

# Inhalation of 2% hydrogen improves survival rate and attenuates shedding of vascular endothelial glycocalyx in rats with heat stroke

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論文題目

Inhalation of 2% hydrogen improves survival rate and attenuates shedding of vascular endothelial glycocalyx in rats with heat stroke

（2%水素吸入は熱中症ラットの生存率を改善し、血管内皮グリコカリックスの脱落を減少させる）

論文の内容の要旨

[Introduction]

Heat stroke is characterized by excessive oxidative stress and inflammatory responses, both of which are implicated in vascular endothelial glycocalyx (EGCX) shedding and the mortality. EGCX plays a crucial role in vascular permeability, mechano-transduction, and the microenvironment. Degradation of the EGCX accelerates systemic inflammation. Although molecular hydrogen has antioxidation and anti-inflammatory potency, its effect on the vascular EGCX in heat stroke has not been examined. Therefore, the aim of this study was to investigate the influence of hydrogen inhalation on the survival and thickness of the vascular EGCX of heat stroked rats.

[Materials and Methods]

This study was approved by the Ethical Committee for Animal Experiments and the Laboratory Animal Facility of Hamamatsu University School of Medicine (2018045). Ninety-eight male Wistar rats, 10 weeks old, were randomly assigned into five groups, seven rats in each, including normothermia (NT); normothermia with hydrogen at 2% (NTH2); heat stroke (HS); heat stroke with hydrogen at 2% (H2); and heat stroke with hydrogen at 4% (H4) in each experiment. A heat-controlled chamber, set at 40°C temperature and 60% humidity, was used to induce heat stroke. After preparation, the anesthetized rats that underwent the heating process were subjected to an hour of stabilization in which 0%, 2% or 4% hydrogen gas was inhaled and maintained until the experiment ended. In addition to survival rate assessments, blood samples and left ventricles were collected to evaluate the thickness of the vascular EGCX and serum level of endotoxin, syndecan-1 (SDC-1), malondialdehyde (MDA), superoxide dismutase (SOD2) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). The permeability of intestine was determined by measuring the leakage amount of fluorescein isothiocyanate-labeled dextran (FITC-dextran).

[Results]

The inhalation of 2% hydrogen significantly improved survival of heat stroked rats (71.4%, compared to 0% of HS,  $P = 0.001$ ). The SDC-1 level was higher in the HS ( $12.5 \pm 3.4$  ng/mL) than the H2 ( $6.9 \pm 2.4$  ng/mL,  $P < 0.05$ ). The cardiac capillary

EGCX was significantly thinner in the HS ( $0.043 \pm 0.007 \mu\text{m}$ ) than H2 ( $0.074 \pm 0.011 \mu\text{m}$ ). Hydrogen gas at 2% significantly diminished TNF- $\alpha$  levels compared to HS ( $251.3 \pm 175.2 \text{ pg/mL}$  and  $555.2 \pm 258.7 \text{ pg/mL}$ , respectively). Similarly, serum MDA of H2 was less than HS, whereas SOD2 activity was higher than HS ( $P < 0.05$ ). In addition, 2% hydrogen inhalation effectively limited the serum endotoxin level ( $P < 0.05$ ) and the leakage amount of FITC-dextran of H2 ( $17.3 \pm 6.0 \mu\text{g/mL}$ ) was significantly lower than HS ( $53.5 \pm 13.4 \mu\text{g/mL}$ ).

[Discussion]

The current study showed that inhalation of 2% hydrogen significantly improved the short-term survival and partially preserved the thickness of the vascular EG CX in heat stroked

rats. Additionally, the decrease in serum SDC-1, MDA, and TNF- $\alpha$  in the presence of increased SOD2 levels revealed the antioxidative and anti-inflammatory effects of hydrogen gas.

The contribution of reactive oxygen species (ROS) and endotoxin in the pathogenesis of heat stroke are linked with systemic inflammation. The increase in intestinal permeability toward FITC-dextran and serum endotoxin level showed that heat stress significantly injured the intestinal wall. However, 2% hydrogen gas effectively improved intestinal barrier function.

The increase in serum MDA indicated that heat stress caused excessive production of ROS, which involved in EG CX shedding. In addition to directly scavenging ROS, molecular hydrogen upregulates the generation of antioxidants including SOD2 via the nuclear factor-E2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1-antioxidant response element pathway. The increase in the serum SOD2 and a decrease in MDA indicates that inhalation of 2% hydrogen was able to ameliorate oxidative stress during heating.

The increase in serum TNF- $\alpha$  and SDC-1 indicated that inflammatory responses were associated with EG CX shedding. TNF- $\alpha$  was reported to mediate the shedding of EG CX after hemorrhagic shock. It is possible that hydrogen gas inactivated the nuclear factor kappa-light chain-enhancer of the activated B (NF- $\kappa$ B), which regulates the transcription of several inflammatory cytokines including TNF- $\alpha$ , during heat stress. A recent study reported that molecular hydrogen upregulated the expression of heat shock protein 70, which inhibited NF- $\kappa$ B activation.

The present study raises the issue of the effect of 4% hydrogen. Inhalation of 4% hydrogen tended to elevate oxidative stress and inflammation, but 2% hydrogen provided the most effective protection. This finding is consistent with that from those previous study on the use of 1.2% hydrogen gas for hemorrhagic shock and 2%

hydrogen gas for liver I/R injury. Apparently, hydrogen gas has a dose-dependent effect. However, insight into the underlying mechanism awaits full elucidation.

[Conclusion]

Inhalation of 2% hydrogen improved the survival of rats with heat stroke and attenuated the shedding of the vascular EGCX related to its anti-oxidative and anti-inflammatory effects.