which cause a shortage of specific effects. The effects of H_2S are dose-, time-, and tissue-dependent. For patients with different diseases, H_2S may need to be administered as different drugs. Therefore, a focus on the general effects of H_2S, such as on cardiovascular protection, is rational.

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Abbreviations

H_2S, Hydrogen sulfide; CO, carbon monoxide; NO, nitric oxide; CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; 3-MST, 3-mercaptopyruvate sulfurtransferase; NaHS, sodium hydrosulfide; AD, Alzheimer's disease; K_{ATP} channels, potassium-dependent ATP-sensitive channels; TRP, transient receptor potential; TRPC, canonical transient receptor potential channel; TRPV, vanilloid transient receptor potential channel; TRPM, melastatin transient receptor potential channel; TRPP, polycystin transient receptor potential channel; TRPML, mucolipin transient receptor potential channel; TRPA, ankyrin transient receptor potential channel; CHO cells, Chinese hamster ovary cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SFKs, SRC family kinases; ICAM-1, intracellular adhesion molecule-1; TRAF1, TNFR-associated factor 1; TRAF2, TNFR-associated factor 2; ROS, reactive oxygen species; RNS, reactive nitrogen species; NSAIDs, nonsteroidal anti-inflammatory drugs; DATS, diallyl trisulfide; DAS, diallyl sulfide; DADS, diallyl disulfide; DATTS, diallyl tetrasulfide; CAR, orphan member of the nuclear steroid hormone receptor superfamily.

References

1 Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation — a tale of three gases! Pharmacol Ther 2009; 123: 386–400.

2 Lagoutte E, Mimoun S, Andriamihaja M, Chaumontet C, Blachier F, Bouillaud F. Oxidation of hydrogen sulfide remains a priority in mammalian cells and causes reverse electron transfer in colonocytes. Biochim Biophys Acta 2010; 1797: 1500–11.

3 Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci 1996; 16: 1066–71.

4 Olson KR. Is hydrogen sulfide a circulating "gasotransmitter" in vertebrate blood? Biochim Biophys Acta 2009; 1787: 856–63.

5 Predmore BL, Lefer DJ. Development of hydrogen sulfide–based therapeutics for cardiovascular disease. J Cardiovasc Translation Res 2010; 3: 487–98.

6 Gong QH, Wang Q, Pan LL, Liu XH, Xin H, Zhu YZ. *S*-propargyl-cysteine, a novel hydrogen sulfide-modulated agent, attenuates lipopolysaccharide-induced spatial learning and memory impairment: involvement of TNF signaling and NF-kappaB pathway in rats. Brain, behavior, and immunity 2011; 25: 110–9.

7 Kawabata A, Ishiki T, Nagasawa K, Yoshida S, Maeda Y, Takahashi T, *et al*. Hydrogen sulfide as a novel nociceptive messenger. Pain 2007; 132: 74–81.

8 Wang R. Signaling pathways for the vascular effects of hydrogen sulfide. Curr Opin Nephrol Hypertens 2011; 20: 107–12.

9 Kimura H. Hydrogen sulfide: its production, release and functions. Amino Acids 2011; 41: 113–21.

10 Hughes MN, Centelles MN, Moore KP. Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide *in vitro* and its measurement *in vivo*: a review. Free Radic Biol Med 2009; 47: 1346–53.

11 Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. Antioxidants Redox Signaling 2009; 11: 205–14.

12 Cunha TM, Dal-Secco D, Verri WA Jr, Guerrero AT, Souza GR, Vieira SM, *et al*. Dual role of hydrogen sulfide in mechanical inflammatory hypernociception. Eur J Pharmacol 2008; 590: 127–35.

13 Liu YY, Sparatore A, Del Soldato P, Bian JS. ACS84, a novel hydrogen sulfide-releasing compound, protects against amyloid β-induced cell cytotoxicity. Neurochem Int 2011; 58: 591–8.

14 Qu K, Lee S, Bian J, Low CM, Wong PH. Hydrogen sulfide: neurochemistry and neurobiology. Neurochem Int 2008; 52: 155–65.

15 Whiteman M, Winyard PG. Hydrogen sulfide and inflammation: the good, the bad, the ugly and the promising. Expert Rev Clin Pharmacol 2011; 4: 13–32. doi: 10.1586/ecp.10.134.

16 Tan BH, Wong PTH, Bian JS. Hydrogen sulfide: A novel signaling molecule in the central nervous system. Neurochem Int 2010; 56: 3–10.

17 Ufnal M, Sikora M, Dudek M. Exogenous hydrogen sulfide produces hemodynamic effects by triggering central neuroregulatory mechanisms. Acta Neurobiol 2008; 68: 382–8.

18 Wang MJ, Cai WJ, Li N, Ding YJ, Chen Y, Zhu YC. The hydrogen sulfide donor NaHS promotes angiogenesis in a rat model of hind limb ischemia. Antioxidants Redox Signaling 2010; 12: 1065–77.

19 Rossoni G, Manfredi B, Tazzari V, Sparatore A, Trivulzio S, Del Soldato P, *et al*. Activity of a new hydrogen sulfide-releasing aspirin (ACS14) on pathological cardiovascular alterations induced by glutathione depletion in rats. Eur J Pharmacol 2010; 648: 139–45.

20 Li YF, Xiao CS, Hui RT. Calcium sulfide (CaS), a donor of hydrogen sulfide (H_2S): A new antihypertensive drug? Med Hypothesis 2009; 73: 445–47.

21 Perna AF, Luciano MG, Ingrosso D, Raiola I, Pulzella P, Sepe I, *et al*. Hydrogen sulfide, the third gaseous signaling molecule with cardiovascular properties, is decreased in hemodialysis patients. J Renal Nutr 2010; 20: S11–S14.

22 Sowmya S, Swathi Y, Yeo AL, Shoon ML, Moore PK, Bhatia M. Hydrogen sulfide: regulatory role on blood pressure in hyperhomocysteinemia. Vasc Pharmacol 2010; 53: 138–43.

23 Zhong G, Chen F, Cheng Y, Tang C, Du J. The role of hydrogen sulfide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. J Hypertens 2003; 21: 1879–85.

24 Qiao W, Chaoshu T, Hongfang J, Junbao D. Endogenous hydrogen sulfide is involved in the pathogenesis of atherosclerosis. Biochem Biophys Res Commun 2010; 396: 182–6.

25 Li W, Tang C, Jin H, Du J. Regulatory effects of sulfur dioxide on the development of atherosclerotic lesions and vascular hydrogen sulfide in atherosclerotic rats. Atherosclerosis 2011; 215: 323–30.

26 Szabó G, Veres G, Radovits T, Gerő D, Módis K, Miesel-Gröschel C, *et al*. Cardioprotective effects of hydrogen sulfide. Nitric Oxide 2011; 25: 201–10.

27 Ji Y, Pang Qf, Xu G, Wang L, Wang JK, Zeng YM. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia–reperfusion injury. Eur J Pharmacol 2008; 587: 1–7.

28 Nicholson CK, Calvert JW. Hydrogen sulfide and ischemia-reperfusion injury. Pharmacol Res 2010; 62: 289–97.

29 Fu Z, Liu X, Geng B, Fang L, Tang C. Hydrogen sulfide protects rat lung from ischemia–reperfusion injury. Life Sci 2008; 82: 1196–202.

30 Sodha NR, Clements RT, Feng J, Liu Y, Bianchi C, Horvath EM, *et al*. Hydrogen sulfide therapy attenuates the inflammatory response in a porcine model of myocardial ischemia/reperfusion injury. J Thorac Cardiovasc Surg 2009; 138: 977–84.

31 Gao Y, Yao X, Zhang Y, Li W, Kang K, Sun L, *et al*. The protective role of hydrogen sulfide in myocardial ischemia–reperfusion–induced injury in diabetic rats. Int J Cardiol 2011; 152: 177–83.

32 Chunyu Z, Junbao D, Dingfang B, Hui Y, Xiuying T, Chaoshu T. The regulatory effect of hydrogen sulfide on hypoxic pulmonary hypertension in rats. Biochem Biophys Res Commun 2003; 302: 810–6.

33 Tang C, Li X, Du J. Hydrogen sulfide as a new endogenous gaseous transmitter in the cardiovascular system. Curr Vasc Pharmacol 2006; 4: 17–22.

34 Hongfang J, Bailin C, Bin Z, Chunyu Z, Xinmin L, Weijin Z, *et al*. Effects of hydrogen sulfide on hypoxic pulmonary vascular structural remodeling. Life Sci 2006; 78: 1299–309.

35 Linden DR, Levitt MD, Farrugia G, Szurszewski JH. Endogenous production of H_2S in the gastrointestinal tract still in search of a physiologic function. Antioxidants Redox Signaling 2010; 12: 1135–46.

36 Ganster F, Burban M, de la Bourdonnaye M, Fizanne L, Douay O, Loufrani L, *et al*. Effects of hydrogen sulfide on hemodynamics, inflammatory response and oxidative stress during resuscitated hemorrhagic shock in rats . Crit Care 2010; 14: R165.

37 Wallace JL. Hydrogen sulfide-releasing anti-inflammatory drugs. Trends Pharmacol Sci 2007; 28: 501–5.

38 Wallace JL, Vong L, McKnight W, Dicay M, Martin GR. Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. Gastroenterology 2009; 137: 569–78.

39 Chávez-Piña AE, Tapia-Álvarez GR, Navarrete A. Inhibition of endogenous hydrogen sulfide synthesis by PAG protects against ethanol-induced gastric damage in the rat. Eur J Pharmacol 2010; 630: 131–6.

40 Zins KA, Ordog T, Bardsley MR, Farrugia G, Levitt MD, Slungaard A, *et al*. T2049 hydrogen sulfide ameliorates murine experimental colitis through specific effects on diverse classes of infiltrating immune cells. Gastroenterology 2010; 138: S–621.

41 Tamizhselvi R, Koh YH, Sun J, Zhang H, Bhatia M. Hydrogen sulfide induces ICAM-1 expression and neutrophil adhesion to caerulein-treated pancreatic acinar cells through NF-κB and Src-family kinases pathway. Exp Cell Res 2010; 316: 1625–36.

42 Morselli-Labate AM, Fantini L, Pezzilli R. Hydrogen sulfide, nitric oxide and a molecular mass 66 u substance in the exhaled breath of chronic pancreatitis patients. Pancreatology 2007; 7: 497–504.

43 Bhatia M, Sidhapuriwala J. M1829 proinflammatory effects of hydrogen sulfide on substance P in caerulein-induced acute pancreatitis. Gastroenterology 2008; 134: A–428.

44 Wee EWL, Bhatia M, Fernandes ML, Madhavan K, Wong JY, Yeo AL, *et al*. T1301 serum hydrogen sulfide and substance P are early clinical predictors of the severity of acute pancreatitis. Gastroenterology 2009; 136: A–543.

45 Tay AS, Hu LF, Lu M, Wong PT, Bian JS. Hydrogen sulfide protects neurons against hypoxic injury via stimulation of ATP-sensitive potassium channel/protein kinase C/extracellular signal-regulated kinase/heat shock protein 90 pathway. Neuroscience 2010; 167: 277–86.

46 Kiyonaga T, Mitsui T, Torikoshi M, Takekawa M, Soejima T, Tada H. Ultrafast photosynthetic reduction of elemental sulfur by Au nanoparticle-loaded TiO_2. J Phys Chem B 2006; 110: 10771–8.

47 Łowicka E BJ. Hydrogen sulfide (H_2S) — the third gas of interest for pharmacologists. Pharmacol Rep 2007; 59 : 4–24.

48 Sun WH, Liu F, Chen Y, Zhu YC. Hydrogen sulfide decreases the levels of ROS by inhibiting mitochondrial complex IV and increasing SOD activities in cardiomyocytes under ischemia/reperfusion. Biochem

Biophys Res Commun 2012; 421: 164–9.

49 Maeda Y, Aoki Y, Sekiguchi F, Matsunami M, Takahashi T, Nishikawa H, *et al*. Hyperalgesia induced by spinal and peripheral hydrogen sulfide: evidence for involvement of Cav3.2 T-type calcium channels. Pain 2009; 142: 127–32.

50 Okubo K, Takahashi T, Sekiguchi F, Kanaoka D, Matsunami M, Ohkubo T, *et al*. Inhibition of T-type calcium channels and hydrogen sulfide-forming enzyme reverses paclitaxel-evoked neuropathic hyperalgesia in rats. Neuroscience 2011; 188: 148–56.

51 Takahashi T, Aoki Y, Okubo K, Maeda Y, Sekiguchi F, Mitani K, *et al*. Upregulation of Ca(v)3.2 T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain. Pain 2010; 150: 183–91.

52 Bauer CC, Boyle JP, Porter KE, Peers C. Modulation of Ca^{2+} signalling in human vascular endothelial cells by hydrogen sulfide. Atherosclerosis 2010; 209: 374–80.

53 Matsunami M, Kirishi S, Okui T, Kawabata A. Chelating luminal zinc mimics hydrogen sulfide-evoked colonic pain in mice: possible involvement of T-type calcium channels. Neuroscience 2011; 181: 257–64.

54 MC M. Editorial comment on distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 2008; 53: 399–400.

55 Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, *et al*. Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder [Research Support, Non-US Gov't]. Eur Urol 2008; 53: 391–9.

56 Miyamoto R, Otsuguro K, Ito S. Time- and concentration-dependent activation of TRPA1 by hydrogen sulfide in rat DRG neurons. Neurosci Lett 2011; 499: 137–42.

57 Chattopadhyay M, Kodela R, Nath N, Barsegian A, Boring D, Kashfi K. Hydrogen sulfide-releasing aspirin suppresses NF-kappaB signaling in estrogen receptor negative breast cancer cells *in vitro* and *in vivo*. Biochem Pharmacol 2012; 83: 723–32.

58 Tamizhselvi R, Koh YH, Sun J, Zhang H, Bhatia M. Hydrogen sulfide induces ICAM-1 expression and neutrophil adhesion to caerulein-treated pancreatic acinar cells through NF-kappaB and Src-family kinases pathway. Exp Cell Res 2010; 316: 1625–36.

59 Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, *et al*. Hydrogen sulfide-linked sulfhydration of NF-kappaB mediates its antiapoptotic actions. Mol Cell 2012; 45: 13–24.

60 Caro A, Kauffman T, Tackett J, Thompson S. Increased oxidative stress and cytotoxicity by hydrogen sulfide in HepG2 cells overexpressing CYP2E1. Cell Biol Toxicol 2011; 27: 439–53.

61 Forgan LG, Forster ME. Oxygen consumption, ventilation frequency and cytochrome *c* oxidase activity in blue cod (*Parapercis colias*) exposed to hydrogen sulphide or isoeugenol. Comp Biochem Physiol Toxicol Pharmacol: CBP 2010; 151: 57–65.

62 Baumgart K, Radermacher P, Wagner F. Applying gases for microcirculatory and cellular oxygenation in sepsis: effects of nitric oxide, carbon monoxide, and hydrogen sulfide. Curr Opin Anaesthesiol 2009; 22: 168–76.

63 Wagner F, Asfar P, Calzia E, Radermacher P, Szabo C. Bench-to-bedside review: hydrogen sulfide — the third gaseous transmitter: applications for critical care. Crit Care 2009; 13: 213.

64 Mancardi D, Penna C, Merlino A, Del Soldato P, Wink DA, Pagliaro P. Physiological and pharmacological features of the novel gasotransmitter: hydrogen sulfide. Biochim Biophys Acta 2009; 1787: 864–72.

65 Whiteman M, Moore PK. Hydrogen sulfide and the vasculature: a novel vasculoprotective entity and regulator of nitric oxide bioavailability? J Cell Mol Med 2009; 13: 488–507.

66 Qu K, Lee SW, Bian JS, Low CM, Wong PT. Hydrogen sulfide: neurochemistry and neurobiology. Neurochem Int 2008; 52: 155–65.

67 Kimura H. Hydrogen sulfide: its production, release and functions. Amino Acids 2011; 41: 113–21.

68 Zhang F, Li X, Stella C, Chen L, Liao Y, Tang C, *et al*. Plasma hydrogen sulfide in differential diagnosis between vasovagal syncope and postural orthostatic tachycardia syndrome in children. J Pediatrics 2012; 160: 227–31.

69 Yang J, Zhao J, Du S, Liu D, Fu C, Li X, *et al*. Postural orthostatic tachycardia syndrome with increased erythrocytic hydrogen sulfide and response to midodrine hydrochloride. J Pediatr 2013. doi: 10.1016/j.jpeds.2013.04.039.

70 Caliendo G, Cirino G, Santagada V, Wallace JL. Synthesis and biological effects of hydrogen sulfide (H_2S): development of H_2S-releasing drugs as pharmaceuticals. J Med Chem 2010; 53: 6275–86.

71 Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. Antioxidants Redox Signal 2009; 11: 205–14.

72 Lee ZW, Zhou J, Chen CS, Zhao Y, Tan CH, Li L, *et al*. The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects *in vitro* and *in vivo*. PloS One 2011; 6: e21077.

73 Zhao Y, Wang H, Xian M. Cysteine-activated hydrogen sulfide (H_2S) donors. J Am Chem Soc 2011; 133: 15–7.

74 Chattopadhyay M, Kodela R, Nath N, Dastagirzada YM, Velazquez-Martinez CA, Boring D, *et al*. Hydrogen sulfide-releasing NSAIDs inhibit the growth of human cancer cells: a general property and evidence of a tissue type-independent effect. Biochem Pharmacol 2012; 83: 715–22.

75 Chattopadhyay M, Kodela R, Olson KR, Kashfi K. NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid is a potent inhibitor of colon cancer cell growth *in vitro* and in a xenograft mouse model. Biochem Biophys Res Commun 2012; 419: 523–8.

76 Jacob C, Anwar A, Burkholz T. Perspective on recent developments on sulfur-containing agents and hydrogen sulfide signaling. Planta Med 2008; 74: 1580–92.

77 Sueyoshi T, Green WD, Vinal K, Woodrum TS, Moore R, Negishi M. Garlic extract diallyl sulfide (DAS) activates nuclear receptor car to induce the sult1e1 gene in mouse liver. PLoS One 2011; 6: e21229.

78 Bonaventura J, Rodriguez EN, Beyley V, Vega IE. Allylation of intraerythrocytic hemoglobin by raw garlic extracts. J Med Food 2010; 13: 943–9.

79 Sidhapuriwala JN, Hegde A, Ang AD, Zhu YZ, Bhatia M. Effects of S-propargyl-cysteine (SPRC) in caerulein-induced acute pancreatitis in mice. Plos One 2012; 7: e32574.

80 Pei Y, Wu B, Cao Q, Wu L, Yang G. Hydrogen sulfide mediates the anti-survival effect of sulforaphane on human prostate cancer cells. Toxicol Appl Pharmacol 2011; 257: 420–8.

81 Gong QH, Shi XR, Hong ZY, Pan LL, Liu XH, Zhu YZ. A new hope for neurodegeneration: possible role of hydrogen sulfide. J Alzheimers Dis 2011; 24: 173–82.