Hydrogen sulfide therapy in brain diseases: from bench to bedside

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Abstract

Hydrogen sulfide (H_2S) has been recognized and studied for nearly 300 years, but past researches mainly focus on its toxicity effect. During the past two decades, the majority of researches have reported that H_2S is a novel endogenous gaseous signal molecule in organisms, and play an important role in various systems and diseases. H_2S is mainly produced by three enzymes, including cystathionine β-synthase, cystathionine γ-lyase and 3-mercaptopyruvate sulfurtransferase along with cysteine aminotransferase. H_2S had been firstly reported as a neuromodulator in the brain, because of its essential role in the facilitating hippocampal long-term potentiation at physiological concentration. It is subsequently reported that H_2S may have relevance to neurologic disorders through antioxidative, anti-inflammatory, anti-apoptotic and additional effects. Recent basic medical studies and preclinical studies on neurologic diseases have demonstrated that the administration of H_2S at physiological or pharmacological levels attenuates brain injury. However, the neuroprotective effect of H_2S is concentration-dependent, only a comparatively low dose of H_2S can provide beneficial effect. Herein, we review the neuroprotevtive role of H_2S therapy in brain diseases from its mechanism to clinical application in animal and human subjects, and therefore provide the potential strategies for further clinical treatment.

Key words: hydrogen sulfide; gaseous signal molecule; neuroprotection; antioxidation; anti-inflammation; anti-apoptosis; therapy; brain diseases

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Introduction

Hydrogen sulfide (H_2S), a gas that smells like rotten eggs, was firstly described in 1731. Since then, most researches about H_2S have been devoted to its toxic effects with little attention paid to its physiological function.[1] H_2S therapy has been an intense subject of interest following the discovery of an endogenous sulfide in mammalian brain by Warenycia et al.[2] in 1989. In 1996, Abe et al.[3] demonstrated that H_2S, as a neuromodulator, facilitates the induction of hippocampal long-term potentiation (LTP) by enhancing the activity of N-methyl D-aspartate (NMDA) receptors. In 2009, Mustafa et al.[4] demonstrated a mode of action for H_2S, suggesting that it physiologically modifies cysteine (Cys) in a large number of proteins by S-sulfhydration. In the same year, Ishigami et al.[5] showed that H_2S is released from bound sulfur, an intracellular store of sulfur, in the presence of physiologic concentrations of endogenous reducing substances glutathione (GSH) and Cys.

Currently, H_2S has been recognized as the third endogenous gaseous signal molecule in organisms, following nitric oxide (NO) and carbon monoxide (CO).[6] Understanding of H_2S biological effect and its mechanism has been deepened, especially the physiopathologic significance of H_2S in various diseases such as neurological diseases, cardiovascular diseases, hematologic diseases, urological diseases, and so on.[7,8] Based on previous studies and literatures, we summarize recent progresses of experimental and clinical researches related to endogenous H_2S, including its formation, metabolism, modulation and mechanism, as well as the role of endogenous H_2S therapy in various brain diseases.

Production, Metabolism and Modulation

H_2S is endogenously generated in mammalian cells *via* enzymatic and nonenzymatic pathways, although the nonenzymatic pathway is less vital in the production of H_2S.[9] On the one hand, there are at least three enzymes in the organisms: β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). H_2S can be produced from L-Cys by CBS and CSE through the transsulfuration pathway.[10] In addition, H_2S also can be produced by 3-MST through the Cys catabolism pathway.[11] The Cys aminotransferase (CAT) catalyzes the transamination of Cys to the 3-mercaptopyruvate, a substrate of 3-MST to produce pyruvate and sulfane sulfur, which may liberate H_2S in the presence of reductants such as dithiothreitol and GSH.[12] Both CBS and CSE are classified as the pyridoxal-5'-phosphate (PLP)-dependent enzymes and use either Cys or Cys together with homocysteine (Hcy) as their principal substrates, while 3-MST is non-PLP dependent enzyme. The distribution of the above enzymes of endogenous H_2S generation is different in different tissues: CBS is mainly expressed in the nervous system, while the cardiovascular system only expresses CSE. Both CBS and CSE are expressed in the liver, ileum, kidney and pancreas. Meanwhile, the 3-MST, a class of zinc dependent enzymes, is active in erythrocytes and heart cells.[13] On the other hand, endogenous H_2S can be produced non-enzymatically and generated either from glucose *via* glycolysis (> 90%) or from phosphogluconate *via* nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (< 10%).[14,15]

The metabolism and modulation of endogenous H_2S are unclear.[16] In general, there are two possible forms of H_2S *in vivo*: one third in the form of gas and two-thirds in the form of sodium bisulfide (NaHS). It is reported that the catabolism of H_2S may involve chemical reactions, including oxidation to sulfate,[17] methylation to methanethiol and dimethyl sulfide, and reactions with Cys-containing proteins.[18,19] The metabolites are excreted mainly from the kidneys, partly from the intestine, and exhaled slightly from the lungs. Eto et al.[19,20] proposed that there are three pathways to regulate the generation of endogenous H_2S: (1) rapid regulation pathway: Ca^{2+}/calmodulin-mediated signal transduction pathway; (2) slow regulation pathway: regulation of testosterone and S-adenosyl-L-methionine (a CBS activator); (3) basic level regulation pathway linked to age and gender.

Mechanisms

Antioxidation

Kimura et al.[21] firstly revealed that the protective effect of H_2S on neurons against oxidative stress by increasing the substrate for the production of the antioxidant GSH, including the cystine/glutamate antiporter and the intracellular concentrations of Cys. It has been subsequently reported at the cellular level that H_2S also is able to enhance the activity of the γ-glutamylcysteine synthase (γ-GCS), a rate-limiting enzyme, which regulates the generation of GSH.[22] In addition, H_2S produced in mitochondria, the major organelle that releases reactive oxygen species (ROS) causing toxic effects and ultimately leading to cell death, also may directly suppress oxidative stress through scavenging ROS.[22] These findings offer evidences for the powerful anti-anti-oxidative role of H_2S.

Anti-inflammation

Thus far, there is no consistent conclusion on the problem whether H_2S is an pro-inflammatory factor or an anti-inflammatory factor.[23] Neuroinflammation can result from nerve injury in a process mediated by inflammatory cells and cytokines.[24] H_2S plays a protective role in inflammation by inhibiting lipopolysaccharide-stimulated tumor necrosis factor-α (TNF-α), the proinflammatory cytokine interleukin-1β (IL-β) and NO release in astrocytes and mi-

croglial cells.[25] Meanwhile, H_2S can increase the release of anti-inflammatory cytokines, such as interleukin-4 (IL-4) or interleukin-10 (IL-10).[26] As a result, H_2S may play an anti-inflammatory role in the central nervous system (CNS).

Anti-apoptosis

According to previous studies, a great deal of evidences described that H_2S may exert its anti-apoptotic role by inhibiting oxidative stress.[27,28] Pretreatment with NaHS (H_2S donor) could significantly suppress hypoxia-induced mouse hippocampal neuronal apoptosis via inhibition of the hydrogen peroxide (H_2O_2)-activated calcium signal pathway.[27] In addition, H_2S can improve mitochondrial dysfunction and inhibit an ROS-mediated caspase-3 pathway in the model of oxygen-glucose deprivation/reoxygenation (OGD/R)-induced neuronal apoptosis.[28] Besides, H_2S could confer its anti-apoptotic effect through regulating nuclear translocation of nuclear factor kappa B (NF-κB), a transcription factor, which is translocated into the nucleus to activate several anti-apoptotic genes.[29]

Additional mechanisms

The underlying mechanisms of H_2S is reflected in other aspects, including acting as a vasculoprotective factor, facilitating hippocampal LTP and regulating ion channel function.[30] Specifically, it has been revealed that H_2S has vasculoprotective properties in endothelial cells and vascular smooth muscle cells, such as eliciting vasorelaxation and decreasing platelet aggregation.[31] Physiological concentrations of H_2S may selectively enhance NMDA receptor-mediated response, which has an essential role in the induction of hippocampal LTP.[32] H_2S possibly activates plasma membrane voltage-gated channels (L-type and T-type Ca^{2+} channels) and mobilized intracellular Ca^{2+} stores.[33,34] In addition, endogenous H_2S was found to activate chloride (Cl^-) channels and potassium (K^+) channels (ATP-sensitive K channels (K_{ATP}) and K_{Ca2+}), which may provide neuroprotective effects.[35,36]

Collectively, the underlying mechanisms of H_2S have been investigated in a large number of studies, and the evidence is summarized in **Table 1**.

Table 1: Summary of experimental evidences for the mechanisms of H_2S

Mechanisms	Evidences	References
Antioxidation	Increasing cystine/glutamate antiporter and the intracellular concentrations of Cys	Kimura et al.[21]
	Enhancing the activity of γ-GCS	Kimura et al.[22]
	Scavenging ROS	Kimura et al.[22]
Anti-inflammation	Inhibiting the TNF-α, IL-β and NO	Seifert and Pennypacker[26]
	Increasing the IL-4 and IL-10	Seifert and Pennypacker[26]
Anti-apoptosis	Inhibiting the H_2O_2-activated calcium signal pathway	Luo et al.[27]
	Improving mitochondrial dysfunction and ROS-mediated caspase-3 pathway	Luo et al.[28]
	Regulating the nuclear translocation of NF-κB	Sen et al.[29]
Vasculoprotection	Eliciting vasorelaxation and decreasing platelet aggregation	Streeter et al.[31]
Facilitating LTP	Enhance NMDA receptor-mediated response	Kimura[32]
Regulating ion channel	Activating L-type channels and T-type Ca^{2+} channels and mobilizing intracellular Ca^{2+} stores	Nagai et al.[33]
	Activating Cl^- channels and K^+ channels (both K_{ATP} and K_{Ca2+})	Lee et al.[34]

Note: H_2S: Hydrogen sulfide; Cys: L-cysteine; γ-GCS: γ-glutamylcysteine synthase; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α; IL-β: interleukin-1β; NO: nitric oxide; IL-4: interleukin-4; IL-10: interleukin-10; H_2O_2: hydrogen peroxide; NF-κB: nuclear factor kappa B; LTP: long-term potentiation; NMDA: N-methyl D-aspartate; K_{ATP}: ATP-sensitive potassium; K_{Ca2+}: Ca^{2+}-sensitive potassium.

Roles of H_2S in Brain Diseases

Traumatic brain injury (TBI)

TBI is defined as a serious public health problem that disrupts the normal function of the brain and can be caused by a bump, blow or jolt to the head, rapid acceleration and deceleration of the calvarium, or a penetrating head injury.[37] There are certain experiments have shown that TBI usually led to brain edema, tissue loss, neurocognitive impairments, and dysfunction of the CNS.[38] Severe traumatic brain injury is a leading cause of increased long-term mortality and reduced life expectancy in the word, and trauma-induced changes in neuronal receptor composition render cells vulnerable to secondary injury.[39] Furthermore, activation of inflammatory reaction and production of ROS are two momentous elements in the early and secondary TBI-induced neuropathology.[40,41]

When TBI occurs in mice, endogenous H_2S in mouse brain cortex and hippocampus exhibits dynamic decrease, in parallel with CBS mRNA and protein expression in the brain.[42] Then, pretreatment with H_2S donor (NaHS, administered intraperitoneally) attenuates TBI-induced lesion volume, suggesting that H_2S is an important neuromodulator in the model of TBI.[42] Other studies have revealed the neuroprotective effects of H_2S on controlled cortical impact injury in rats: neurologic dysfunction is improved, endog-

enous antioxidant enzymatic (superoxide dismutase (SOD) and catalase) activities increase and the levels of oxidative products (malondialdehyde (MDA) and 8-iso-prostaglandin F2α) decrease, the blood-brain barrier (BBB) permeability increases and the brain edema is attenuated. Furthermore, the K_{ATP} channel blocker 5-hydroxydecanoate further proves that mitochondrial adenosine triphosphateesensitive potassium (mitoK_{ATP}) channels are activated and oxidative stress is reduced following exogenous H_2S therapy.[43]

Stroke

As the society ages rapidly, stroke has become the devastating disease second only to ischemia myocardial as a cause of disability and death worldwide, and also become a major threat to human health and life.[44] Although a great deal of factors can lead to the stroke, its main causes include cerebral vasospasm, obstacles in cerebral blood circulation, and the rupture of cerebral vessels.[45] A stroke is usually defined as one of two types: ischemic stroke caused by a blockage in an artery and hemorrhagic stroke caused by a tear in the arterial wall that produces bleeding into or around the brain. Either in the early brain injury (EBI) phase or in the late repair stage, the key factors of stroke pathobiology are oxidative stress and immunity.[46,47]

One study on rats revealed that H_2S provides potent nerve protection against a severe cerebral injury induced by transient middle cerebral artery occlusion.[48] It is regarded as the evidence that declination of the post-ischemic cerebral edema and the infarct volume as well as the improvement of behavior function after treatment with H_2S donor (NaHS). In the same experiment, researchers also demonstrated that H_2S could act as an antioxidant and significantly increase SOD activity in brain tissues. In contrast, the MDA content was selectively reduced and the mRNA levels of p47phox and gp91phox subunits of NADPH oxidase were up-regulated. Meanwhile, the expression of the anti-inflammatory cytokine IL-10 and the anti-apoptotic marker Bcl-2 was markedly induced in NaHS-tread group compared with ischemia/reperfusion (I/R) group.[49] In conclusion, H_2S has potent neuroprotective effect in the model of cerebral I/R through its anti-oxidative, anti-inflammatory, and anti-apoptotic effects. There is a point we have to say, however, that different concentration of H_2S may result in different outcomes, and even get the opposite conclusion. It means that the neuroprotective effect of H_2S is concentration-dependent in the model of I/R, only a comparatively low dose of H_2S can provide beneficial effect.[50]

The hemorrhagic stroke is generally divided into subarachnoid hemorrhage and intracerebral hemorrhage, two major types with high morbidity and mortality.[51] According to previous study, hemorrhagic strokes are typically more dangerous than ischemic strokes.[52] In a rat model of subarachnoid hemorrhage, treatment with NaHS attenuates EBI *in vivo*, including brain edema, BBB disruption, brain cell apoptosis, inflammatory response, and cerebral vasospasm. Further more, H_2S protects neurons and endothelial function *via* functioning as an antioxidant and antiapoptotic mediator *in vitro*.[53] In conclusion, H_2S may improve EBI and secondary brain injury in the subarachnoid hemorrhage model. In another study, treatment with NaHS reduced tissue plasminogen activator-induced the hemorrhagic transformation following ischemic stroke possibly by inhibiting the Akt-vascular endothelial growth factor-metalloproteinase 9 cascade.[54] However, it is unclear whether H_2S has potential application value in brain injury induced by intracerebral hemorrhage.

Neurodegenerative diseases

Neurodegenerative disease is an umbrella term for a range of conditions that primarily affect the neurons in human brain, which are incurable and debilitating conditions that result in progressive degeneration and death of nerve cells. In general, there are two main types of neurodegenerative diseases: impacting mental functioning (called dementias) and affecting movement (called ataxias).

Alzheimer's disease (AD), a form of dementia, is the most common progressive neurodegenerative disease, which may cause a series of clinical symptoms, such as memory impairment, logagnosia, personality changes and other neuropsychiatric symptoms. As previously described, AD usually damages neurons through activated neuroinflammation, oxidative stress and neuron apoptosis.[55] Admittedly, Hcy, a potential risk factor for AD, has harmful effects on cognitive function. Recent study have demonstrated that H_2S improved Hcy-induced cognitive dysfunction, which may play a benificial role through inhibiting reactive aldehydes accumulation, preserving glutathione homeostasis, and upregulating aldehyde-dehydrogenase 2 activity and expression in the hippocampus of Hcy-exposed rats.[56] Moreover, the beta-amyloid peptides (Aβ) cascade theory is regarded as a major pathogenese that may induce AD through oxidative stress and the change of synapsis.[57] However, H_2S can reverse Aβ-induced cognitive deficits *via* attenuating the production of Aβ and suppressing the down-regulation of CBS and 3-MST.[58] In addition, one study found that the progression of AD can be deterred through treatment with H_2S donors or spa-waters rich in H_2S content targeting multiple pathophysiological mechanismsappropriate.[59] In that study, a significant decrease in TNF-α and Bcl-2 expression increased, resulting in a attenuation of hippocampus morphological alterations and improved the ability to spatial learning and memory.[59] In other AD models, the cytotoxic lipid oxidation product 4-hydroxynonenal was scavenged with H_2S therapy, which provides a novel hope against AD through the neuroprotection effects of H_2S.[60]

Parkinson's disease (PD) is an age-related neurodegenera-

tive disease histopathologically characterized by progressive degeneration of dopaminergic neurons in substantia nigra of midbrain and formation of the Louis bodies in cytoplasm of residual neurons.[61] At present, clinical treatment of PD is levodopa (L-DOPA) replacement therapy to improve symptoms, but it not only may induce side effects like dyskinesia, also can not obstruct the development of PD. According to previous studies, plasma Hcy levels are significantly elevated in PD when patients are treated with L-DOPA group compared to other groups.[62] Furthermore, recent studies demonstrated that treatment with NaHS is able to significantly reduce the loss of substantia nigra neurons and slow down the development of motor dysfunction in 6-hydroxydopamine hydrobromide-induced and rotenone-induced PD models.[63] Moreover, inhalation of H_2S prevented the movement disorder from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced PD.[64] Therefore, H_2S is expected to provide new ideas for the pathogenesis and clinical treatment of PD.

In order to facilitate simple comprehension, we systematically analyzed the latest researches concerned with the use of H_2S in the above brain diseases and the relevant conclusions are described through illustration (**Figure 1**).

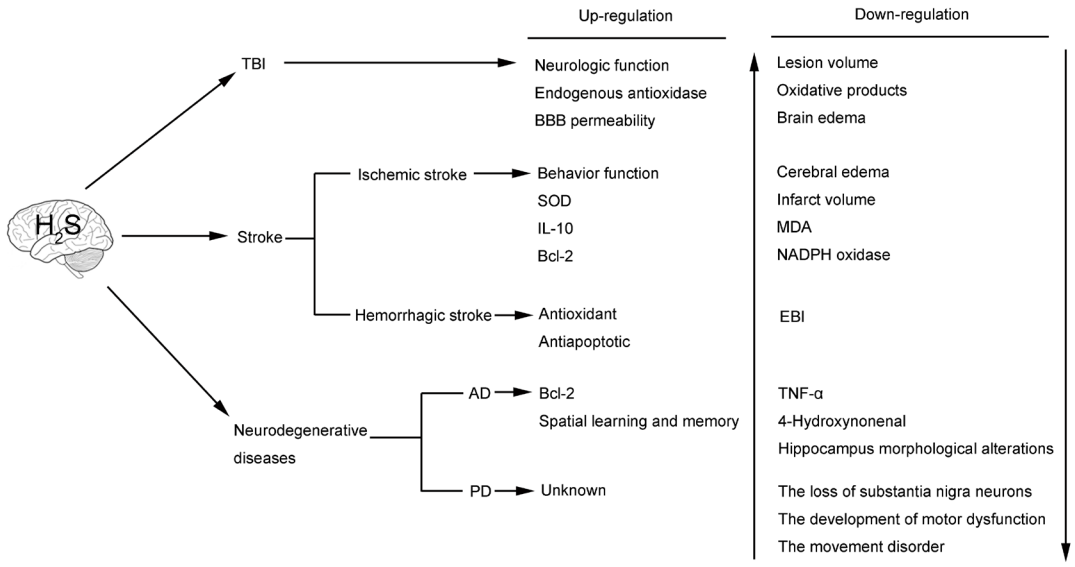

Figure 1: The application of H_2S therapy in brain diseases.
Notes: H_2S: hydrogen sulfide; TBI: traumatic brain injury; BBB: blood-brain barrier; SOD: superoxide dismutase; IL-10: interleukin-10; AD: Alzheimer's disease; PD: Parkinson's disease; MDA: malondialdehyde; TNF-α: tumor necrosis factor-α; EBI: early brain injury; NADPH: nicotinamide adenine dinucleotide phosphate.

Clinical Studies

Until now, no direct clinical studies have confirmed the neuroprotection of H_2S in brain disease. However, it is reported that there is a close touch between the plasma H_2S level and the long-term clinical outcome in stroke patients. A growing number of evidence has suggested that hyperhomocysteinaemia is a risk factor for stroke, although several meta-analyses have not came to an agreement.[65-67] In another study, results indicated that increased plasma Cys in patients with acute stroke may show an increase in the production of H_2S in the brain, thus leading to poor clinical outcomes.[68] Generally speaking, indirect evidence proves that it is no doubt that H_2S exerts neuroprotective effects in clinical trials and has a close association with brain injury caused by acute stroke, but its underlying mechanisms are needed to be further studied.

Conclusion

As endogenous gas signal molecules, NO, CO and H_2S have extensive tissue distribution and diverse bioeffects. Over the past two decades, H_2S has been proved to be the third gas signal molecule that plays an important role in physiology and pathology. Furthermore, a unique gas signal network may come into being among the three gaseous systems, which are not only independent of each other but also jointly participate in the regulation and control of diseases. Increasing studies have shown that H_2S has been regarded as a neuromodulator in the brain, rather than the previously described toxic effects. It is noteworthy that only the appropriate dose of H_2S may provide neuroprotective effects, because H_2S is toxic to the body when it is higher than the physiological dose. However, it is poor that our understanding of the underlying mechanisms of H_2S actions in the CNS. And there are still many controversies that are needed to further explore. H_2S therapy has only entered a preliminary stage whether in basic medical research or preclinical research. Along with the deepening of research, we firmly believe that the clinical application of H_2S therapy will become a potent treatment regimen in the near future.

Author contributions

JYZ and YPD were responsible for writing the manuscript. ZW and YK were responsible for its revision. RG and GC were responsible for its drafting and revision. All authors read and approved the final version of the paper for publication.

Conflicts of interest

The authors declare that they have no competing interests.

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