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Trauma and hemorrhagic shock activate molecular association of 5-lipoxygenase and 5-lipoxygenase–activating protein in lung tissue

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ABSTRACT

Background: Post-traumatic lung injury following trauma and hemorrhagic shock (T/HS) is associated with significant morbidity. Leukotriene-induced inflammation has been implicated in the development of post-traumatic lung injury through a mechanism that is only partially understood. Postshock mesenteric lymph returning to the systemic circulation is rich in arachidonic acid, the substrate of 5-lipoxygenase (ALOX5). ALOX5 is the rate-limiting enzyme in leukotriene synthesis and, following T/HS, contributes to the development of lung dysfunction. ALOX5 colocalizes with its cofactor, 5-lipoxygenase–activating protein (ALOX5AP), which is thought to potentiate ALOX5 synthetic activity. We hypothesized that T/HS results in the molecular association and nuclear colocalization of ALOX5 and ALOX5AP, which ultimately increases leukotriene production and potentiates lung injury.

Materials and methods: To examine these molecular interactions, a rat T/HS model was used. Post-T/HS tissue was evaluated for lung injury through both histologic analysis of lung sections and biochemical analysis of bronchoalveolar lavage fluid. Lung tissue was immunostained for ALOX5 and ALOX5AP with association and colocalization evaluated by fluorescence resonance energy transfer. In addition, rats undergoing T/HS were treated with MK-886, a known ALOX5AP inhibitor.

Results: ALOX5 levels increase and ALOX5/ALOX5AP association occurred after T/HS, as evidenced by increases in total tissue fluorescence and fluorescence resonance energy transfer signal intensity, respectively. These findings coincided with increased leukotriene production and with the histological changes characteristic of lung injury. ALOX5/

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ALOX5AP complex formation, leukotriene production, and lung injury were decreased after inhibition of ALOX5AP with MK-886.

Conclusions: These results suggest that the association of ALOX5/ALOX5AP contributes to leukotriene-induced inflammation and predisposes the T/HS animal to lung injury.

Introduction

Despite advances in the management of trauma, shock, and postinjury critical care, post-traumatic lung injury leading to acute respiratory distress syndrome (ARDS) continues to result in compelling morbidity and high cost of care.[1] Prior investigation has shown that lung dysfunction following trauma and hemorrhagic shock (T/HS) is exacerbated by the presence of proinflammatory molecules derived from arachidonic acid (AA). Specifically, postshock mesenteric lymph contains free AA that has been shown to activate the leukotriene biosynthetic pathway.[2-4] The rate-limiting enzyme in this pathway is arachidonate lipoxygenase-5 (ALOX5), which ultimately results in the production of bioactive leukotrienes. The inhibition of the ALOX5 pathway has been shown to reduce lung damage following T/HS in a murine model.[5] Arachidonate lipoxygenase-5 activating protein (ALOX5AP) is a cofactor of ALOX5 that is thought to have two primary roles: (1) potentiating ALOX5 catalysis and (2) anchoring complexed ALOX5/ALOX5AP to the nuclear membrane.[6] In addition, selective inhibition of ALOX5AP attenuates leukotriene synthesis.[7]

Although recent evidence has suggested a mechanism for ALOX5/ALOX5AP binding at the nuclear membrane in the presence of AA,[6] the role of the localized ALOX5/ALOX5AP complex in the development of lung injury after T/HS has not been firmly established. Furthermore, it is not known whether inhibition of ALOX5AP results in the inhibition of ALOX5/ALOX5AP association and colocalization. Finally, the inhibition of ALOX5/ALOX5AP association as a method to curtail the development of post-traumatic lung injury has not been explored. Therefore, we hypothesize that molecular association of ALOX5 and ALOX5AP is a necessary condition for the production of the proinflammatory leukotrienes that contribute to the development of post-traumatic lung injury.

Materials and methods

All animal experiments were performed using the recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and approved by the University of Colorado—Denver Institutional Animal Care and Use Committee. Adult male Sprague—Dawley rats (Harlan Laboratories, Indianapolis IN) of uniform weight (range 350-425 g) were supplied with food and water ad libitum, maintained in climate-controlled facilities with 12-h light/dark cycles, and allowed to acclimate for at least 1 wk before experimentation. No female rats were included in this study due to their resistance to end organ dysfunction following T/HS.[8] Sample size calculation was performed with two-tailed tests with α set to 0.05, power at 80%, and an estimated standard deviation of 20%. We wanted to be able to detect an effect size of 35%-40%, giving us a sample size of 6-8 animals per group.

Control, trauma/sham shock, and trauma/hemorrhagic shock models

All treatment groups contain 6-8 animals, unless otherwise stated. Control animals were overdosed with sodium pentobarbital and immediately sacrificed. Trauma/sham shock (T/SS) animals were anesthetized with 50 mg/kg intraperitoneal sodium pentobarbital (Abbot Labs, Chicago IL). Surgical cannulation of the femoral artery and vein was performed using PE-50 polyethylene tubing (Baxter Healthcare, Deerfield, IL). Heart rate and blood pressure were continuously monitored via the arterial cannula. Core temperature was measured via rectal probe, and normothermia was maintained using a radiant warmer throughout the experiments. A tracheostomy was created, and the animals were allowed to spontaneously ventilate on 30% FiO_2 at 2 L/min. After an observation period of 45 min, a midline laparotomy was performed to simulate mild traumatic injury. These animals were subsequently maintained under anesthesia with of pentobarbital redosing as needed (1 mg/kg) for 3 h before sacrifice (Fig. 1).

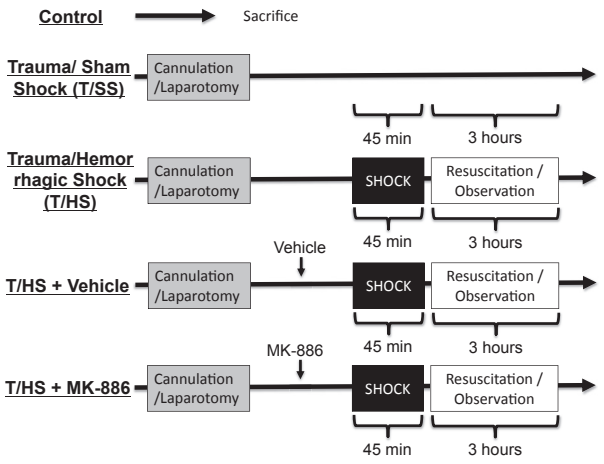

Fig. 1 – Experimental design. A standardized rodent model of trauma/sham shock (T/SS) and trauma/hemorrhagic shock (T/HS) was used. Control animals were immediately sacrificed. After anesthesia, tracheostomy and femoral arterial and venous cannulation was performed. Next, a laparotomy was created to simulate trauma. Hemorrhagic shock was induced to achieve a mean arterial pressure of 30-32 mmHg for 45 min. Animals were then resuscitated with crystalloid followed by a period of observation. Vehicle and the ALOX5AP inhibitor MK-886 were administered before initiation of shock.