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Effects of hydrogen-rich saline on aquaporin 1, 5 in septic rat lungs

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ABSTRACT

Aquaporin 1(AQP1) and AQP5 have an important role in eliminating extravascular lung water, an increase of which contributes to lung injury in patients with sepsis and its consequent mortality. It has been reported that hydrogen-rich saline (HRS) has protective effects against sepsis-related lung injury. In this study, we hypothesized that the protective effect occurred by preserving the expression of AQP1 and AQP5. To test this hypothesis, male Sprague–Dawley rats received intratracheal administration of lipopolysaccharide (LPS) followed by intraperitoneal injection of HRS. Lung function, wet-to-dry weight ratio, and histopathology scores were determined. The expression of AQP1 and AQP5 at the messenger RNA and protein levels, as well as the involved pathways, was explored by quantitative polymerase chain reaction and Western blot. LPS significantly impaired lung function and downregulated the expression of AQP1 and AQP5 in the rat lung, all of which were attenuated by HRS treatment. Moreover, HRS treatment inhibited LPS-induced activation of p38 mitogen-activated protein kinase and jun N-terminal kinase, which is associated with LPS-induced downregulation of AQP1 and AQP5.

Introduction

Increased capillary permeability is one of the characteristics of septic shock, and the subsequent increase in extravascular lung water (ELW) from capillary leakage is believed to result in hypoxemia and acute respiratory distress syndrome [1]. High ELW is associated with a high mortality rate and becomes an independent prognostic factor in patients with acute respiratory distress syndrome [2]. ELW is treated by targeting water transport across biological membranes to remove extracellular water. Recent studies showed that, in the lung, water channel aquaporin (AQP) has an important role in transporting water across the air space–capillary barrier [3]. In mammals, 13 different types of AQPs have been identified [4], four of which are found in the lung [5]. AQP1 and AQP5, expressed in the pulmonary capillary endothelium and type I pneumocytes of the alveoli respectively, are important AQPs for water transport in the distal lung [5], disruption of which affects water movement across the alveolar and capillary membrane in a rat model of acute lung injury [6]. A recent study has shown that lipopolysaccharide (LPS) can decrease the expression of AQP1 and AQP5 in mouse alveolar epithelial cells [7]. Besides LPS, factors causing acute lung injury, such as drugs, transfusion and radiation, can downregulate the expression of AQPs in the lung [8].

In the past few years, studies on hydrogen have shown that hydrogen gas or hydrogen-rich saline (HRS) has protective effects in the therapy of disorders such as ischemic–reperfusion injury, stroke, sepsis, and organ transplantation [9–11]. In a mouse model of LPS-induced acute lung

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injury, HRS administration can ameliorate acute lung injury and decrease ELW [12]. Although reducing inflammation and apoptosis accounts for the protective effect of HRS, the mechanisms of decreasing ELW remain undetermined. In the present study, rats were challenged with LPS followed by HRS administration. We hypothesized that HRS decreases ELW by upregulating the expression of AQP1 and AQP5 in an LPS-induced acute lung injury model.

Material and methods

Animals

Animals were handled as approved by the Institutional Animal Ethics Committee of China Medical University in accordance with the National Institutes of Health (Bethesda, MD) guidelines for the care and use of laboratory animals.

Male Sprague–Dawley rats (body weight: 200–250 g, age: 8–10 wk) were provided by the Experimental Animal Center of Shengjing Hospital affiliated with China Medical University (Shenyang, Liaoning, China). Conditions for animal housing included temperatures of 20°C–22°C, 12:12-h light-dark cycle, and pathogen-free enclosures.

Acute lung injury model

Acute lung injury model was induced by intratracheal administration of LPS as described previously [13]. Briefly, after anaesthetized by intraperitoneal (IP) injection of sodium pentobarbital (40 mg/kg), rats were orally intubated with a sterile plastic catheter, and a single dose of LPS (*Escherichia coli*; Sigma, St. Louis, MO; 50 μg/rat, dissolved in 100 μL of phosphate-buffered saline [PBS]) was given through this catheter. After the administration of LPS, the catheter was removed, and rats breathed spontaneously. Control rats were intratracheally administrated with 100 μL of PBS. No more anesthetics were given to rats until 8 h after LPS challenge.

Preparation of HRS

HRS was prepared as in previous studies [14]. Briefly, hydrogen gas was dissolved in normal saline (NS) under 0.4 MPa (MPa) pressure for 6 h and stored in an aluminum bag without dead volume at 4°C under atmospheric pressure followed by γ-radiation sterilization. To make sure the hydrogen level in the NS was at least 0.6 mmol (mmol)/L, gas chromatography was performed following the method described by Ohsawa *et al.* [15].

Animal groups

Forty rats were randomly divided into four groups ($n = 10$ per group): the NS group received an intratracheal administration of only NS; the NS + HRS group received an intratracheal administration of NS followed by post 1-h and 4-h IP injection of HRS (10 mL/kg); the LPS group rats received an intratracheal administration of LPS; the LPS + HRS group received HRS (10 mL/kg IP) at 1 h and 4 h after the LPS challenge.

To investigate which cell signaling pathways were involved in regulation of AQP1 and AQP5 expression in LPS-challenged rats, an additional 30 rats were randomly assigned to three more groups (10 rats per group); they were given the p38 inhibitor SB203580 (Calbiochem, San Diego, CA) and the JNK inhibitor SP600125 (Sigma, St. Louis, MO), respectively. Both inhibitors were dissolved in dimethyl sulfoxide and injected intravenously 30 min before the LPS challenge. Rats in the control group were injected with dimethyl sulfoxide alone.

After 8 h of LPS challenge, rats were anesthetized by IP injection of sodium pentobarbital (150 mg/kg) and killed by opening chest and drawing blood from hearts, bronchoalveolar lavage fluid (BALF) was obtained by flushing the lung with 5 mL of PBS and centrifuged at 1500 g for 10 min at 4°C. After resuspending the cell pellet in PBS, a hemocytometer (Beckman Coulter, Brea, CA) was used to investigate the number of total cells. The slides prepared from BALF cell suspension were visualized after Wright–Giemsa staining (Fisher Scientific Co, Middletown, VA), and polymorphonuclear neutrophils (PMNs) were identified and counted by two certified laboratory technologists who were blind to animal treatment. Bio-Rad protein assay (Bio-Rad, Hercules, CA) was performed to investigate total protein concentration in the BALF. The lung tissue was removed and used for evaluating histopathologic changes, wet-to-dry weight ratio (W/D), and expression of AQPs 1 and 5.

Arterial blood gas analyses

To evaluate the lung function of the rats, blood was drawn from the femoral artery at 8 h after LPS administration. A RAPIDLab 400 gas analyzer (Siemens Healthcare, Berlin, Germany) was used to measure the pH value, the partial pressure of oxygen (PaO2), and the partial pressure of carbon dioxide (PaCO2).

Wet-to-dry weight ratio

Wet weight was obtained by weighing the freshly harvested left lung tissue. The dry weight was obtained after placing the lung tissue in an oven for 24 h at 55°C. The W/D was calculated.

Histopathologic examination

After being fixed in a 10% formalin buffer for 24 h at 4°C, the upper and middle lobes of the right lung were then embedded in paraffin. Hematoxylin–eosin staining was introduced to a series of microsections (4 μm) for investigating histologic changes. The histopathologic score was evaluated following the method described by Keliang *et al.* [9].

RNA quantification

For total RNA isolation, we used a reagent by Trizol (Ambion, Carlsbad, CA) according to the manufacturer's protocol on 100 mg of rat lung tissue. RNA concentrations were determined by spectrometry. A SuperScript III First-Strand Synthesis kit (Invitrogen, Carlsbad, CA) was used to reverse-transcribe 1 μg of total RNA into complementary DNA and quantified in an ABI 7500 fast real-time PCR machine (Applied