The role of hydrogen sulfide in stroke

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Abstract

Stroke is a kind of acute cerebrovascular disease characterized by the focal lack of neurological function, including ischemic stroke and hemorrhagic stroke. As society ages rapidly, stroke has become the second leading cause of disability and death, and also become the main threat to human health and life. In recent years, findings from increasing animal and clinical trials have supplied scientific evidences for the treatment of stroke. Hydrogen sulfide (H_2S), which has always been seen as a toxic gas, now has been thought to be the third gaseous signaling molecule following nitric oxide and carbon monoxide. Accumulating evidences indicate that H_2S plays an important role in stroke. Given that its neuroprotective effect is dose-dependent, only when its concentration is relatively low, H_2S can yield the neuroprotection, while high dose may lead to neurotoxicity. All these study results suggest that H_2S may offer a new promising application for the therapy of stroke. Here, our review will present the role of H_2S in stroke from its mechanism to animal and clinical studies.

Key words: hydrogen sulfide; stroke; anti-oxidation; anti-inflammatory effects; anti-apoptosis; dose-dependent; neuroprotection; neurotoxicity.

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Introduction

Stroke is a kind of acute cerebrovascular disease characterized by high morbidity, disability and death rates. It is reported that stroke has become one of the most frequently-occurring neurological injuries in USA and China, about 800,000 and 2,000,000 people every year respectively (Roger et al., 2011). Stroke includes ischemic stroke and hemorrhagic stroke, and a large number of clinical studies and animal experiments have shown that the cerebral injury and ischemia-reperfusion injury caused by stroke are complex physiopathologic processes, involving toxic effects of excitatory amino acids, inflammatory reaction, oxidative stress, the generation of free radical, brain edema, neuronal apoptosis/death (Zhu et al., 2015). Although the mechanisms of the injury are diverse, our treatment theory of neuroprotective strategies mainly focus on two aspects: on the one hand, we can activate the endogenous protective mechanisms of patients' bodies to protect the brain from injury; on the other hand, it is advisable to try our best to minimize secondary brain injury and promote the recovery of the damaged tissues. For many years, people have been trying to understand and search effective treatments for stroke from every conceivable angle. For now, for ischemic stroke, thrombolytic and anticoagulant therapy has become regular treatments (Mandava et al., 2015), and for hemorrhagic stroke, hematomas cleaning operation and the stable control of intracranial pressure are conductive to improve the prognosis of patients (Siler et al., 2014). In addition, hypothermia has been considered to be a valuable clinical treatment for centuries (Thome et al., 2005), and

mild hypothermia (33−36°C) has been demonstrated to play neuroprotective role (Antonic et al., 2014; Wang et al., 2014). Its mechanisms mainly involve decreasing the metabolism rates of brain tissues and alleviating brain edema (Hobbs et al., 2008). Besides, a variety of methods are used for the treatment of stroke, including stroke unit, neuronal electrophysiological treatment, and even the possibility of psychological therapy (Pohjasvaara et al., 1998; Patel et al., 2004; Sheffler and Chae, 2007). Our previous studies suggested that pramipexole-induced hypothermia could effectively inhibit subarachnoid hemorrhage-induced early brain injury in rats *via* phosphoinositide 3-kinase/protein kinase B/glycogen synthase kinase-3 signaling pathway (Ma et al., 2016). However, the present theories for stroke are still unsatisfactory and needed to be further improved, although the development of evidence-based medicine is ongoing. Specifically, in recent years, medical gas, including oxygen and hydrogen, have become issues of concern. No matter ischemic stroke or hemorrhagic stroke, the final outcome is hypoxic-ischemic damage of brain tissues, hyperbaric oxygen therapy can significantly increase the oxygen pressure and blood oxygenation content, accelerate collateral circulation to protect neurons, repair the damaged microvessels, stimulate angiogenesis and neurogenesis, and accordingly, play neuroprotective role (Gill and Bell, 2004; Sanchez, 2013). Hydrogen has also been found play roles of anti-inflammation, anti-oxygenation and anti-apoptosis when a stroke occurs. Hydrogen sulfide (H_2S) has always been considered as a toxic gas with its odor of rotten eggs, because it could play the cytotoxic role through inhibiting cytochrome C oxidase and mitochondrial respiration (Furne et al., 2001; Partlo et al., 2001). However, in recent years, accumulating evidences have suggesting that H_2S is an another important gaseous signaling molecule in biological systems following nitric oxide (NO) and carbon monoxide and in central nervous system (CNS) (Wang, 2002; Li and Moore, 2008), a larger number of researches have shown that it has neuroprotective effects as neurotransmitter (Kimura and Kimura, 2004; Whiteman et al., 2004), and researchers hope to provide a new direction for the therapy of stroke through studying H_2S. After the endogenous H_2S was first found in the brain in the end of 20th century (Warenycia et al., 1989), researchers gradually found that the production of H_2S in CNS predominantly involve three particular enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST), which could convert to H_2S from L-cysteine (Cys) in combination with cysteine aminotransferase (CAT) (Kolluru et al., 2013; Jung and Jeong, 2014). That is to say, CSE primarily induce the production of H_2S in cardiovascular system, while CBS mainly is responsible for producing H_2S in the CNS (Qu et al., 2008; Kimura et al., 2012), and CBS uses Cys and homocysteine (Hcy) that have been known the risk factors for stroke and heart disease as substrates (Abe and Kimura, 1996; Chen et al., 2004; Singh et al., 2009). Interestingly, although there are amounting evidences showing that H_2S participates in the regulation of neuronal function and signaling pathway, concerning the neuroprotective or neurotoxic effects of H_2S still remains controversial. In our review, we will discuss the main mechanism and the possible role of H_2S in stroke.

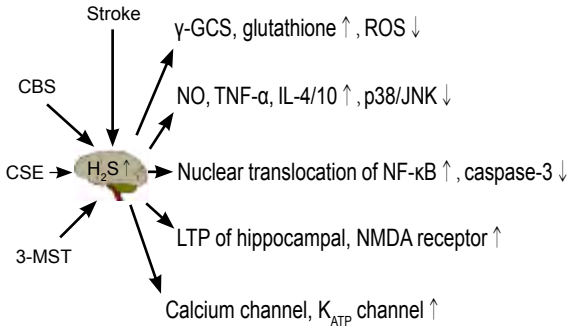

Figure 1: The mechanisms of H_2S in CNS.
Note: H_2S: Hydrogen sulfide; CNS: central nervous system; CBS: cystathionine-β-synthase; CSE: cystathionine-γ-lyase; 3-MST: 3-mercaptopyruvate sulfurtransferase; γ-GCS: γ-glutamyl-cysteine synthetase; ROS: reactive oxygen species; NO: nitric oxide; TNF-α: tumor necrosis factor-α; IL-4/10: interleukin-4/10; JNK: c-Jun N-terminal kinase; NF-κB: nuclear factor kappa B; LTP: long-term potentiation. NMDA: N-methyl-D-aspartic acid; K_{ATP}: ATP sensitive potassium.

Mechanisms of H_2S in stroke

Because of its toxicity, people have always thought that the concentration of H_2S in animal tissues is very low for a long time. However, as what we have known, H_2S could be produced endogenously, and further studies indicate that H_2S could exist in mammalian tissues at very high concentration, up to 50–160 mM (Goodwin et al., 1989; Warenycia et al., 1989), which is dramatically higher than that in the peripheral blood (0–46 mM) (Zhao et al., 2003), suggesting that H_2S may have some effects and show potential therapeutic value in some CNS diseases, including stroke, traumatic brain injury, Alzheimer's disease and Parkinson's disease.

Here, we give a brief description on the mechanisms of H_2S in the CNS as follows (**Figure 1**): (1) anti-oxidation: facing the oxidative stress injury after stroke occurring, H_2S could promote the activation of cystine/glutamate antiporter and increase the intracellular concentrations of Cys that it is a kind of substrate for the generation of glutathione. In addition, in this process, γ-glutamyl-cysteine synthetase, a kind of rate-limiting enzyme, could also be activated by H_2S and regulates the production of glutathione (Kimura and

Table 1: The neuroprotective and neurotoxic effects of hydrogen sulfide (H_2S) in stroke

Role of H_2S in stroke	Reference
Neuroprotection	
1) Protect hippocampal neurons, increase synaptic plasticity, decrease the brain edema, at 0.01 mM concentration	Kimura et al., 2010
2) Reduce infarct volume, decrease nuclear factor kappa B expression	Seifert and Pennypacker, 2014
3) The concentration of endogenous H_2S increased at 12 hours and decreased at 24 hours after stroke	Hu et al., 2007
4) Sodium hydrosulfide protect the brain against oxygen glucose deprivation injury	Liu et al., 2014
5) Promote angiogenesis by up-regulating vascular endothelial growth factor and angiopoietin-1 expression	Luo et al., 2012
6) Increase the expression of superoxide dismutase, interleukin-10 and Bcl-2	Luo et al., 2013
7) Decrease the blood brain barrier damage	Sen et al., 2012
Neurotoxicity	
1) Increase infarct volume at 0.18 mM concentration	Kimura, 2002
2) Cause cell death in the cerebral cortex	Kimura, 2002
3) Induce necrosis and cause brain damage if sodium hydrosulfide concentration is < 200 μM	Kimura, 2000

Kimura, 2004; Kimura et al., 2010), which is an important intracellular antioxidant. Thus, the intracellular levels of glutathione increases and could clear the reactive oxygen species (ROS) existing in the mitochondria to achieve the goal of protecting the neurons from oxidative stress injury. (2) Anti-inflammatory effects: the major sites that H_2S plays the role of fighting inflammatory response are astrocytes and microglia (Seifert and Pennypacker, 2014), where H_2S could inhibit the release of NO, tumor necrosis factor-α and the proinflammatory cytokine interleukin-1β, and decrease the activation of p38/c-Jun N-terminal kinase, conversely, increase the release of anti-inflammatory cytokines, for instance, interleukin-4/10. It is reported that SB 203580, a kind of p38 mitogen-activated protein kinase (MAPK) inhibitor, could mimic the anti-inflammatory effects, indicating that H_2S may regulate the immune function by MAPK signaling pathway (Hu et al., 2007), but concrete mechanisms still need to be investigated. (3) Anti-apoptosis: a growing body of evidence points that H_2S exerts its role of anti-apoptosis probably *via* mitochondrial apoptotic pathway, H_2S could inhibit the caspase-3 signaling pathway mediated by ROS and calcium pathway activated by hydrogen peroxide (Liu et al., 2014), accordingly, prevent the apoptosis induced by oxygen glucose deprivation/reoxygenation (Luo et al., 2012, 2013). In addition, H_2S could promote the nuclear translocation of nuclear factor kappa B (NF-κB), which activates the anti-apoptotic gene (Sen et al., 2012). (4) Facilitating long-term potentiation: It has always been demonstrated that H_2S can facilitate hippocampal long-term potentiation *via* activating N-methyl-D-aspartic acid (NMDA) receptor (Kimura, 2002). Subsequently, researchers found that unlike carbon monoxide or NO, H_2S promotes the accumulation of cyclic adenosine monophosphate rather than cyclic guanosine monophosphate (Kimura, 2000), and NMDA receptor activation induced by H_2S could be inhibited by adenylate cyclase inhibitor (Kimura, 2002). (5) Regulating calcium concentration: H_2S can activate calcium channel to increase calcium influx, whereas decrease the release of calcium stored in the cells, thus induce the production of calcium waves in primary cultures of astrocytes, and this process can be inhibited by calcium channel antagonist (Nagai et al., 2004). In addition, H_2S has been known as an ATP sensitive potassium (K_{ATP}) channel activator in secondary brain injury after stroke or other diseases, H_2S could play its neuroprotection through mimicking K_{ATP} channel (Jiang et al., 2013). However, the problem of calcium overload cannot be ignored (O'Bryant et al., 2014). There are accumulating evidence that the neuroprotection or neurotoxicity of H_2S depends on the concentration of it, which needs further investigations, including animals and clinical trials.

Animal studies

As we have known, a large number of animal studies must be tested before clinical application for anything, H_2S is no exception. In view of the toxicity and high solubility of H_2S, it is even uncommon to inhale it directly; therefore, sodium hydrosulfide (NaHS, a H_2S donor) is widely applied in animal studies concerning cerebral injury after stroke. At present, researchers gradually reach a consensus that the neuroprotection of H_2S in stroke is dose-dependent, that is, the relatively low-dose of H_2S in brain may play neuroprotective effect in stroke, whereas high concentration of H_2S may cause neurotoxicity, suggesting that we must view H_2S from a comprehensive perspective, and enhance its advantage, while avoid its disadvantage, to apply H_2S in clinical treatment more effectually. In our review, we will show our analysis for animal studies about the role H_2S in stroke (**Table 1**).

The neuroprotection of H_2S is exhibited as follows: (1) Li et al. (2011) reported that H_2S, whose concentration is 0.1 mM, has neuroprotective effects by protecting hippocampal neurons, improving the deficiency of learning and memory, and increasing synaptic plasticity, decreasing the brain edema around pyramidal neurons and their nuclear shrink in a rat brain model of stroke. (2) In a rat

model of reversible occlusion of the right middle cerebral artery, H_2S was found to reduce infarct volume through long-term hypothermia and the decrease of the expression of NF-κB, thus protecting the brain against hypoxic injury due to stroke (Florian et al., 2008). (3) After stroke, the endogenous concentration of H_2S increases at 12 hours and decreases at 24 hours. In addition, if pretreated by NaHS at a relatively low dose (25 mmol/kg), the brain injury could be attenuated at 7 days after stroke (Ren et al., 2010). NaHS is also reported to protect brain against oxygen glucose deprivation injury (Tay et al., 2010). (4) By stimulating the phosphorylation of protein kinase B and extracellular signal regulated kinases, up-regulating vascular endothelial growth factor and angiopoietin-1 expression, H_2S could promote angiogenesis around the infarct area, and show better functional outcomes in a rat model of middle cerebral artery occlusion (MCAO) (Jang et al., 2014). (5) H_2S has anti-oxidative, anti-inflammatory, and anti-apoptotic effects in stroke; and in cerebral ischemia/reperfusion injury, it is also reported H_2S could increase the superoxide dismutase activity, anti-inflammatory interlukin-10 and anti-apoptotic protein Bcl-2, accordingly, play the role of neuroprotection (Yin et al., 2013). In addition, H_2S, or its donor, can reduce the infarct size and decrease the blood brain barrier damage in a model of cerebral ischemia-reperfusion injury (Gheibi et al., 2014).

However, different models, administration paradigms and concentration of H_2S may cause different results, even get the opposite conclusion: (1) Using the same model of MCAO, Qu et al. (2006) found that if the H_2S concentration is raised to 0.18 mM, the infarct volume will increase, instead of decrease, and this process can be ameliorated by H_2S synthesis inhibitors or NMDA receptor channel inhibitors, suggesting that H_2S has different effects on brain in different dose-concentration. (2) The concentration of H_2S increases in the cortex, and reach the peak at 12 hours after cerebral ischemia, then it will decrease (Ren et al., 2010). A recent research showed that H_2S, at a high dose level, can cause cell death in cerebral cortex based on the model of permanent MCAO, and in this process, the infarct volume can be reduced by the inhibitors of H_2S synthesis enzymes, on the contrary, decreased by the administration of NaHS (Qu et al., 2006). (3) In addition, when the concentration of NaHS is below 200 μM, the apoptosis can be induced, while the concentration is higher than 200 μM, NaHS will induce necrosis and cause brain damage (Cheung et al., 2007).

In general, the neuroprotective effect of H_2S in stroke is concentration-dependent, only a relatively low dose of H_2S can yield beneficial effect. However, there is a point we have to say, the most of research on H_2S at present mainly focus on ischemic stroke, while study concerning the hemorrhagic stroke is very few, which need us to do more research to reveal the role of H_2S in entire stroke.

Clinical studies

At present, there is no direct clinical studies about neuroprotection of H_2S being reported. However, the plasma H_2S level has been reported to be related to long-term clinical outcome in stroke patients. A clinical study involving 36 patients showed that high plasma Cys levels on admission and before treatment tend to poor clinical outcome when assessed at 3 months after stroke, and the patients who have early stroke deterioration are usually correlated significantly with high plasma level of Cys (Wong et al., 2006), suggesting that H_2S may be indirectly responsible for this effect. Furthermore, as accumulating evidence shows that hyper Hcy is a risk factor for stroke (Abbate et al., 2003; Kim et al., 2003; Hassan et al., 2004), Parnetti et al. (2004) found that the concentration of Hcy increased in all stroke subtypes in a case-control study including 313 participants (161 patients and 152 controls), indicating that Hcy may be used as a marker for the clinical outcome of stroke as well. However, the definite relationship between hyper Hcy and stroke has not been found (Fallon et al., 2001), and several meta-analyses have not reached an agreement (Homocysteine Studies Collaboration, 2002; Fallon and Ben-Shlomo, 2003). Above all, although there is no direct evidence indicating that H_2S has neuroprotective effect in clinical trials, the close touch with brain injury caused by stroke is no doubt, and needs us to make great efforts to study.

Neurotoxicity of H_2S

As what we have mentioned above, the high dose-concentration of H_2S can cause systemic toxicity reacts (Nakata et al., 2015; Wu et al., 2015), especially neurotoxic effect, because it inhibits cytochrome C oxidase and mitochondrial respiration. The CNS is sensitive to hypoxia, H_2S poisoning can lead to respiratory paralysis rapidly and cause a series of nerve dysfunction, including brain edema, disturbance of consciousness, and others. Therefore, the following strategies are extremely important, including oxygen uptake, keeping the airway open, and symptomatic and supportive treatments.

Conclusion

Although H_2S is known as a toxic substance, increasing evidences have indicated that H_2S plays an important role of neuroprotection in stroke, while its neurotoxicity cannot be neglected. So, we need to view H_2S in the point of dialectics, and we believe that further research on H_2S,

including animal studies and clinical studies, may provide a new insight into the treatment of stroke and other CNS diseases, such as traumatic brain injury and neurodegenerative diseases.

Author contributions

YD and ZW were responsible for writing the paper, and GC 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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