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Regional pulmonary inflammation in an endotoxemic ovine acute lung injury model

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ABSTRACT

The regional distribution of inflammation during acute lung injury (ALI) is not well known. In an ovine ALI model we studied regional alveolar inflammation, surfactant composition, and CT-derived regional specific volume change (sVol) and specific compliance (sC). 18 ventilated adult sheep received IV lipopolysaccharide (LPS) until severe ALI was achieved. Blood and bronchoalveolar lavage (BAL) samples from apical and basal lung regions were obtained at baseline and injury time points, for analysis of cytokines (IL-6, IL-1β), BAL protein and surfactant composition. Whole lung CT images were obtained in 4 additional sheep. BAL protein and IL-1β were significantly higher in injured apical *vs.* basal regions. No significant regional surfactant composition changes were observed. Baseline sVol and sC were lower in apex *vs.* base; ALI enhanced this cranio-caudal difference, reaching statistical significance only for sC. This study suggests that apical lung regions show greater inflammation than basal ones during IV LPS-induced ALI which may relate to differences in regional mechanical events.

1. Introduction

Sepsis-induced acute lung injury (ALI), including its most severe form the acute respiratory distress syndrome (ARDS), is a common and frequently fatal disease triggered by a wide array of insults that lead to a severe refractory hypoxemia. Despite greater awareness and research efforts, mortality has stabilized around 30–40%, probably due to the complex and progressive pathophysiology and lack of effective therapies (Brun-Buisson et al., 2004; Rubenfeld et al., 2005). It is well known that inflammation is a central component of this complex pathophysiology. Inflammatory mediators (i.e., TNF-α, IL-6, and IL-1β) have been widely studied in ALI, and have been found significantly increased in BAL fluid from septic ALI patients (Mokart et al., 2003; Pugin et al., 1996). Increases in plasma and bronchoalveolar lavage (BAL) levels of both IL-6 and IL-1β have been identified as predictors of poor outcome in patients with septic or non-septic ALI (McClintock et al., 2008; Meduri et al., 1995; Parsons et al., 2005; Ware et al., 2010). Lung inflammation and resultant capillary leak and edema cause a decrease in lung compliance and increase in shunt fraction that compromises the ventilatory management of these patients. Lung strain triggers a local release of inflammatory mediators (Dos Santos and Slutsky, 2000; Halbertsma et al., 2010; Pinheiro de Oliveira et al., 2010; Simon et al., 2006) and impacts the release and effectiveness of alveolar surfactant (Hillman et al., 2011; Ito et al., 1997; Majumdar et al., 2012; Veldhuizen et al., 2002), potentially propagating the mechanical injury. In summary, mechanical ventilation is both life-saving and an iatrogenic source of further alveolar inflammation and surfactant damage.

CT imaging has proven useful as a tool for exploring the pathophysiology of ALI. CT imaging techniques have increased our understanding of the diffuse and heterogeneous inflammatory

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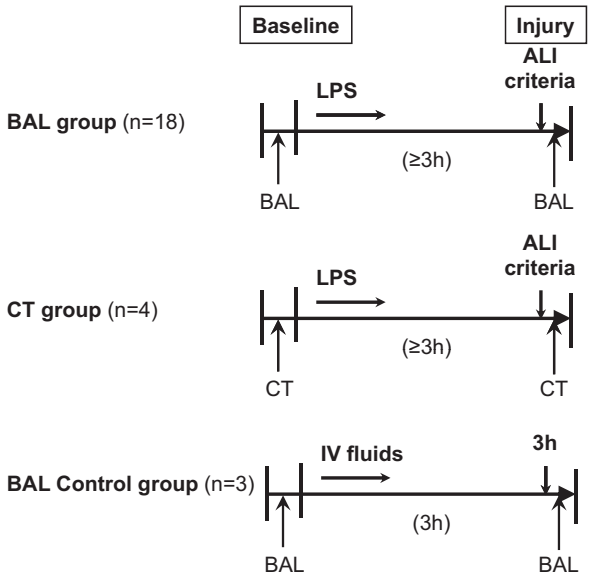

Fig. 1. Schematic description of experimental groups.

process of the lung during ALI (Fernandez-Bustamante et al., 2009; Gattinoni et al., 2001; Kaczka et al., 2005), hypoxemia mechanisms (Easley et al., 2006), PEEP and recruitment maneuvers (Gattinoni et al., 2006; Vieira et al., 1998), and positioning (Galiatsou et al., 2006; Gattinoni et al., 1991). CT scans in patients and experimental ALI have demonstrated cranio-caudal (Gattinoni et al., 1986; Puybasset et al., 1998) and ventral-dorsal (Fernandez-Bustamante et al., 2009; Hedlund et al., 1985; Slutsky et al., 1984) gradients in aeration and recruitment, and a recent PET-CT study associated regional parenchymal stretch with regional inflammation (Bellani et al., 2011). However, to our knowledge no studies have explored whether regional differences in CT-derived measurements of regional lung mechanics reflect differences in underlying regional alveolar inflammation during septic ALI.

In this study we examined the cranio-caudal (apex vs. base) changes of: (1) inflammatory mediators (IL-6, IL-1β) and surfactant content and composition in the bronchoalveolar lavage fluid and (2) CT-derived parameters of regional aeration and lung mechanics (specific volume change, sVol, and specific compliance, sC), in a severe endotoxemic ALI ovine model. Our hypothesis was that CT-derived regional sVol and sC would reflect regional differences in pulmonary inflammation during endotoxemia and mechanical ventilation.

2. Methods

LPS-induced severe ALI was induced in a total of 22 anesthetized sheep. In 18 sheep we studied the composition of plasma and bronchoalveolar lavage (BAL) fluid at the apex and the base (2 samples per region) before ("Baseline") and after the LPS-induced ALI ("Injury"). The other 4 sheep underwent exactly the same animal preparation and injury protocol, but instead of BAL we performed whole lung inspiratory and expiratory CT images at the same baseline and injury time points. CT images were analyzed for mean aeration of the whole lung and of apex and base regions, and regional tidal specific volume change (sVol) and specific compliance (sC) (Fuld et al., 2008) were calculated as a measure of biomechanical stretch during the respiratory cycle. Finally, to study the potential effect of dilution from repeated BALs at the same location, repeated BALs were performed in 3 additional uninjured sheep at the same apex and base locations at baseline and after 3 h of mechanical ventilation (BAL Control group). Fig. 1 shows a schematic description of the experimental protocol in the 3 groups.

All studies were performed by the same team; the CT imaging studies were performed at the University of Iowa Comprehensive Lung Imaging Center (iCLIC) while all other studies were performed in the research laboratory at the Johns Hopkins University School of Medicine.

2.1. Animal preparation

The animal protocol was approved by both the Johns Hopkins University and the University of Iowa Institutional Animal Care and Use Committees. Adult farm bred sheep weighing 25–45 kg were sedated with intramuscular ketamine (30 mg/kg) and anesthetized with halothane by mask. Sheep were instrumented with percutaneous external jugular central venous and pulmonary artery catheters, a femoral arterial catheter was placed via cut down, and a tracheotomy performed. Subsequent anesthesia was maintained with pentobarbital (1–3 mg/kg IV every hour and when indicated) and pancuronium (0.1 mg/kg and 0.5–1 mg hourly IV). Animals were mechanically ventilated with the following parameters: tidal volume (VT) 10 ml/kg, frequency titrated for a target end-tidal CO_2 partial pressure (PET_{CO_2}) 30–40 mmHg, 5 cmH_2O positive end-expiratory pressure (PEEP), and inspired oxygen fraction (Fi_{O_2}) 1.0. Mechanical ventilation was performed with a Drager Evita 2 ventilator (Drager Medical, Germany). Oxygen saturation (SaO_2), PET_{CO_2}, airway pressure (Paw), heart frequency (fH), arterial blood pressure (BP), and pulmonary artery pressure (PAP) were continuously monitored. Arterial and mixed venous blood samples were periodically obtained during the experiment for assessment of gas exchange per protocol. An infusion of lactated ringers (14 ml/kg/h) was given for maintenance fluid replacement after an initial bolus of 40 ml/kg. PA temperature was maintained at ≥36 °C with radiant heat lamps. At the end of the experiment, all animals were euthanized with an extra dose of pentobarbital followed by a bolus of saturated KCl.

2.2. Acute lung injury (ALI) model

Arterial blood samples were analyzed before the induction of ALI ("Baseline" time point) to assure the adequate initial health status of the animal, considering unacceptable any subject with a baseline arterial oxygen partial pressure to inspired oxygen fraction ratio (Pa_{O_2}/Fi_{O_2}) less than 400.

For induction of ALI, *E. coli* lipopolysaccharide 055:B5 (Sigma L4005; Sigma Chemical, St. Louis, MO) was intravenously infused at 10 μg/kg/h and stopped when venous admixture (Qs/Qt) ≥ 40% and $Pa_{O_2}/Fi_{O_2} \leq 200$ were obtained. ALI was considered established when these endpoints persisted for 20 min ("Injury" time point). Based on pilot studies designed to promote a uniform distribution of LPS in the pulmonary circulation, all subjects were positioned prone for the first 3 h of LPS infusion and turned supine from that moment on or when injury endpoints were reached. This LPS administration protocol was chosen to achieve greater homogeneity of ventral/dorsal LPS vascular distribution and consequent injury, but the position changes confound interpretation of ventral/dorsal regional analysis of the resulting injury.

We compared parameters of regional (apex *vs.* base) lung inflammation and CT-derived lung aeration and lung mechanics before the induction of ALI (Baseline) and after ALI endpoints were reached (Injury).

2.3. Regional lung inflammation

Bronchoalveolar lavage (BAL) was performed in each sheep in the supine position at four lung locations: two in the left apex (gravitationally non-dependent and dependent segments) and two in