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High tidal volume ventilation does not exacerbate acid-induced lung injury in infant rats

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ABSTRACT

The impact of mechanical ventilation with high V_T-low PEEP in infant rats with preinjured lungs is unknown. After tracheal instillation of saline or acid, two week old rats were ventilated with V_T 7 mL/kg and PEEP 5 cm H_2O or V_T 21 mL/kg and PEEP 1 cm H_2O for 4 h. Airway resistance and the coefficient of tissue elastance, measured via low-frequency forced-oscillation technique, and quasi-static pressure-volume curves deteriorated less with high V_T-low PEEP when compared with low V_T-high PEEP. IL-6 concentration in bronchoalveolar lavage fluid (BALF) did not differ between all ventilated groups. Moreover, differences in BALF protein concentration and histological lung injury scores were independent of applied ventilation strategies. In contrast to experimental studies with adult rats, short-term mechanical ventilation with high V_T-low PEEP is not deleterious when compared to low V_T-high PEEP in both healthy and pre-injured infant rat lungs. Our results call for caution when extrapolating data from adult studies and highlight the need for age-specific animal models.

1. Introduction

Almost 4 decades ago Webb and Tierney (1974) showed that volutrauma during mechanical ventilation with large tidal volume (V_T) induced acute lung injury (ALI) in adult rats with healthy lungs. Further experimental studies demonstrated that additional mechanisms such as repetitive opening and closing of peripheral lung units, shear forces, and inflammation caused by mechanical stressors were involved in the development of ventilator-induced lung injury (VILI) in animal models (Dreyfuss and Saumon, 1998; Slutsky, 1999).

Clinical studies avoiding high V_T ventilation, low positive end-expiratory pressure (PEEP), and high peak inspiratory pressure (PIP) resulted in reduced mortality, improved lung function, and more ventilator-free days in adult humans (Hickling et al., 1990; Amato et al., 1995; ARDS network, 2000). Hence, confirmation of pre-clinical results led to implementation of lung protective ventilation strategies using low V_T, high PEEP, limited PIP, and tolerance towards moderate hypercapnia in clinical practice.

Based on clinical adult studies protective ventilation strategies were also recommended for infants and children with respiratory failure (Mehta and Arnold, 2004; Dahlem et al., 2007; Mesiano and Davis, 2008). Adoption of this concept is mainly due to a lack of both age-specific animal models and large clinical trials in the field of paediatrics (Khemani and Newth, 2010; Kneyber and Rimensberger, 2012) and does not take into account physiological and developmental age differences.

Results from the few infant animal models on VILI clearly demonstrated that younger rats with healthy lungs better tolerate high V_T ventilation when compared to adult rats (Copland et al., 2004; Kornecki et al., 2005). However, it is not known whether infant rats also tolerate a supposedly injurious high V_T-low PEEP ventilation strategy in the context of an ongoing inflammatory process. A key characteristic of adult VILI is that injurious ventilation more heavily affects pre-injured lungs of various etiologies when compared to healthy lungs (Frank et al., 2002; Quinn et al., 2002; Gurkan et al., 2003; Altemeier et al., 2004; Yang et al., 2008). Intratracheal acid instillation is often used to model ALI since it produces direct injury of airways and alveolar epithelium, patchy areas of neutrophilic inflammation, and impairment of lung function (Matute-Bello et al., 2008). Thus, the acid instillation model offers a possibility to investigate mechanical ventilation strategies in both healthy lungs and those with an ongoing inflammatory process.

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The aim of this study was to test how so-called protective and injurious ventilation strategies affect lung function and inflammatory response in infant rats exposed to acid instillation. We hypothesised that acid-induced lung function impairment and inflammatory response are both aggravated by high V_T-low PEEP ventilation.

2. Methods

Experimental protocols were approved by the local Animal Experimentation Ethics Committee and were performed in accordance with Australian guidelines. Rat pups were kept under 12 h light and dark cycle and were housed with their parents and littermates. A total of 50 infant rats were allocated to 2 non-ventilated ($n = 5$ each) and 4 ventilated ($n = 10$ each) study groups. Piebald-Virol-Glaxo (PVG) rats used in this study are generally docile, have good health conditions, and show good breeding performance.

2.1. Acid instillation and animal preparation before mechanical ventilation

After inhalational anaesthesia with methoxyflurane 2 week old infant PVG rats (25.8 ± 1.5 g) were weighed and underwent oral intubation for intratracheal application of 75 μl saline or hydrochloric acid solution. Then, infant rats recovered in a warm environment and had to show adequate behaviour and activity before being returned to the parental cage.

Twenty-four hours later, infant rats were weighed to both ensure normal food intake and to enter current body weight for V_T calculation in the *flexiVent*® system. Subsequently, infant rats were anaesthetised with an i.p. injection of a solution containing ketamine (80 μg/g) and xylazine (13 μg/g). A tracheostomy was performed and a 10 mm polyethylene cannula (ID: 0.86 mm) inserted. The rat was then placed in supine position on a heating mat and connected to a computer-controlled ventilator (*flexiVent*®, Scireq, Montreal, Canada) using the following settings: inspired oxygen fraction (FiO_2) 0.5, respiratory rate (RR) 90/min, V_T of 7 mL/kg, and PEEP 3 cm H_2O. Oxygen saturation (SpO_2) was monitored via pulse oximeter (MouseOx™, STARR Life Sciences Corporation™, Oakmont PA, USA) by placing a sensor on the tail.

2.2. Respiratory system mechanics, pressure-volume curves, allocation to study groups

Lung volume history was standardised by application of 2 lung volume recruitment manoeuvres with 40 mL/kg over 16 s within 2 min. A pressure–volume (*PV*) curve, consisting of a slow continuous ramp inflation from 3 to 20 cm H_2O and a deflation back over a total of 12 s, was then recorded to measure quasi-static compliance. Subsequently, baseline measurement of respiratory system input impedance (Z_{rs}) was performed using the low-frequency forced oscillation technique provided by *flexiVent*® system. Z_{rs} was obtained with a 4 s broadband signal between 1.0 and 20.5 Hz during a pause from mechanical ventilation. The "constant-phase" model, consisting of a single compartment comprising airway and tissue impedance elements connected in series, was fitted to the resulting Z_{rs} (Hantos et al., 1992), allowing the estimation of airway resistance (R_{aw}) and inertance, and the coefficients of tissue damping (G) and elastance (H). At each time point 4 respiratory system input impedance (Z_{rs}) spectra were collected within 120 s and the corresponding values were averaged. Inertance values got insignificantly low and hence are not reported. Since acid instillation leads to changes in airway resistance and lung compliance (Matute-Bello et al., 2008), we report R_{aw} and H, supposed to approximate airway resistance and lung elastance, respectively, to characterise alterations in lung function. Only after baseline measurements, rats ($n = 10$ per group) were randomly allocated to one of the following ventilation strategies.

SLV and ALV: low V_T ventilation with 7 mL/kg, PEEP of 5 cm H_2O, RR of 90/min, and FiO_2 of 0.5, following pre-treatment with saline or acid instillation the day before; SHV and AHV: high V_T ventilation with 21 mL/kg, PEEP of 1 cm H_2O, RR of 30/min, and FiO_2 of 0.5, following pre-treatment with saline or acid. Animals were then ventilated for 4 h with Z_{rs} measurements every 60 min. In order to avoid dehydration 0.3 mL of saline solution was given i.p. after the measurements at 1 and 3 h. Body temperature was maintained at 36.5–37.5 °C with a heating pad.

After the last Z_{rs} measurement at 4 h, ventilator settings were returned to baseline conditions, i.e. V_T of 7 mL/kg, PEEP of 3 cm H_2O, and RR of 90/min in all animals to allow for comparison between *PV* curves obtained at baseline, after 4 h of ventilation, and after final recruitment. Hence, 1 min later, a second *PV* curve ("after 4 h") was performed. Subsequently, a third and final *PV* curve, preceded by 2 lung volume recruitment manoeuvres with 40 mL/kg within 2 min ("following recruitment"), was applied before the animal was disconnected from the ventilator.

Except for mechanical ventilation, non-ventilated controls (SNV and ANV, $n = 5$ per group) underwent the same procedures (pre-treatment, anaesthesia, and tracheostomy) as the ventilated groups.

2.3. Sampling and processing of bronchoalveolar lavage fluid (BALF) and serum

Direct cardiac puncture at the end of the protocol was carried out under general anaesthesia and monitoring of SpO_2 and heart rate. Blood samples were allowed to clot before centrifugation. Serum was frozen for later analysis of macrophage inflammatory protein-2 (MIP-2), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α). BALF was collected from each animal by lavaging the lungs 3 times with 1.0 mL saline through the endotracheal tube. The supernatant was stored at −80 °C until the measurement of IL-6, MIP-2, and TNF-α using ELISA kits (BD Biosciences, San Diego, California). Total protein was analysed using a colorimetric protein assay (Bio-Rad, Regents Park, NSW, Australia). Samples obtained from serum and BALF were processed and analysed individually, i.e. not pooled within groups, to give a measure of variability. Next, individual results were expressed as group mean values with standard deviation.

2.4. Morphologic analysis of lung tissues

Lungs were fixed by buffered 10% formalin instillation via the endotracheal tube at a pressure of 10 cm H_2O and then embedded in paraffin with the caudoventral aspect down. Sections were cut at 5-μm and stained with haematoxylin–eosin for light microscopy. A pathologist (PN) assessed slides ($n = 6$ per group) in a blinded manner. Inflammation was assessed by first counting the number of inflamed foci as judged from a low power scan of the entire lung section. For each focus, the intensity of alveolar neutrophil and macrophage inflammation was scored on an arbitrary scale from 0 to 3. An assessment of the total inflammatory burden was then made by multiplying the number of inflamed foci by the intensity of alveolar neutrophil and macrophage inflammation at each focus, and calculating the mean for each group.

2.5. Statistical analysis

For group comparisons of SpO_2, peak airway opening pressure (P_{ao}), R_{aw}, H, *PV* curves, and cytokines and protein concentrations two-way ANOVA (factor 1: pre-treatment strategy, i.e. saline and acid; factor 2: ventilation strategy, i.e. low and high tidal) with