

## Hydrogen Inhalation Reverses Brain Injury in Mice Submitted to Sepsis by Cecal Ligation and Puncture

**Authors:** Ling-ling Liu, Ke-liang Xie, Hong-guang Chen, Xiao-qing Dong, Guo-lin Wang, Yong-hao Yu

Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin Research Institute of Anesthesiology, Tianjin 300052, China

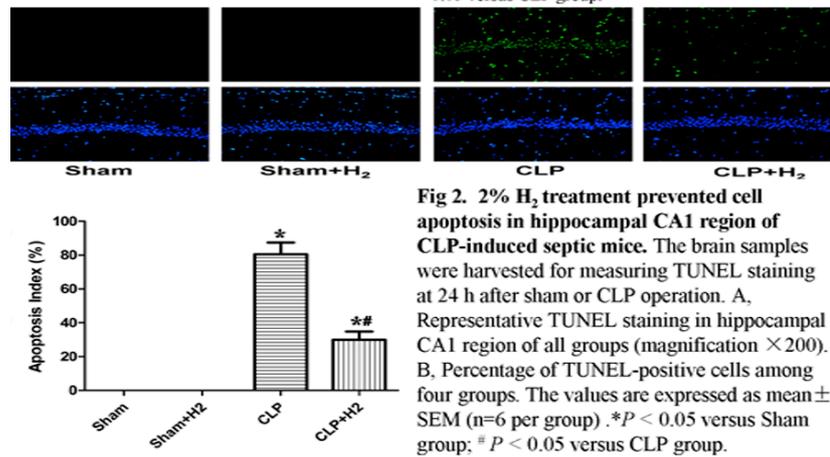
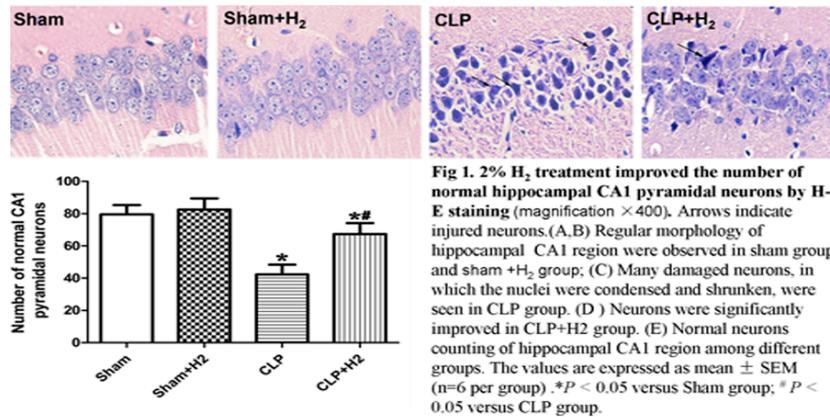
**Fundings:** This study was supported by research grants from the National Natural Science Foundation of China (81071533,81101409), Natural Science Foundation of Tianjin (11JCYBJC12900), Key Projects in the Tianjin Science & Technology Pillar Program (13JCQNJC11400), Science & Technology Foundation of Tianjin Health Bureau (2011KZ108)

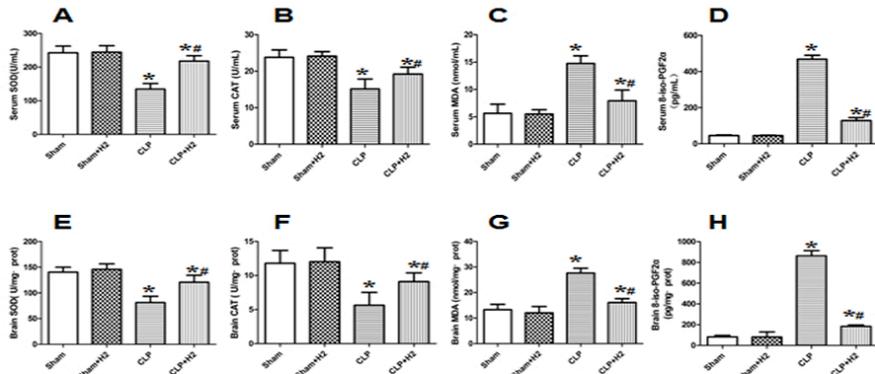
**Background:** During sepsis, Central nervous system (CNS) complication occurs frequently in septic patients often before failure of other organs, which can obviously increase mortality. Several studies have demonstrated that 80% sepsis survivors present long-term cognitive impairment including alterations in memory, attention, concentration and/or global loss of cognitive function. We previously reported that molecular hydrogen could markedly improve the survival rate of septic mice and organ damage, such as heart, liver, lung and kidney. This study aims to investigate the effect of hydrogen (H<sub>2</sub>) inhalation on brain injury in septic mice.

**Methods:** Mice were randomly divided into 4 groups: sham group, sham +H<sub>2</sub> group, CLP group and CLP+H<sub>2</sub> group. Sepsis was produced by cecal ligation and puncture (CLP). 2% H<sub>2</sub> inhalation were received for 1 h at 1 h and 6 h after sham operation or CLP operation, respectively. The histopathologic changes and neuron apoptosis in the hippocampus were evaluated by hematoxylin-eosin staining and the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling assay. The activities of superoxide dismutase (SOD) and catalase (CAT), as well as the levels of malondialdehyde (MDA) and 8-iso-prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>) in serum and hippocampus were observed at 24 h after sham or CLP operation to evaluate the oxidative stress levels. Western blot analysis was used to detect the levels of expression of Nrf2 in hippocampus. Cognitive function were observed by Y-maze test and Fear conditioning test at 3 d、5 d、7 d and 14 d after sham or CLP operation.

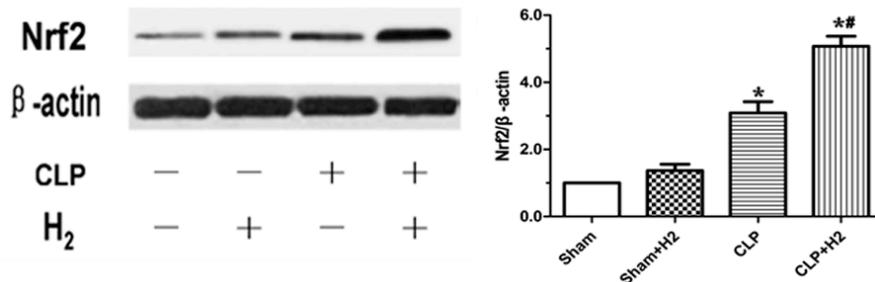
**Results:** H<sub>2</sub> inhalation could significantly mitigate pathological damage and neuron apoptosis in hippocampus, increase the activities of SOD and CAT, decrease the levels of MDA and 8-iso-PGF<sub>2α</sub> in serum and hippocampus, as well as up-regulate the levels of expression of Nrf2 in hippocampus of septic mice ( $P < 0.05$ ). Besides, H<sub>2</sub> inhalation could obviously improve short- and long-time cognitive dysfunction of septic mice ( $P < 0.05$ ).

**Conclusion:** H<sub>2</sub> inhalation can ameliorate brain injury and cognitive dysfunction of septic mice, associated with up-regulating Nrf2 to increase the activities of antioxidant enzymes and decrease the levels of oxidative products.

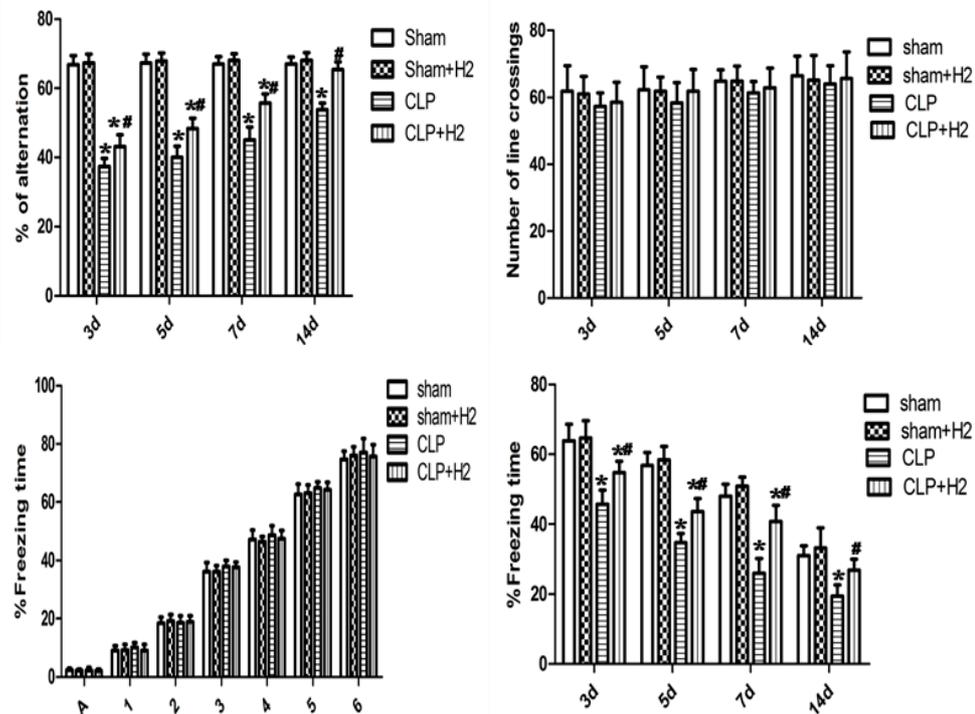




**Fig 3.** 2% H<sub>2</sub> treatment increased the activities of antioxidant enzymes and reduced the levels of oxidative products in serum and hippocampus of septic mice. The values are expressed as mean ± SEM (n = 6 per group). \**P* < 0.05 versus Sham group; # *P* < 0.05 versus CLP group.



**Fig 4.** 2% H<sub>2</sub> treatment upregulated the protein expression of Nrf2 in hippocampus of septic mice. The values are expressed as mean ± SEM (n = 6 per group). \**P* < 0.05 versus Sham group; # *P* < 0.05 versus CLP group.



**Fig 5.** 2% H<sub>2</sub> treatment ameliorated the short-time working memory and hippocampal-dependent memory of septic mice. (A, B) Y-maze spontaneous alternation test. (C, D) Fear Conditional test. The values are expressed as mean ± SEM (n=8 per group). \**P* < 0.05 versus Sham group; # *P* < 0.05 versus CLP group.

**Summary:**

Several studies have demonstrated that 80% sepsis survivors present long-term cognitive impairment including alterations in memory, attention, concentration and/or global loss of cognitive function. We previously reported that molecular hydrogen could markedly improve the survival rate of septic mice and organ damage, such as heart, liver, lung and kidney. In this study, it was demonstrated that H<sub>2</sub> inhalation can ameliorate brain injury and cognitive dysfunction of septic mice, associated with up-regulating Nrf2 to increase the activities of antioxidant enzymes and decrease the levels of oxidative products.