Evaluation of different treatment protocols for combined injury-induced lung injury in rabbits

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ABSTRACT

BACKGROUND: This study aims to evaluate the effectiveness of different treatment regimens on combined injury-induced lung injury.

METHODS: Rabbits were subjected to non-lethal closed-chest bilateral lung contusion followed by a 30% total body surface area scald burn. The rabbits were randomly assigned to resuscitation groups that maintained a minimum mean arterial blood pressure of 70 mmHg using one of the following three methods: normal saline plus polygeline injection in a ratio of 1:1 (1:1G), normal saline plus polygeline injection in a ratio of 1:2 (1:2G), and normal saline plus polygeline injection in a ratio of 1:3 (1:3G). After injury, lung injury was assessed using lung wet-to-dry (W/D) weight ratio, enzyme-linked immunosorbent assay, and real-time PCR.

RESULTS: In the 1:3 fluid resuscitation group, rabbits exhibited significantly reduced lung W/D ratio, alveolar hemorrhage, myeloperoxidase activity, and IL-8 and TNF-α levels in the serum compared with the 1:1 or 1:2 fluid resuscitation groups. The 1:3 fluid resuscitation-treated rabbits also attenuated ultrastructural changes in the lung 24 h after the combined injury.

CONCLUSION: This study demonstrated the impact of fluid resuscitation on combined injury-induced lung injury. Further, 1:3 fluid resuscitation treatment at the early stage of lung injury after combined lung contusion and burn injury was found to be more effective.

Keywords: Acute lung injury; burn injury; fluid resuscitation; lung contusion.

INTRODUCTION

Combined lung contusion (LC) and burn injuries increases morbidity and mortality by promoting severe lung injury and hemodynamic shock.[1–3] Burn injury alone, even in the absence of smoke inhalation, often causes damage to the lung tissue.[4] In recent years, increasing evidence has suggested that neutrophils play an important role in the pathophysiology of burn-induced lung injury.[5,6] LC is associated with acute respiratory failure, exhibiting as clinical acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).[7,8]

Proper fluid resuscitation is critical for the survival of the victim of a major burn injury.[9,10] With adoption of weight and injury size-based formulas for resuscitation, multiple organ dysfunctions and inadequate resuscitation have become uncommon. However, LC is sensitive to fluid resuscitation after trauma. Such treatment can increase the lung water content and lead to desaturation.[11] The decision to use colloid or crystalloid solutions for fluid resuscitation of critically ill patients remains controversial.

In the light of these findings, we hypothesized that fluid resuscitation can provide protection against combined LC and burn injury-induced pulmonary damage. We aimed to investigate whether and to what extent fluid resuscitation reduces this damage by determining the presence of oxidative tissue injury using biochemical and histological parameters.

MATERIALS AND METHODS

Animals

Eighty healthy adult male New-Zealand rabbits weighing between 3.1 and 3.3 kg were used for all experiments. Rabbits were housed in microisolator cages under specific pathogen-free conditions. All animal experiments were approved by the Institutional Animal Care and Use Committees at first center hospital.
Combined Lung Contusion and Burn Injury

Rabbits were anesthetized with 50 mg/kg intramuscular ketamine hydrochloride. Each rabbit’s right hemithorax was drawn, and a 350-g metal cylinder was dropped from a height of 150 cm in the supine position, as described in the study by Raghavendran et al.[12] Trauma was standardized by applying 5.14 J of energy on the chest region according to the formula. The impact energy (E) of the falling weight was calculated using the following equation: 

\[ E = m \times g \times h \]

(150 cm).[13]

Rabbits subjected to combined injury were subjected to LC, followed by induction of 30% TBSA full-thickness burns on the back with 90°C water for 9 s.

The rabbits were randomly assigned to resuscitation groups that maintained a minimum mean arterial blood pressure of 70 mmHg using one of the following three methods: normal saline plus polygeline injection in a ratio of 1:1 (1:1G) (n=20), normal saline plus polygeline injection in a ratio of 1:2 (1:2G) (n = 20), normal saline plus polygeline injection in a ratio of 1:3 (1:3G) (n=20), and control rabbits which were anesthetized, but were not injured and did not receive treatment (n=20).

Lung Histopathology

The lung was excised and perfused with 10% PBS buffered formalin. After fixation, lung tissues were embedded with paraffin and sectioned (5 μm sections). The sections were stained with hematoxylin & eosin.

Electron Microscopy

Lung tissues were post-fixed in 1% osmium tetroxide in sodium phosphate buffer and then processed and embedded in epoxy resin. Thin sections were installed on copper grids and observed on a transmission electron microscope (TEM).[14]

Wet/Dry Weight Determinations

Lung samples were separated and weighed immediately after removal to determine the wet weight. The sample was oven-dried at 65°C for 24 h and re-weighed. The lung wet-to-dry weight ratio was calculated as the index of lung water content.

Measurement of Myeloperoxidase (MPO) Activity

Lungs of the rabbits from all groups were separated and homogenized. The homogenates were used to observe MPO activity. Briefly, weighed lungs were thawed and homogenized in a homogenate medium. The homogenates were then performed according to the manufacturer’s instructions.

Measurement of Cytokine Levels in the Serum

TNF-α and IL-8 serum levels in the rabbits were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

Total RNA Extraction and Real-Time PCR

Total RNA was isolated from the lung samples using a QIAGEN RNeasy Mini Kit (Dusseldorf, Germany) and treated with RNase-free DNase. RNA was reverse transcribed, and cDNA was subjected to PCR for analyzing the expression of IL-8 and TNF-α. IL-8 sense, 5’-CAA ACC TTTCCA CCC CAA AT-3’ and IL-8 anti-sense, 5’-ATT GCA TCT GCC AAC CCT AC-3’, amplified fragment length 572 bp; and TNF-α sense, 5’-TTATCT CTC AGC TCC ACG CC-3’ and TNF-α anti-sense, 5’-TGC GCA CTG AAA GCA TGA TC-3’, amplified fragment length 383 bp.

RESULTS

Fluid Resuscitation Reduces Pulmonary Edema Induced By Combined Injury

Pulmonary edema is one of the most characteristic pathologic changes in burn-induced lung injury.[15] Meanwhile, LC leads to hypoxemia severe enough to qualify as ALI/ARDS. The lung W/D ratio was examined to determine the effect of combined injury on pulmonary edema. As shown in Figure 1, the lung W/D ratio in 1:3 fluid resuscitation-treated rabbits was substantially higher than that in the control group (p<0.05) and lower than that in the 1:2 fluid resuscitation group. The lung W/D ratio in the 1:2 fluid resuscitation group was lower than that in the 1:1 fluid resuscitation group (p<0.05), which suggests that pulmonary edema alleviated when rabbits with combined injury were treated with fluid resuscitation.

Fluid Resuscitation Protects Against Histopathologic Changes Induced By Combined Injury

As illustrated in Figure 2b, c, the lungs in rabbits with combined injury show apparent proinflammatory changes characterised...
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terized by lung edema, alveolar hemorrhage and neutrophil infiltration, and destruction of the epithelial and endothelial cell structure. Furthermore, 1:3 fluid resuscitation-treated rabbits showed significant alleviation of interstitial edema formation at 24 h (Fig. 2d). Conversely, no destructive changes were observed in lung tissues from the control group (Fig. 2a).

Figure 2. Histological evaluation of fluid resuscitation-treated rabbits following combined injury. These images are representative of the treatment groups at 24 h post injury. (a) control group, (b) 1:1 fluid resuscitation group, (c) 1:2 fluid resuscitation group, and (d) 1:3 fluid resuscitation group. (H&E staining ×200) (n=10 rabbits per group, CG: control group, 1:1G: 1:1 fluid resuscitation group, 1:2G: 1:1 fluid resuscitation group, and 1:3G: 1:1 fluid resuscitation group).

Figure 3. Electron microscopic findings of the lamellar body. (a) Electron micrograph of the lungs of the control animal that did not have an injury and did not receive any treatment. (b) TEM image of the lamellar body in the 1:1 fluid resuscitation group at 24 h after injury. (c) TEM image of the lamellar body in the 1:2 fluid resuscitation group at 24 h after injury. (d) TEM image of the lamellar body in the 1:3 fluid resuscitation group at 24 h after injury. (n=10 rabbits per group, CG: control group, 1:1G: 1:1 fluid resuscitation group, 1:2G: 1:1 fluid resuscitation group, and 1:3G: 1:1 fluid resuscitation group).
Ultrastructural Changes in Combined Injury-Induced Lung Injury Compared With Controls

The degree of lung injury was further observed by TEM examination of the different experimental groups. TEM exhibited that the control group had a normal lung tissue structure. Compared with other groups, we identified lamellar bodies with few cavitations in the 1:3 fluid resuscitation group (Fig. 3).

Lung MPO Activity in Lung Injury

Neutrophil accumulation in the lungs was determined using MPO activity assays. MPO expression was markedly up-regulated in fluid resuscitation-treated rabbits (Figure 4). This increase in MPO levels was clearly weakened in 1:3 fluid resuscitation-treated rabbits and differed obviously from that in MPO levels in 1:1 or 1:2 fluid resuscitation treatment after injury (p<0.05) (Figure 4). MPO estimates in the control group and at 24 and 72 h after combined injury were significantly diverse from those at all other time points in both groups (p<0.05) (Figure 4).

Figure 4. The MPO activity in lung homogenates. Fluid resuscitation inhibits myeloperoxidase (MPO) activity in combined injury-induced ALI. After 24 and 48 h interventions, rabbits were sacrificed, and their lungs were removed. The MPO activity was measured to assess the accumulation and activation of neutrophils in the lung tissues. (Data are denoted as means±s.e.m, n=10 rabbits per group, *p<0.05 compared with CG. *p<0.05 compared with 1:1G. *p<0.05 compared with 1:2G. CG: control group, 1:1G: 1:1 fluid resuscitation group, 1:2G: 1:1 fluid resuscitation group, and 1:3G: 1:1 fluid resuscitation group).

Figure 5. Effects of fluid resuscitation on inflammatory cytokine expression in combined injury-induced ALI rabbits. Serum and lung samples were obtained 24 or 48 h after combined injury to analyze inflammatory cytokine, including IL-8 (A), TNF-α (B), IL-8 mRNA (C), and TNF-α mRNA (D), levels. (Data are denoted as means±s.e.m, n=10 rabbits per group, *p<0.05 compared with CG. *p<0.05 compared with 1:1G. and *p<0.05 compared with 1:2G. CG: control group, 1:1G: 1:1 fluid resuscitation group, 1:2G: 1:1 fluid resuscitation group, and 1:3G: 1:1 fluid resuscitation group).
Fluid Resuscitation Decreases Local and Systemic Inflammatory Mediator Levels Induced By Combined Injury

Inflammatory mediators play an important role in the pathogenesis of burn-induced ALI. TNF-α and IL-8 levels in the serum were determined by ELISA. Combined injury showed elevated TNF-α and IL-8 levels in the serum in 1:1 and 1:2 fluid resuscitation-treated rabbits. 1:3 fluid resuscitation treatment lowered these mediator levels in the blood (Figure 5a, b). In line with the measurements of the serum, 1:3 fluid resuscitation-treated rabbits had substantially lower TNF-α mRNA and IL-8 mRNA expression levels in the lungs (Figure c, d).

Statistical Analyses

We used Student's t-test for comparing differences between groups. P<0.05 was considered to be statistically significant. Data are expressed as mean±standard error of the mean (s.e.m.).

DISCUSSION

We were able to demonstrate a reduction in the severity of combined injury-induced lung injury in 1:3 fluid resuscitation-treated rabbits. 1:3 fluid resuscitation-treated rabbits with combined injury exhibited lower pulmonary edema, alveolar hemorrhage, and neutrophil invasion after injury. The generation of proinflammatory mediators, IL-8 and TNF-α, was markedly downregulated in the 1:3 fluid resuscitation group. Under conditions of combined injury, we also demonstrated lower neutrophil recruitment in the lung.

Fluid resuscitation following burn injury must support organ perfusion with the least amount of fluid necessary and the least physiological cost. Resuscitation fluids are broadly categorized into colloid and crystalloid solutions. However, it is unclear whether fluid resuscitation is closely associated with combined injury. To the best of our knowledge, this study was the first to compare strategies for fluid resuscitation after combined injury in rabbits over a midterm period of 48 h with histopathology and pathophysiology. Our results suggest that fluid resuscitation attenuates lung injury in rabbits after combined injury. This is supported by three findings. First, colloids are more effective than crystalloids in early resuscitation of patients in shock when administered during surgery. Our data are in agreement with these findings. However, we showed that an increase in the MPO activity was clearly diminished in 1:3 fluid resuscitation-treated rabbits and significantly differed from that in 1:1 and 1:2 fluid resuscitation-treated rabbits at 24 or 48 h. Third, patients with ALI/ARDS had persistent elevations in inflammatory cytokines (TNF-α, IL-1β, and IL-6) levels in the plasma, hypothalamic-pituitary-adrenal axis hormones, and similar severity of organ dysfunction scores. Colloids have a possible advantage over crystalloids when used for initial hemodynamic stabilization of critically ill patients. Similarly, 1:1 fluid resuscitation treatment increased the release of IL-8 (Figure 5a) and TNF-α (Figure 5b) in the lung compared with 1:3 fluid resuscitation treatment. In the air space, an alveolar macrophage secretes cytokines, IL-8 and TNF-α, which act locally to stimulate chemotaxis and activate neutrophils. Our research also shows that IL-8 and TNF-α levels were significantly affected at 24 h post injury in the 1:3 fluid resuscitation group compared with those in the 1:1 fluid resuscitation group (Figure 5c, d). Collectively, these findings suggest that fluid resuscitation significantly attenuates lung injury in rabbit. In conclusion, we were able to show that after combined injury, 1:3 fluid resuscitation-treated animals develop a less severe lung injury than different fluid resuscitation-treated animals. Further studies are needed to better understand the effects of fluid resuscitation on both neutrophil development and function in pulmonary inflammation.

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Conflict of interest: None declared.

REFERENCES

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