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Effects of lipopolysaccharide-induced inflammation on initial lung fibrosis during open-lung mechanical ventilation in rats

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ABSTRACT

This study aimed to assess the impact of pulmonary inflammation on early fibrotic response in rats challenged with increasing doses of lipopolysaccharide (LPS). Twenty-four rats were randomized and infused with three different increasing doses of continuous LPS infusion ($n = 8$/group) while being ventilated with low tidal volumes and open-lung positive end-expiratory pressure. Another eight animals served as uninjured control group. Hemodynamics, gas exchange, respiratory system mechanics, lung histology, α-smooth muscle actin, plasma cytokines, and mRNA expression of cytokines and type I and III procollagen in lung tissue were assessed. We found impaired hemodynamics and gas exchange as well as higher histological lung injury scores and α-smooth muscle actin expressions in the medium LPS dose compared to control and the lower LPS dose. The highest LPS dose did not cause further aggravation of these findings. In all LPS groups type I and III procollagen decreased compared to controls and there was a negative correlation between type III procollagen-RNA expression and proinflammatory mediators.

1. Introduction

The acute respiratory distress syndrome (ARDS) is characterized by acute onset of bilateral pulmonary infiltrates and hypoxemia caused by different pulmonary and extrapulmonary diseases (Force et al., 2012). Histomorphological post-mortem examinations reveal a distinctive chronological pattern of inflammation and fibrosis (Thille et al., 2013) resulting in an accumulation of intraparenchymatous collagen (Zapol et al., 1979) in lung tissue.

In the late stages of the disease, type I collagen (PCI) fibers, which are thick and exhibit cross-linked fibrils, are prevalent (Pelosi and Negrini, 2008). In the initial phase of lung injury, however, there is a predominance of type III collagen (PCIII) fibers, which are more flexible and susceptible to breakdown. This is an important observation, as there appears to be a correlation between the initial detection (Madtes et al., 1998) and amount (Marshall et al., 2000) of PCIII and ARDS-related mortality.

The initial synthesis of PCIII can be modulated experimentally through various treatment strategies. Silva et al. found an increase of PCIII RNA expression in hypervolemic rats with acute lung injury

Abbreviations: α-SMA, α-smooth muscle actin; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BL, baseline; CINC-1, cytokine-induced neutrophil chemoattractant 1; Ecw, chest wall static elastance; EL, lung static elastance; ELISA, enzyme-linked immunosorbent assay; Ers, respiratory system static elastance; FiO_2, fraction of inspired oxygen; g, gram; h, hour; H_2O, water; HR, heart rate; I:E, inspiratory/expiratory ratio; IL-1β, interleukin-1β; IL 6, interleukin 6; i.p., intraperitoneal; Kg, kilogram; LPS, lipopolysaccharide; MAP, mean arterial pressure; mg, milligram; mRNA, messenger RNA; OL-MV, open lung mechanical ventilation; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen; PaO_2/FiO_2, ratio of partial pressure arterial oxygen/fraction of inspired oxygen; PC I, type I procollagen; PC III, type III procollagen; PCR, polymerase chain reaction; PEEP, positive end expiratory pressure; pH, negative logarithm of the molar concentration of dissolved hydronium ions; Pinsp, ed-inspiratory plateau pressure; Pmean, mean airway pressure; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; UI, uninjured control group; V_T, tidal volume.

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induced by cecal ligation and puncture compared to normovolemic animals (Silva et al., 2010). Other authors reported effects on early PCIII RNA expression caused by different recruitment maneuvers to open up atelectatic lung regions (Rzezinski et al., 2009; Santiago et al., 2010; Silva et al., 2013), different positive end-expiratory pressure (PEEP) levels to keep these atelectatic lung regions open during end-expiration (Farias et al., 2005; Passaro et al., 2009), as well as different applied tidal volumes (de Carvalho et al., 2007; Krebs et al., 2010). Finally, the application of assisted compared to controlled mechanical ventilation (Saddy et al., 2010) reduced PCIII RNA expression in experimental ARDS.

Therefore, knowing the time course of lung fibrosis from the early to late phases of ARDS, as well as the effects of different ventilatory strategies that may affect pulmonary fibrosis, might be a valuable tool to reduce ARDS morbidity and mortality.

In recent studies from our group (Krebs et al., 2010, 2011), reduced expression of PCI and PCIII mRNA was found in rats ventilated with open-lung mechanical ventilation (OL-MV), consisting of an inflating maneuver followed by individualized titrated PEEP and low tidal volumes. These findings were consistent in uninjured animals and animals challenged with a continuous infusion of either lipopolysaccharide (LPS) (extrapulmonary injury model) or a saline washout model (pulmonary injury model) and treated with conventional or high-frequency oscillatory ventilation.

The aim of the present study was to evaluate the effects of increasing LPS doses on systemic and pulmonary inflammation and on the early fibrotic response. We hypothesized that, when using a lung-protective ventilator strategy, the progressive LPS-induced inflammatory response suppresses the early fibrotic response.

2. Materials and methods

The study was approved by the Institutional Review Board for the care of animal subjects of the University of Heidelberg, Mannheim, Germany. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the U.S. National Academy of Sciences.

2.1. Animal preparation

A total of 32 male Wistar rats (450–500 g) were housed in standard conditions with food and water ad libitum. Twenty-four rats were randomized and infused with three different increasing doses of continuous *Escherichia coli* LPS (O55:B5, Sigma–Aldrich, Hamburg, Germany) infusion [0.075 (LPS 0.075), 0.75 (LPS 0.75) or 1.5 (LPS 1.5) mg/kg/h] ($n = 8$/group). All animals in the three LPS groups were primed with 1 mg/kg LPS intraperitoneally (i.p.) 24 h prior to the experiment, as previously described (Krebs et al., 2010). Another eight animals received 1 mL saline i.p. 24 h prior to the experiment, but no LPS during the experimental period, and served as uninjured (UI) control group. Anesthesia was induced by i.p. injection of ketamine hydrochloride (50 mg/kg; Ketanest 10%®, Pfizer, Karlsruhe, Germany) and xylazine (2 mg/kg; Rompun®, BayerVital, Leverkusen, Germany) and maintained with ketamine as needed. One femoral artery was cannulated for continuous measurement of mean arterial pressure (MAP) and heart rate (HR) and intermittent collection of blood samples for blood gas analysis. Both femoral veins were cannulated with polyethylene catheter tubing for maintenance of anesthesia and continuous infusion of LPS. Esophageal pressure was measured using an appropriate catheter whose correct positioning was confirmed as previously described (Talmor et al., 2006). Animals were then transferred to a heating blanket, placed in the supine position, tracheotomized, intubated with a 14G catheter (Kliniject, KLINIKA Medical GmbH, Usingen, Germany), and mechanically ventilated with a neonatal respirator (Babylog 8000, Dräger, Lübeck, Germany). Initially, a pressure-controlled mode was used with a PEEP of 2 cm H_2O, an inspiratory/expiratory ratio (I:E) of 1:1 and a fraction of inspired oxygen (FiO_2) of 0.5. FiO_2 was maintained constant throughout the entire experimental period. End-inspiratory plateau pressure (Pinsp) was adjusted to maintain a V_T of 6 mL/kg. Respiratory rate was adjusted to keep $PaCO_2$ within physiological range. MAP was kept above 60 mmHg during the experiment using additional fluid boluses of balanced electrolyte solution (Deltajonin, Deltaselect GmbH, Munich, Germany) and norepinephrine as needed. Fluid volume, weight gain, and norepinephrine requirements were recorded for the 6-h experimental period.

2.2. Experimental protocol

After a stabilization period of 15 min, the LPS infusion in the LPS 0.075, LPS 0.75 and LPS 1.5 groups was started. Measurements of hemodynamic, respiratory system mechanics and gas exchange parameters were obtained (baseline, UI BL and LPS BL), after which all groups were ventilated for 6 consecutive hours using OL-MV (Krebs et al., 2010, 2011, 2014). Briefly, lungs were inflated with a continuous positive airway pressure of 25 cmH_2O for 40 s, followed by a decremental PEEP titration starting at 10 cmH_2O which was reduced every 10 min in steps of 2 cmH_2O, until the respiratory system static elastance (Ers) no longer decreased while V_T was kept at 6 mL/kg. "Optimal" PEEP (defined as PEEP at minimal Ers) was applied throughout the 6-h experimental period after re-inflation. Hemodynamics, respiratory mechanics and the results of a blood gas analysis were noted hourly. At the end of the experiment (END), PEEP was again reduced to 2 cmH_2O to ensure comparability to BL. Again, a full set of hemodynamics, respiratory mechanics and blood gas measurements were taken. Body weight was noted as well. Heparin (1000 IU) was then injected intravenously and the lungs were excised. During the procedure, the trachea was clamped at 5 cmH_2O. The right lungs were snap-frozen in nitrogen for mRNA extraction and real-time quantitative polymerase chain reaction (PCR) analysis. The left lungs were immersed in 4% formalin and embedded in paraffin for histology.

2.3. Respiratory system, lung and chest wall mechanics

Respiratory mechanics were calculated as previously described (Krebs et al., 2010, 2011, 2014). Respiratory system static elastance (Ers) was computed as the difference between end-inspiratory and end-expiratory tracheal pressure divided by V_T. Chest wall static elastance (Ecw) was calculated as the difference between end-inspiratory and end-expiratory esophageal pressure divided by V_T, and lung static elastance (EL) as Ers–Ecw. All measurements were taken during 3–4 s of end-inspiratory and end-expiratory airway occlusion, respectively.

2.4. Histological examination

For histological examination, hematoxylin–eosin stains were prepared from 4 μm-thick slices of the left lung. Two experienced investigators blinded to group allocation evaluated 10 random non-coincident fields of view using a conventional light microscope at 100× magnification. A well-established (Krebs et al., 2010, 2011, 2014) five-point (ranging from 0 to 4) scoring system (see online supplement for further details) was used to describe the amount of intra- and extra-alveolar hemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and