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Hydrogen-rich saline resuscitation alleviates inflammation induced by severe burn with delayed resuscitation

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ARTICLE INFO

Article history:
Accepted 22 July 2014

Keywords:
Hydrogen-rich saline resuscitation
Inflammatory reaction
Severe burns
Delayed resuscitation

ABSTRACT

Severe burns with delayed resuscitation are associated with high morbidity which is attributed to ischemia–reperfusion injury. This study was undertaken to investigate the effect of hydrogen-rich saline known as a significant selective antioxidant on the inflammatory reaction induced by severe burns with delayed resuscitation. By establishing the model of severe burns with delayed resuscitation in rats, we recorded improvement on the mortality, secretion of cytokines and reaction of oxidative stress of rats treated with hydrogen-rich saline. We found that resuscitation by hydrogen-rich saline alleviated inflammation significantly. We further detected the change of the key nuclear factor NF-κB contributed to inflammation. The expression of both NF-κB and phosphorylated NF-κB in rats having severe burns with delayed resuscitation by hydrogen-rich saline was lower than that in rats with delayed resuscitation with Ringers' solution. Our data imply that hydrogen-rich saline significantly improves the inflammatory reaction in rats with severe burns with delayed resuscitation, possibly by inhibiting activation of NF-κB.

1. Introduction

Burn shock resuscitation is one of the key therapies in burn care, and severe burns with delayed resuscitation, defined as fluid resuscitation begun 6 h after injury, are associated with high morbidity and mortality [1,2]. There is no effective treatment clinically, so it is urgent to solve this sophisticated problem [2].

Mounting evidence suggests that ischemia–reperfusion injury contributes to the tissue or organ damage, and the mainstay underlying mechanism is oxidative stress evoking

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http://dx.doi.org/10.1016/j.burns.2014.07.012
0305-4179/

inflammatory reaction. Persistent hypoperfusion resulted in hindrance of cellular energy metabolism which is involved in hypoxanthine as a substrate for xanthine oxidase. The process of reperfusion initiates the reaction between hypoxanthine and xanthine oxidase which produce abundant deleterious free radicals, for example, superoxide anion radical ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2). Furthermore, adherent activated neutrophils are another important resource of free radicals in burn trauma. These free radicals contribute to additional tissue injury by upregulating the transcription factor nuclear factor kappa B (NF-κB) to promote transcription and translation of numerous inflammatory cytokines [3].

Recently, we found that hydrogen exhibited significant antioxidant effects with certain unique properties in varied disease models of oxidative stress. Research demonstrated that H_2, acting as selective antioxidant is permeable to cell membranes and acts within cells [4,5]. However, the effect of hydrogen on burn shock with delayed resuscitation has not been reported [4,6,7].

The purpose of the study was to determine whether hydrogen diminishes the inflammatory reaction induced by severe burns with delayed resuscitation in a rat model that is of particular clinical significance when discussing the treatment for severe burns with delayed resuscitation.

2. Materials and methods

2.1. Animals

All animal experimental procedures and protocols in this study were carried out according to the National Institutes of Health guidelines on the use of experimental animals and were approved by Institutional Animal Care and Use Committees (IACUC) at the Third Military Medical University. Male Wistar rats weighing 200–250 g were purchased from the Experimental Animal Department of the Third Military Medical University, Chongqing, China. All animals were maintained under specific pathogen-free conditions. They were divided into four groups: sham: Ringer solution injection; immediate resuscitation: severe burns + immediate resuscitation with Ringers' solution; Ringer' resuscitation: severe burns + delayed resuscitation with Ringers' solution; and of hydrogen resuscitation: severe burns + delayed resuscitation by hydrogen saline.

Preparation of Hydrogen-Rich Saline: H_2 was dissolved in normal saline under 0.4 Mpa pressure to a supersaturated level and stored at 4 °C. Hydrogen water was freshly prepared every week in order to maintain the final concentration of hydrogen above 0.6 mmol/L and gas chromatography was used to confirm the content of hydrogen in saline [7].

Severe burns with delayed resuscitation: all animals were deprived of any food and drinking water for 12 h before injury and anesthetized with 3% pentobarbital sodium (30 mg/kg) by intraperitoneal injection (IP) injection. After shaving the hair of dorsal area and abdomen, 40% total body surface area (TBSA) burn was induced for 18 s by a desktop temperature controller (YLS-QS) maintaining a temperature of 98 °C. The depth of the burn wound was confirmed by pathology. According to the Parkland Formula, 4 ml/kg/TBSA Ringer solution or hydrogen-rich saline was injected by femoral vein for resuscitation at 6 h after burn. During 6–8 h after burn, half of the volume of fluid infusion was given and the residual volume was given at between 9 and 24 h after burn.

2.2. Detection of Malondialdehyde and 8-hydroxy-2′-deoxyguanosine levels

Malondialdehyde (MDA) content in blood was detected by chemical method kits (Nanjing Jiancheng Biochemistry, Nanjing, China) according to the manufacturer's instructions. Absorbance at 532 nm was measured using a spectrophotometer. The level of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was also determined by the method of enzyme-linked immunosorbent assay (ELISA) (Cell BioLabs). All standards and samples were run in duplicate.

2.3. Measurements of interleukin (IL)-1β, interleukin (IL)-6, tumor necrosis factor (TNF)-α

Blood was collected by femoral vein at different experimental time points. After standing 30 min, blood was centrifuged at 500 × *g* for 5 min at room temperature. The supernatant was collected and stored at −80 °C. The levels of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α were measured by ELISA according to the instruction of the manufacturer (R&D Systems).

2.4. Isolation and counting of monocytes

Blood was collected into a heparinized tube by the femoral vein at different experimental time points and was diluted with phosphate buffered saline (PBS) at the ratio of 1:2. The blood was then added into rat cellular segregation slowly, and centrifuged at 300 × *g* for 40 min at 4 °C. The pellet containing monocytes and red blood cells was collected and then red blood cells were eliminated by red blood cell lysis solution.

2.5. Western analysis of NF-κB and phosphorylated NF-κB

Total protein was extracted using the total protein extraction kit (KeyGen), which contained protease and phosphatase inhibitors. The concentration of protein was determined using Pierce BCA Protein Assay Kit (Thermo Scientific). The primary antibodies were used as follows: anti-NF-κB p65 antibody (1:2000) (Abcan) and anti-NF-κB p65 (phospho S529) (1:1000) (Abcan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the loading reference for data analysis.

2.6. Statistical analysis

Results were expressed as means ± standard deviation (SD). Data were analyzed with one-way analysis of variance by Statistical Package for the Social Sciences (SPSS) 19.0. *P* value <0.05 was considered significant.