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Mechanical ventilation increases the inflammatory response induced by lung contusion

Karlijn J.P. van Wessem, MD,[a,*] *Marije P. Hennus, MD,*[b] *Linda van Wagenberg, MD,*[a] *Leo Koenderman, PhD,*[c] *and Luke P.H. Leenen, MD, PhD*[a]

[a] *Department of Trauma Surgery, University Medical Center Utrecht, Utrecht, The Netherlands*
[b] *Department of Pediatric Intensive Care, Wilhelmina Children's Hospital, Utrecht, The Netherlands*
[c] *Department of Respiratory Medicine, University Medical Center Utrecht, Utrecht, The Netherlands*

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ABSTRACT

Background: Posttraumatic lung contusion is common after blunt chest trauma, and patients often need ventilatory support. Lung contusion induces an inflammatory response signified by primed polymorph neutrophil granulocytes (PMNs) in blood and tissue. Mechanical ventilation (MV) can also cause an inflammatory response. The aim of this study was to develop an animal model to investigate the effect of high-volume ventilation on the inflammatory response in blunt chest trauma.

Materials and methods: We assigned 23 male Sprague-Dawley rats to either MV or bilateral lung contusion followed by MV. We used three extra rats as controls. Lung contusion was induced by a blast generator, a device releasing a single pressure blast wave centered on the chest. We determined tissue and systemic inflammation by absolute PMN numbers in blood and bronchoalveolar lavage fluid (BALF), myeloperoxidase, interleukin (IL)-6, IL 1β, growth-related oncogene–KC, and IL-10 in both plasma and BALF.

Results: Survival after blunt chest trauma was correlated to the distance to the blast generator. Compared with controls, both MV and blast plus MV rats showed increased systemic and pulmonary inflammation, expressed by higher PMNs, myeloperoxidase levels, and cytokine levels in both blood and BALF. Blast plus MV rats showed a higher systemic and pulmonary inflammatory response than MV rats.

Conclusions: The blast generator generated reproducible blunt chest trauma in rats. Mechanical ventilation after lung contusion induced a larger overall inflammatory response than MV alone, which indicates that local damage contributes not only to local inflammation, but also to systemic inflammation. This emphasizes the importance of lung protective ventilation strategies after pulmonary contusion.

1. Introduction

Trauma is the leading cause of death in men under 40 y of age [1]. Improvements in seatbelts, airbags, and vehicle constructions have led to a decline in the number of accidental deaths. However, they have resulted in an increased number of surviving victims with blunt chest trauma. In addition, trauma represents the second leading cause of life-threatening acute lung injury [1]. After initial nonfatal injury (thoracic), trauma can induce a systemic

* *Corresponding author*. Department of Trauma Surgery, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Tel.: +31 88 75 598 82; fax: +31 88 75 550 15.
E-mail address: kwessem@umcutrecht.nl (K.J.P. van Wessem).
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inflammatory response by activating the immune system [2]. This activation is mainly caused by primed polymorphonuclear leukocytes, which are prone to home and become activated in the tissues when they encounter additional local inflammatory stimuli. The lung is a preferred site for homing because of the large and narrow microvascular bed and long transit time [3,4]. Excessive immune activation can result in a systemic inflammatory response syndrome and multiple organ dysfunction syndrome [2–5].

Blast injuries are a special form of blunt trauma that cause serious internal injuries, often without evidence of external lesions. Blast injury occurs after a sudden change in pressure, originating from an explosion, for example. The organs most affected by blast injuries are the hollow, gas-filled organs such as ears, lungs, and gastrointestinal tract, and to a lesser extent, the cardiovascular and central nervous system. The lungs are almost always affected by blast injuries.

Treatment of blunt thoracic injury includes resuscitative fluids and respiratory support by mechanical ventilation (MV). Mechanical ventilation in itself can also cause an inflammatory response in the lung by activating the immune system [6–8]. In addition, several studies have demonstrated that when MV was applied in the presence of a pulmonary infection, pulmonary inflammation was further enhanced [9,10]. This phenomenon is also known as a second hit model.

To investigate a possible synergistic inflammatory response between traumatic lung injury and MV, we designed a rat model combining isolated bilateral pulmonary contusion with MV. We hypothesized that a combination of pulmonary contusion and MV would cause a higher inflammatory response than MV alone. A possible synergy is clinically relevant because an increased inflammatory response might cause more inflammation-related complications. This could be influential in choosing MV strategies.

2. Materials and methods

The Animal Care and Use Committee of the University Medical Center Utrecht, Utrecht, The Netherlands, approved this. All animal procedures were carried out in compliance with national and international standards for use of laboratory animals.

2.1. *Animal preparation*

We performed experiments with healthy adult male Sprague-Dawley rats (Harlan, Zeist, The Netherlands) weighing 300–480 g. The animals acclimated for at least 7 d in our animal facility with free access to standard rodent food and water.

2.2. *Experimental design*

We subjected 23 rats to either MV alone (six rats) or MV after blast-induced pulmonary contusion (blast plus MV, 17 rats). Three extra rats were directly killed after induction anesthesia and served as controls to obtain physiological baseline levels.

At the start of the experiment, we anesthetized all rats using inhalation anesthesia (5% isoflurane [Pharmachemie BV, Haarlem, The Netherlands], 1 L/min oxygen, and 1 L/min room air). Next, we maintained anesthesia using a mix of 0.9 mL/kg ketamine (100 mg/mL; AST Farma BV, Oudewater, The Netherlands), 0.5 mL/kg dexmedetomidine (0.5 mg/mL; Orion Corporation, Espoo, Finland) and 0.05 mL/kg atropine (1.0 mg/mL; American Regent, Inc, Shirley, NY) intraperitoneally. We maintained body temperature between 36°C and 38°C with a heating pad to prevent hypothermia. We inserted a silicone catheter (0.02 × 0.037 inches; Degania Silicone Ltd, Hatzor HaGlilit, Israel) into the right carotid artery of all rats to monitor blood pressure and draw blood. Pain was relieved by 0.3 mL buprenorphine, 0.3 mg/mL/h, 10% intramuscularly (AST Farma BV). We performed blood gas analysis at $t = 0$ (only MV rats), 30, 90, 180, 240 and 300 min using a pH/blood gas analyzer (ABL 505; Radiometer, Copenhagen, Denmark). Rats subjected to blast plus MV had no blood gas analysis at $t = 0$ min because that was the time of the blast. Their first blood gas analysis was done after 30 min.

2.3. *Pulmonary contusion*

Animals randomized for pulmonary contusion were subjected to a pressure blast. We induced blunt chest trauma by a single blast wave centered on the chest. For these experiments, we used a blast wave generator previously described by Jaffin *et al.* [11]. The blast generator consisted of two parts. The upper section served as a pressure reservoir and was separated from the lower nozzle by a 50-μm Mylar polyester film (Du Pont, Bad Homburg, Germany). The pressure reservoir was connected to a storage tank of compressed air. Between both components, an electronically releasable high-speed valve and a pressure-reducing valve set to 15 bar were interposed. In the experiments, the blast wave generator was directed with the nozzle toward the animal's chest. The distance between the nozzle and animal's chest varied from 3.5 to 6.0 cm. By opening the high-speed valve, compressed air was delivered into the upper section of the generator. When the pressure in this compartment exceeded the resistance of the polyester diaphragm, the film rapidly ruptured toward the nozzle, releasing a reproducible single blast wave. The reproducibility of each blast wave was assessed by two pressure transducers (TDS3032B; Tektronix, Maarssen, The Netherlands) on both sides of the animal's chest. We recorded both the maximal pressure and the duration of the blast wave.

2.4. *Mechanical ventilation*

We tracheotomized rats and inserted a metal cannula. After the blast, the cannula was connected to a ventilator (Servo Ventilator 900C; Siemens-Elema, Solna, Sweden) and rats were ventilated for 5 h in a pressure-controlled, time-cycled mode (positive end-expiratory pressure 5 cm H_2O, pressure control +20 cm H_2O, FiO_2 0.50, frequency 10–20/min). We made ventilator adjustments only in frequency to aim for normocapnia. Muscle relaxation was attained with 2 mg/kg/90 min pancuronium bromide (Pavulon; Organon Technika, Boxtel, The Netherlands).

To prevent hemodynamic instability during mechanical ventilation, we gave all rats 10 ml/kg/h normal saline, as proposed by Vreugdenhil *et al.* [12].