Therapeutic applications of hydrogen sulfide and novel donors for cerebral ischemic stroke: a narrative review

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Abstract

Ischemic stroke happens when the blood supply to the brain is obstructed and it is associated with numerous complex mechanisms, such as activated apoptosis genes, oxidative stress and reaction of inflammation, which finally result in neurological deficits. Several gases have been proved to have neuroprotective roles, even the classic gases that are thought to be toxic such as hydrogen sulfide (H_2S). H_2S is the third identified endogenous gas signaling molecule following carbon monoxide and nitric oxide. H_2S plays a significant role in stroke. Inhalation of H_2S can attenuate cerebral infarct volume and promote neurological function in a rat model of middle cerebral artery occlusion to reduce ischemic stroke-induced injury in vivo and in vitro as a result. Therefore, H_2S can be clinically used to reduce ischemic stroke-induced injury. This review introduces the toxic mechanisms and effects of H_2S on cerebral ischemic stroke

Key words: apoptosis; clinical application; donors; hydrogen sulfide; ischemic stroke; neuroinflammatory; oxidative stress; potential mechanism

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Introduction

Ischemic stroke is defined as a precipitate loss of blood circulation in a part of the brain which leads to neurological deficits and cognitive decline.[1,2] Thrombotic or embolic occlusion of cerebral arteries causes acute ischemic stroke.[3,4] Males aged over 40 years old have great chance to suffer from ischemic stroke. What's more, ischemic stroke can result in high morbidity, mortality and disability rates and may cause serious social and economic burdens.[5] Ischemia/reperfusion (I/R) injury causes severe damage to most organs and may occur in a variety of tissues including the brain, kidney, heart, and liver.[6,7] Reactive oxygen species (ROS), as one of the main hazards, is excessively generated after I/R injury, leading to severe internal tissue damage, and further induces cell damage through reactions of inflammation.[8] Numerous studies have been conducted to improve the prognosis of stroke and many therapies have been discovered and applied.[9]

Hydrogen sulfide (H_2S) is garnering increasing attention for its neuroprotective function.[10,11] H_2S is a small gaseous compound that acts as a gas transmitter along with carbon monoxide and nitric oxide.[12] It influences physiological and pathological processes throughout the body. However, increasing studies have shown that H_2S has anti-inflammatory, anti-oxidative, and protective effects on neurological diseases. Sodium hydrosulfide (NaHS) as an H_2S donor can protect the nerve system after I/R based on the data from *in vitro* studies and animal experiments.[13,14] In recent years, other H_2S donors have also been proved to have the neuroprotective activity to ischemic stroke. A great concern has been concentrated on inorganic H_2S donors such as NaHS, which may generate H_2S instantaneously at high concentrations and cause neurotoxic effects.[15,16] Hence, we should pay attention to its beneficial effects that could address clinical problems of neuronal death caused by ischemia stroke. To explore the feasibility of H_2S for clinical treatment, we analyze relevant experimental and clinical studies in this review and discuss the effects of H_2S on ischemic stroke injury and possible neuroprotective mechanisms.

Experimental Studies of Hydrogen Sulfide Donors in Ischemic Stroke

Before clinical applications, numerous animal experiments have been carried on. Animal models of ischemic stroke have been successfully established to explore the role of H_2S in ischemic stroke.[16] It is impractical to inhale the gas directly due to its toxicity. Therefore, other reagents have been used in animals to simulate the effects of H_2S. For example, NaHS which can form HS^- to react with H^+ to form H_2S is commonly used in experiments as a donor of H_2S to explore the potential physiologic functions of H_2S.[17] Inorganic H_2S donors such as NaHS have been paid great attention which may generate H_2S instantaneously at high concentrations and cause neurotoxic effects. Therefore, it is needed to develop quantitative and durable H_2S release agents to maintain a defined concentration range of H_2S. For example, 8e, as an H_2S releasing derivative of 3-n-butylphthalide, reduced neural apoptosis, focal infarction, cerebral edema and sensorimotor deficits 72 hours after transient occlusion of middle cerebral artery significantly.[18] GYY4137 is also a new drug that can slowly release low concentrations of H_2S in water for several days at physiological pH and temperature.[16] AP39 (50 nmol/kg),

a H_2S delivery molecule that can release slowly and targeted at mitochondria, reveals its neuroprotective activity and may reduce infarct volume and neurological deficits in the experimental AP39 groups.[15] These agents are applied to the model of ischemic stroke to explore the potential mechanisms of H_2S. By comprehensive analysis of these experiments, we found that H_2S plays a protective or deleterious role in the ischemic brain depending on its concentration, H_2S is deleterious at a high concentration and protective at a low concentration. In some experiments, exogenously administered H_2S in the form of NaHS at 180 mmol/kg but not at 90 mmol/kg increased infarct volume in permanent middle cerebral artery occlusion rats. N-methyl-D-aspartate receptor antagonist could attenuate this increase. Importantly, administration of cystathionine β-synthase inhibitors contributed to the reduction of infarct volume, suggesting that the production of endogenous H_2S help ameliorate ischemic injuries.[19] Wen et al.[20] also reported that after $1 \times 10^{-5} – 1 \times 10^{-7}$ mol/kg NaHS supplements, H_2S could help upregulate cerebral vascular function in terms of contraction and dilation which may depend on endothelium cells via activating potassium channel. H_2S could therefore play an important role in the protection of cerebral I/R injury. In this review, the major mechanism and potential role of H_2S in the treatment of ischemic stroke are discussed.

Mechanisms of Hydrogen Sulfide in Ischemic Stroke

H_2S plays a protective role via several mechanisms such as inhibiting oxidative stress, inflammation, endoplasmic reticulum stress, cell death and apoptosis. Here, we describe the mechanisms of H_2S in ischemic stroke briefly (**Figure 1** and **Additional Table 1**).

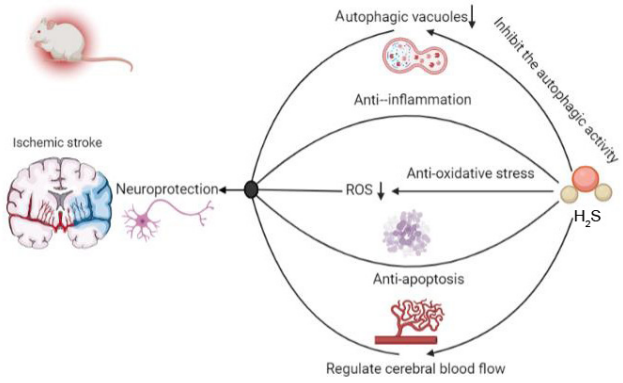

Figure 1: Therapeutic applications of hydrogen for cerebral ischemic stroke.
Note: H_2S: Hydrogen sulfide; ROS: reactive oxygen species.

Inhibition of autophagic activity

Exogenous H_2S suppressed the elevation of microtubule-associated protein light chain 3-II and the decrease of p62, but had no notable effect on Beclin-1 complex of cerebral I/R injury model mice, which indicated that exogenous H_2S decreased autophagosome accumulation to inhibit autophagy.[21] Jiang et al.[22] reported that H_2S can attenuate brain injury by inhibiting the autophagic activity of cells. They built a middle cerebral artery occlusion model *in vitro* by oxygen-glucose deprivation/reoxygenation in PC12 cells and finally proved that NaHS treatment can alleviate injury in cells and inhibit autophagy overactivated by oxygen-glucose deprivation/reoxygenation in PC12 cells. Furthermore, the accumulation of autophagic vacuoles in mouse brain after I/R injury can be decreased by exogenous H_2S.[23]

Anti-oxidative stress

Oxidative stress is a significant mechanism during the process of cerebral I/R injury. Oxidative stress can lead ROS accumulation and excessive ROS will damage neurons. Oxidative stress can activated Mitogen-activated protein kinase (MAPK) pathway and H_2S may function as a neuroprotector by protecting against neuronal damage caused by oxidative stress biologically.[24] Exogenous H_2S can inhibit p38MAPK and extracellular-regulated kinase 3 signaling pathway and regulate MAPK signaling pathway to protect neurons against injury from oxidative stress.[16,25] Thus, it indicated that exogenous H_2S provides a protective effect against oxygen-glucose deprivation/reoxygenation-induced injury by enhancing the activation of the ERK3, p38MAPK and nuclear factor-erythroid factor 2-related factor 2 mRNA.

Regulation of cerebral blood flow

H_2S can upregulate the contraction and dilation function of cerebral vessels to change its blood flow partially via activating potassium channel. Phosphatidylinositol bisphosphate can activate ion channels directly, which potassium channels are also involoved. H_2S can regulate potassium channel activity by altering channel-phosphatidylinositol bisphosphate interaction.[18] Shi et al.[26] reported that cerebral blood flow increases while the resistance of cerebral vessels, blood viscosity, and thrombogenesis decrease after treatment with NaHS. NaHS can also promote angiogenesis in the peri-infarct area after ischemic stroke, possibly through augmenting AKT and ERK phosphorylation and increasing angiopoietin-1 and vascular endothelial growth factor expression.[27] These results indicated that H_2S performs its protective effect on ischemic stroke by improving the endothelium-dependent function of cerebral vessels in terms of contraction and dilation and promoting angiogenesis.

Anti-inflammation

The nuclear factor kappa B (NF-κB) signaling pathway can be activated by ROS produced by oxidative stress within the cell, where NF-κB production can lead to increased levels of cytokines such as interleukin-6 and interleukin-1β to trigger inflammation. The anti-inflammatory effect of H_2S can be mediated by inhibiting NF-κB.[28,29] SB203580, a kind of p38MAPK inhibitor, significantly attenuates lipopolysaccharide-induced tumor necrosis factor-alpha secretion, another inflammatory indicator. H_2S can play the same role as SB203580.[13] Hu et al.[13] confirmed that H_2S is able to reduce inflammation by suppressing nitric oxide synthase and p38MAPK signaling pathways.

Anti-apoptosis

Accumulating evidence points out that H_2S may play its role in anti-apoptosis via multiple apoptotic pathways. H_2S can inhibit ROS-mediated caspase-3 signaling pathway via the calcium pathway and promote the nuclear translocation of NF-κB that mediates apoptosis pathways.[30,31] In addition, exogenous H_2S such as GYY4137 can inhibit p38MAPK and ERK1/2 pathways and regulate MAPK signaling pathway against neuronal injury from oxidative stress.[16] By regulating p38MAPK, ERK1/2 and c-Jun N-terminal kinase signaling pathways can inhibit apoptosis and protect neurons.[16]

Additional mechanism

H_2S preconditioning could protect mice against cerebral I/R injury through activating heat shock protein-70 and phosphoinositide 3-kinase/Akt/nuclear factor-erythroid factor 2-related factor-2 pathway.[18] In addition, inhalation of H_2S can activate protein kinase C and then downregulate the expression of aquaporin-4 to exert its protective effects.[14]

Clinical Applications

The current study on H_2S is still in the experimental stage and no clinical application has been reported. More clinical trials are needed to explore the value of H_2S.

Limitations

Most studies focused on the protective effects of H_2S. However, they ignored the long-term protective effects of H_2S. Therefore, it is important for us to investigate the effects of H_2S on long-term stroke. Inorganic H_2S donors may generate H_2S instantaneously at a high concentration and may thus result in a neurotoxic effect. Therefore, new H_2S donors for the quantitative and persistent release of H_2S are needed, to ensure their safety in the treatment of cerebral ischemic stroke.

Conclusions

H_2S may exert a protective role in cerebral ischemic stroke. The role of H_2S is somewhat inconsistent with those mentioned above that may depend on its concentration. More studies are needed to explore the possible role and mechanisms of H_2S in neurofunctional protection and to explore how to optimize the use of this gas in ischemic stroke treatment. Finally, we confirm that H_2S will blaze a new trail in the treatment of cerebral ischemic stroke.

Author contributions

Manuscript writing: DJS; manuscript revision: ZY; manuscript drafting: WTY and LX. All authors read and approved the final version of the manuscript for publication.

Conflicts of interest

The authors declare that they have no competing interests.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Open access statement

Additional file

Additional Table 1: Experimental studies of hydrogen sulfide in ischemic stroke of recent years (until 2021).

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Additional Table 1: Experimental studies of hydrogen sulfide in ischemic stroke of recent years (until 2021)

Study	Year	Model	Animals/cells	Main results
Jiang et al.[20]	2017	MACO OGD/R	Rats PC cells	Inhibition of overactivated autopagy may contribute to the attenuation of MCAO - induced cerebral ischemia/reperfusion injury in rats and OGD/R - induced cellular injury in PC12 cells by exogenous supplementation of NaHS.
Woo et al.[23]	2017	tMCAO	Rats	The NaHS-1 group (NaHS delivered at 1 min before reperfusion, respectively) had the lowest apoptosis rate compared with other group such as sham and NaHS30 groups (NaHS delivered at 30 min before reperfusion, respectively).
Zhu et al.[21]	2017	OGD/R Cerebral I/R	SH-SY5Y cells Mice	NaHS (intraperitoneal) shows best protection at 2 mg/kg, less protection at 1 or 4 mg/kg, and no protection at 8 or 16 mg/kg against mouse cerebral I/R injury through single injection. The neuroprotectiive effects of exogenous H_2S on ischemia/hypoxia and reperfusion/reoxygenation injury is mediated by enhanced autophagic degradation.
Wen et al.[18]	2018	MCAO	Rats Endothelial cells	1×10^{-5} - 1×10^{-7} mol/kg NaHS supplement: H_2S has the protective effects on brain I/R injury by upregulation of endothelium-dependent vasoconstriction and dilation function of cerebral vessels, which may be associated with activating potassium channel.
Bai et al.[25]	2019	tGCI	Rats	NaHS (24 μmol/kg) postconditioning effectively protected hippocampal CA1 neurons from tGCI-induced injury, at least in part by activating ERK1/2 signaling pathway.
Song et al.[22]	2020	MCAO	Rats	NaHS (28 μmol/kg) could down-regulate the phosphorylation of p38 by reducing the assembly of CaMKII with the ASK1-MKK3-p38 signal module, thus inhibiting brain I/R injury.
Tao et al.[27]	2020	MCAO	mice	Exogenous H_2S treatment suppressed inflammation and reduced behavioral impairment. The anti-inflammatory effect of H_2S was mediated by inhibiting NF-κB.
Wang et al.[16]	2018	MCAO	Rats	8e, a H_2S derivation released by 3-n-butylphthalide, significantly reduced neural apoptosis, focal infarction, brain edema and sensorimotor deficits within 72 h after transient middle cerebral artery occlusion.
Han et al.[14]	2020	MCAO	Rats	H_2S sustained release agent GYY4137 inhibited apoptosis by regulating p38MAPK, ERK1/2 and JNK signaling pathways, improved neural function after brain I/R injury,

				and reduced infarct area.
Pomiernyet al.[13]	2021	MCAO	Rats	AP39 (50 nmol/kg), an H_2S delivery molecule which can release slowly and target at mitochondria. After administration, this compound was found to have the neuroprotective activity and the notably reduced infarct volume and neurological deficit in the experimental groups treated with AP39 and subjected to MCAO.

Note: ASK1: Apoptosis signal-regulating kinase 1; CaMKII: calmodulin-dependent protein kinase II; ERK: extracellular-regulated kinase; H_2S: hydrogen sulfide; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; MCAO: middle cerebral artery occlusion; MKK3: mitogen-activated protein kinase kinase 3; NaHS: sodium hydrosulfide; NF-κB: nuclear factor kappa B; tGCI: transient global cerebral ischemia.