RESPIRATORY MECHANICS IN INFANT AND ADULT MICE MODELLING VENTILATOR-INDUCED LUNG INJURY

PhD Thesis

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SUMMARY

Though critical in the management of patients with respiratory failure, mechanical ventilation has also the potential to aggravate or induce lung injury. This injury is referred to as ventilator-induced lung injury (VILI). To better understand different aspects and mechanisms involved in VILI age-specific animal models are desirable.

This thesis aimed at investigating determinants of VILI, namely high tidal volume (V_T), positive end-expiratory pressure (PEEP), high oxygen concentrations, lung volume recruitment maneuvers (RM), and stress and strain-induced release of inflammatory mediators. Different ventilation strategies in infant and adult mice were compared in an interventional controlled manner. The following outcome variables were assessed: a) respiratory system impedance, b) thoracic gas volume, c) pressure-volume curves, d) inflammatory response, and e) histology. While high-V_T ventilation produced lung injury in infant mice presumably via overdistension and loss of lung volume, high oxygen concentrations had no impact on respiratory system mechanics in either age group. In adult mice frequent application of substantial RMs on top of elevated PEEP levels produced stable lung mechanics without signs of lung injury.

These findings underline the need for age-specific small animal models and require that specification of ventilator settings are reported in all studies investigating effects of mechanical ventilation in mice.

__List of papers included in the thesis:__

1. Cannizzaro V, Zosky GR, Hantos Z, Turner DJ, Sly PD. High tidal volume ventilation in infant mice. *Respir Physiol Neurobiol* 2008; 162: 93-9.

2. Cannizzaro V, Berry LJ, Zosky GR, Turner DJ, Hantos Z, Sly PD. Impact of supplemental oxygen in mechanically ventilated adult and infant mice. *Respir Physiol Neurobiol* 2009; 165: 61-6.

3. Cannizzaro V, Berry LJ, Nicholls PK, Zosky GR, Turner DJ, Hantos Z, Sly PD. Lung volume recruitment maneuvers and respiratory system mechanics in mechanically ventilated mice. *Respir Physiol Neurobiol* 2009; 169: 243-51.

__Paper related to the subject of this thesis:__

4. Zosky GR, Janosi TZ, Adamicza A, Bozanich EM, **Cannizzaro V**, Larcombe AN, Turner DJ, Sly PD, Hantos Z. The bimodal quasi-static and dynamic elastance of the murine lung. *J Appl Physiol* 2008; 105: 685-92.

INTRODUCTION

Ventilator-induced lung injury (VILI)

Clinical and experimental studies have demonstrated that artificial ventilation can promote lung injury. As a result, research on the field of VILI has led to 'protective' ventilation strategies, which comprise the application of low tidal volumes (V_T), limitation of peak pressures, and adequate setting of positive end-expiratory pressures (PEEP). This approach is supposed to minimize the adverse influences involved in VILI, including overdistension of alveolar units, repeated opening and closing of peripheral lung units resulting in shear stress at the interface between aerated and non-aerated airspaces, and damage due to release of inflammatory mediators.

VILI in age-specific animal models

The potential harm of mechanical ventilation is magnified in infants because of a more compliant chest wall with greater risk of volutrauma, small lung volumes promoting atelectasis, and developing lung structures. Given the difficulties in performing mechanical ventilation studies in human infants, animal models have emerged as useful tools for studying VILI. Rodent models are widely used because of their availability and well-characterized respiratory mechanics. These models have provided important insights into the mechanisms of VILI, but have predominantly used adult animals with fewer studies in infants. Recent advances in techniques for measuring respiratory system mechanics in small animals have the potential to provide further insights for the specific age group of infants. Establishment and study of an infant mouse model of VILI is particularly promising because of the potential to use genetically altered mice in future studies that may allow more specific mechanisms to be explored. In terms of lung development 2-week-old mice can be compared to 2-year-old human infants.

Ventilation protocols in small animals – supplemental oxygen

Supplemental oxygen is often used to improve survival during prolonged periods of ventilation. However, hyperoxia may release pro-inflammatory mediators, hasten atelectasis, and alter the function of the pulmonary surfactant system, both leading to a decrease in lung compliance. Hence, a high fraction of inspired oxygen ($F_{I_{O_2}}$) may impair lung mechanics, contribute to additional lung injury and confound

interpretation of the main study design. Injurious mechanical ventilation, i.e. high V_T and low PEEP, and hyperoxia resulted in lung injury and increased inflammatory response in adult and infant rodents. However, it is not known how exposure to hyperoxia affects respiratory system mechanics during 'protective' mechanical ventilation with low-V_T and PEEP.

Ventilation protocols in small animals – recruitment maneuvers (RM)

Since ventilator settings have the potential to affect lung function, V_T, RR, $F_{I_{O_2}}$, airway pressure levels, and inspiratory to expiratory time ratio are commonly reported in mechanical ventilation studies using rodent models to investigate lung diseases. However, only few experimental studies using mechanical ventilation protocols provide detailed information on application, frequency, and type of lung volume RM. Given that the mechanical properties of the respiratory system are specific to the lung volume at which their measurements are made and to the lung volume history, it is surprising that details of RMs are not always reported. In lung function studies conducted in murine models of respiratory diseases, RMs aim at establishing similar lung volume history and often precede baseline measurement of lung function. Generally, these RMs consist of a series of inflation maneuvers that are either volume- or pressure-controlled and do not include elevation of PEEP. Application of different types of RMs reflects different views on how to best recruit non-aerated lung units without producing lung injury.

Aims and hypotheses of the present thesis

Study 1: High tidal volume ventilation in infant mice

The aim of this study was to investigate the effects of high-V_T ventilation in a novel *in vivo* infant mouse model for VILI. We hypothesized that high-V_T ventilation without PEEP may cause the most significant changes in lung mechanical parameters and lung injury.

Study 2: Impact of oxygen in mechanically ventilated adult and infant mice

Study 2 was undertaken to determine whether high oxygen concentrations alter respiratory system mechanics and inflammatory response, and whether this interaction differs between infant (2 week old) and adult (8 week old) rodents.

Study 3: Lung volume recruitment maneuvers and respiratory system mechanics in mechanically ventilated mice

The respective impacts of PEEP elevation, inflation maneuvers without PEEP elevation, and RMs (i.e. inflation maneuvers plus PEEP elevation) on lung function are not clear in mice. The aim of study 3 was to establish how these maneuvers affect respiratory system mechanics and whether they induce or exacerbate lung injury in mechanically ventilated mice. We hypothesized that frequent and large RMs provide stable respiratory system mechanics, but at the expense of lung injury.

METHODS

Study animals and animal preparation

Two and 8 week old female BALB/c mice were purchased from the Animal Resource Centre in Murdoch, Western Australia. The experimental procedures were approved by the Telethon Institute for Child Health Research Animal Experimentation and Ethics Committee and conform to the guidelines of the National Health and Medical Research Council of Australia. After inducing surgical level of anaesthesia, a tracheotomy was performed and a 10 mm long polyethylene cannula (ID: 0.86 mm) or a metal cannula (21-gauge) for 8 and 2 week old mice, respectively, was inserted and secured with suture. Mice were then connected to a computer-controlled ventilator (*flexiVent,* Scireq, Montreal, Canada).

Measurement of lung volume and respiratory system mechanics via whole body plethysmography and wave-tube technique, respectively (Study 1)

After lung volume history standardisation, baseline measurements of end-expiratory lung volume (EELV) and respiratory system impedance (Z_{rs}) were taken. EELV was measured using the whole-body plethysmographic technique based on Boyle's law. Briefly, the airway was occluded at 0 cmH_2O transrespiratory pressure (P_{rs}) and breathing efforts were induced using electrical stimulation of the intercostal muscles. Z_{rs} was measured using the miniature wave-tube version of the low-frequency forced oscillation technique (FOT). A pseudorandom oscillatory signal ranging from 4 to 38 Hz was delivered by a loudspeaker-in-box system to the tracheal cannula via a wave-tube at P_{rs} = 0 cmH_2O, and Z_{rs} was measured as the load impedance on the wave-tube. The constant-phase model was then fitted to the resulting Z_{rs}, allowing the estimation of airway resistance (R_{aw}) and inertance (I_{aw}), and the coefficients of tissue damping

(G) and elastance (H): $Z_{rs} = R_{aw} + j\omega I_{aw} + (G-jH)/\omega^{\alpha}$: where $\alpha=(2/\pi)\tan^{-1}(H/G)$, j is the imaginary unit, and ω is angular frequency. Values of R_{aw} and I_{aw} were corrected for the resistance and inertance, respectively, of the tracheal cannula. After the subtraction of the impedance of the tracheal cannula the values of I_{aw} became insignificantly low and hence not reported.

Measurement of respiratory system mechanics with *flexiVent*® (Studies 2 and 3)

Following lung volume history standardisation, baseline measurement of Z_{rs} was performed using the low-frequency FOT provided by the *flexiVent*® system. In study 2, Z_{rs} was obtained during a 16 s pause from mechanical ventilation during which a broadband signal of 19 mutually prime frequencies from 0.25 to 20 Hz was applied to the airway opening of the mouse. In study 3, Z_{rs} was obtained by a 4-s oscillation signal of 13 mutually prime frequencies from 1.0 to 20.5 Hz. During Z_{rs} measurement PEEP level remained unchanged at the pre-measurement level. The resulting input impedance data were analysed using the constant-phase model, which allows distinction between central and peripheral respiratory mechanics. R_{aw}, G, and H were determined by fitting the model to the Z_{rs} data.

Study designs and experimental protocols

Study 1: High tidal volume ventilation in infant mice

After baseline measurements, mice were randomized to receive: (1) high V_T with zero end-expiratory pressure (HVZ): RR = 150/min, V_T of 20 mL/kg, no sighs (n=12), (2) high V_T with PEEP (HVP): RR = 150/min, V_T of 20 mL/kg, PEEP of 3 cmH_2O, and no sighs (n=12), and (3) low-V_T with PEEP (LVP): RR = 360/min, V_T of 8 mL/kg, PEEP of 3 cmH_2O, and 2 sighs of 20 mL/kg which did not exceed 20 cmH_2O inspiratory peak pressure, applied shortly after and 5 min before each measurement of respiratory mechanics (n=12). Mice were ventilated for 60 min with measurements of EELV and Z_{rs} at baseline and every 10 min. Ventilation frequencies in each strategy were matched for minute ventilation.

Study 2: Impact of oxygen in mechanically ventilated adult and infant mice

Adult mice were ventilated with room air at a RR = 300/min, PEEP of 3 cmH_2O, and V_T of 7 mL/kg. Infant mice were ventilated with room air, RR of 240/min, PEEP of 3

cmH_2O, and V_T of 9 mL/kg. Minute ventilation was similar in both age groups and high enough to ensure that mice remained apneic during lung function measurements. After baseline measurement, mice were assigned to: (1) $F_{I_{O_2}}$=0.21 (controls), (2) $F_{I_{O_2}}$=0.3, (3) $F_{I_{O_2}}$=0.6, and (4) $F_{I_{O_2}}$=1.0; n = 8 mice per group. Mice were ventilated for 120 min. Moderate RMs, designed to prevent extensive atelectasis were delivered shortly after and 5 min before each Z_{rs} measurement. The RM was pressure-limited and lasted 6 s, partitioned into 3 s ramp duration and 3 s hold at 20 cmH_2O, followed by passive deflation to a PEEP of 3 cmH_2O.

Study 3: Lung volume recruitment maneuvers and respiratory system mechanics in mechanically ventilated adult mice

Lung volume recruitment was achieved by a combination of changes in PEEP level (i.e. 2 and 6 cmH_2O) and application of inflation maneuvers (IM) at different time points (i.e. every 5 or 75 min) during the 150-min protocol. IMs were delivered either in a volume-controlled manner without pressure limit (20 mL/kg or 40 mL/kg) or in a pressure-controlled mode (25 cmH_2O). The IMs consisted of a 3-s ramp duration to reach preset volume or plateau pressure and a 3-s hold followed by passive deflation to the predefined PEEP level. Animals (n=7 mice/group) were allocated as follows: six groups (1a-6a) received different IMs while the PEEP level was unchanged at 2 cmH_2O: 20 mL/kg every 5 min (Group 1a) or every 75 min (Group 2a), 40 mL/kg every 5 min (Group 3a) or every 75 min (Group 4a), and IMs to 25 cmH_2O every 5 min (Group 5a) or every 75 min (Group 6a). In another six groups (1b-6b) PEEP was increased from 2 to 6 cmH_2O shortly before application of the first IM and then remained at 6 cmH_2O throughout the study: 20 mL/kg every 5 min (Group 1b) or every 75 min (Group 2b), 40 mL/kg every 5 min (Group 3b) or every 75 min (Group 4b), and IMs to 25 cmH_2O every 5 min (Group 5b) or every 75 min (Group 6b). Two additional groups were ventilated at a PEEP of 2 cmH_2O (Group 7a) or 6 cmH_2O (Group 7b) without any application of IMs. In the latter group (Group 7b) PEEP was increased from 2 to 6 cmH_2O shortly after the first baseline measurement.

Z_{rs} measurements were performed every 15 min and included pre- and post-IM measurements for the study groups 1a-6a and 1b-6b. Heart rate and transcutaneous oxygen saturation were monitored via a small animal pulse oximeter (MouseOx[TM], STARR Life Sciences Corporation[TM], Oakmont PA, USA).

Sampling and processing of blood and bronchoalveolar lavage fluid (BALF)

Blood, obtained by cardiac puncture at the end of the study, was allowed to clot, centrifuged, and serum frozen for later analyses of interleukin-6 (IL-6) and macrophage inflammatory protein-2 (MIP-2). Then, lungs were lavaged with 0.9% saline solution via tracheostomy. BALF was centrifuged and supernatant collected and frozen for later analysis of IL-6, MIP-2, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and total protein. The cell pellet was resuspended in phosphate buffered saline and an aliquot was stained with Trypan blue to obtain a total cell count using a haemocytometer. A second aliquot was centrifuged onto a slide and stained with Leishmann's to obtain a differential cell count using light microscopy by counting 300 cells from each slide. Cytokine and total protein concentrations were measured by using ELISA and a colorimetric protein assay, respectively.

Analysis of lung tissue by morphology and morphometry (Study 3)

Inflammatory cells were counted by blindly selecting ten fields at x100 from each section. In each of these ten fields, the number of erythrocytes, alveolar macrophages, alveolar neutrophils, and septal neutrophils was counted. To determine the degree of lung inflation by morphometry, ten fields from each lung section were blindly selected and digitally captured under the x40 objective. A 100-point grid was superimposed over each image, and the number of grid intersection points that coincided with an alveolar wall was determined. For each animal, the sum of the grid counts over the ten digitized images was taken as the relative inflation score.

RESULTS

Study 1: High tidal volume ventilation in infant mice

End-expiratory lung volume (EELV) in both the HVP and LVP groups did not change throughout the ventilation period. In contrast, the HVZ group showed a significant reduction in EELV after 20 min of ventilation, which was sustained for the duration of the study period and approximated to a volume loss of one third.

A significant increase in R_{aw} was found in the HVZ group whereas R_{aw} significantly dropped after 10 min in the HVP group and remained unchanged until the end of the experiment. G and H increased significantly after 10 min in the HVZ group and by 20 min for the HVP and LVP groups when compared with their respective

baselines. At the end of the study G and H in the HVZ group increased by 86% and 148%, respectively, which was significantly greater than in the HVP (38% and 56%, respectively) and LVP groups (62% and 88%, respectively).

After switching to the allocated ventilation pattern peak airway opening pressure (P_{ao}) levels of the LVP, HVP and HVZ groups increased to 11.8, 16.9, and 13.3 cmH$_2$O, respectively. Thereafter, P_{ao} values of the study groups with PEEP (LVP and HVP) rose to 14.4 and 20.2 cmH$_2$O, respectively, whereas peak P_{ao} in the HVZ group increased to 20.6 cmH$_2$O by the end of the experiment. Periodically applied sighs in the LVP group were below P_{ao} levels of 20 cmH$_2$O.

There was no difference between any of the ventilation groups and the non-ventilated controls for total cells and differential cell counts obtained from the BALF. Similarly, we found no significant differences in levels of IL-6 in BALF (p = 0.09). In contrast, there was a significant increase in total protein levels of mice ventilated with the HVP strategy compared to all other groups. The level of IL-1ß was below the range of detection for control and ventilated mice. The serum concentration of IL-6 was significantly higher in the HVZ group when compared with all other groups.

Study 2: Impact of oxygen in mechanically ventilated adult and infant mice

Baseline measurements for R_{aw}, G, and H were not different between $F_{I_{O_2}}$ groups in adult and infant mice. We found no difference in R_{aw}, G, and H between $F_{I_{O_2}}$ groups after 120 min of ventilation in both age groups. When compared with baseline values, changes in R_{aw} were small and considered to be physiologically insignificant, whereas G and H significantly increased after 30 and 10 min, respectively. At the end of the study G increased on average by 27 and 29% while H increased by 49 and 41% in 8 and 2 week old mice, respectively. We found no difference in total and differential cell counts obtained from the BALF between $F_{I_{O_2}}$ groups in both age groups (p > 0.41 in all cases). There also was no significant difference between oxygen concentrations in both age groups for IL-6, MIP-2, and total protein concentrations.

Study 3: Lung volume recruitment maneuvers and respiratory system mechanics in mechanically ventilated mice

At baseline no differences were found for R_{aw}, G, H, and peak P_{ao} levels between study groups (p>0.49 in all cases).

R_{aw}, G and H after lung volume recruitment at PEEP level of 2 cmH$_2$O

Compared with baseline values R_{aw} statistically significantly increased at 150 min in the control group. IMs with 20 mL/kg resulted in steady R_{aw} values (p>0.17 in both cases) while frequent (i.e. every 5 min) application of IMs to 25 cmH$_2$O or with 40 mL/kg produced a physiologically unimportant decrease in R_{aw} over time. At the end of the protocol we found small but statistically significantly higher R_{aw} in controls when compared to groups receiving pressure controlled IMs or IMs of 40 mL/kg. G steadily and significantly increased from baseline to the time points 75 and 150 min in controls and study groups receiving IMs of 20 mL/kg. Application of more substantial and frequent IMs (25 cmH$_2$O and 40 mL/kg) resulted in stable G values. Similarly, to significantly decrease the steady rise of G in groups receiving intermittent IMs, large IMs were necessary. A general pattern of progressive increase in H over time can be seen in all groups apart from those given large IMs (40 mL/kg or 25 cmH$_2$O) every 5 min. Where a single IM was given every 75 min, an abrupt decrease in H was seen; however H subsequently increased along the same trajectory.

R_{aw}, G and H after lung volume recruitment with PEEP level of 6 cmH$_2$O

When PEEP was increased from 2 to 6 cmH$_2$O R_{aw} decreased in all groups, regardless of the timing of the increase in PEEP. From the time point 15 min on, R_{aw} statistically significantly rose in the control group with 6 cmH$_2$O PEEP. Frequent IMs with 20 mL/kg and 40 mL/kg produced a physiologically unimportant rise and fall, respectively, in R_{aw}. After 150 min R_{aw} values of controls were significantly higher when compared to all other groups, except for infrequent IMs with 20 mL/kg (p=0.08). In the control group G steadily and significantly increased until the end of the protocol. After the time point 15 min, irrespective of the magnitude, frequent application of IMs produced stable G values. When a sporadic IM was given every 75 min, large IMs (40 mL/kg) were required to significantly decrease G values. IMs given every 5 min on top of a PEEP of 6 produced a stable H over the ventilation period, regardless of the type of magnitude of the IM. Less frequent IMs were associated with a progressive increase in H up until the time the IM was applied; with a subsequent increase in H following a similar trajectory.

Pressure-volume relation during the 3-s ramp inflation

Delayed application of the first RM at the time point 75 min resulted in lower values of inflation volume at a given P_{ao} when compared with early application of RMs. Irrespective of the time point of first RM application and the PEEP level, inflation

above ~25 cmH$_2$O was followed by another steep rise of the inflation limb without signs of flattening up to a peak P$_{ao}$ of 35 cmH$_2$O.

Heart rate and transcutaneous oxygen saturation

No differences were found between study groups at baseline and at the time points 75 and 150 min (p>0.13 in all cases). Over time heart rate significantly decreased when compared to baseline values.

Cell counts, total protein and cytokines concentrations in BALF

BALF analysis for cell counts, cytokines, and protein concentrations produced no significant difference between groups (p>0.10 in all cases).

Analysis of lung tissues by morphology and morphometry

Differences between numbers of alveolar macrophages and erythrocytes, and alveolar and septal neutrophils, as well as lung inflation scores were not significant (p>0.40 in all cases).

DISCUSSION

Major findings emerging from this thesis

First, alterations of respiratory system mechanics during artificial ventilation without PEEP can be attributed to loss of lung volume. Second, application of PEEP during short-term high-V$_T$ ventilation prevents atelectasis but induces lung injury in infant mice. Third, short-term exposure to levels of oxygen up to 100% does not increase changes in respiratory system mechanics induced by mechanical ventilation. Fourth, frequent application of substantial IM on top of elevated PEEP levels results in stable respiratory system mechanics without causing lung injury after short-term ventilation with low V$_T$. Lastly, both an increase in PEEP (without use of IMs) and application of IMs resulting in peak P$_{ao}$ below 25 cmH$_2$O are insufficient to prevent or reverse increases in R$_{aw}$, G, and H during low-V$_T$ ventilation.

Forced oscillation technique and estimates of respiratory system mechanics

Assessment of respiratory system mechanics is essential because of the potential to gain insight into development and mechanisms involved in VILI. The dynamic measurement of respiratory system mechanics via application of forced oscillations provides information about resistive and elastic properties of the respiratory system. Impedance data generated by the low-frequency FOT has best been described by the constant-phase model. Parameters estimated from model fitting include Newtonian

resistance (R_N), G, and H. In species with relatively low chest wall impedance, such as mice, R_N is essentially equal to R_{aw} and basically reflects a change in central airway calibre, whereas G and H can be considered as parenchymal parameters and characterize dissipative and elastic properties, respectively, of the respiratory system. A proportionate increase of G and H results from lung volume derecruitment, while a disproportionate, i.e. higher rise in G compared to H reflects an increase in regional heterogeneity. Hence, the parameters obtained from low-frequency Z_{rs} data allow one to differentiate changes in airway diameter from changes related to loss of lung volume or regional ventilation heterogeneities.

Assessment of inflammatory response

The relevance of cytokine production in the context of VILI has been challenged. This is not surprising since several factors such as species, pre-treatment, and ventilation strategy influence duration and outcome of experiments. Furthermore, it is evident that short-lived and clinically relevant, that is less extreme, animal study protocols using primary healthy lungs will not cause massive cytokine and protein production. Nonetheless, more recent studies provide evidence that cytokines are important mediators in the development of VILI.

High tidal volume ventilation in infant mice (Study 1)

Based on adult rodent data we hypothesized that a HVZ strategy would cause lung injury and significant changes in lung mechanics. Our data only partially support this hypothesis. HVZ caused the fastest and steepest rise in G and H. The proportionate increase in G and H in the HVZ group is indicative of a peripheral process, namely atelectasis. Application of PEEP in the HVP and LVP groups prevented loss of lung volume. Periodic delivery of mild RMs with V_T of 20 mL/kg in the LVP group resulted in a pronounced increase of G and H without signs of lung injury.

In adult mice high-V_T ventilation with or without PEEP produced no change in R_{aw}, G, and H after 60 min, whereas infant mice showed an increase in R_{aw} with HVZ and a decrease with HVP and an elevation in G and H after ventilation with both HVZ and HVP. Apart from developmental and structural differences between lungs of infant and adult mice we cannot exclude that differences in mouse strain, lung volume history standardization, RR, and slightly higher V_T may have caused more alveolar recruitment in adult mice and hence different patterns of R_{aw}, G, and H.

Experimental studies with infant rats exposed to extremely high V_T ventilation and low PEEP found that total lung compliance assessed by constructed static pressure-volume curves was not even altered after 90 min of ventilation with peak pressures of 30 cmH$_2$O. Clearly, this is in contrast to our findings in infant mice ventilated with both the HVZ and HVP strategy. A comparison of the effects of high-V_T ventilation between infant mice and rats is difficult and may be biased by different ventilation modes and lung function techniques used. However, infant mice and rats produce a different pattern of respiratory system mechanics and inflammation and lung injury when compared with adult rodents after artificial ventilation.

Inflammatory response and lung injury

We only found increased values of IL-6 in serum of the HVZ group. A similar result was also shown in adult rats after a high pressure zero PEEP strategy. We assume that the reason for elevated serum IL-6 levels was largely related to substantial atelectasis leading to ventilation-perfusion-mismatch, deterioration in cardiac output and eventually a systemic inflammatory response. In addition, HVP strategy caused increased total protein concentrations in BALF. High V_T was delivered on top of PEEP and may have caused overstretching of lung units resulting in lung injury. This is in line with results from adult animal studies where alveolar overdistension has been shown to be an important mechanism in the pathogenesis of VILI. However, one may wonder why our HVZ strategy did not lead to elevated protein levels, such as with comparable HVZ strategies in adult animal studies, even though similar peak P_{ao} levels were achieved as within the HVP group. The degree of lung distension and with it the risk of VILI does not depend on peak P_{ao}, but rather on transpulmonary pressure (P_{tp}), tissue elastance, and lung volume history. Due to the relatively small contribution of the chest wall on respiratory system mechanics reported in mice, it is unlikely that a higher state of inflation (as in HVP) may have attenuated alveolar distension in infant mice, where chest wall tissue is even thinner. Furthermore, the use of PEEP also resulted in consistently higher mean P_{ao} values throughout the study, probably reflecting the higher state of inflation. Consequently, higher EELV, lower R_{aw}, and better lung compliance most likely resulted in alveolar overdistension during HVP ventilation when compared with HVZ ventilation. Conversely, despite similar peak P_{ao} values at the end of the study, lower EELV, high lung elastance, and increased R_{aw} is likely to have resulted in less alveolar distension in the HVZ group.

Impact of oxygen in mechanically ventilated adult and infant mice (Study 2)

We found no physiologically significant oxygen-related changes of R_{aw} in either age group. Also, the increase in G was associated with a similar elevation in H and reflects development of atelectasis rather than an increase in ventilation heterogeneity as a result of inhomogeneous regional airway narrowing. In addition, we did not find elevations in either cytokine in response to increased $F_{I_{O_2}}$. These data are consistent with results from adult rodents and newborn rats exposed to hyperoxia and mechanical ventilation. Moreover, the lack of increase in total protein in BALF we observed in the present study suggests that short-term exposure to high $F_{I_{O_2}}$ *per se* does not compromise epithelial or endothelial barrier function. Taken together, our findings underscore the lack of significant oxygen-associated pulmonary inflammation and lung injury after non-injurious short-term mechanical ventilation.

Lung volume recruitment maneuvers and respiratory system mechanics in mechanically ventilated mice (Study 3)

Mechanical ventilation with low V_T and low PEEP resulted in a significant increase in G and H over time. The almost linear rise of G and H, reflecting gradual airway closure, is consistent with a progressive loss of lung volume secondary to atelectasis. In order to prevent progressive atelectasis we applied IMs in a volume-controlled manner without pressure limit or in a pressure-controlled mode. Volume-controlled IMs delivered a fixed V_T and resulted in peak P_{ao} determined by lung compliance. In contrast, pressure-controlled IMs ensured that a selected peak P_{ao} was not exceeded, but provided different V_T depending on respiratory mechanics.

Only large IMs resulting in peak P_{ao} above 30 cmH$_2$O produced overall improvements in respiratory mechanics, whereas IMs reaching peak P_{ao} values at or below 25 cmH$_2$O did not reverse ventilation-induced increases in R_{aw}, G, and H. By contrast, repetitive IMs with 40 mL/kg superimposed on high PEEP, producing peak P_{ao} values above 35 cmH$_2$O, provided the most significant improvement in lung function and stable respiratory system mechanics with little intra-group variability. This finding is closely linked to the development of a "secondary" pressure-volume sigmoid with lung inflation beyond 20 cmH$_2$O, which makes total lung capacity difficult to define in mice. This bi-modal pressure-volume behaviour was demonstrated in some small species long time ago; however, its importance was not

recognised until recently. Our findings therefore support the view that the improvement of estimates of respiratory system mechanics after large RMs is due to fundamental changes in quasi-static and dynamic lung compliance, as substantiated by the inflation PV curve and the values of R_{aw}, G, and H, respectively. Though the mechanisms responsible for the increased lung compliance above P_{ao} of 25 cmH_2O are not clear, alveolar unfolding and surfactant redistribution have been proposed as possible explanations, while others suggest that alveolar mouths, previously closed by a surfactant-lined liquid film, open during recruitment of peripheral lung units at high P_{tp} providing a new population of available alveoli. A structural reorganization of peripheral lung units resulting in an increased number of alveoli and homogeneous ventilation may explain why our initial hypothesis, i.e. frequent and large RMs would induce lung injury via overdistension, could not be confirmed in this study.

Application of RMs producing transient peak P_{ao} elevations did not increase cytokine concentrations in BALF. Similarly, total protein concentration did not differ between groups, indicating that RM-induced stretch of airways and alveoli did not adversely affect epithelial-endothelial barrier function. Also, absence or application of RMs had no impact on transcutaneous oxygen saturation; however, it should be noted that healthy lungs were ventilated over a short period of time and $F_{I_{O_2}}$ of 0.5 was delivered in order to avoid survival problems.

CONCLUSIONS

The presented *in vivo* infant and adult mouse studies involving measurements of airway and tissue mechanics, absolute lung volumes, construction of PV curves, and assessment of inflammatory response show that infant mice lungs behave differently from adult and neonatal rodent lungs. This finding underlines the need for age-specific animal models and asks for caution when data and conclusions from adult animal research are extrapolated for infant animals and eventually human infants.

Our first study provides evidence that mechanical ventilation with high V_T is deleterious in infant mice. We propose that overdistension of peripheral lung units and atelectasis are main mechanisms involved in the development of VILI.

Also, our results show that short-time exposure to high $F_{I_{O_2}}$ in the presence of non-injurious mechanical ventilation does not exacerbate lung injury in either infant or adult mice. This finding may not have a direct translational value for clinical practice,

but has valuable implications for experimental VILI studies, which are often performed in mice and use short-term mechanical ventilation protocols.

Finally, we demonstrated that infrequent application of large RMs is sufficient to reverse increases in bronchial resistance and lung elastance in healthy mice. To maximize lung volume recruitment throughout study protocols using ventilation strategies of low V_T and "adequate" PEEP, repetitive IMs reaching peak P_{ao} values >25 cmH_2O are required to provide stable respiratory mechanics. This is particularly useful in animal model studies where similar baseline conditions after standardized procedures are desirable. Furthermore, frequent application of substantial RMs resulting in peak P_{ao} of 35 cmH_2O and above provides stable respiratory mechanics without inflammatory and histological signs of lung injury during short-term mechanical ventilation in mice. Given the impact of RMs on respiratory system mechanics documented by the present thesis requires that the specifications of PEEP and IMs used are reported in all studies investigating the effects of mechanical ventilation in mice.

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