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 (H₂S). H₂S is the third identified endogenous gas signaling molecule following carbon monoxide and nitric oxide. H₂S plays a significant role in stroke. Inhalation of H₂S 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, H₂S can be clinically used to reduce ischemic stroke-induced injury. This review introduces the toxic mechanisms and effects of H₂S 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 (H₂S) is garnering increasing attention for its neuroprotective function.¹⁰,¹¹ H₂S 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 H₂S has anti-inflammatory, anti-oxidative, and protective effects on neurological diseases. Sodium hydrosulfide (NaHS) as an H₂S 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 H₂S donors have also been proved to have the neuroprotective activity to ischemic stroke. A great concern has been concentrated on inorganic H₂S donors such as NaHS, which may generate H₂S 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 H₂S for clinical treatment, we analyze relevant experimental and clinical studies in this review and discuss the effects of H₂S 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 H₂S 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 H₂S. For example, NaHS which can form HS⁻ to react with H⁺ to form H₂S is commonly used in experiments as a donor of H₂S to explore the potential physiologic functions of H₂S.¹⁷ Inorganic H₂S donors such as NaHS have been paid great attention which may generate H₂S instantaneously at high concentrations and cause neurotoxic effects. Therefore, it is needed to develop quantitative and durable H₂S release agents to maintain a defined concentration range of H₂S. For example, 8e, as an H₂S 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 H₂S in water for several days at physiological pH and temperature.¹⁶ AP39 (50 nmol/kg),
a H$_2$S 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$_2$S. By comprehensive analysis of these experiments, we found that H$_2$S plays a protective or deleterious role in the ischemic brain depending on its concentration, H$_2$S is deleterious at a high concentration and protective at a low concentration. In some experiments, exogenously administered H$_2$S 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 $\beta$-synthase inhibitors contributed to the reduction of infarct volume, suggesting that the production of endogenous H$_2$S help ameliorate ischemic injuries.$^{16}$ Wen et al.$^{20}$ also reported that after $1 \times 10^{-5} – 1 \times 10^{-7}$ mol/kg NaHS supplements, H$_2$S could help upregulate cerebral vascular function in terms of contraction and dilation which may depend on endothelial cells via activating potassium channel. H$_2$S 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$_2$S in the treatment of ischemic stroke are discussed.

**Mechanisms of Hydrogen Sulfide in Ischemic Stroke**

H$_2$S 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$_2$S in ischemic stroke briefly (Figure 1 and Additional Table 1).

**Figure 1:** Therapeutic applications of hydrogen for cerebral ischemic stroke. Note: H$_2$S, Hydrogen sulfide; ROS, reactive oxygen species.

**Inhibition of autophagic activity**
Exogenous H$_2$S 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$_2$S decreased autophagosome accumulation to inhibit autophagy.$^{21}$ Jiang et al.$^{22}$ reported that H$_2$S 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$_2$S.$^{23}$

**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$_2$S may function as a neuroprotector by protecting against neuronal damage caused by oxidative stress biologically.$^{24}$ Exogenous H$_2$S 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$_2$S 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$_2$S 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 involved. H$_2$S 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$_2$S 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$_2$S 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$_2$S can play the same role as SB203580.$^{13}$ Hu et al.$^{13}$ confirmed that H$_2$S is able to reduce inflammation by suppressing nitric oxide synthase and p38MAPK signaling pathways.

**Anti-apoptosis**
Accumulating evidence points out that H$_2$S may play its role in anti-apoptosis via multiple apoptotic pathways. H$_2$S 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$_2$S 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

H₂S preconditioning could protect mice against cerebral I/R injury through activating heat shock protein-70 and phosphoinosito 3-kinase/Akt/nuclear factor-erythroid factor 2-related factor-2 pathway. In addition, inhalation of H₂S can activate protein kinase C and then downregulate the expression of aquaporin-4 to exert its protective effects.

Clinical Applications

The current study on H₂S is still in the experimental stage and no clinical application has been reported. More clinical trials are needed to explore the value of H₂S.

Limitations

Most studies focused on the protective effects of H₂S. However, they ignored the long-term protective effects of H₂S. Therefore, it is important for us to investigate the effects of H₂S on long-term stroke. Inorganic H₂S donors may generate H₂S instantaneously at a high concentration and may thus result in a neurotoxic effect. Therefore, new H₂S donors for the quantitative and persistent release of H₂S are needed, to ensure their safety in the treatment of cerebral ischemic stroke.

Conclusions

H₂S may exert a protective role in cerebral ischemic stroke. The role of H₂S 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 H₂S in neurofunctional protection and to explore how to optimize the use of this gas in ischemic stroke treatment. Finally, we confirm that H₂S will blaze a new trail in the treatment of cerebral ischemic stroke.

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  | Rats          | Inhibition of overactivated autophagy 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 | SH-SY5Y cells | 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 neuroprotective effects of exogenous H$_2$S on ischemia/hypoxia and reperfusion/reoxygenation injury is mediated by enhanced autophagic degradation. |
| Wen et al. 18  | 2018 | MCAO  | Rats          | 1 × 10$^{-5}$ - 1 × 10$^{-7}$ mol/kg NaHS supplement: H$_2$S 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$_2$S treatment suppressed inflammation and reduced behavioral impairment. The anti-inflammatory effect of H$_2$S was mediated by inhibiting NF-κB. |
| Wang et al. 16 | 2018 | MCAO  | Rats          | 8e, a H$_2$S derivate released by 3-n-butylphthalalide, 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$_2$S sustained release agent GYY4137 inhibited apoptosis by regulating p38MAPK, ERK1/2 and JNK signaling pathways, improved neural function after brain I/R injury. |
AP39 (50 nmol/kg), an H₂S 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; H₂S: 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.