Experimental and Molecular Pathology 98 (2015) 268–276

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Review

Hydrogen-rich saline ameliorates lung injury associated with cecal ligation and puncture-induced sepsis in rats

Yu Zhai [a,1], Xiaohong Zhou [a,1], Qingchun Dai [b,1], Yamin Fan [c], Xinli Huang [c,*]

[a] Department of basic medicine, Hebei University of Chinese Medicine, Shijiazhuang 050200, PR China
[b] Department of intensive care unit, Cangzhou Central Hospital, Cangzhou 061001, PR China
[c] Department of Pathophysiology, Hebei Medical University, Shijiazhuang 050017, PR China

ARTICLE INFO

Article history:
Received 13 September 2014
and in revised form 16 December 2014
Accepted 2 March 2015
Available online 4 March 2015

Keywords:
Hydrogen
Acute lung injury
Oxidative stress
Inflammation
Cecal ligation and puncture

ABSTRACT

Aims: Although hydrogen has been proved to be a novel therapeutic medical gas in several lung injury animal models, to our knowledge, it has not been tested yet in acute lung injury (ALI) induced by cecal ligation and puncture (CLP). This study was to investigate the hypothesis that hydrogen could ameliorate CLP-induced lung injury in rats.

Methods and results: Our experiments exhibited that gas exchange dysfunction and lung tissue inflammation were observed in animals exposed to CLP. Hydrogen-rich saline treatment significantly attenuated lung injury as indicated by significantly improved gas exchange and histological changes in the lung and significantly reduced lung water content (LWC) and neutrophil infiltration 8 h after CLP. Lipid peroxidation and DNA oxidation in the lung tissue were significantly reduced along with a decreased nitrotyrosine content and maintained superoxide dismutase activity in the presence of hydrogen, demonstrating antioxidant role of hydrogen in CLP-induced ALI. Importantly, hydrogen-rich saline treatment significantly inhibited the activation of p-p38 and NF-κB while suppressing the production of several proinflammatory mediators.

Conclusions: This observation indicated that hydrogen-rich saline peritoneal injection improves histological and functional assessment in rat model of CLP-induced ALI. The therapeutic effects of hydrogen-rich saline may be related to antioxidant and anti-inflammatory actions.

Contents

* Corresponding author at: No. 361, Zhongshan East Road, Department of Pathophysiology, Hebei Medical University, Shijiazhuang 050017, PR China.
E-mail address: 422708842@qq.com (X. Huang).
[1] Co-first author.

http://dx.doi.org/10.1016/j.yexmp.2015.03.005
0014-4800/

1. Introduction

Acute lung injury (ALI), as manifested by acute respiratory distress syndrome (ARDS), which frequently occurs after hemorrhage, trauma, burn, or abdominal surgery, is a serious illness of which the incidence and mortality is very high. It is a leading cause of morbidity and mortality in severely ill patients (Raghavendran and Napolitano, 2011a,b; Ware and Matthay, 2000). The major pathological changes of ALI include impaired gas exchange, neutrophil accumulation, increased vascular permeability and parenchyma injury (Wu et al., 2009; Lee and Downey, 2001; Suda et al., 2010). Endotoxin (LPS), derived from the cell wall of gram-negative bacteria, is thought to be the most important pathogen that leads to the development of ALI. Cecal ligation and puncture (CLP)-induced ALI model seems to resemble qualitatively as well as quantitatively the clinical observations of vascular reactivity and inflammation in the lung during polymicrobial peritonitis, bacteremia, and systemic sepsis (Riedemann et al., 2003).

Hydrogen, the simplest element in the universe, is a colorless, odorless, and tasteless reducing agent composed of two atoms. Hydrogen, which has low solubility, cannot be absorbed easily by the human body. Thus the biological effects of hydrogen in high organism have been overlooked. However, in 2007, Ohsawa et al. first provided evidence of hydrogen selectively reducing •OH and $ONOO^-$, both of which are important mediators of ALI (Ohsawa et al., 2007). Further studies demonstrated similar protective effects of hydrogen on injuries caused by oxidative stress in the brain (Cai et al., 2008; Sun et al., 2011), liver (Liu et al., 2010), heart (Q. Sun et al., 2009), and intestines (Zheng et al., 2009). Hydrogen also provides anti-inflammatory effect in acute pancreatitis (Chen et al., 2010), colon inflammation, and liver inflammation (Kajiya et al., 2009). Although hydrogen has been proved to be a novel therapeutic medical gas in several animal models of lung injury (Huang et al., 2010a; Kawamura et al., 2010; Zheng et al., 2010), it has not been tested in the CLP-induced ALI. Therefore, the aim of this study is to evaluate the feasibility and efficacy of hydrogen therapy applied to CLP-induced ALI in an animal model.

2. Materials and methods

2.1. Animal model of induced ALI

Adult male Sprague–Dawley rats (250 ± 20 g in weight) were provided by the Experimental Animal Center of Hebei Medical University. The study design was approved by the Animal Ethics Committee of Hebei Medical University, and all experiments were carried out in compliance with established guidelines for animal research. The previously described model of CLP-induced ALI was adopted with minor modifications (Baker et al., 1983). The rats were lightly anesthetized with a mixture of ketamine and medetomidine [0.75 ml ketamine (100 mg/ml) and 1 ml medetomidine (1 mg/ml) dissolved in 8.25 ml distilled water; 7.5 ml/kg] under aseptic conditions. With abdominal fur shaved and topical disinfectant applied, a small midline incision was made through the skin and peritoneum of the abdomen to expose the cecum. The cecal appendage was ligated utilizing a Silkam 4-0 thread at 3–5 mm below the ileocecal valve without occluding the bowel passage and then perforated with an 18-gauge needle in two locations distal to the point of ligation. After this, a small amount of stool was squeezed out through both holes. Finally, the bowel was repositioned, and the abdominal cavity was stitched up with a sterile Premilene 5-0 thread. Animals with sham operation underwent the same procedure without CLP (n = 12 per group). Both sham and CLP operation groups were given saline or hydrogen-rich saline simultaneously (n = 12 per group). Rats were euthanized 8 h after the surgery. When the right lung was isolated and tied off with a micro clamp at the right bronchus, the left lung was douched with bronchoalveolar lavage fluid (BALF). The right lower lobe was used for wet/dry (W/D) ratio measurement, the right middle lobe was fixed in 4% (vol/vol) neutral phosphate-buffered formalin and prepared for histological and immunohistochemical examinations, and the other portion of the right lung was immediately snap-frozen in liquid nitrogen for oxidative stress variables and Western blotting. In another set of experiments, tail artery blood was taken 8 h after sham or CLP operation. Partial pressures of gases, including PaO_2 and $PaCO_2$ were determined by automatic blood gas analyzer (CoHb IL Synthesis 25, IL1629; Instrumentation Laboratory, Lexington, MA).

2.2. Drugs

Hydrogen was dissolved in physiologic saline for 6 h under high pressure (0.4 MPa) to a supersaturated level accomplished by a hydrogen-rich saline-producing apparatus constructed in this lab. The saturated hydrogen saline was stored under atmospheric pressure at 4 °C in an aluminum bag with no dead volume and was sterilized by γ-radiation. The saline hydrogen level was measured by gas chromatography following the method described by Ohsawa et al. (2007). The average level was 0.86 mmol/L. Hydrogen saline was freshly prepared every week to ensure that a concentration of >0.6 mmol/L was maintained. The normal saline containing undetectable hydrogen was stored and handled in a same manner as the hydrogen saline.

2.3. BALF analysis

Bronchoalveolar lavage fluid was collected from the left lung after in situ instillation with sterile saline (8 ml/delivery × 3) via a cannula directly inserted into the left bronchus. Twenty milliliters of BALF was recovered from each rat. Then bronchoalveolar lavage fluid passed through a mesh (200 μm) to remove mucus, followed by centrifuging at 1200 g for 10 min, at 4 °C. The supernatant was collected to measure total protein concentrations utilizing Coomassie (Bradford) protein assay kit (Bio-Rad Laboratories, Hercules, CA). The cell pellet was resuspended, and viable cells were counted using trypan blue dye exclusion. The sediment was used for counting neutrophil numbers after Giemsa staining according to the method described by Feng et al. (2008).

2.4. Assessing pulmonary edema

The right lower lobe was weighed immediately after collection and placed into an 85 °C oven to dry out for 48 h, at which point their dry weight was determined. Lung edema was evaluated by lung water content (LWC) calculated as below (Ohkuda et al., 1982).

$$\text{LWC(mg/mg lung water content)} = (\text{[wet lung weight]} - \text{[dry lung weight]})/\text{dry lung weight}.$$