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Endomicroscopic analysis of time- and pressure-dependent area of subpleural alveoli in mechanically ventilated rats

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ABSTRACT

We investigated the effects of recruitment maneuvers on subpleural alveolar area in healthy rats. 36 mechanically ventilated rats were allocated to either ZEEP-group or PEEP – 5 cmH_2O – group. The subpleural alveoli were observed using a transthoracal endoscopic imaging technique. Two consecutive low-flow maneuvers up to 30 cmH_2O peak pressure each were performed, interrupted by 5 s plateau phases at four different pressure levels. Alveolar area change at maneuver peak pressures and during the plateau phases was calculated and respiratory system compliance before and after the maneuvers was analyzed. In both groups alveolar area at the second peak of the maneuver did not differ significantly compared to the first peak. During the plateau phases there was a slight increase in alveolar area. After the maneuvers, compliance increased by 30% in ZEEP group and 20% in PEEP group. We conclude that the volume insufflated by the low-flow recruitment maneuver is distributed to deeper but not to subpleural lung regions.

1. Introduction

A lot of effort has been made to investigate respiratory mechanics under mechanical ventilation and to link global pulmonary mechanics with the status of alveolar recruitment (DiRocco et al., 2007; Gatto and Fluck, 2004; Schiller et al., 2003). Hence the relationship between global and local respiratory mechanics came into the focus of interest.

In the severely injured lung, alveoli may open and collapse with almost every breath, leading to an exaggeration of lung injury during necessary mechanical ventilation. The reason is transfer of too high mechanical energy from the ventilator to the lung's alveolar tissue. The mechanical behavior of alveoli in the mechanically ventilated healthy lung is largely unexplained. Open alveoli are essential for an efficient gas exchange. From the lack of knowledge about dynamic alveolar mechanics follows that the mechanism of intrapulmonary volume changes during tidal ventilation is not clear. Different mechanisms are being discussed, such as distension and unfolding of the alveolar walls, opening and closing of single alveoli or volume changes of the airways (Hajari et al., 2012; Nieman, 2012; Smaldone and Mitzner, 2012). Supposedly more than one of these mechanisms is involved at the same time.

Unlike in the injured lung, opening and closing of single alveoli does probably not contribute significantly to volume changes in the healthy lung (Mertens et al., 2009). However, there are still mechanisms that can contribute to injury in healthy lungs during mechanical ventilation. While unfolding of alveolar walls happens without a rise in mechanical wall tension at normal tidal breathing, further increase in pulmonary gas volume may lead to an increase in strain of the alveolar walls, which is likely a main cause for ventilator associated lung injury (Gattinoni et al., 2003).

Because of the inconsistent results of past studies, further knowledge of alveolar mechanical behavior is important to estimate its dependence on ventilator strategies and is a prerequisite to developing numerical models of the lung which can help to improve lung-protective ventilation strategies.

Recently, several methods to visualize alveoli have been established. Some of them use open chest preparations (Carney et al., 1999; Pavone et al., 2007), others lung window preparations, for

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example in combination with dark-field intravital microscopy and optical coherence tomography (Mertens et al., 2009).

For analysis of intravital properties of subpleural alveoli we introduced a method based on endoscopic microscopy, allowing direct optical analysis of subpleural alveoli via a minimally opened thorax (Schwenninger et al., 2010).

It is well known that respiratory system compliance increases with pressure or volume respectively up to the range of pulmonary gas volume where overdistension occurs (Gattinoni and Pesenti, 2005; Grasso et al., 2004; Schumann et al., 2011). In the present study we wanted to investigate if in the healthy mechanically ventilated lung an intrapulmonary volume increase (and lung recruitment respectively) comes along with an increase in subpleural alveolar area. To differentiate which factor, pressure or time, is more significant for imposing stress and strain on alveolar walls in the healthy lung, we investigated the dynamic compliance within small volume portions of the tidal volume and the alveolar area within small time-windows during 5-s-periods of relatively low static pulmonary pressure.

2. Methods

The experiments were approved by the review board for the care of animal subjects of the government executive (Regierungspräsidium, Freiburg, Germany, G-12-078) and were carried out in accordance with the German law for animal protection and in compliance with the animal care guidelines of the European Community (86/609/EC).

2.1. Protocol

36 male Wistar rats (Charles River, Sulzfeld, Germany) with an average body weight of 411 ± 42 g were anesthetized with an intraperitoneal injection of 100 mg/kg ketamine (Ketavet®, Pfizer, Karlsruhe, Germany) plus 0.2 mg/kg medetomidine (Domitor®, Pfizer, Karlsruhe, Germany). A venous catheter was placed in a dorsal pedal vein for volume resuscitation (3 ml/h 0.9% NaCl). The animals then were tracheotomised and tracheally intubated. For measurement of arterial blood pressure and for blood gas sampling (i-STAT portable clinical analyzer, Heska Corporation, Loveland, CO), a catheter (Portex Non Sterile Polythene Tubing, 0.58 mm ID, 0.96 mm OD, SIMS Portex Ltd., Kent, UK) was placed in the arteria carotis communis. Subsequently, volume-controlled, pressure-limited ventilation was applied via a small animal ventilator (FlexiVent, Scireq, Montreal, Canada). Ventilation was started with 70 breaths per minute and a tidal volume of 10 ml/kg bodyweight. FiO_2 was 1.0 and positive end-expiratory pressure was set to one of the randomized levels 0 or 5 cmH_2O. Inspiratory and expiratory gas flow rates were measured via two separate flow sensors (Fleisch pneumotachograph 000, Dr. Fenyves and Gut GmbH, Hechingen, Germany). Pressure and flow values were recorded with a sampling rate of 500 Hz using custom software.

For introduction of the endoscope, the intercostal space between the fifth and sixth rib was opened dorsally at the left side of the thorax and a trocar for guiding the endoscopic system was inserted. This trocar was anchored inside the thorax between the ribs and fixed with a screw nut from outside the thorax. The animal was then placed into supine position, and the endoscopic system was inserted through the trocar until its tip touched the surface of the lung (Fig. 1).

In both groups, PEEP 5 cmH_2O and ZEEP group, following a 10 min period for hemodynamic and respiratory stabilization, measurement maneuvers to analyze mechanics of subpleural alveoli and respiratory mechanics of the whole lung were performed every 30 min. O_2 partial pressure (paO_2) was analyzed before and after each respiratory maneuver and the mean arterial pressure was noted.

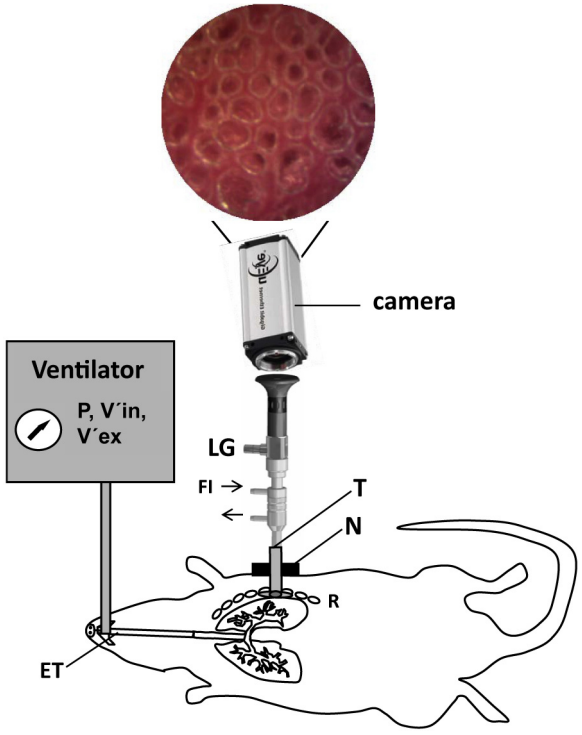

Fig. 1. Schematic view of the experimental setup of the endoscopic in vivo recording of subpleural alveoli in the rat model. The lungs are ventilated via tracheotomy tube by a small animal ventilator. Inspiratory (*V*´in) and expiratory flow (*V*´ex) are measured separately. Airway pressure (*P*) is measured at the proximal end of the endotracheal tube (ET). The endoscope is introduced through a tube (*T*) that is mechanically fixed with a locknut (*N*) between two ribs (*R*). The endoscope has channels for pressure measurement in the field of view (not displayed) and for fluid flushing (FI) and fluid removal (FO), and a connector for a fiber-optic light guide (LG).

At the end of the protocol, after a total experimental time of 120 min, the rat was killed by exsanguination.

2.2. Measurement maneuvers

A "double low-flow-manoeuvre" was programmed in the FlexiVent software, consisting of two consecutive pressure controlled low-flow-maneuvers with a peak pressure of 30 cmH_2O, interrupted by a 5 s plateau phase at different pressures (2, 4, 8 and 12 cmH_2O) (Fig. 2). The same maneuvers were conducted in both groups. In the FlexiVent system ventilation is driven by a piston pump connected to a pressure transducer, measuring the absolute pressure applied and controlling the piston position. The expiratory valve was closed during the maneuver, making plateau pressures lower than PEEP possible. The maneuvers were performed every 30 min in a randomized order.

2.3. Endoscopic system

The endoscopic system consists of a rigid endoscope (Schölly Fiberoptic GmbH, Denzlingen, Germany) inserted in two concentric trocars (6.5 mm o.d.). The system was designed to guide a controlled fluidic flow from the outer toward the inner trocar to create a defined negative pressure at its tip (Fig. 1). Images were recorded using a charge-coupled device camera (UI-5550HEC-HQ, iDS, Obersulm, Germany) connected to the eyepiece of the endoscope. The pressure in the endoscope's field of view was adjusted by controlled flushing and suction through the endoscope so that subpleural