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Research Report

Inhalation of hydrogen gas attenuates brain injury in mice with cecal ligation and puncture via inhibiting neuroinflammation, oxidative stress and neuronal apoptosis

Lingling Liu[a,b], *Keliang Xie*[a,b], *Hongguang Chen*[a,b], *Xiaoqing Dong*[a,b], *Yuan Li*[a,b], *Yang Yu*[a,b], *Guolin Wang*[a,b], *Yonghao Yu*[a,b,*]

[a]*Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin 300052, PR China*
[b]*Tianjin Research Institute of Anesthesiology, China*

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ABSTRACT

During the development of sepsis, the complication in central nervous system (CNS), appearing early and frequently relative to other systems, can obviously increase the mortality of sepsis. Moreover, sepsis survivors also accompany long-term cognitive dysfunction, while the ultimate causes and effective therapeutic strategies of brain injury in sepsis are still not fully clear. We designed this study to investigate the effects of 2% hydrogen gas (H_2) on brain injury in a mouse model of sepsis. Male ICR mice were underwent cecal ligation and puncture (CLP) or sham operation. 2% H_2 was inhaled for 60 min beginning at both 1 and 6 h after sham or CLP operation, respectively. H_2 concentration in arterial blood, venous blood and brain tissue was detected after H_2 inhalation separately. The survival rate was observed and recorded within 7 days after sham or CLP operation. The histopathologic changes and neuronal apoptosis were observed in hippocampus by Nissl staining and TUNEL assay. The permeability of brain–blood barrier (BBB), brain water content, inflammatory cytokines, activities of antioxidant enzymes (SOD and CAT) and oxidative products (MDA and 8-iso-PGF2α) in serum and hippocampus were detected at 24 h after sham or CLP operation. The expressions of nucleus and total nuclear factor erythroid 2-related factor 2 (Nrf2) and cytoplasmic heme oxygenase-1(HO-1) in hippocampus were measured at 24 h after sham or CLP operation. We assessed their cognitive function via Y-maze and Fear Conditioning test on day 3, 5, 7 and 14 after operation. H_2 treatment markedly improved the survival rate and cognitive dysfunction of septic mice. CLP mice showed obvious brain injury characterized by aggravated pathological damage, BBB disruption and brain edema at 24 h after CLP operation, which was markedly alleviated by 2% H_2 treatment. Furthermore, we found that the beneficial effects of H_2 on brain injury in septic mice were linked to the decreased

*Correspondence to: Department of Anesthesiology, Tianjin Medical University General Hospital, 154 Anshan Road, Heping District, Tianjin 300052, PR China.
E-mail addresses: liulingling2007024@126.com (L. Liu), xiekeliang2009@hotmail.com (K. Xie), daguang521521@163.com (H. Chen), dongxiaoqing2011@126.com (X. Dong), 821609938@qq.com (Y. Li), 174543746@qq.com (Y. Yu), wang_guolin@hotmail.com (G. Wang), yuyonghao@126.com (Y. Yu).

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levels of inflammatory cytokines and oxidative products and the increased activities of antioxidant enzymes in serum and hippocampus. In addition, 2% H_2 inhalation promoted the expression and transposition of Nrf2 and the expression of HO-1 to mitigate brain injury in sepsis. Thus, the inhalation of hydrogen gas may be a promising therapeutic strategy to relieve brain injury in sepsis.

1. Introduction

Sepsis is a systemic inflammatory response syndrome caused by various infectious factors, which has turned into a primary cause of mortality in critical patients and a main public health burden worldwide (Cheng et al., 2007; Nguyen and Smith, 2007). In septic patients, the complication in central nervous system (CNS) appears to be earlier and more frequently than those in other systems. 9–71% septic patients have been found to present the encephalopathy of various severity (Gofton and Young, 2012; Pytel and Alexander, 2009). Sepsis-associated encephalopathy (SAE) is found to be the most pervasive encephalopathy in intensive care units, which is related to a higher mortality (Flierl et al., 2010).

Many studies have proved that sepsis survivors still suffer from long-term cognitive impairment, which ranges from alterations in attention and concentration to cognitive dysfunction (Comim et al., 2009; Iwashyna et al., 2010). The pathogenesis on brain injury in septic patients are still not fully clear, which possibly arises from the release of inflammatory cytokines, oxidative stress, neuronal apoptosis and blood–brain barrier damage in brain tissue(Dal-Pizzol et al., 2010; Flierl et al., 2010).

Nuclear factor-erythroid 2-related factor 2 (Nrf2), as a transcriptional factor sensitive to redox in the cytoplasm, remains stable via binding to kelch-like ECH-associated protein 1 (Keap1) in normal cells. Under the oxidative stress, Nrf2 can enter into the nuclear to bind to antioxidant response element (ARE) to activate its downstream molecules, such as SOD and CAT. Heme oxygenase-1(HO-1), as one of vital downstream molecule of Nrf2, has been found to regulate oxidative stress and inflammatory responses in various diseases, such as neurodegenerative disease (Kim et al., 2013). Therefore, the Nrf2-ARE pathway is indentified as a key target for treatment to inflammation- and oxidative stress-related disorders.

Recently, hydrogen gas or hydrogen-rich saline are widely accepted to exert protective effects in many diseases including ischemia-reperfusion injury, stroke,sepsis, traumatic brain injury and neurodegenerative diseases via regulating oxidative stress, inflammatory response and neuronal apoptosis (George and Agarwal, 2010; Ohta, 2014). Besides, our previous researches have indicated that H_2 inhalation dramatically reduced the mortality and ameliorate crucial organ damage in septic mice. Moreover, the protective effects of H_2 treatment on sepsis were related to the inhibition of oxidative stress and inflammation in different tissues (Xie et al., 2014). H_2 is non-inflammable nor non-explosive when its concentration is below 4.1% in pure oxygen or 4.6% in air (Ohsawa et al., 2007). Therefore, hydrogen gas can be widely applied to clinic medicine.

Based on these previous studies, this study was aimed to investigate the potential protective effects of H_2 on brain injury in the mouse model of CLP and whether the beneficial effects was linked to the up-regulation of the Nrf2 pathway.

2. Results

2.1. H_2 concentration in arterial blood, venous blood and brain tissue was detected at the beginning and the end of 2% H_2 inhalation

After 2% H_2 inhalation at 1 h after sham or CLP operation, we investigated H_2 concentration in arterial blood, venous blood and brain tissue of each group. Due to unobvious changes in H_2 concentration in arterial blood, venous blood and brain tissue in mice of Sham group, the data was not listed. In Sham+H_2 group, H_2 concentration in arterial blood was much higher than that in CLP group at minute 10, 20, 30, 45 and 60, respectively (68.50±6.24 vs. 24.08±3.27, 91.25±7.28 vs. 23.70±4.48, 129.01±8.55 vs. 22.30±3.73, 131.67±8.91 vs. 21.95±3.17, 132.39±9.67 vs. 21.50±3.38: $P<0.001$).Compared with CLP group, H_2 concentration in arterial blood was significantly increased in CLP+H_2 group at minute 10, 20, 30, 45 and 60, respectively (61.84±6.09 vs. CLP, 81.16±7.28 vs. CLP, 119.08±8.07 vs. CLP, 129.67±9.37 vs. CLP, 131.17±8.62 vs. CLP: $P<0.001$).After stopping inhaling H_2, H_2 concentration in arterial blood in Sham+H_2 group was 56.75±7.24, 40.01±5.61, 30.70±5.96, 27.42±4.21 at minute 5, 15, 30 and 45, respectively. H_2 concentrations in arterial blood in CLP group were still lower than that in Sham+H_2 group at minute 5, 15, 30, 45 after stopping inhalation, respectively (21.32±2.62 vs. Sham+H_2, 21.05±2.79 vs. Sham+H_2: $P<0.001$; 20.67±2.71 vs. Sham+H_2, 20.34±2.54 vs. Sham+H_2: $P<0.01$). However, H_2 concentrations in arterial blood in CLP group was lower than that in CLP+H_2 group at minute 5 and 15 but not 30 and 45, respectively(48.09±5.70 vs. CLP, $P<0.001$; 29.08±5.62 vs. CLP, $P<0.05$) (Fig. 1A).

Simultaneously, in Sham+H_2 group, H_2 concentrations in venous blood was higher than that in CLP group at minute 10, 20, 30, 45 and 60, respectively (30.76±4.85 vs. 11.22±3.05, 45.58±6.36 vs. 11.09±2.48,61.01±6.86 vs. 10.68±2.01,84.26±8.59 vs. 10.38±2.32,87.77±8.75 vs. 10.05±2.05: $P<0.001$). In contrast with CLP group, H_2 concentration in venous blood was significantly increased in CLP+H_2 group at minute 10, 20, 30, 45 and 60, respectively (24.17±4.25 vs .CLP, 39.58±4.05 vs. CLP, 52.08±7.72 vs. CLP, 77.36±7.50 vs. CLP, 78.69±8.23 vs. CLP: $P<0.001$). After stopping inhaling H_2, H_2 concentration in venous blood in Sham+H_2 group was 38.53±6.31, 24.17±4.79, 18.42±5.00,13.58±3.46 at minute 5, 15, 30 and 45, respectively.