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Treatment of ventilation-induced lung injury with exogenous surfactant

Abstract  Objective: It has been demonstrated that pulmonary surfactant plays a role in the pathophysiology of ventilation-induced lung injury (VILI). Therefore, we investigated whether exogenous surfactant might restore lung function and lung mechanics in an established model of VILI.  Design: Prospective, randomized, animal study.  Setting: Experimental laboratory of a university.  Subjects: Twenty-four adult male Sprague-Dawley rats.  Interventions: First, a group of six animals were killed immediately after induction of anesthesia and used as healthy controls. Then, in 18 rats, VILI was induced by increasing peak inspiratory pressure (PIP) to 45 cmH₂O without positive end-expiratory pressure (PEEP) for 20 min. Thereafter, animals were randomly divided into three groups of six animals each: one group was killed immediately after VILI and served as VILI-control. In the other two groups, ventilator settings were changed to a PIP of 30 cmH₂O and a PEEP of 10 cmH₂O, and a respiratory rate of 40 bpm. One group received a bolus of surfactant and the other group received no treatment.  Measurements and results: Blood gas tension and arterial blood pressures were recorded every 30 min for 2 h. After the study period, a pressure-volume curve was recorded. Then, a broncho-alveolar lavage (BAL) was performed to determine protein content, minimal surface tension, and surfactant composition in the BAL fluid. Oxygenation, lung mechanics, surfactant function and composition were significantly improved in the surfactant-treated group compared to the ventilated and non-ventilated control groups.  Conclusion: We conclude that exogenous surfactant can be used to treat VILI.

Key words  Ventilation-induced lung injury · Mechanical ventilation · Pulmonary surfactant · Animal · Rat

Introduction

It is known that modes of mechanical ventilation which allow alveolar end-expiratory collapse and/or end-inspiratory alveolar overstretching lead to decreases in lung compliance [1, 2, 3, 4] and gas exchange [5], and result in atelectasis, pulmonary edema, pneumonitis, and fibrosis [6, 7]. Development of intra-alveolar edema in healthy rats subjected to intermittent positive pressure ventilation at high inflation pressures, without positive end-expiratory pressure (PEEP), was first demonstrated by Webb and Tierney, and was later confirmed by Drey-
fuss and colleagues who suggested that high inspiratory lung volumes induce endothelial and epithelial overstretching leading to microvascular injury [8, 9]. However, it is increasingly realized that impairment of the surfactant system plays a key role in the mechanism of ventilation-induced lung injury (VILI) in the above-mentioned model [5, 10, 11, 12]; further, it has been shown that surfactant function is impaired by pulmonary edema constituents [13, 14, 15]. Loss of surfactant function will increase the surface tension at the air-liquid interface of the alveolar walls [1, 2, 3], which will lead, amongst other things, to alveolar collapse and to an increased suction force on the pulmonary interstitium resulting also in alveolar edema [5, 8, 9, 10, 11, 12]. Continuous re-expansion and collapse during the ventilatory cycles causes epithelial and endothelial damage mainly due to shear forces [5, 10]. In addition, we have shown that exogenous surfactant administration preceding mechanical ventilation with high peak inspiratory lung volumes without PEEP, could partially prevent VILI which is characterized, for example, by impaired gas exchange and lung mechanics [11]. In this study, we wanted to investigate whether exogenous surfactant is able to restore gas exchange and lung mechanics in VILI.

**Experimental design**

In order to produce VILI, PIP was increased to 45 cmH₂O and PEEP was decreased to zero for 20 min; other settings were not changed. Thereafter, PIP was decreased to 26 cmH₂O and PEEP was increased to 6 cmH₂O for 5 min, in order to increase arterial CO₂ tension. These ventilator settings were chosen based on a pilot study (unpublished data) in which we observed that when animals were ventilated at 450 cmH₂O (PIP/PEEP, respectively) for 20 min and then ventilated at 30/10 cmH₂O, the animals died from severe hypocapnia. Then, the animals were disconnected from the ventilator to allow some edema fluid (1 ± 0.5 ml) to flow from the lungs; after this procedure the animals were randomized.

**Experimental groups**

The animals were randomized to one of three groups (n = 6). The first group (surfactant), received a bolus of exogenous surfactant (100 mg/kg) intratracheally. The surfactant used was isolated from minced pig lungs, that were processed as previously described [16]. The surfactant suspension, at a concentration of 40 mg/ml, was administered as a bolus followed by a bolus of air 28 ml/kg, directly into the endotracheal tube via a syringe, and was immediately followed by re-connection to the ventilator. Mechanical ventilation was continued at a PIP of 30 cmH₂O, PEEP of 10 cmH₂O, I/E ratio of 1:2, FiO₂ 1.0, and respiratory rate of 40 bpm for 2 h. These ventilator settings, were chosen based on results of a preliminary study which showed that applied ventilation pressures of 26/6 cmH₂O (PIP, PEEP, respectively) and 28/8 cmH₂O were too low to keep animals alive for an observation period of 2 h. The second group (ventilated) did not receive exogenous surfactant, but received a sham bolus of air 28 ml/kg intratracheally and was mechanically ventilated at the same settings as the surfactant group. The third group of animals (VILI-control) was killed after the 5-min ventilation period of 26/6 with an overdose of pentobarbital and was used as a non-treated, non-ventilated control group.

**Material and methods**

**Animal preparation**

This study was approved by the local Animal Committee at the Erasmus University Rotterdam, and the care and handling of the animals conformed with European Community guidelines (86/609/EC).

The study was performed in 24 adult male Sprague-Dawley rats (body weight 280–350 g). Anesthesia was induced with 2% enflurane and 65% nitrous oxide in oxygen, and a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples and continuous monitoring of arterial blood pressure. Immediately after induction of anesthesia, six animals were killed, the thorax was opened, and static pressure-volume curves (P-V curves) were recorded and a bronchoalveolar lavage (BAL) was performed. These animals served as a healthy non-ventilated control (healthy). In the remaining animals, before tracheostomy, the animals received 30 mg/kg pentobarbital sodium, i.p. (Nembutal, Algin, Maassluis, The Netherlands). After tracheostomy, muscle relaxation was induced by pancuronium bromide 0.6 mg/kg, i.m. (Pavolon, Organon Teknika, Boxtel, The Netherlands) immediately followed by connection to a ventilator and a pressure transducer for continuous monitoring of arterial blood pressure. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an inspired oxygen concentration (FiO₂) of 1.0, frequency of 30 breaths per minute (bpm), peak inspiratory pressure (PIP) of 12 cmH₂O, positive end-expiratory pressure (PEEP) of 2 cmH₂O, and inspiratory/expiratory (I/E) ratio of 1:2. Anesthesia was maintained with pentobarbital sodium 30 mg/kg per hour, i.p.; muscle relaxation was maintained with pancuronium bromide 0.6 mg/kg per hour, i.m. Body temperature was kept within normal range by means of a heating pad.

**Gas exchange and hemodynamics**

Arterial blood gas samples were taken in all groups before, after VILI, and at 5 min after the 26/6 period, and in the surfactant and ventilated control groups at 5 min after the 30/10 period, and every 30 min for 2 h. The samples were analyzed for arterial oxygen tension (PaO₂) and arterial carbon dioxide tension (PaCO₂) by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). At the same time points, arterial pressure was recorded. Hemodynamic support was provided by infusion of 1 ml of saline 0.9% (to a maximum of 2 ml per hour) when mean arterial pressure (MAP) decreased below 60 mmHg.

**Pressure-volume curves**

At 120 min after exogenous surfactant therapy all animals were killed with an overdose of pentobarbital sodium injected through the penile vein. Then static P-V curves were recorded. After the thorax and diaphragm were opened, the tracheostomy catheter was connected to a pressure transducer (Validyne model DP 45–32, Validyne Engineering, Northridge, Calif., USA) with a syringe attached to it, and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument, Quincy, Mass., USA). Using a syringe filled with nitrogen (N₂) the lungs were first inflated (within 10 s) to an airway pressure of 35 cmH₂O, which was main-