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Effect of hydrogen-rich water on acute peritonitis of rat models

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ABSTRACT

Objective: To study the effect of hydrogen-rich water (HRW) on acute peritonitis with three different rat models.
Methods: Acute peritonitis was induced by three methods including intraperitoneal injection of lipopolysaccharide (LPS), rats' feces or cecal ligation and puncture (CLP) operation. For each model, male Sprague Dawley rats were used and distributed into saline control group, HRW control group, saline plus model group, and HRW plus model group. Saline or HRW (3 ml per rat) was orally administered by gavage for 7 days beforehand and 3 days after modeling. The efficacy was tested by detecting concentrations of white blood cells (WBCs), plasma endotoxin, interleukin (IL)-6 and tumor necrosis factor (TNF)-α. The activities of malondialdehyde (MDA), myeloperoxidase (MPO) and glutathione (GSH) in visceral peritoneum tissues were also evaluated. Meanwhile, histopathology examination of visceral peritoneum was performed using hematoxylin and eosin staining. The expression and location of nuclear factor kappaB (NF-κB) in the visceral peritoneum were detected by immunohistochemistry.
Results: Three models showed the same result that hydrogen-rich water had an efficient protective effect on acute peritonitis. HRW could significantly lower the levels of WBCs, plasma endotoxin and cytokines, enhance GSH activity and reduce MPO and MDA activities in the peritoneum tissue when compared with that of groups with only saline treated. Simultaneously, we found that HRW could also decrease the NF-κB expression in the peritoneum tissues.
Conclusion: Hydrogen-rich water could alleviate the severity of acute peritonitis, and it might perform this function by its anti-inflammation, anti-oxidation and anti-bacterial effects and reducing NF-κB expression in the peritoneum tissues.

1. Introduction

Peritonitis is a common postoperative complication that can develop into lethal sepsis in case of delayed diagnosis or inappropriate treatment, whose mortality doubled in the past decades [1,2]. In the United States, about 750,000 people developed into sepsis each year, with a mortality rate of 28.6% and an approximate cost of $16.7 billion [3,4]. Although high-class antibiotics and advanced intensive care have proven to be effective on the treatment, the morbidity and mortality remain kept at a high level. The pathophysiology of peritonitis is complicated and is involved in various processes, of which, the most important one is the inflammatory reaction [5]. Local intra-abdominal focus of inflammation caused by the microorganisms can promote the synthesis and secretion of massive inflammatory cytokines, which would destroy the endothelial junctions and provide access for bacteria into the systemic circulation leading to lethal bacteremia [6,7]. A more severe inflammatory response process usually indicates a much higher mortality. Meanwhile, the oxidative stress induced by the direct effect of bacteria and indirect effect of inflammation also contributes to the severity of peritonitis [8]. Overproduction of reactive oxygen can not only result in the direct organ injury but also exacerbate the inflammatory reaction simultaneously [9]. In terms of the deeper molecule mechanisms, it is supposed that the microorganisms and their components can immediately activate the transcription factors—nuclear factor-κB (NF-κB). NF-κB can initiate gene expression of cytokines, adhesion molecules, chemokines, and cytotoxic enzymes, which are considered to be directly responsible for the organ injury and death [10–13]. During the pathological process of the peritonitis, NF-κB plays an activating role in the inflammatory reaction, which might be a potential therapeutic target in the future clinical work [14].

Hydrogen therapy is a new medical approach which has gotten a rapid development in the past several years [15]. In 2007, Ohsawa et al. found that inhalation of hydrogen gas significantly suppressed brain injury by buffering the effects of oxidative stress in an acute focal ischemia and reperfusion rat model [16]. And then in 2008,

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Buchholz et al. found that the hydrogen therapy could inhibit the inflammatory reaction in the rat model of small intestinal transplantation [17]. Contemporarily, Chen et al. proved that the hydrogen therapy had a protective effect against acute pancreatitis for its ability to inhibit oxidative stress, apoptosis, NF-κB activation, etc [18]. In 2010, Xie et al. found that hydrogen had the protective effects on sepsis and sepsis-associated organ damage mainly relied on its anti-oxidative property [19]. Up to now, three main methods including inhalation, oral intake of hydrogen-rich water (HRW) and injection of hydrogen-saturated saline have been developed and proved to be valid and reliable to deliver the hydrogen.

The most common experimental models used in acute peritonitis research generally pursue three strategies, including injection of lipopolysaccharide (LPS) into the abdominal cavity [20], injection of feces into the peritoneal cavity [21], and cecal ligation and puncture (CLP) operation [22]. Our study was designed to investigate the potential therapeutic effects of hydrogen on the peritonitis in three above-mentioned rodent models and try to find out the possible mechanism.

2. Materials and methods

2.1. Experimental animals and HRW

The study was conducted using male Sprague Dawley rats (210–260 g) (Animal Feeding Center of Xi'an Jiaotong University Medical School). All rats were housed (5 per cage) in conventional animal facilities with 12:12 light/dark cycle. The study was approved by the Animal Research Committee in Xi'an Jiaotong University Medical School. Animals received care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals. The hydrogen-rich water adopted Izumio drinking water (Naturally Plus Japan International Co.). The main technology of this product is dissolving the hydrogen in water under high pressure to the supersaturated level using a gas-rich water-producing apparatus, and storing it under atmospheric pressure at 4 °C in an aluminum bag with no dead volume. The gas chromatography was used to confirm the content of hydrogen (hydrogen concentration of the HRW we used in this research: 0.62–0.82 mM).

2.2. Experimental design

Acute peritonitis was induced by three different experimental methods: 1) by intraperitoneal administration of LPS, 2) by intraperitoneal administration of rats' fecal suspension and 3) by cecal ligation and puncture (CLP) operation.

Model 1. Acute peritonitis was induced by intraperitoneal injection with LPS (10 mg/kg, LPSL2880, from *Escherichia coli* 055:B5, Sigma Chemical Co, St. Louis, MO, USA) [20,23].

Model 2. Acute peritonitis was induced by intraperitoneal injection of fecal slurry (6.25 ml/kg), which was prepared from the bowel contents of a rat from the same batch, suspended in saline and filtered to remove fibrous material [24,25].

Model 3. Acute peritonitis was induced by cecal ligation and puncture (CLP) operation. All animals were anesthetized, shaved and prepared with iodine. Through a midline laparotomy, the cecum was filled with feces by milking the stools back from the descending colon and then ligated just below the ileocecal valve with a 3-0 silk ligature. The anterior mesenteric cecal surface was punctured twice with a 23-gauge needle below the ligature, the bowel was placed back into the peritoneal cavity, and the abdomen was closed in two layers. The operative procedure was done under aseptic conditions [22,26].

Overall, for each model, male Sprague Dawley rats were divided into four groups randomly, consisting of 6–17 animals each: saline control group, HRW control group, model group and HRW plus model group. Saline or HRW (6 ml/kg per rat) was orally administered by gavage for 7 days beforehand and 3 days after modeling (daily at 10:00 AM). On the 8th day, acute peritonitis was induced by the aforementioned three methods. Normal control and HRW control groups were given intraperitoneal injection of saline (models 1 and 2) or suture following laparotomy only (model 3). One milliliter of preheat sterile saline was administered s.c. for fluid resuscitation in models. On the 10th day, rats were sacrificed by euthanasia to collect the blood and tissue samples. The detailed experimental protocol was shown in the Supplementary Fig. 1.

2.3. Analytical measurements

Blood samples were collected from the cut tail (6 h, 24 h, and 48 h after modeling) and cardiac puncture (72 h after modeling) and divided into whole blood and plasma for further assays. The concentration of circulating white blood cells (WBCs) was determined using a hematology analyzer. Plasma concentrations of TNF-α and IL-6 were measured using enzyme-linked immunosorbent assays. Endotoxin concentrations were measured by the Limulus Amebocyte Lysate test (Dakewe Biotech Co.)

2.4. Visceral peritoneum enzymatic activity assay

Activities of malondialdehyde (MDA), myeloperoxidase (MPO) and glutathione (GSH) from the visceral peritoneum (greater omentum) tissue were measured with the activity assay kits from NanJing JianCheng Bioengineering. The harvested greater omentum was homogenized with 10 volumes of potassium phosphate buffer (20 mmol/L, 0.1 M, pH 7.4) containing potassium chloride (30 mmol/L) and then centrifuged at 1500 *g* for 15 min. The supernatants were collected, liquoted, and stored at −80 °C until the following analysis. The detection was conducted following the reference manual.

2.5. RNA isolation and quantitative reverse transcription–polymerase chain reaction (qRT–PCR) analysis

Visceral peritoneum tissue samples from each group were snap-frozen in liquid nitrogen and stored at −70 °C until the experiments. Total RNA was isolated from cells using the RNAfast200 Kit (Fastagen Biotech, Shanghai, China). Reverse transcription was performed using the PrimeScript RT reagent Kit (TaKaRa Biotechnology, Dalian, China). The mRNA expression was assayed in triplicate and normalized to the β-actin mRNA expression. The relative levels were calculated using the Comparative-Ct Method (ΔΔCt method). The following primers were used for qRT–PCR, NF-κB:

5′-CAGCCTTCCCCACTAAAATAACC-3′ (sense) and
5′-ACCCACAAAAACCCTGCTCTG-3′ (antisense); β-actin:
5′-ATCGTGCGTGTGACATTAAGGAG-3′ (sense) and 5′-AGGAAGGA AGGCTGGAAGAGTG-3′ (anti-sense); All primer pairs were synthesized by TaKaRa.

2.6. Histological and immunohistochemistry study

Samples from the visceral peritoneum were fixed in 10% formalin solution and embedded in paraffin after completion of the routine follow-up. Serial sections of 5-μm thickness were obtained and stained with hematoxylin/eosin (HE) to evaluate gastric morphology. To establish the immunolocalization of NF-κB, a mouse polyclonal antibody (Beijing Biosynthesis Biotechnology Co., LTD) was used at a working dilution of 1:50. The antibody was applied directly to sections, and slides were incubated overnight at 4 °C in a humidified chamber. Immune complexes were subsequently treated with the secondary antibody (containing anti-rabbit and anti-mouse immunoglobulins) and detected via application of streptavidin peroxidase treatment for 20 min at room temperature. After rinsing sections with three changes