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Seawater immersion aggravates burn-associated lung injury and inflammatory and oxidative-stress responses

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ABSTRACT

With the increasing frequency of marine development activities and local wars at sea, the incidence of scald burns in marine accidents or wars has been increasing yearly. Various studies have indicated that immersion in seawater has a systemic impact on some organs of animals or humans with burn. Thus, for burn/scald injuries after immersion in seawater, it is desirable to study the effects and mechanisms of action on important organs. In the present study, we aimed to investigate the effect of immersion in seawater on lung injury, inflammatory and oxidative-stress responses in scalded rats. The structural damage to lungs was detected by hematoxylin and eosin staining and the results showed that seawater immersion aggravated structural lung injury in scalded rats. The expression of HMGB1 in lung tissues was detected by immunohistochemical analysis and the results showed that seawater immersion increased HMGB1 expression in lung tissues of scalded rats. Apoptosis in lung tissues was detected by terminal deoxynucleotidyl transfer-mediated dUTP nick end-labeling (TUNEL) staining and the results showed that seawater immersion increased apoptosis rate in lung tissues of scalded rats. In addition, the expression levels of TNF-α, IL-6, IL-8, SOD, and MDA in serum were analyzed by enzyme-linked immunosorbent assays (ELISAs) and the results showed that seawater immersion induced secretion of proinflammatory factors (TNF-α, IL-6, and IL-8), increased MDA protein level, and suppressed SOD activity in the serum of scalded rats. Furthermore, measurement of plasma volume and pH showed that seawater immersion decreased plasma volume and pH value. Overall, the results indicated that all effects induced by immersion in seawater in scalded rats are more pronounced than those induced by freshwater. In conclusion, seawater immersion may aggravate lung injury and enhance inflammatory and oxidative-stress responses after burn.

1. Introduction

Burn is a major cause of premature death or disability among military personnel or civilians involved in accidents, conflicts, and terrorist attacks worldwide [1]. Severe burns over 20% of the total body surface area (TBSA) result in inflammatory response and oxidative stress [2,3]. Increased civil and military activities at sea will likely augment the incidence of burns occurring at sea. Wound surface is likely to be polluted by

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seawater. Seawater has such characteristics as low temperature, alkalinity, high permeativeness, and high concentrations of sodium, potassium, calcium, magnesium, and other electrolytes [4]. When a wound is immersed into hypertonic seawater, this procedure increases vascular leakage, cell dehydration, and exacerbates microcirculation and energy metabolic aberrations in the injured tissue [4]. After immersion of lesions of burn into seawater, the damaged vessels are exposed to a hypertonic environment, and seawater infiltrates the damaged blood vessels. One study showed that seawater immersion after a burn appears to be associated with severe hemodynamic changes and results in progressive dysfunction of the circulatory system, leading to a fatal outcome and decreasing the survival period [5]. In addition, seawater immersion aggravates endothelial dysfunction caused by burn, thereby increasing the risk of thrombosis [6]. It is sure that immersion in seawater has a systemic impact on some organs of animals or humans with burn. Thus, for burn/scald injuries immersed in seawater, it is desirable to study the effects and mechanisms of action on the relevant organs.

The respiratory injuries are one of the main factors responsible for high mortality in burned patients [7–9]. Lungs are key organs of the respiratory system. Burn can induce pulmonary fibrosis, pulmonary edema, even lung failure [10,11]. In addition, one study showed that lung injury induced by seawater is more severe than that caused by freshwater [12]. Nonetheless, the effect of immersion in seawater after a scald burn on lung injury has not been reported to date. In the present study, we aimed to investigate the effects of immersion in seawater on lung injury and inflammatory and oxidative-stress responses in scalded rats. The structural damage of lungs was detected by hematoxylin and eosin (H&E) staining. The expression of High-mobility group box 1 (HMGB1), functioned as a proinflammatory protein [13], in lung tissues was detected by immunohistochemical analysis. Apoptosis in lung tissues was detected by terminal deoxynucleotidyl transfer-mediated dUTP nick end-labeling (TUNEL) assay. In addition, we measured the expression levels of TNF-α, IL-6, IL-8, SOD, and MDA in serum by enzyme-linked immunosorbent assays (ELISAs) to detect inflammatory and oxidative-stress responses.

2. Materials and methods

2.1. Animals and the model implemented

Sixty-three Sprague-Dawley (SD) rats (4–6 weeks old, 90–120 g) were obtained from the Animal Experiment Center of Southern Medical University (Guangzhou, China). SD rats were kept in separate cages under standard conditions and were supplied with water *ad libitum*. Anesthesia was induced with 3% sodium thiopentone by intraperitoneal injection. After the backside of the rats was depilated, heated water maintained at 95°C was applied for 12 s to create a model of 20% total body surface area (TBSA) scalded deep II° in the back. Then, all SD rats were immediately subdivided into three groups: control scalding group (control), scalding plus freshwater immersion group (freshwater), and scalding plus seawater immersion group (seawater). Rats were fixed to avoid inhale water. After immersion for 2, 4, or 8 h, seven rats from each group were euthanized. Before euthanasia, arterial blood was collected for ELISAs and measurement of plasma volume and pH. After euthanasia, left lung tissues were excised for histopathological analysis. All the experimental work was carried out in an animal room at 24°C±2°C maintained by air-conditioning. The deep part of the wound was identified by histopathological analysis. All animal experiments were approved by the ethics committee of Southern Medical University. All animal experiments complied with the ARRIVE guidelines and was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Measurement of the lung injury score

For histopathological examination, lung tissues of each group were quickly fixed in 10% formalin for 24 h. After dehydration and paraffin embedding, lung tissues were sectioned into 7-μm thickness slices. After that, slices were stained with H&E for histopathological examination. The lung injury score of each slide was assessed by two pathologists as previously described [14]. The lung injury score of each slide are the average of the scores produced by these pathologists. Each section was scored according to the following four items: alveolar septal congestion, (ii) alveolar hemorrhage, (iii) intra-alveolar cell infiltrates, (iv) intra-alveolar fibrin deposition. Each item was scored from 0 to 3 in an injury field. Points were assigned according to the criteria as previously described [14]: alveolar septal congestion, 0: all septae are thin and delicate, 1: congested alveolar septae occur in <1/3 of the field, 2: congested alveolar septae occur in 1/3–2/3 of the field, 3: congested alveolar septae occur in >2/3 of the field; alveolar hemorrhage, 0: no hemorrhage, 1: at least 5 erythrocytes per alveolus in 1–5 alveoli, 2: at least 5 erythrocytes in 5–10 alveoli, 3: at least 5 erythrocytes in >10 alveoli; intra-alveolar fibrin deposition, 0: no intra-alveolar fibrin, 1: fibrin deposition in <1/3 of the field, 2: fibrin deposition in 1/3–2/3 of the field, 3: fibrin deposition in >2/3 of the field; intra-alveolar cell infiltrates, 0: 0–5 intra-alveolar cells per field, 1: 5–10 intra-alveolar cells per field, 2: 10–20 intra-alveolar cells per field, 3: >20 intra-alveolar cells per field. All of the points for each item were weighted according to their relative importance. The total injury score was calculated in accordance with below formula: total lung injury score = [(points of alveolar septal congestion/number of fields) + (points of alveolar hemorrhage/number of fields) + 2 × (points of intra-alveolar cell infiltrates/number of fields) + 3 × (points of intra-alveolar fibrin deposition/number of fields)] ÷ total number of alveoli counted.

2.3. HMGB1 immunohistochemical analysis

Endogenous peroxidases in paraffin slices (7 μm thick) of lung lesions were quenched with H_2O_2 (3% in methanol) for 10 min, and samples were boiled in sodium citrate buffer for 10 min. After blockage with 1% goat serum, slides were incubated with an anti-HMGB1 antibody (1:100, Affinity Biosciences, Cincinnati, OH, USA) diluted in phosphate-buffered saline (PBS) overnight at 4°C. After washed thrice with PBS with 1‰ Triton

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