Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Effects of hydrogen sulfide on cognitive dysfunction and NR2B in rats

Fa-Ping Tu, MD,[a] *Jun-Xiang Li, MD,*[a] *Qiang Li, MD,*[b] *and Ji Wang, MD*[a,*]

[a] *Department of Anesthesiology, Affiliated Hospital of North Sichuan Medical College, Nanchong, China*
[b] *Hepatobiliary Research Institute of North Sichuan Medical College, Nanchong, China*

ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form
28 April 2016
Accepted 27 June 2016
Available online 5 July 2016

Keywords:
Hydrogen sulfide
Hepatic ischemia/reperfusion
Cognitive dysfunction
NR2B

ABSTRACT

Background: Hepatic ischemia/reperfusion (hepatic I/R) has been found to induce cognitive dysfunction. The NR2B subunit of N-methyl-D-aspartate (NMDA) receptors is a major factor in memory and learning processes, and hydrogen sulfide (H_2S) may modulate this NMDA receptor. Therefore, in this study, sodium hydrosulfide (NaHS, a donor of H_2S) was administered in an animal model of hepatic I/R to investigate the effects of H_2S on cognitive impairment and expression of NR2B.

Materials and methods: NaHS (5 mg/kg) or normal saline was administered intraperitoneally once a day for 11 consecutive days, during which a rat model of 70% hepatic I/R was established on the fourth day. Cognitive function was evaluated using a Morris water maze, mRNA and protein levels of the NR2B subunit were detected in the hippocampus by RT-PCR and Western blotting. All these tests were performed on postoperative days 1, 3, 5, and 7.

Results: Cognitive dysfunction was detected in the hepatic I/R group, and this dysfunction was associated with a decrease in the mRNA and protein levels of the NR2B subunit of the NMDA receptors in the hippocampus. In contrast, treatment with NaHS significantly ameliorated the impairment of cognitive function caused by hepatic I/R, and an increase in mRNA and protein levels of the NR2B subunit was detected in the corresponding hippocampus tissues.

Conclusions: The present data suggest that H_2S exerts a protective effect on hepatic I/R-induced cognitive impairment, and this effect may be associated with the NR2B subunit of the NMDA receptors. H_2S may represent a novel therapeutic agent for the treatment of postoperative cognitive dysfunction after liver surgery.

Introduction

Hepatic ischemia/reperfusion (hepatic I/R) that often occurs during liver surgery can involve the release of reactive oxygen species and pro-inflammatory cytokines, as well as neutrophil-mediated inflammatory responses. As a result, liver injury and subsequent dysfunction have been observed.[1] Other organs can develop secondary dysfunctions as well.[2–4] In a recent study, hepatic I/R was found to affect the function of central nervous system, leading to postoperative cognitive dysfunction (POCD).[5] POCD was reported to represent a serious complication after surgery and was found to occur primarily 1 wk after surgery.[6] POCD was also found to be associated with increased morbidity and mortality.[7,8]

* *Corresponding author*. Department of Anesthesiology, Affiliated Hospital of North Sichuan Medical College, Wen Hua Rd 63#, Nanchong, China. Tel./fax: +86 0817 2262086.
E-mail address: wangjinsmc@sina.com (J. Wang).
0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2016.06.071

Consequently, POCD has been recognized as an important clinical problem and has gained much attention. However, the exact mechanisms of POCD remain unclear.

N-methyl-D-aspartate (NMDA) receptors are the major excitatory glutaminergic receptors that are expressed in the brain. Functional NMDA receptors are composed of an NR1 subunit and modulatory subunits NR2A-D.[9] In the hippocampus, NR2B is one of the major regulatory subunits, and it plays a key role in learning and memory.[10] Overexpression of NR2B in the forebrain has been found to enhance learning and memory in rats,[11] whereas lower levels of NR2B expression in the hippocampus impaired NMDA-dependent long-term potentiation (LTP), as well as memory and learning.[12]

In mammalian tissues, hydrogen sulfide (H_2S) is produced, and it serves as a gaseous signaling molecule. However, H_2S is also highly toxic and can inhibit mitochondrial respiration.[13] H_2S may inhibit cytochrome c oxidase and stop ATP production at high concentrations, thereby mediating adverse effects at both the cellular and organism levels.[14] On the other hand, at lower concentrations, H_2S has the capacity to preserve mitochondrial function by decreasing the production of reactive oxygen species.[15] In an animal model of cardiac arrest and cardiopulmonary resuscitation, administration of H_2S was accompanied by a reduction in intracellular reactive oxygen species levels and an increase in ATP levels.[16] Thus, mitochondrial function and neurological outcome after resuscitation were improved. In another study, treatment with H_2S increased the uptake of glutamate, and neural damage was alleviated.[17] Furthermore, a recent report revealed that H_2S attenuated partial hepatectomy (not hepatic I/R)–induced cognitive impairment, and the hippocampus presented lower expression levels of pro-inflammatory cytokines.[18] However, it remains unclear whether H_2S is able to mediate a neuroprotective role after hepatic I/R. Therefore, in the present study, the effects of H_2S on cognitive dysfunction and expression of the NR2B subunit of the NMDA receptors in rats undergoing hepatic I/R were investigated.

Materials and methods

Animals

Male Sprague–Dawley rats (200-250 g) were housed in a standard environment with controlled temperature (23°C-24°C) and humidity (50 ± 5%) and a 12-h light/dark cycle. Water and food were provided *ad libitum*. Rats were obtained from the Laboratory Animal Center of North Sichuan Medical College (Sichuan, China). The experimental procedures of the present study were approved by the Ethics Review Board for Animal Studies of North Sichuan Medical College, and they complied with the guidelines for the use of laboratory animals by the National Institutes of Health.

Experimental design and hepatic I/R model

The rats received four training sessions daily for five consecutive days in a Morris water maze (MWM). The rats that exhibited abnormal behaviors were not used. A total of 72 rats were randomly divided into three groups (n = 24/group): sham-operated group (sham group), hepatic I/R group (HIR group), and hepatic I/R + NaHS group (HIR + NaHS group). The rats in the HIR + NaHS group received a peritoneal administration of 5 mg/kg/d NaHS (Sigma, MO), whereas the rats in the sham group and the HIR group received saline as a peritoneal injection. Daily administration of NaHS or saline was performed over 11 consecutive days. Rats in the HIR group and the HIR + NaHS group underwent 70% hepatic ischemia followed by reperfusion on the fourth day as previously described.[19] Briefly, after the rats received an injection of sodium pentobarbital (50 mg/kg, i.p.), a midline laparotomy was performed, and a microvascular clip was applied to occlude the hepatic artery and portal vein to induce ischemia of the left and median lobes of the liver (70% hepatic ischemia). The clip was then released 60 min later to allow reperfusion. The sham group underwent the same laparotomy procedure without clipping of the hepatic artery and portal vein. The wound was perfused with 0.25% bupivacaine and then was closed by sterile suture. Rectal temperature of the rats undergoing surgery was maintained between 36°C and 37°C. After the rats recovered from anesthesia, the rats were individually housed.

Morris water maze (MWM) task

The MWM test was performed as previously described.[20] The maze was a circular pool with a diameter of 120 cm and a height of 50 cm. The pool was filled with water (22 ± 2°C) to a depth of 25 cm. The addition of white nontoxic paint to the pool provided an opaque surface. Visual cues were positioned around the pool, which was divided into four equal quadrants. An escaping platform (10 cm in diameter) was fixed at 2 cm below the water surface in one quadrant of the pool, and this position of the platform remained unchanged for all the training sessions. The swimming behaviors of the rats were analyzed with a computer tracking system. For five consecutive days, the rats completed four training sessions each day. For each trial, the rats were positioned to face the pool wall and then were gently put into the water. The rats were timed for their ability to navigate to the platform for up to 90 s. On reaching the platform, the rats were left to rest for 20 s. If a rat failed this task, the rat would be guided to the platform and given 20 s to rest. The escape latency, the distance traveled to the platform, and swimming speed were collected. A probe trial was performed on postoperative days 1, 3, 5, and 7. For these trials, the platform was not present, and the rats could swim freely for 90 s. The three parameters that were recorded during the probe trials were: percentage of time spent in the target quadrant, the number of times the rats crossed over the platform, and swimming speed. All the trials were performed between 8:00 AM and 4:00 PM. After finishing the probe trials, the rats were killed, and the hippocampus was collected and stored at −80°C until used.

Reverse transcription-polymerase chain reaction (RT-PCR analysis)

Hippocampal NR2B mRNA levels of six rats extracted randomly from each group were measured by using RT-PCR analyses. RNeasy Mini Kits (Qiagen, Hilden, Germany) were