Hydrogen Gas Inhibits Oxidative Stress in Lungs of Septic Mice by Nrf2/HO-1 Pathway in Vivo

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Background: Sepsis is a kind of systemic inflammatory response syndrome (SIRS) which is caused by severe infection. Hydrogen (H_2) has a magical effect in the treatment of sepsis. In the present study, we investigated whether the protective effect of H_2 on septic lung Injury in mice was through the activation of nuclear factor-erythroid 2 p45-related factor 2 (Nrf2)/ heme oxygenase-1(HO-1) pathway in vivo.

Methods: Male ICR mice were subjected to sepsis by cecal ligation and puncture (CLP) with the presence or absence of H_2. 2%H_2 was inhaled for 1 h at 1 and 6 h after CLP or sham operation. We also employed the inhibitor of HO-1——ZnPPIX (40mg/kg) 1h before CLP by intraperitoneal injection. To assess the severity of septic lung injury induced by CLP, we observed the 7d survival rate, W/D weight ratio of lung, lung histologic score, oxygenation index, etc. The serum and tissue homogenates of lung were obtained from mice at 24 h after the CLP or sham operation and used for measuring the level of inflammatory cytokine—— high mobility group box 1 (HMGB1). Furthermore, the protein and mRNA expressions of Nrf2, HO-1 and HMGB1 were measured at 6h, 12h and 24h after the CLP or sham operation.

Results: Mice in the severe sepsis group had a low survival rate and the lung injury was much heavier than the sham group. However, therapy with H_2 increased survival rate and alleviated the lung injury, attenuated the expression of HMGB1 in the serum and lung at 6 h, 12 h and 24 h after CLP operation, stimulated the expression of HO-1 and Nrf2. In addition, the inhibitor of HO-1——ZnPPIX may eliminate the protective effect of H_2 on septic lung injury.

Conclusion: Hydrogen plays an important role in regulating the release of inflammatory cytokine -HMGB1 in severe septic mice, and this effect is at least partly mediated by the expression and activation of HO-1 which is the downstream molecule of Nrf2.

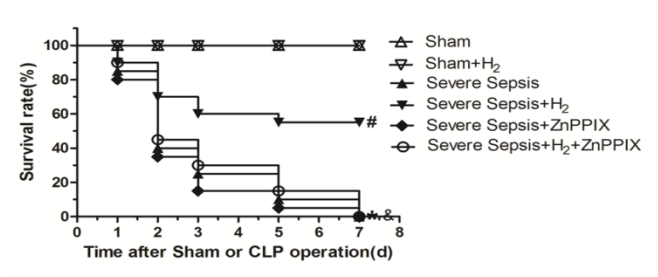


Figure 1: Effects of H_2 on survival rate of mice.

Survival rate (n=20). Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. Survival rate of mice was monitored for 7d. *P < 0.05 versus the sham group, #P < 0.05 versus the severe sepsis group. &P < 0.05 versus the severe sepsis+H_2 group.

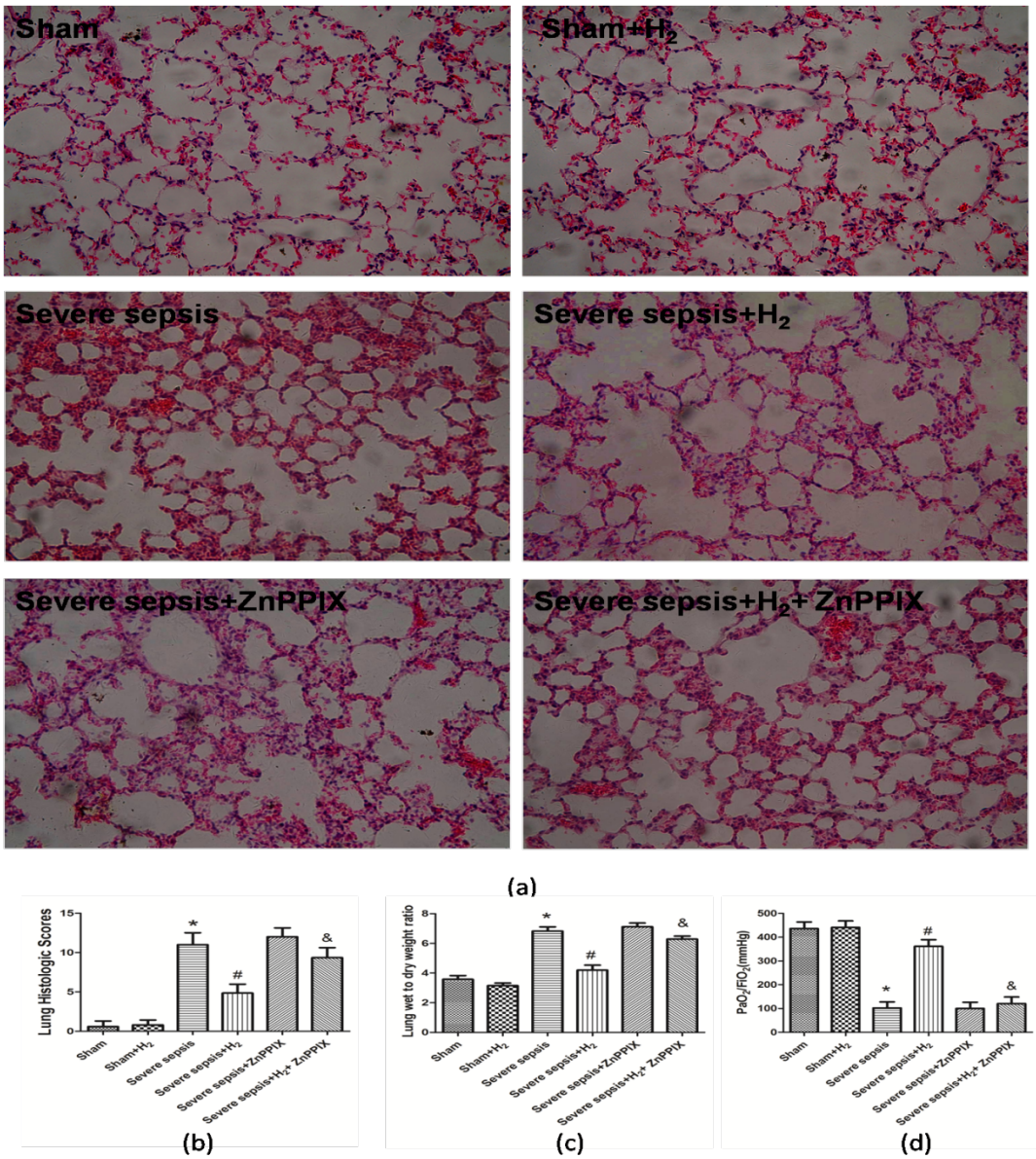


Figure 2: Effects of H_2 on histology changes in the lungs of the mice.

Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. The lung tissues were obtained from mice which were killed at 24h after CLP or sham operation. (a) Hematoxylin and eosin-staining. (original magnification, 400x) (b) Histology scores. (c) The wet/dry weight ratio of lung tissues. (d) PaO_2/FIO_2 ratio. Results were expressed as mean ± SD (n=6). *P < 0.05 versus the sham group, #P < 0.05 versus the severe sepsis group. &P < 0.05 versus the severe sepsis+H_2 group.

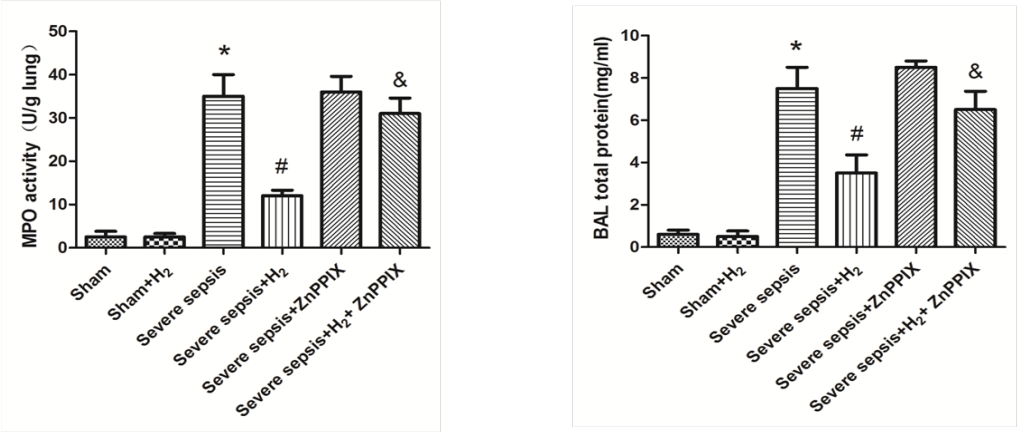


Figure 3: Effects of H_2 on lung injury in septic mice with severe sepsis.

Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. At 24h after CLP or sham operation, BALF were collected for measurement of MPO activity and total protein. (a) Lung MPO activity. (b) Lung BAL total protein. Results were expressed as mean ± SD (n=6). *P < 0.05 versus the sham group, #P < 0.05 versus the severe sepsis group. &P < 0.05 versus the severe sepsis+H_2 group.

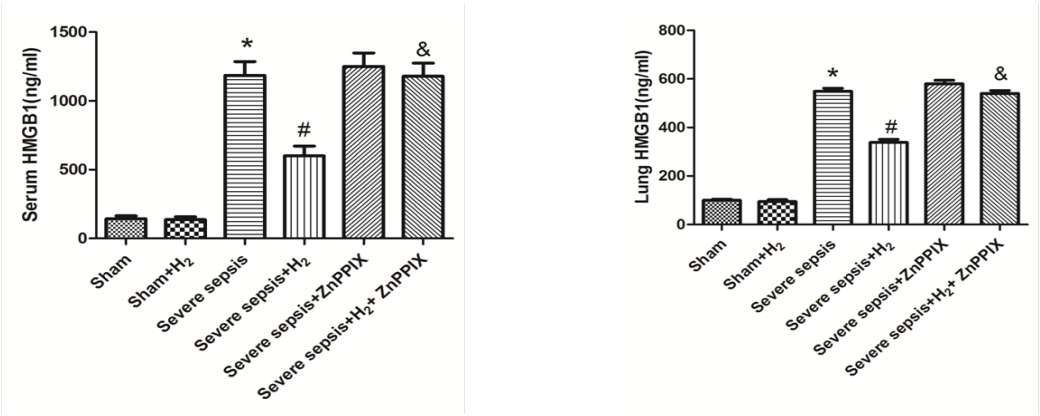


Figure 4: Effects of H_2 on the levels of HMGB1 in serum and lung tissues of mice.

Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. Serum and lung tissues were collected at 24h after CLP or sham operation, HMGB1 was analyzed by ELISA. (a) HMGB1 in serum. (b) HMGB1 in lungs. Results were expressed as mean ± SD (n=6). *P < 0.05 versus the sham group, #P < 0.05 versus the severe sepsis group. &P < 0.05 versus the severe sepsis+H_2 group.

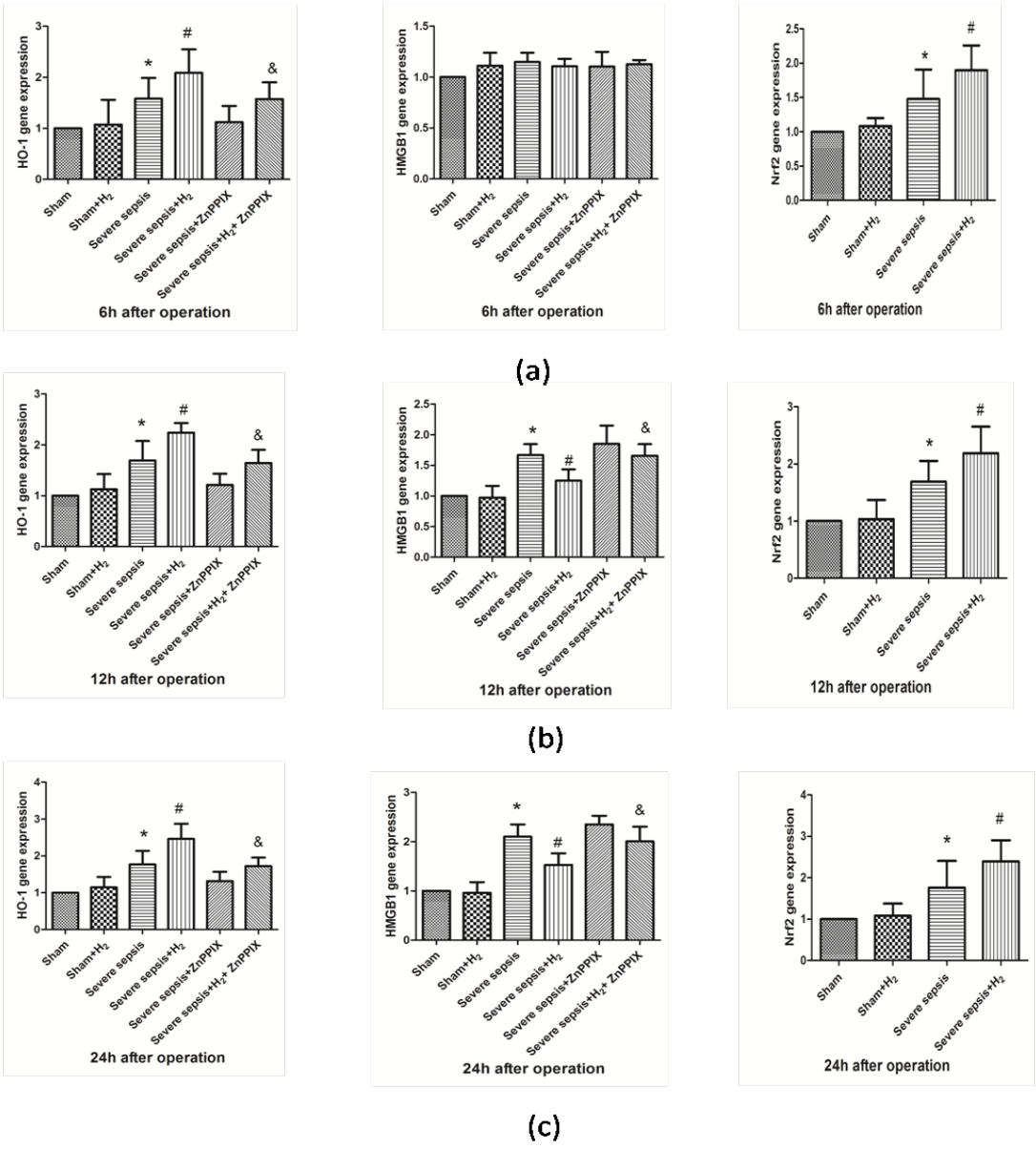
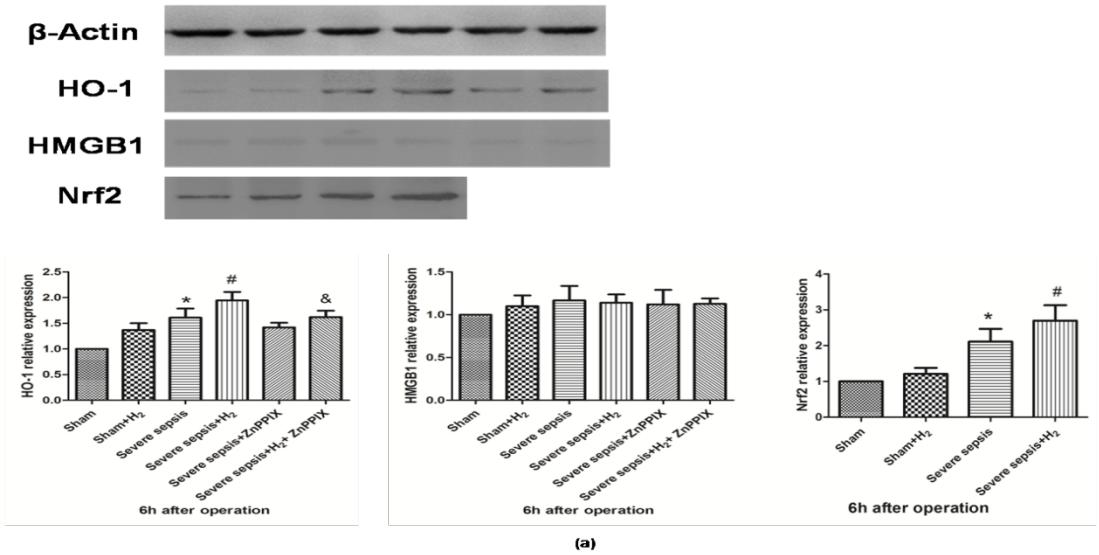
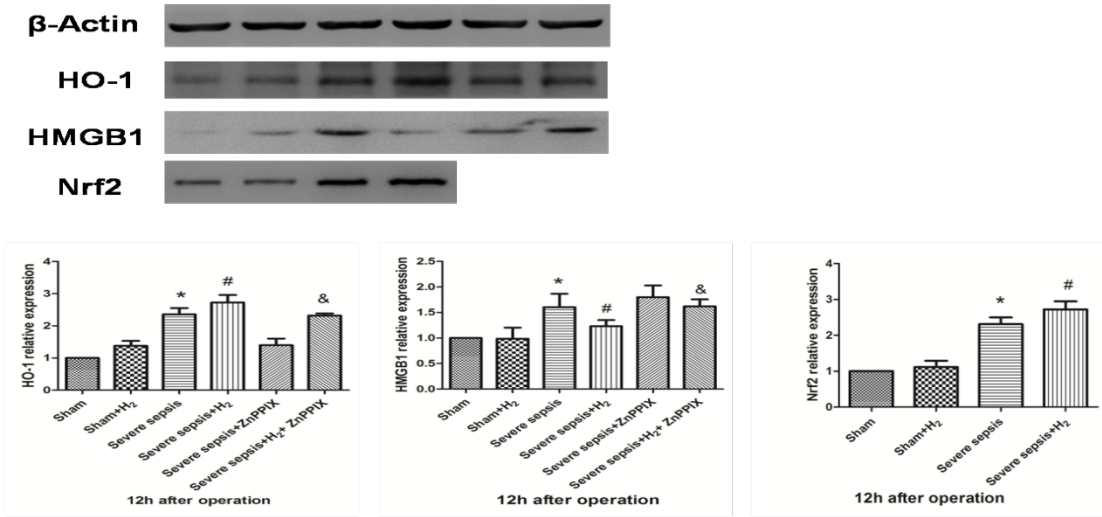


Figure 5: Effects of H_2 on the mRNA expression of HO-1、HMGB1 and Nrf2 in the lungs of mice.

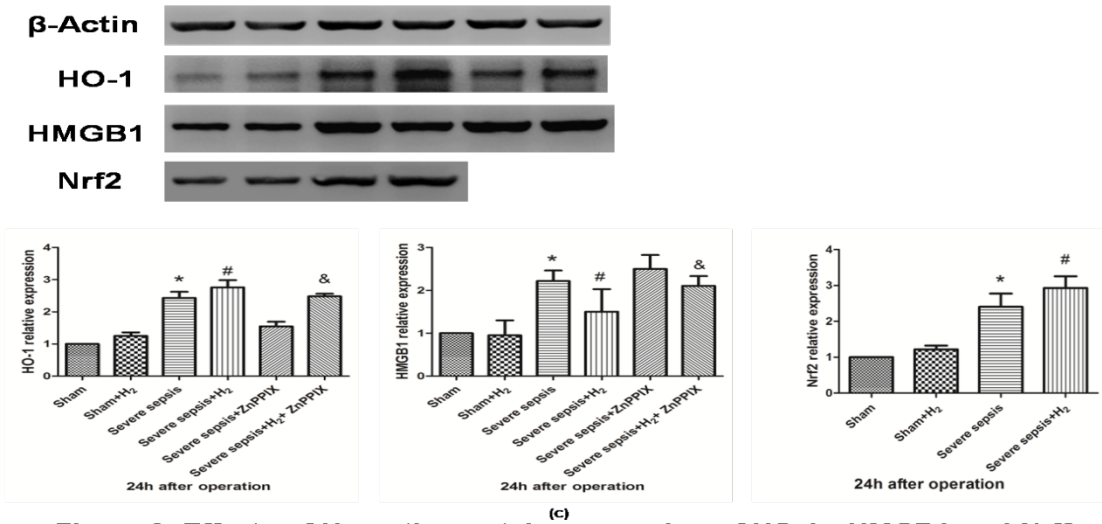
Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. The lung tissues were obtained 6h、12h and 24h after CLP or sham operation for the evaluation of the three kinds of mRNAs. (a) The mRNA expressions of HO-1、HMGB1 and Nrf2 in lungs at 6h. (b) The mRNA expressions of HO-1、HMGB1 and Nrf2 in lungs at 12h. (c) The mRNA expressions of HO-1、HMGB1 and Nrf2 in lungs at 24h. Results were expressed as mean± SD (n=6). *P < 0.05 versus the sham group, #P < 0.05 versus the severe sepsis group. &P < 0.05 versus the severe sepsis+H_2 group.



(a)



(b)



(c)

Figure 6: Effects of H_2 on the protein expression of HO-1、HMGB1 and Nrf2 in the lungs of mice.

Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. The lung tissues were obtained at 6h、12h and 24h after CLP or sham operation for the evaluation of the three kinds of proteins. (a) The expressions of HO-1、HMGB1 and Nrf2 in lungs at 6h. (b) The expressions of HO-1、HMGB1 and Nrf2 in lungs at 12h. (c) The expressions of HO-1、HMGB1 and Nrf2 in lungs at 24h. Results were expressed as mean± SD (n=6). *P < 0.05 versus the sham group, #P < 0.05 versus the severe sepsis group. &P < 0.05 versus the severe sepsis+H_2 group.

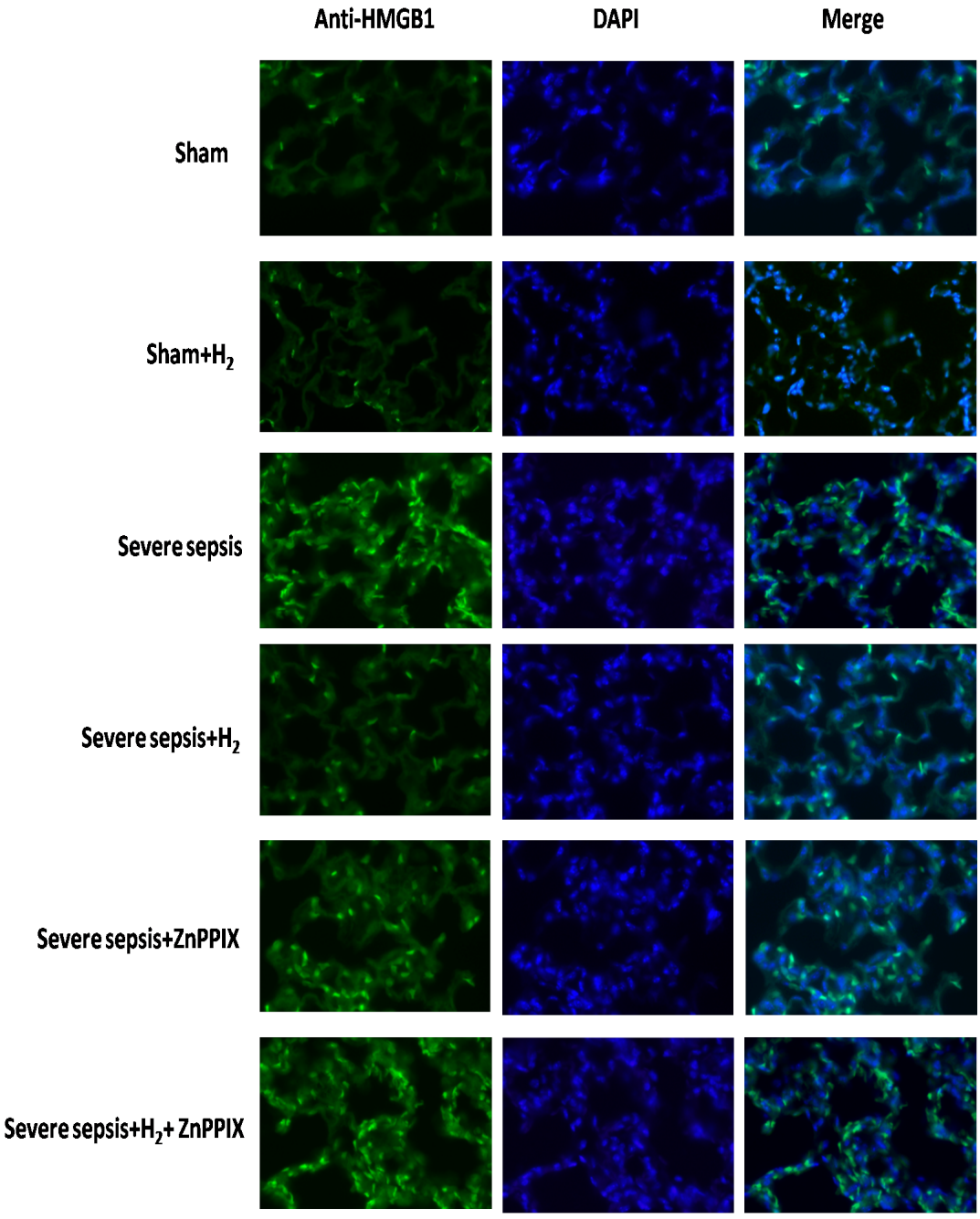


Figure 7: Effects of H_2 on the HMGB1 in the lungs of mice.

Mice were treated with 2% H_2 (1h and 6h after CLP or sham operation) in the presence or absence of ZnPPIX (40mg/kg) 1h prior to CLP. The lung tissues were obtained at 24h after CLP or sham operation for the evaluation of the expression of HMGB1. Immunostaining with HMGB1 (Green) antibody showed HMGB1 accumulation in the lungs of mice at 24h. Nuclei were visualised by Hoechst staining (Blue). The tissues were observed by epifluorescence microscopy.