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Research Report

Hydrogen sulfide induces neuroprotection against experimental stroke in rats by down-regulation of AQP4 via activating PKC

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ABSTRACT

Hydrogen sulfide (H_2S) is now known as an important neuromodulator in the central nervous system. The aim of the current study was to investigate whether exogenous H_2S gas can attenuate brain edema induced by experimental stroke and to clarify the potential mechanisms. Rats underwent 2-h middle cerebral artery occlusion (MCAO) and received 40 ppm or 80 ppm H_2S inhalation for 3 h at the beginning of reperfusion. The effects of H_2S were investigated by evaluating neurological function, infarct size, brain edema volume, and aquaporin4 (AQP4) protein expression at 24 h after reperfusion. Moreover, to explore the possible mechanisms for the neuroprotective effects of H_2S, protein kinase C (PKC) activity was detected and a PKC inhibitor, Go6983, was used via intracerebral ventricular injection. Our results showed that 40 ppm or 80 ppm H_2S inhalation significantly reduced neurological deficits, infarct size, and brain edema after MCAO. The expression of AQP4 in the peri-infarct area of brain was also inhibited after inhalation of H_2S. PKC was activated by H_2S treatment and the PKC inhibitor attenuated the neuroprotection of H_2S with an increased AQP4 expression at the same time. In conclusion, H_2S inhalation attenuates brain edema, reduces infarct volume, and improves neurologic function in a rat experimental stroke model. The therapeutic benefits of H_2S inhalation are associated with down-regulation of AQP4 expression via activating PKC.

Abbreviations: H_2S, hydrogen sulfide; MCAO, middle cerebral artery occlusion; AQP4, aquaporin 4; PKC, protein kinase C; rCBF, regional cerebral blood flow; BBB, blood–brain barrier

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1. Introduction

Ischemic stroke, which is one of the leading causes of death worldwide, is characterized by complex and devastating neurological conditions (Moskowitz et al., 2010). The resulting cerebral edema and subsequent increase in intracranial pressure contribute to further progressive deterioration in these patients. Brain edema induced by stroke accounts for more severe disabilities and mortality (Manley et al., 2004). To relieve the subsequent brain edema and minimize the disabling sequelae of ischemic stroke, a promising focus of research is to modulate the expression of aquaporin4 (AQP4). AQP4 is a bidirectional water channel which expresses strongly in astroglia and cerebrospinal fluid–brain interfaces (Amiry-Moghaddam et al., 2003; Kovalenko et al., 2006). Previous study has shown that AQP4 plays an important role in cytotoxic edema in central nervous system (Papadopoulos et al., 2002), and AQP4-deficient mice experiences significantly reduced brain edema and less severe neurological dysfunctions after stroke (Manley et al., 2000). Protein kinase C (PKC) has recently been reported to play a vital role in modulating the activity of AQP4. Tang et al. (2012) reported that the thrombin-induced down-regulation of AQP4 expression was mediated by the PKC pathway. McCoy et al. (2010) also revealed that activation of PKC enhanced AQP4 phosphorylation and reduced water permeability. Therefore, modulation of PKC–AQP4 may provide a new therapeutic option for improvement of neurological function after stroke.

In recent years, hydrogen sulfide (H_2S) has been known as a novel type of neural regulatory factor and gaseous mediator (Lowicka and Beltowski, 2007). The neuroprotective effects of H_2S have been confirmed in animal models in vivo (Joseph et al., 2012; Yin et al., 2013) and in vitro (Kimura and Kimura, 2004; Marutani et al., 2012). However, the underlying mechanisms of H_2S are still poorly understood. Previous study from Li et al. (2011) proposed that exogenous H_2S inhibited the edema around pyramidal neurons and the nuclear shrink induced by ischemia. Wang et al. (2014) further revealed that H_2S donors (5-p-hydroxyphenyl-1, 2-dithiole-3-thione and NaHS) reduced vasogenic cerebral edema induced by stroke via maintaining the integrity of blood–brain barrier (BBB). As we know, the edema after stroke is characterized by the coexistence of vasogenic edema and cytotoxic edema. But the effect of H_2S on cytotoxic edema following stroke is still lacking. The aim of the present study was to investigate the effect of H_2S on cytotoxic edema after stroke and to determine whether the PKC–AQP4 pathway was involved in this process.

2. Results

The experiments were performed with minimal complications and no procedure-related mortality was observed during the ischemia and reperfusion period. In our preliminary experiment, the variables from continuous monitoring of blood pressure and repeated blood gas did not show any significant differences and stayed within physiological limits throughout middle cerebral artery occlusion (MCAO) procedure (details in Supplementary data).

2.1. *H_2S inhalation improved neurological function after MCAO*

The median neurobehavioral scores evaluated by the Garcia system are shown in Fig. 1. No neurological deficits were observed in sham-operated animals. There was a significant decrease in Garcia scores in the control group compared to the sham group ($P<0.05$). Compared with the control group, the Garcia score was significantly improved both in the H_2S 40 group and H_2S 80 group (both $P<0.05$). However, no significant differences were observed between the H_2S 40 group and H_2S 80 group ($P=0.699$). To exclude the possible impairment induced by cerebral ventricular injection, the $CI_{PBS}+H_2S$ group was designed in this study. At 24 h after 2-h MCAO, Garcia scores in $CI_{PBS}+H_2S$ group showed no significant differences compared to the H_2S 40 group ($P>0.05$). However, Go6983 used in $CI_{Go6983}+H_2S$ group was found to significantly reduce the Garcia scores when compared with $CI_{PBS}+H_2S$ group ($P<0.05$), which demonstrated that PKC involved in the protection of H_2S.

In the tape removal test, all sham animals removed the adhesive tapes rapidly [18.5 s, (14–24 s)]. However, a significant sensorimotor deficit was observed at 24 h after reperfusion, and all rats in the control group failed to remove the tapes within 180 s (Fig. 2). The time needed in the tape removal test was significantly reduced in the H_2S 40 group (135 s (121.5–169.5 s, $P<0.05$) and H_2S 80 group (138 s (115.25–169.5 s, $P<0.05$) when compared with the control group. There were no significant differences between the H_2S 40 and H_2S 80 group ($P>0.05$). The time in $CI_{PBS}+H_2S$ group (131.5 s (110.75–161.75 s) showed no significant differences compared to that in the H_2S 40 group ($P>0.05$). However, the removal time in the $CI_{Go6983}+H_2S$ group was significantly increased (more than 180 s) compared with that in the $CI_{PBS}+H_2S$ group ($P<0.05$).

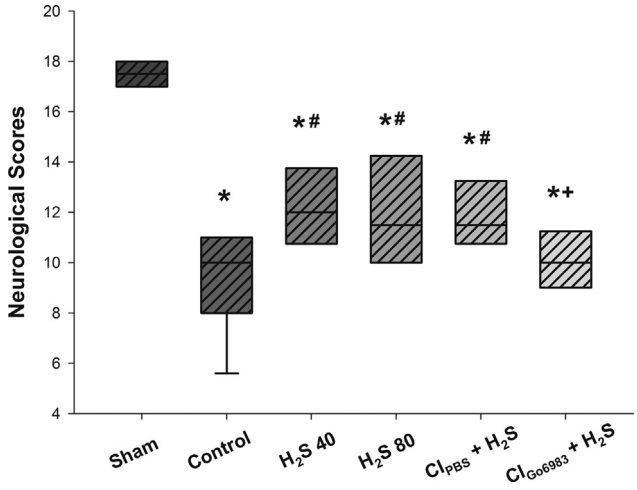

Fig. 1 – Neurobehavioral scores assessed using the Garcia system. All animals developed neurobehavioral deficits following MCAO injury. Treatment with H_2S inhalation markedly improved the neurobehavioral scores at 24 h after reperfusion. PKC inhibitor prevented the improvement by H_2S inhalation. $^{*}P<0.05$ vs sham group, $^{\#}P<0.05$ vs control group and $^{+}P<0.05$ vs $CI_{PBS}+H_2S$ group.