Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres
Brain Research

Research Report

Exogenous hydrogen sulfide protects against global cerebral ischemia/reperfusion injury via its anti-oxidative, anti-inflammatory and anti-apoptotic effects in rats

Jun Yin[a,1], *Chao Tu*[a,1], *Jie Zhao*[b,1], *Danmin Ou*[c], *Guangwen Chen*[c], *Ying Liu*[c,*], *Xianzhong Xiao*[c]

[a]*Clinical Medicine for Eight-year-program, Xiangya School of Medicine, Central South University, Changsha, Hunan 410078, China*
[b]*Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha, Hunan 410078, China*
[c]*Laboratory of Shock, Department of Pathophysiology, Xiangya School of Medicine, Central South University, Changsha, Hunan 410078, China*

ARTICLE INFO

Article history:
Accepted 25 October 2012
Available online 1 November 2012

Keywords:
Hydrogen sulfide (H_2S)
Ischemia–reperfusion
Brain
Oxidative stress
Inflammation
Apoptosis

ABSTRACT

The present study was undertaken to study the effects of exogenous hydrogen sulfide (H_2S) on global cerebral ischemia–reperfusion(I/R) and the underlying mechanisms. After a 24 h I/R, administration of NaHS, an exogenous donor for H_2S, at the dose of 0.2 or 0.4 μmol/kg significantly decreased the apoplexy index, neurological symptom scoring, and brain infarcted area as compared to the I/R group in a dose dependent manner. At the same time, NaHS-treated rats displayed significant reduction of MDA content, and striking increase of SOD activity in the brain tissues compared with I/R group. The up-regulated mRNA levels of $p47^{phox}$ and $gp91^{phox}$ subunits of NADPH oxidase were also suppressed by NaHS treatment. In this study, the pro-inflammatory markers TNF-α and MCP-1 in I/R group were markedly increased by 24 h I/R, which were significantly attenuated by NaHS in a dose-dependent manner. In contrast, the anti-inflammatory factor IL-10 was markedly induced by NaHS administration. In addition, the expression of the anti-apoptotic protein Bcl-2 was significantly decreased in I/R group compared with the sham-operated group. This reduction was significantly blunted in NaHS-treated group. On the contrary, the pro-apoptotic protein Bax content in brain tissues of I/R group was markedly elevated compared with sham-operated animals. And such an induction of Bax content was significantly ameliorated by NaHS. Taken together, our results suggest that hydrogen sulfide has potent protective effect against a severe cerebral injury induced by a global I/R possibly through the inhibition of oxidative stress, inflammation and apoptosis.

*Correspondence to: Department of Pathophysiology, Xiangya School of Medicine, Central South University, 110 Xiangya Road, Changsha, Hunan 410008, China. Fax: 86 731 82355019.
E-mail address: liu1977ying@126.com (Y. Liu).
[1] These authors contributed equally to this work.

0006-8993/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.10.046

1. Introduction

Hydrogen sulfide (H_2S) has been well known as a toxic gas and environmental pollutant with an offensive odor of rotten eggs for many decades (Elsey et al., 2010). However, emerging evidences indicated the important physiological effects of H_2S as a novel type of endogenous neural regulatory factor and gaseous mediator (Lowicka and Beltowski, 2007). In particular, it has been demonstrated that the administration of H_2S significantly ameliorates ischemia–reperfusion (I/R) injury in multiple organs. In myocardial I/R injury (Johansen et al., 2006), sodium hydrosulfide (NaHS), an exogenous donor for H_2S, can not only reduce the mortality rate of rats after myocardial I/R injury, improve left ventricular systolic function and diastolic function, but also reduce the adhesion of white blood cells, hyperplasia and hypertrophy of cardiac fibroblast, and lipid peroxidation. In an intestinal I/R rat model (Liu et al., 2009), H_2S can significantly protect the intestinal mucosal injury associated with a reduction of malondialdehyde (MDA) activity and the increased activity of superoxidase dismutase (SOD) and GSH-Px in Sprague-Dawley (SD) rats. As for the hepatic I/R injury (Kang et al., 2009), administration of NaHS significantly attenuated the severity of liver injury and inhibited the oxidative stress, inflammation and apoptosis.

A recent study interestingly showed that H_2S at a low concentration significantly attenuated the injury in a mild focal cerebral ischemia rat model (Florian et al., 2008). Most recently, Kimura et al. reported that H_2S can improve the glutathione(GSH) levels of brain in an intrauterine I/R (5 min/24 h) model (Kimura et al., 2010). Minamishima et al. also reported that Na_2S can effectively benefit neurological function in parallel with a reduction of caspase-3 in hippocampus and enhancement of anti-apoptotic protein GSK-3β in brain cortex in a mouse model of cardiac arrest/cardiopulmonary resuscitation (CA/CPR) (Minamishima et al., 2009). All these studies convincingly demonstrated the neuro-protective role of H_2S during a relatively moderate brain injury. However, it is worth to examine if H_2S is still potently beneficial under a much more severe cerebral injury status. In present study, the authors employed a rat cerebral I/R model with longer time global ischemia and investigated the effect of H2S in this particularly severe injury model, as well as the underlying mechanisms.

2. Results

2.1. *NaHS attenuated global cerebral I/R injury*

In this study, two behavioral tests (apoplexy index and neurological symptom scoring) were performed to determine the neurological outcome. As shown in Tables 1 and 2, the apoplexy index and neurological symptom scoring were much higher at 6 h, 12 h and 24 h after global cerebral I/R than sham-operation group. Treatment with NaHS (0.2 and 0.4 μmol/kg, ip) significantly decreased the apoplexy index and neurological symptom scoring as compared to that of I/R group in a dose dependent manner.

By TTC staining (Fig. 1), no infarcted area of the global cerebral tissue in SD rats was seen in sham-operation group. While the infarcted areas in 0.2 μmol/kg and 0.4 μmol/kg NaHS groups were (23±2)% and (9±2)% respectively, which were both lower than that in I/R group [(55±4)%]. Higher dose of NaHS (0.4 μmol/kg)

Table 1 – Effects of NaHS on the apoplexy index after global cerebral I/R.

Group	*n*	Time after ischemia–reperfusion			
		0 h	6 h	12 h	24 h
Sham	6	0	0	0	0
I/R	6	0	6.83±0.75**	6.33±0.52**	5.83±0.75**
NaHS (0.2 μmol/kg)+I/R	6	0	5.67±0.82**#	5.00±0.63**#	4.50±0.55**#
NaHS (0.4 μmol/kg)+I/R	6	0	3.83±0.75**##	3.17±0.75**##	2.67±0.52**##

** $p<0.01$ vs. Sham group.
# $p<0.05$ vs. I/R group.
$p<0.01$ vs. I/R group by single-measures ANOVA.

Table 2 – Effects of NaHS on the neurological symptom scoring after global cerebral I/R.

Group	*n*	Time after ischemia–reperfusion		
		6 h	12 h	24 h
Sham	6	0	0	0
I/R	6	17.67±1.37**	16.17±1.17**	14.83±1.17**
NaHS (0.2 μmol/kg)+I/R	6	14.00±0.89**#	13.00±0.63**#	11.00±1.41**#
NaHS (0.4 μmol/kg)+I/R	6	8.67±0.52**##	7.17±1.17**##	6.33±0.82**##

** $p<0.01$ vs. Sham group.
# $p<0.05$ vs. I/R group.
$p<0.01$ vs. I/R group by single-measures ANOVA.