Hydrogen inhalation protects against acute lung injury induced by hemorrhagic shock and resuscitation

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Introduction. *Hemorrhagic shock followed by fluid resuscitation (HS/R) triggers an inflammatory response and causes pulmonary inflammation that can lead to acute lung injury (ALI). Hydrogen, a therapeutic gas, has potent cytoprotective, antiinflammatory, and antioxidant effects. This study examined the effects of inhaled hydrogen on ALI caused by HS/R.*
Methods. *Rats were subjected to hemorrhagic shock by withdrawing blood to lower blood pressure followed by resuscitation with shed blood and saline to restore blood pressure. After HS/R, the rats were maintained in a control gas of similar composition to room air or exposed to 1.3% hydrogen.*
Results. *HS/R induced ALI, as demonstrated by significantly impaired gas exchange, congestion, edema, cellular infiltration, and hemorrhage in the lungs. Hydrogen inhalation mitigated lung injury after HS/R, as indicated by significantly improved gas exchange and reduced cellular infiltration and hemorrhage. Hydrogen inhalation did not affect hemodynamic status during HS/R. Exposure to 1.3% hydrogen significantly attenuated the upregulation of the messenger RNAs for several proinflammatory mediators induced by HS/R. Lipid peroxidation was reduced significantly in the presence of hydrogen, indicating antioxidant effects.*
Conclusion. *Hydrogen, administered through inhalation, may exert potent therapeutic effects against ALI induced by HS/R and attenuate the activation of inflammatory cascades. (Surgery 2015;158:399-407.)*

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Hemorrhage contributes to mortality after trauma accounting for 30–40% of deaths caused by trauma worldwide despite recent advances in resuscitation and critical care.[1] Hemorrhagic shock followed by fluid resuscitation (HS/R) triggers an inflammatory response characterized by upregulation of proinflammatory cytokines and adhesion molecules and induces pulmonary inflammation that can lead to acute lung injury (ALI). ALI often predicates multiple organ failure and mortality.[2] Thus, despite recent advances in intensive care, lung injury after HS/R is still among the most common causes of death after trauma.[3,4]

Hydrogen is an important physiologic regulatory molecule and exerts antioxidant, antiinflammatory, and antiapoptotic protective effects on cells and organs.[5] Because hydrogen can be administered via a ventilation circuit, inhaled hydrogen therapy is considered clinically applicable for patients with shock. Additionally, although hydrogen has therapeutic benefits in multiple organs, the lungs are an ideal target organ for hydrogen therapy, because inhalation is a straightforward delivery method. In fact, hydrogen treatment ameliorated lung injury in several model systems, including ventilator-induced lung injury,[6] ischemia–reperfusion injury during lung transplantation,[7] and hyperoxic lung injury.[8] Although the therapeutic efficacies of hydrogen have been studied, there is limited information on processes regulated by the hydrogen molecule. We hypothesized that, because of its antiinflammatory and antioxidant

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properties, inhaled hydrogen therapy could ameliorate ALI after HS/R.

METHODS

Animals. Male Sprague-Dawley rats weighing 300–500 g (6–9 weeks old) were purchased from Clea Japan, Inc. (Tokyo, Japan) and were kept in individual stainless steel cages in a temperature, humidity, and light-controlled room (23 ± 3°C; 55 ± 15%; 12-hour light–dark cycle) for 2–5 weeks before the experiments. During this period, all animals were provided standard food (AIN-93G diet; Oriental Kobo Corporation, Tokyo, Japan) and free access to water. All procedures involving rats were conducted in accordance with the guidelines of the Animal Care and Use Committees of the Hyogo College of Medicine and complied with the National Research Council's Guide for the Humane Care and Use of Laboratory Animals.

Animal model. Under general anesthesia with isoflurane, a catheter for monitoring arterial pressure was placed into the right femoral artery. To create a shock, blood was withdrawn through this catheter, and blood pressure was maintained at 30 ± 5 mmHg for 60 minutes. After 60 minutes, shed blood was reinfused (with saline if necessary for adequate volume restoration), the femoral artery was ligated, and the wound was closed (Fig 1). In sham control rats, we did not induce shock or resuscitate with fluid restoration; we simply placed the catheter, removed it 60 minutes later, ligated the femoral artery, and closed the wound. To maintain body temperature, 37°C warming pads were used throughout this procedure. After the procedure, the rats were kept in an air/gas exposure box for 1–6 hours. Blood samples were collected. Rats were humanely killed 1, 3, or 6 hours after HS/R by isoflurane overdose (Fig 1). The lungs were excised and divided into 2 sections. The right lobe was snap frozen immediately with liquid nitrogen for further analysis. The left lobe of the lungs was used for histologic examination.

Hydrogen treatment. For hydrogen gas treatment, cylinders with nitrogen-based, high-pressure, premixed gases were purchased (Japan Fine Products, Kanagawa, Japan). The manufacturer confirmed the concentrations of H_2 (1.3%), O_2 (21%), and N_2 (77.7%). In Japan, 1.3% is the highest concentration of H_2 that can be mixed and bottled under high pressure with 21% oxygen for clinical use. As a control, additional N_2 was administered instead of H_2 (O_2, 21%; N_2, 79%). The premixed gases were delivered to the rats via a gas exposure chamber (Natsume Seisakusho Co. Tokyo, Japan). Hydrogen or control gas (designated N_2) was administered during shock and for 1, 3, or 6 hours before killing and tissue collection. The sham control rats were placed in the gas exposure chamber with control (N_2) gas for 1 hour.

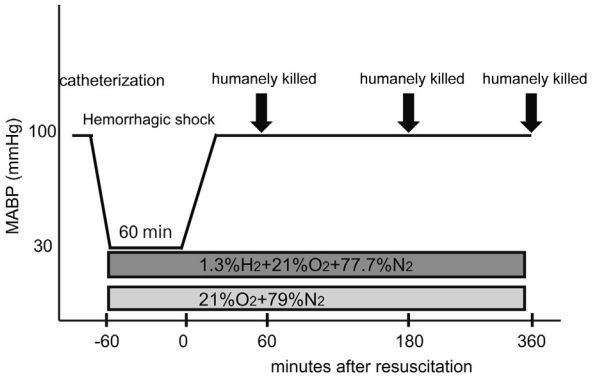

Fig 1. Experimental protocol. Blood was withdrawn, and blood pressure was maintained at 30 ± 5 mmHg for 60 minutes. After 60 minutes, shed blood was reinfused. Rats were then placed in an exposure chamber filled with either 1.3% H_2 (H_2, 1.3%; O_2, 21%; N_2, 77.7%) or with only oxygen and nitrogen (O_2, 21%; N_2, 79%). *MABP*, Mean arterial blood pressure.

Assessment of gas exchange function and blood lactate levels. Partial pressure of oxygen (Po_2), partial pressure of carbon dioxide (Pco_2), and blood lactate levels were assessed by analysis of blood gases (iSTAT, Abbott Point Care Inc., Princeton, NJ) before killing. Blood gases were assessed on a fraction of inspired oxygen (Fio_2) of 1.0 in blood drawn from the abdominal aorta 3 minutes after oxygen inspiration was initiated.

Measurement of malondialdehyde. Lung tissues were harvested after 3 hours of gas exposure and kept at -80°C until analysis. The tissue was homogenized, and tissue malondialdehyde (MDA) concentration was determined according to the manufacturer's instructions (Kit MDA-586; Oxisresearch, Portland, OR).

Histopathology. Lungs were fixed by inflation with buffered 4% paraformaldehyde for 24 hours. After embedding in paraffin, the sections were prepared and stained with hematoxylin and eosin. Polymorphonuclear neutrophils (PMNs) were stained using a naphthol AS-D chloroacetate esterase staining kit (Sigma Diagnostics) and identified by nuclear morphology stained in bright red. PMN were counted in 10 high-power fields (×400) per sample. ALI was scored with the samples' identities masked according to previously described criteria,[9] specifically (1) thickness of the alveolar wall, (2) infiltration or aggregation of neutrophils in air space, alveolar wall, or vessel wall,