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Hyperoxygenated hydrogen–rich solution suppresses shock- and resuscitation-induced liver injury

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ABSTRACT

Background: It is not known whether simultaneous delivery of hydrogen and oxygen can reduce injury caused by hemorrhagic shock and resuscitation (HSR). This study investigated the therapeutic potential of hyperoxygenated hydrogen–rich solution (HHOS), a combined hydrogen/oxygen carrier, in a rat model of HSR-induced liver injury.

Materials and methods: Rats ($n = 60$) were randomly divided into 5 groups ($n = 6$ per group at each time point). One group underwent sham operation, and the others were subjected to severe hemorrhagic shock and then treated with lactated Ringer's solution (LRS), hydrogen-rich solution, hyperoxygenated solution, or HHOS. At 2 and 6 h after resuscitation, blood samples ($n = 6$) were collected from the femoral artery and serum concentrations of alanine aminotransferase and aspartate aminotransferase (AST) were measured. Rats were then sacrificed, and histopathological changes in the liver were evaluated by quantifying the percentage of apoptotic cells by caspase-3 immunohistochemistry and terminal deoxynucleotidyl transferase dUTP nick-end labeling. Inflammation was assessed by assessing malondialdehyde content and tumor necrosis factor-α, and interleukin (IL)-6 expression.

Results: Compared to lactated Ringer's solution, hydrogen-rich solution, or hyperoxygenated solution groups, serum AST and alanine aminotransferase levels and IL-6, tumor necrosis factor-α, and malondialdehyde expression in liver tissue were decreased by HHOS treatment. The number of caspase-3- and terminal deoxynucleotidyl transferase

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dUTP nick end labeling-positive cells was decreased ($P < 0.05$) by HHOS treatment, 2 and 6 h after resuscitation.

Conclusions: HHOS has protective effects against liver injury in a rat model of HSR.

Introduction

Hemorrhagic shock resulting from massive loss of blood is one of the most common complications associated with trauma or with gynecological diseases involving bleeding, peptic ulcer bleeding, and esophageal varicose vein rupture.[1] It can also cause pathologic changes in cellular metabolism and contribute to organ dysfunction leading to multiple organ failure.[2]

Liver injury caused by hemorrhagic shock and resuscitation (HSR) is a common clinical emergency that is often secondary to lung and gastrointestinal injury[3]; the mortality rate can reach 100% owing to the lack of effective prevention and treatment measures. It is therefore essential to manage liver injury by suppressing the systemic inflammatory response and thereby improve patient prognosis.

Hemorrhagic shock is associated with a reduction in effective circulating blood volume, which can be improved by timely replenishment of blood.[4] However, reperfusion can lead to the production of cytotoxic substances such as peroxides, H_2O_2, and hydroxyl radicals that cause ischemia/reperfusion (I/R) injury to vital organs.[5] To date, there are no specific strategies for mitigating the resultant cellular damage. When blood products are unavailable or the control of bleeding is delayed, the type of fluid used for resuscitation can determine patient outcome. Ideally, this should be a solution that not only restores oxygen to tissue but also prevents reperfusion-induced cell injury.

Recent studies have investigated the possibility of suppressing inflammation and I/R injury using hydrogen-rich liquids[6] and treating hemorrhagic shock by administering liquids with high oxygen content.[7-9] However, it is unclear whether simultaneous treatment with hydrogen and oxygen can effectively reduce injury caused by HSR.

To address this issue, the present study evaluated the effects of hyperoxygenated hydrogen–rich solution (HHOS), a combined hydrogen/oxygen carrier, in a rat model of HSR-inducted liver injury. Our results indicate that HHOS treatment is a promising strategy for preventing liver damage following HSR.

Materials and methods

Animals

Male Sprague-Dawley rats weighing 250-300 g were purchased from the laboratory animal center of The Fourth Military Medical University and housed at 22°C-26°C under a 12:12-h light/dark cycle at 40%-70% humidity with free access to regular chow and tap water. Animals were handled in accordance with the guidelines of the Animal Care and Use Committees of The Fourth Military Medical University of Medicine and complied with the National Research Council's Guide for the Humane Care and Use of Laboratory Animals.

Rat model of HSR-induced liver injury

The rat model of HSR-induced liver injury was generated as previously described.[10] Briefly, under 1% sodium pentobarbital anesthesia (40 mg/kg by intraperitoneal injection), a catheter connected to an arterial pressure monitoring system (CW-PM9000 E,Shenzhen Mindray electronics, China) was inserted into the left femoral artery. Heparin (100 U/kg) was administered via the left femoral vein. To lower blood pressure to 30-40 mmHg (1 mmHg = 0.133 kPa), blood was drawn via the catheter at a rate of 3 mL/kg/min for 15 min, and blood pressure was maintained at 35 ± 5 mmHg for 60 min via exsanguination or autologous blood transfusion. The extracted blood was preserved after adding heparin (5 U/ml). After 60 min, lactated Ringer's solution (LRS), hydrogen-rich solution (HHS), hyperoxygenated solution (HOS), or HHOS was reinfused. Rectal temperature was maintained at 36.5°C-37.5°C by illumination with a lamp and by means of a WD-type IIA type constant temperature control operating table (Siemens AG, Germany). Rats were placed in an air/gas exposure box for 2 or 6 h postresuscitation. Blood samples were collected at each of these time points before the rats were sacrificed. The liver was excised and divided into two parts—the right lobe was immediately flash frozen in liquid nitrogen and the left lobe was used for histologic examination.

Experimental stages and groups

Animals were randomly divided into five groups ($n = 6$ each): sham, LRS, HHS, HOS, and HHOS. Sham animals were not subjected to HSR but were anesthetized, and the catheter was into the left femoral artery. Rats in the LRS, HHS, HOS, and HHOS groups were subjected to HSR and administered a volume of blood 2-fold greater than the volume that was lost. It contained 50 mL/kg HHS, HOS, HHOS, or LRS and was delivered via the femoral vein, and the remaining volume was supplemented with LRS to achieve a 2-fold volume of blood loss with a constant-speed pump for 20 min 1 h after the shock.

Preparation and storage of HHS, HOS, and HHOS

Oxygen was dissolved in LRS or colloidal solution for 15 min using a homemade GY high-oxygen liquid apparatus, with 2-3 L/min oxygen flow to achieve 20.0-30.0 mg/L HOS.[8] Hydrogen was dissolved in LRS for 10 min, with 1 L/min flow rates to obtain 0.50- to 1.00-mmol/L HHS.[11] We dissolved hydrogen in HOS for 10 min at a 1 L/min flow rate to obtain HHOS in which the hydrogen and oxygen contents were >0.50 mmol/L and >20 mg/L, respectively. HHOS and HHS were stored in an