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A model of recovery from inhalation injury and cutaneous burn in ambulatory swine

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ABSTRACT

Inhalation injury commonly accompanies thermal injury, increasing the likelihood of mortality and multiple organ dysfunction (MOD). Large animal models have given important insight into the pathophysiology of this injury; however recapitulating late MOD has remained difficult. The current report describes experiments using a smoke inhalation and burn model, with follow-up of ambulatory swine for 14 days with bronchoscopy, CT scanning, and bronchoalveolar lavage fluid (BALF)/blood collection. Clinically, animals cleared airway damage in the first several days after-injury. This was mirrored with erythematous airways on day 2 after-injury, which resolved by the end of the experiment, as did parenchymal damage seen on CT. An initial rise in the protein content of BALF immediately after-injury was followed by a dramatic increase in the concentration of leukocytes. Circulating neutrophils increased while lymphocytes decreased; both correlated with cell counts in BALF. IL8 levels in BALF increased 30-fold and remained elevated throughout the experiment. IL1ra increased circulation immediately after-injury, and afterwards in BALF. Other cytokines (TNFα, IL12) transiently increased in BALF (and decreased in circulation) on day 2. Taken together, these results display a remarkable capability for the lungs to recover in the absence of intubation, with further evidence of the role of cytokines such as IL8 and IL1ra. The possible exacerbating effects of clinical practices such as ventilation and bronchoscopies should be considered.
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Abbreviations: MOD, multiple organ dysfunction; ICU, intensive care unit; CT, computed tomography; BALF, broncheoalveolar lavage fluid; IL, interleukin; TNF, tumor necrosis factor; TBSA, total body surface area; ARDS, acute respiratory distress syndrome; BL, baseline; PI, post-injury; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COhb, carboxyhemoglobin.

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1. Introduction

Burns are a common occurrence, with an estimated 11 million people worldwide seeking medical treatment for burns [1]. Smoke inhalation injury occurs frequently with burns, and an estimated 10–20% of patients present with inhalation injury [2]. Along with age and percent total body surface area burned (% TBSA), inhalation injury is one of the most powerful predictors of mortality, especially at the mid-range levels of age and burn size [3,4]. In treatment of inhalation injury, intubation increases the risk of ventilator-associated pneumonia and subsequent acute respiratory distress syndrome (ARDS) [2,5]. Indeed, it has recently been shown in a military population that approximately one-third of all intubated burn patients have inhalation injury, and that mortality increased in a step-wise fashion with severity of acute respiratory distress syndrome (ARDS) [6].

Damage to the lungs (i.e., ARDS) represents one organ system implicated in the development of multiple organ dysfunction (MOD) which also includes hepatic, renal, cardiovascular, and hemodynamic and nervous systems depending on the MOD scoring equations used [7]. Burn patients are over 2.5 times likely to develop severe MOD if they have inhalation injury [8]. While there is a significant cohort of these patients that die early after-burn due to the initial injury, another population of patients die later after injury due to MOD, usually within 2 weeks [9]. The mechanisms for both inhalation injury and late MOD remain unclear, but have been linked to several different inflammatory mediators [4,10].

Animal models have afforded invaluable insight into burn pathophysiology [11], with porcine models being regarded as a superior clinical surrogate for burn research [12,13]. However, few studies have examined systemic inflammation in conscious, extubated swine due to burn and inhalation injuries over a recovery period of weeks [14]. Manipulation of the inflammatory response after combined burn and inhalation injuries is of great interest, highlighted by a recent study examining the utility of extracorporeal cytokine removal in a porcine model [15]. That study was among others that examine survival and inflammation in an ICU setting over 1–4 days. By contrast, the purpose of the study described herein was to examine the feasibility of creating an animal model of late MOD due to combined smoke and inhalation injury, with specific emphasis on pulmonary function. In this study we sought to preserve natural compensatory mechanisms by, for example, avoiding mechanical ventilation and deep sedation during the recovery period.

2. Materials and methods

2.1. Animals

Four female Yorkshire swine (Midwest Research Swine, Gibbon, MN) weighing 42.3±1.1 kg were used in this study. Animals were followed up for 14 days until euthanasia for harvesting of tissues. Animals were singly housed with ad libitum access to water, and were allowed to acclimate to the facilities for at least 7 days prior to any procedures. This protocol was approved by the Animal Care and Use Committee, US Army Institute of Surgical Research under protocol A15-026. This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.

2.2. Anesthesia

Animals were fasted the night before any anesthetic event to avoid emesis, but were given access to Laboratory Mini-Pig Grower (LabDiet, St. Louis, MO, Cat #5081) upon recovery from anesthesia. Anesthesia was induced with an intramuscular (IM) injection of tiletamine–zolazepam (Telazol, 6 mg/kg), at which time vitals (body temperature, pulse, respiratory rate) were taken at 5 min intervals for 30 min. On day 0, as well as burn days 2, 7, and 14 (for CT/bronchoscopy) the animals were then intubated with an endotracheal tube and placed on a ventilator with the initial tidal volume at 10 mL/kg and respiration rate of 8–12 breaths per minute. The ventilator settings were adjusted to maintain an end tidal PCO_2 of 40 ±5 mmHg, and anesthesia was maintained with 1%–3% isoflurane and 100% oxygen.

While under anesthesia, analgesics were given in the form of IM injection of buprenorphine extended release (Buprenex, 0.25 mg/kg) which was repeated every 72 h. Additionally, hair was removed from the dorsum, flanks, and legs with shaving cream and a razor. These areas were cleaned with chlorohexidine. On days 1, 2, 3, 7, 10 and 14 after-burn, the animals were also briefly sedated with an IM injection of tiletamine-zolazepam (Telazol, 6 mg/kg) in order to obtain vitals, blood samples, and to perform bronchoscopies and CT scanning (on days 2, 7, and 14 only).

2.3. CT Scanning and bronchoscopies

Chest CT scans were acquired at both pre (BL-baseline) as well as on days 2, 7, and 14 in both end-inspiratory and end expiratory phases with a Toshiba Aquilion Prime 160 scanner (model TSX-303A, Toshiba America Medical Systems, Irvine, CA) at the following settings: 140 mAs, tube voltage 120 kVp, 0.5 s rotation time; 32 mm-0.5 mm collimation; pitch 0.85, reconstruction matrix of 512×512, pixel size 0.625 mm×0.625 mm; no tube current modulation; 5 mm section width and 5 mm interval; body standard axial filter. For bronchoscopies, an Olympus Evis Exera II, BF Type Q180 pediatric video bronchoscope (Olympus America, Inc., Center Valley, PA) was used at the timepoints listed above. During the procedure, digital pictures of the main carina, and the trachea at the level of the accessory lobe were taken.

During each bronchoscopy procedure, 60 mL of saline was instilled through the first sub-segmental bronchus of the right main bronchus lobe, and collected as bronchoalveolar lavage fluid (BALF) for cell and protein analysis. All BALF samples were stained with Protocol® HEMA3® differential stain (Fisher Scientific Company, LLC)for cell analysis, and protein content was determined with a BCA assay (Thermo Fisher). After each bronchoscopy, animals were extubated and returned to their home cages for recovery.

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