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Hydrogen gas inhibits high-mobility group box 1 release in septic mice by upregulation of heme oxygenase 1

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ABSTRACT

Background: Sepsis is a potentially fatal whole-body inflammation caused by severe infection. Hydrogen gas (H_2) is effective for treating sepsis. In this study, we hypothesized that the protective function of H_2 in mice with septic lung injury occurred through the activation of heme oxygenase 1 (HO-1) and its upstream regulator nuclear factor-erythroid 2 p45-related factor 2 (Nrf2).

Materials and methods: Male institute of cancer research mice were subjected to sepsis by cecal ligation and puncture (CLP) with the presence or absence of H_2. Beginning at 1 and 6 h after CLP or sham operation, respectively, 2% H_2 was inhaled for 1 h. We intraperitoneally injected the HO-1 inhibitor zinc protoporphyrin IX (40 mg/kg) 1 h before CLP. To assess the severity of septic lung injury, we observed the 7-d survival rate, wet/dry weight ratio of lung, lung histopathologic score, oxygenation index, and so forth. Serum and homogenates from the lung, liver, and kidney were acquired for measuring the levels of high-mobility group box 1 (HMGB1) at 6, 12, and 24 h after CLP or sham operation. Furthermore, the protein and messenger RNA expression of Nrf2, HO-1, and HMGB1 was measured at 6, 12, and 24 h.

Results: Septic mice had a lower survival rate and more severe lung injury compared with the sham group. However, therapy with H_2 increased the survival rate and alleviated the severity of lung injury, reduced the HMGB1 level, and increased the HO-1 and Nrf2 levels in septic mice. Moreover, the HO-1 inhibitor zinc protoporphyrin IX significantly eliminated the protective effect of H_2 on septic lung injury.

Conclusions: H_2 plays a significant role in regulating the release of the inflammatory cytokine HMGB1 in septic mice, which is partially mediated through the activation of HO-1 as a downstream molecule of Nrf2.

1. Introduction

Sepsis is a potentially fatal whole-body inflammation caused by severe infection [1]. Because of its rapid progress and high mortality, it has attracted widespread attention in the medical field [2,3]. Sepsis is closely connected to acute lung injury, acute respiratory distress syndrome, and multiple organ dysfunction syndrome. Sepsis is a life-threatening and

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common medical condition, for more than 50% of patients who receive therapy in intensive care units develop acute lung injury [4]. In the event of sepsis, a considerable number of inflammatory mediators and lipid metabolites enter into the blood circulation and stimulate the recruitment and activation of inflammatory cells in lung tissues. Cytokines, chemokines, and oxygen free radicals, which are produced by inflammatory cells, magnify the inflammatory response and form a "waterfall-like chain reaction" [5]. The lung injury that is caused by sepsis is related to the excessive release of proinflammatory mediators, such as tumor necrosis factor α, interleukin 1β, and interleukin 6 [6]. Once homeostasis between the anti-inflammatory and proinflammatory cytokines is disturbed and the proinflammatory cytokines predominate, the detrimental septic effects on the lung cannot be inhibited.

It is widely accepted that hydrogen gas (H_2) exerts an effective therapeutic role in sepsis. The mechanisms of H_2 treating sepsis may be closely related to its anti-inflammatory, antioxidant, and antiapoptosis effects. Our previous studies have shown that H_2 inhalation significantly improves the prognosis of sepsis in mice through its association with the inhibition of oxidative stress or the expression of high-mobility group box 1 (HMGB1) [7]. Furthermore, our recent research has certified that heme oxygenase 1 (HO-1) mediates the anti-inflammatory effect of H_2 in RAW264.7 macrophages stimulated by lipopolysaccharide [8]. However, the mechanism of the anti-inflammatory effect of H_2 *in vivo* remains unknown. After evaluating the results of all the studies, we hypothesized that HO-1 may be a crucial protective factor that mediates the anti-inflammatory function of H_2 *in vivo*.

HO-1, which is also called heat shock protein 32, is an antioxidant enzyme that represents an antagonist of oxidative stress. It is an inducible enzyme in the process of oxidative degradation that converts heme to biliverdin, free iron, and carbon monoxide [9]. It is distributed widely in body tissues, especially in the microsomes of the mononuclear phagocyte system. In this study, the pharmacologically competitive inhibitor zinc protoporphyrin IX (ZnPPIX) was used to confirm the protective effect of HO-1.

It is well known that cecal ligation and puncture (CLP) causes sepsis because of a polymicrobial infection [10]. Therefore, this study was designed to investigate the therapeutic effect of H_2 on sepsis-induced lung injury in a CLP model. Additionally, we aimed to demonstrate the protective effect of a specific HO-1 induction with H_2 on sepsis-induced lung injury.

2. Materials and methods

2.1. Animal model

Adult male institute of cancer research mice weighing 18–25 g were purchased from the Laboratory Animal Center of the Military Medical Science Academy of the Chinese People's Liberation Army. All the mice were raised in polypropylene cages (five mice per cage) under standard controlled conditions (22°C–25°C, 12-h light–dark cycle) with food and water freely available. All the experimental protocols were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University and were implemented in accordance with the National Institutes of Health guidelines for the care and use of experimental animals. Anesthesia was induced using intraperitoneal injection of 2% sodium pentobarbital (50 mg/kg) in saline. CLP was performed as previously described [10]. Under sterile surgical conditions, the cecum was exposed through a 1-cm midline abdominal incision and was subsequently ligated below the ileocecal valve. To induce severe sepsis, the distal three quarters of the cecum were ligated and punctured using a 20-gauge needle. The fecal contents were gently squeezed through the puncture point. Finally, the cecum was returned into the abdomen, and the incision was closed using a sterile 3-0 silk suture. The animals with sham operation were subjected to laparotomy without CLP. All the animals underwent a subcutaneous injection of 1-mL prewarmed saline solution (0.9% NaCl, 37°C) for resuscitation.

2.2. H_2 treatment

According to our previous studies, the animals were placed in a sealed Plexiglas box with inflow and outflow outlets for H_2 treatment [11,12]. H_2 was administered through a TF-1 gas flowmeter (Yutaka Engineering Corp, Tokyo, Japan) and was mixed with air at a rate of 4 L/min in the box. The H_2 concentration in the box was continuously monitored using a detector (HY-ALERTA Handheld Detector Model 500; H_2 Scan, Valencia, CA) and was maintained at 2% throughout the therapy. Carbon dioxide was removed from the box with Baralyme. The mice that were not given the H_2 treatment inhaled room air in the box. The H_2 treatment was given via 2% H_2 inhalation for a 1-h period at 1 and 6 h after CLP or sham operation.

2.3. Experimental design

2.3.1. Experiment 1: effects of H_2 treatment or ZnPPIX on the survival rate of septic mice

A total of 120 animals were randomly divided into the six groups ($n = 20$ per group) as follows: sham, sham + H_2, sepsis, sepsis + H_2, sepsis + ZnPPIX, and sepsis + H_2 + ZnPPIX groups. The sepsis + ZnPPIX and sepsis + H_2 + ZnPPIX groups received an intraperitoneal injection of ZnPPIX (40 mg/kg) 1 h before CLP. ZnPPIX (Millipore, Darmstadt, Germany) was dissolved in N,N-dimethylformamide. The animals in the sham + H_2, sepsis + H_2, and sepsis + H_2 + ZnPPIX groups maintained 2% H_2 inhalation for 1 h at 1 and 6 h after CLP or sham operation, respectively. As a control, the mice in the sham, sepsis, and sepsis + ZnPPIX groups received room air at similar time points. The survival rate was observed on days 1, 2, 3, 5, and 7 after CLP or sham operation.

2.3.2. Experiment 2: effects of H_2 treatment or ZnPPIX on lung injury in mice with sepsis

An additional 36 animals were randomly assigned to the six groups ($n = 6$ per group) as follows: sham, sham + H_2, sepsis, sepsis + H_2, sepsis + ZnPPIX, and sepsis + H_2 + ZnPPIX groups. The detailed experimental protocols were the same as previously described. Using hematoxylin and eosin-staining, we observed the lung injury of mice in these groups. Lung histopathology, wet/dry (W/D) weight ratio,