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EXPERIMENTAL

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Effects of frequency and inspiratory plateau pressure during recruitment manoeuvres on lung and distal organs in acute lung injury

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Abstract *Purpose:* To evaluate the effects of frequency and inspiratory plateau pressure (Pplat) during recruitment manoeuvres (RMs) on lung and distal organs in acute lung injury (ALI). *Methods:* We studied paraquat-induced ALI rats. At 24 h, rats were anesthetized and RMs were applied using continuous positive airway pressure (CPAP, 40 cmH_2O/40 s) or three-different sigh strategies: (a) 180 sighs/h and Pplat = 40 cmH_2O (S180/40), (b) 10 sighs/h and Pplat = 40 cmH_2O (S10/40), and (c) 10 sighs/h and Pplat = 20 cmH_2O (S10/20). *Results:* S180/40 yielded alveolar hyperinflation and increased lung and kidney epithelial cell apoptosis as well as type III procollagen (PCIII) mRNA expression. S10/40 resulted in a reduction in epithelial cell apoptosis and PCIII expression. Static elastance and alveolar collapse were higher in S10/20 than S10/40. *Conclusions:* The reduction in sigh frequency led to a protective effect on lung and distal organs, while the combination with reduced Pplat worsened lung mechanics and histology.

Keywords Acute lung injury · Sigh · Ventilator-induced lung injury · Transpulmonary pressure · Apoptosis

Introduction

The use of low tidal volumes and limited inspiratory plateau pressure has been proposed to ventilate acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) patients and prevent lung and distal organ injury [1–4]. The reduction in tidal volume may result in alveolar derecruitment if not enough positive end-expiratory pressure (PEEP) is applied to prevent alveolar collapse [5]. On the other hand, high PEEP levels may be associated with excessive lung parenchyma stress and strain [6] and negative hemodynamic effects resulting in systemic organ injury [7].

Several recruitment manoeuvres (RMs) have been used in clinical and experimental ALI/ARDS to open the lung [8, 9]. In this line, continuous positive airway pressure (CPAP) of 40 cmH_2O for 40 s (RM-CPAP) is a well-known method of RM since it improved respiratory

function in several experimental models of ALI and in patients with ALI/ARDS. However, RM-CPAP failed to induce a sustained improvement of oxygenation [10, 11] and may result in lung injury [12, 13]. In this line, a RM at a frequency of 180 sighs/h (3 sighs/min) showed a beneficial [14, 15] but short-lived effect [16] on lung mechanics and oxygenation. Additionally, RM with high frequency and inspiratory plateau pressure may yield shear and tensile stresses resulting in lung damage [17]. There may be a certain level of frequency and inspiratory plateau pressure during RM which maintains the benefits on pulmonary function with less lung and distal organ injury.

In this study, we hypothesized that RM with high frequency and inspiratory plateau pressure may yield lung parenchyma and distal organ injury in healthy and ALI rats. We evaluated the effects of different levels of frequency and inspiratory plateau pressure during RM on arterial blood gases, lung static elastance and histology (light and electron microscopy), lung and distal organ epithelial cell apoptosis, and type III procollagen mRNA expression in lung tissue (an early marker of lung parenchyma remodelling). Some of the results of this study have been previously reported in abstract form [18].

Materials and methods

Detailed methods are described in the Electronic supplementary material (ESM) accompanying this article, and briefly summarized here.

Animal preparation and experimental protocol

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Centre, Federal University of Rio de Janeiro. Eighty Wistar rats (250–300 g) were randomly assigned to two groups. In Control (CTRL, $n = 40$), sterile saline solution (0.9% NaCl, 1.0 ml) was intraperitoneally (*ip*) injected and in acute lung injury (ALI, $n = 40$), paraquat (15 mg/kg, *ip*) was administered.

Twenty-four hours after saline or paraquat administration, animals were sedated (diazepam 1 mg, *ip*), anaesthetised (thiopental sodium 20 mg/kg, *ip*), tracheotomised, paralysed (pancuronium 2 mg/kg intravenously), and mechanically ventilated (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) with the following parameters: tidal volume (V_T) = 4 ml/kg, airflow = 6 ml/s, respiratory rate (RR) = 100 breaths/min, inspiratory to expiratory ratio = 1:2, fraction of inspired oxygen (FiO_2) = 0.21, and PEEP = 5 cmH_2O. The dosage, duration and level of anaesthesia were similar in all groups. A polyethylene catheter was introduced into the femoral artery to collect blood sampling and measure arterial pressure. CTRL and ALI animals were then randomized as follows: (1) non-recruited (NR) ($n = 8$/group); (2) recruitment manoeuvre (RM) consisting of one continuous positive airway pressure of 40 cmH_2O for 40 s (RM-CPAP) ($n = 8$/group), (3) RM with 180 sighs/h (3 sighs/min) and inspiratory plateau pressure (Pplat) of 40 cmH_2O (S180/40) ($n = 8$/group), (4) RM with 10 sighs/h (one sigh every 6 min) and Pplat of 40 cmH_2O (S10/40) ($n = 8$/group), or (5) RM with 10 sighs/h and Pplat of 20 cmH_2O (S10/20) ($n = 8$/group). We delivered a sequence of 180 sighs/h, in volume control mode, since it is the closest to the clinical setting [14, 15]. The other levels of pressure or frequency were decided based on some pilot studies. All experiments were performed at the same time. The frequency of non-sigh tidal volume was reduced to maintain minute ventilation constant among groups. Before randomization (BASELINE) and after 1-h ventilation period (END), arterial blood gases (i-STAT, Abbott Laboratories, IL, USA) and lung static elastance were analysed. After ventilation period, animals were then killed, lungs and distal organs were prepared for histology, and type III procollagen (PCIII) mRNA expression in lung tissue was measured.

Mechanics

Airflow, tracheal and oesophageal pressures were measured. Tidal volume (V_T) was calculated by digital integration of flow signal. Transpulmonary pressures were calculated by the difference between tracheal and oesophageal pressures [13]. To calculate lung static elastance (Est,L), airways were occluded at end-inspiration until a transpulmonary plateau pressure (Pplat,L) was reached (at the end of 5 s), after which this value was divided by V_T [13].

Histology

Light microscopy

A laparotomy was done immediately after the determination of lung mechanics (END). Lungs, liver, kidneys, and small intestine were removed, fixed in 3% buffered formaldehyde, paraffin embedded, and stained with haematoxylin–eosin. The volume fraction of the lung occupied by hyperinflated structures (alveolar ducts, alveolar sacs or alveoli wider than 120 μm) or collapsed alveoli or normal pulmonary areas were determined by the point-counting technique [13, 19].

Transmission electron microscopy

Three slices 2 × 2 × 2 mm were cut from three different segments of the left lung and fixed for electron microscopy analysis. The following structural damages were analyzed: (a) alveolar capillary membrane, (b) type II epithelial cells, and (c) endothelial cells, and then graded according to a 5-point semi-quantitative severity-based scoring system: 0 = normal lung parenchyma, 1 = changes in 1–25%, 2 = changes in 26–50%, 3 = changes in 51–75%, and 4 = changes in 76–100% of examined tissue [13]. Two investigators, unaware of the origin of the material, examined the samples microscopically. The slides were coded and examined only at the end of all measurements.

Apoptosis assay of lung and distal organs

Apoptotic cells of lung, kidney, liver, and small intestine villi were quantified using Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) assay [20] and immunohistochemical staining for Fas and FasL protein [21] in a blinded fashion by two pathologists. A 5-point semiquantitative severity-based scoring system was used and graded as: 0 = no apoptotic cells; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of apoptotic cells in the examined tissue.

Semi-quantitative reverse-transcription and polymerase chain reaction

Lung parenchyma strips (3 × 3 × 10 mm) were longitudinally cut from left lungs. Total RNA was isolated from the frozen lung tissue and the relative expression of PCIII was obtained by semi-quantitative Reverse-Transcription and Polymerase Chain Reaction (RT-PCR). In the PCIII mRNA detection by RT-PCR, the rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) primers were used as internal positive control [12, 13].

Statistical analysis

The normality of the data (Kolmogorov–Smirnov test with Lilliefors' correction) and the homogeneity of variances (Levene median test) were tested. If both conditions were satisfied, the effects of different ventilatory strategies in CTRL and ALI groups were analysed by using Two-way ANOVA followed by Tukey's test. Otherwise, two-way ANOVA on ranks followed by Dunn's post hoc test was selected instead. These two tests were used to compare lung histological data and PCIII mRNA expression. The effects of ventilatory strategies on CTRL and ALI groups at BASELINE and END (time) were assessed by using a three-way ANOVA with repeated measures on the time factor. This test was used to compare lung static elastance and arterial blood gases. The significance level was set at 5%. The parametric data were expressed as mean ± SEM, while the non-parametric data were expressed as median (Interquartile range). All tests were performed using SigmaStat 3.0 statistical software package (Jandel Corporation, San Raphael, CA, USA).

Results

Mean arterial pressure was maintained stable and at adequate levels during the experiments (see ESM).

In ALI, PaO_2 and pH were lower while $PaCO_2$ was higher than in CTRL (Table 1). The percentage of increase of PaO_2 from BASELINE to END was 13 and 48% after RM-CPAP and S180/40, respectively (Table 1). S10/40 and S10/20 groups presented greater increase in PaO_2 (58 and 54%, respectively) compared to S180/40. $PaCO_2$ and pH were lower in S180/40 and S10/40 compared to the other groups with no significant changes between them (Table 1).

As shown in Fig. 1, Est,L significantly increased in ALI compared to CTRL. RM-CPAP did not modify Est,L while sigh (S180/40, S10/40, and S10/20) significantly decreased Est,L. Est,L was lower in S10/40 than S10/20.

The fraction area of alveolar collapse was higher in ALI compared to CTRL. S180/40 showed a reduction of alveolar collapse and increased hyperinflated areas compared to RM-CPAP (Fig. 2). S10/40 led to a decrease in alveolar collapse and hyperinflation, while the amount of alveolar collapse was higher in S10/20 than S10/40 (Fig. 2).

CTRL animals showed no lung ultrastructural modifications independent of RM. All ALI animals presented cytoplasmatic degeneration of type II pneumocyte (PII) and endothelial damage (Table 2). However, in S180/40, type II pneumocyte and endothelial damage was higher with a detachment of alveolar epithelium and denudation of epithelial basement membrane. In S10/40 and S10/20 these ultrastructural changes were minimized (Table 2).

The present model of ALI led to lung and distal organ epithelial cell apoptosis, while in CTRL the amount of epithelial cell apoptosis in lung and distal organs was similar among the groups. Lung and kidney apoptosis were increased only in S180/40 (Table 3). Photomicrographs of light and electron microscopy and immunohistochemistry are shown in ESM.

PCIII mRNA expression was higher in ALI than CTRL. In both ALI and CTRL, S180/40 increased PCIII mRNA expression (Fig. 3) while RM-CPAP caused no alteration. PCIII mRNA expression was significantly

Table 1 Arterial blood gases and pH

	Ventilatory strategies	NR		RM-CPAP		S180/40		S10/40		S10/20	
	Groups	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**
PaO_2 (mmHg)	BASELINE	82 ± 3	65 ± 2	80 ± 3	62 ± 6	82 ± 2	61 ± 3	84 ± 4	60 ± 4	81 ± 5	59 ± 6
	END	83 ± 5	57 ± 2	88 ± 2*	70 ± 6*	93 ± 3*	90 ± 2*†	96 ± 3*	95 ± 3*†**	94 ± 5*	91 ± 2*†#
$PaCO_2$ (mmHg)	BASELINE	40 ± 2	50 ± 4	39 ± 2	51 ± 7	40 ± 2	52 ± 3	39 ± 2	50 ± 7	38 ± 2	51 ± 4
	END	43 ± 1	64 ± 8*	38 ± 2	57 ± 6	35 ± 1†	41 ± 6*†	36 ± 1†	45 ± 6*†	41 ± 2	57 ± 6†#§
pH	BASELINE	7.31 ± 0.02	7.22 ± 0.02	7.35 ± 0.01	7.19 ± 0.03	7.34 ± 0.01	7.24 ± 0.05	7.36 ± 0.01	7.24 ± 0.06	7.34 ± 0.02	7.21 ± 0.01
	END	7.28 ± 0.01	7.13 ± 0.06	7.36 ± 0.01	7.16 ± 0.04	7.39 ± 0.01	7.31 ± 0.07†‡	7.37 ± 0.02	7.29 ± 0.05†‡	7.31 ± 0.02	7.18 ± 0.03#§

Arterial oxygen partial pressure (PaO_2, mmHg), arterial carbon dioxide partial pressure ($PaCO_2$) and pH immediately before (BASELINE) and at 1 h mechanical ventilation (END) in control (CTRL) and acute lung injury (ALI). CTRL and ALI groups were randomized as follows: (a) non-recruited (NR), (b) a 40-s inflation to a peak airway pressure of 40 cmH_2O (RM-CPAP), (c) 180 sighs/h and plateau pressure of 40 cmH_2O (S180/40), (d) 10 sighs/h and plateau pressure of 40 cmH_2O (S10/40), and (e) 10 sighs/h and a plateau pressure of 20 cmH_2O (S10/20). Values are means (±SEM) of five rats in each group. *Significantly different from BASELINE ($P < 0.05$). †Significantly different from NR ($P < 0.05$). ‡Significantly different from RM-CPAP ($P < 0.05$). #Significantly different from S180/40 ($P < 0.05$). §Significantly different from S10/40 ($P < 0.05$). **All data from ALI groups were significantly different from CTRL ($P < 0.05$)

lower in S10/40 compared to S180/40, with no significant difference between S10/40 and S10/20 (Fig. 3).

Discussion

In the present experimental model of moderate ALI, a RM with standard sigh [180 sighs/h and Pplat = 40 cmH_20 (S180/40)] improved oxygenation and decreased $PaCO_2$, Est,L, and alveolar collapse; nevertheless, it yielded hyperinflation, ultrastructural changes in alveolar capillary membrane, increased lung and kidney epithelial cell apoptosis, and PCIII mRNA expression in lung tissue. On the other hand, RM with 10 sighs/h and Pplat = 40 cmH_20 (S10/40) diminished Est,L and improved oxygenation, with a marked decrease in alveolar hyperinflation, PCIII mRNA expression in lung tissue, and apoptosis in lung and kidney epithelial cells. However, this sigh frequency associated with a lower Pplat of 20 cmH_2O (S10/20) worsened Est,L, histology and oxygenation, increased $PaCO_2$ but did not modify PCIII mRNA expression in lung tissue and epithelial cells apoptosis of distal organs. Therefore, RM with high frequency or low plateau pressure should be avoided.

To our knowledge this is the first study investigating the role of frequency and Pplat during RMs on lung and distal organ injury in an experimental model of ALI.

ALI was induced by paraquat, a herbicide which yields a moderate lung injury [22] with epithelial cell apoptosis in distal organs [23]. In our study the degree of alveolar collapse in non-recruited (NR) ALI was around 26% (Fig. 2) similar to that observed in previous experimental [12, 22] and human studies in ALI/ARDS [24]. After 1-h ventilation, Est,L increased while oxygenation decreased in NR animals. This deterioration may be caused by the use of limited V_T and/or an insufficient PEEP level to open collapsed alveoli. We decided to use low V_T and 5 cmH_2O PEEP to minimize the possible interactions between conventional mechanical ventilation and different recruitment strategies on ventilator-induced lung injury (VILI) [25, 26]. Besides, animals were ventilated in air to prevent reabsorption atelectasis [27] and reduce possible hyperoxia-induced lung injury [28].

Comparison between RM-CPAP and S180/40

RM-CPAP did not significantly change Est,L, but improved oxygenation and reduced atelectasis after 1-h ventilation period. We cannot rule out the fact that RM-CPAP may lead to an early improvement in Est,L and its beneficial effects wore off with time. Furthermore, the association of 5 cmH_2O PEEP with V_T of 4 ml/kg

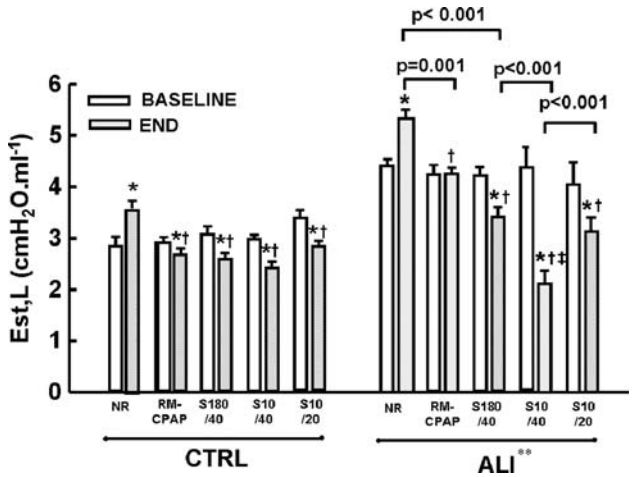

Fig. 1 Lung static elastance (*Est,L*) immediately before (BASELINE) and 1 h after (END) recruitment manoeuvre (*RM*) in control (*CTRL*) and acute lung injury (*ALI*). CTRL and ALI groups were randomized as follows: (a) non-recruited (*NR*), (b) a 40-s inflation to a peak airway pressure of 40 cmH_2O (RM-CPAP), (c) 180 sighs/h and plateau pressure of 40 cmH_2O (S180/40), (d) 10 sighs/h and plateau pressure of 40 cmH_2O (S10/40), and (e) 10 sighs/h and a plateau pressure of 20 cmH_2O (S10/20). Values are mean ± SEM of eight animals in each group (ten determinations per animal). *Significantly different from BASELINE ($P < 0.05$). †Significantly different from NR group ($P < 0.05$). ‡Significantly different from RM-CPAP group ($P < 0.05$). **All data from ALI groups were significantly different from CTRL ($P < 0.05$)

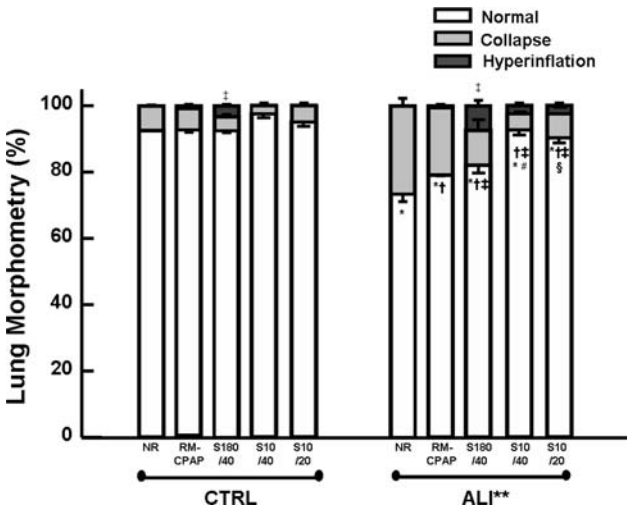

Fig. 2 The volume fraction of the lung occupied by hyperinflated structures (*dark gray*) or collapsed alveoli (*gray*) or normal pulmonary areas (*white*) in control (*CTRL*) and acute lung injury (*ALI*) groups. All values were computed in ten random, non-coincident fields per rat. Values are mean ± SEM of eight animals in each group. CTRL and ALI groups were randomized as follows: (a) non-recruited (NR), (b) a 40-s inflation to a peak airway pressure of 40 cmH_2O (RM-CPAP), (c) 180 sighs/h and plateau pressure of 40 cmH_2O (S180/40), (d) 10 sighs/h and plateau pressure of 40 cmH_2O (S10/40), and (e) 10 sighs/h and a plateau pressure of 20 cmH_2O (S10/20). *Significantly different from CTRL-NR ($P < 0.05$). †Significantly different from NR group ($P < 0.05$). ‡Significantly different from RM-CPAP group ($P < 0.05$). #Significantly different from S180/40 ($P < 0.05$). §Significantly different from S10/40 ($P < 0.05$). **All data from ALI groups were significantly different from CTRL ($P < 0.05$)

may preclude positive effects of 1-h mechanical ventilation.

Est,L and the amount of alveolar collapse was lower while PaO_2 higher in S180/40 compared to RM-CPAP, in accordance with previous studies in ALI/ARDS patients both in supine [14] and prone positioning [15]. In contrast, S180/40 group presented alveolar hyperinflation, severe epithelial and endothelial injury (Table 2), associated with an increase in PCIII mRNA expression (Fig. 3). The high pressure of sigh used to re-expand and open collapsed lung units may expose the alveoli to tensile and shear stresses stimulating fibroblasts and macrophages to synthesize collagen fibres [29, 30]. Our results are in accordance with previous reports demonstrating increased procollagen mRNA expression in lungs submitted to high airway pressures [31, 32], high inflation [12, 13, 32] or cyclic mechanical strain [30]. Additionally, sigh led to higher lung and kidney epithelial cell apoptosis, in line with other studies, which reported epithelial cell apoptosis in distal organs during injurious mechanical ventilation [7, 33]. In this context, kidney has been described as the first distal organ to be damaged during injurious mechanical ventilation [34, 35]. Distal organ injury process may be related to the (1) release of circulating soluble Fas ligand and cytokines [7] and/or (2) hypotension or depressed cardiac function resulting in decreased organ perfusion. Although mean arterial pressure was maintained at adequate levels during the experiments, we cannot rule out possible cardiac output reduction. Furthermore, even though we did not measure FasL or cytokines, lung and kidney apoptosis may be associated with increased PCIII expression. Therefore, we may hypothesize that the release of inflammatory mediators induced by excessive pulmonary stress and strain could have contributed to lung and kidney injury. However, the degree of lung stress induced by sigh was probably not high enough to cause small intestine villi and liver epithelial cell apoptosis [36]. We also found an important dissociation between the improvement in the clinical parameters, i.e. oxygenation and lung elastance, and molecular (Fig. 3) and ultra-structural (Table 2) damages in lung parenchyma. This observation is important since the measurements of oxygenation and lung mechanics are most commonly used to optimize mechanical ventilation at the bedside; however, our experimental data suggest that they do not necessarily represent the optimal parameters to monitor the possible RMs effects on lung and peripheral organ injury [25].

Table 2 Semi-quantitative analysis of electron microscopy

Ventilatory strategies	NR		RM-CPAP		S180/40		S10/40		S10/20	
Groups	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**
Alveolar capillary membrane	0 (0–0)	2 1–2.5	0 (0–0)	2 2–2	0 (0–1)	4* (3.5–4)	0 (0–0)	2 (2–3)	0 (0–0)	2 (1.5–2.5)
Type II epithelial cells	0 (0–0)	2 2–2.5	0 (0–0)	2 1.5–2.5	0 (0–1)	3* (2.5–3.5)	0 (0–0)	2 (1.5–2)	0 (0–0)	1.5 (1–2)
Endothelial cells	0 (0–0)	1.5 1–2.5	0 (0–0)	2 2–2.5	0 (0–0)	3.5* (2.5–4)	0 (0–0)	1.5 (1–2.5)	0 (0–0)	1 (1–1.5)

Lung tissue score was done independently by two different investigators. The pathologic findings were graded according to a 5-point semi-quantitative severity-based scoring system: 0 = normal lung parenchyma, 1 = changes in 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of the examined tissue. Electron microscopy of lung parenchyma in CTRL and ALI groups. *NR* non-recruited, RM-CPAP: a 40-s inflation to a peak airway pressure of 40 cmH_2O, S180/40: 180 sighs/h and plateau pressure of 40 cmH_2O, S10/40: 10 sighs/h and plateau pressure of 40 cmH_2O, and S10/20: 10 sighs/h and a plateau pressure of 20 cmH_2O. Values are median (25 percentile–75 percentile) of four to five rats in each group.*Significantly different from NR group ($P < 0.05$). **All data from ALI groups were significantly different from CTRL ($P < 0.05$)

Table 3 Epithelial cells apoptosis in lung and distal organs

Ventilatory strategies	NR		RM-CPAP		S180/40		S10/40		S10/20	
Groups	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**	CTRL	ALI**
Lung	0 (0–0)	2 (1.5–2.5)	0 (0–0)	2 (1.5–2.5)	0 (1–2)	3.5† ‡ (3–4)	0 (0–0)	2# (1.5–2)	0 (0–0)	2 # (1.5–2.5)
Kidney	0 (0–0)	2 (2–3)	0 (0–0)	3 (2.5–3.5)	0 (0–1)	4 † (3.5–4)	0 (0–0)	2.5# (1.5–3)	0 (0–0)	2 # (1.5–2)
Villi	0 (0–0)	1.5 (1–2.5)	0 (0–0)	2 (1.5–3)	0 (0–0)	2.5† (1.5–3.5)	0 (0-0)	2.5 (1.5–2.5)	0 (0–0)	2 (1.5–2.5)
Liver	0 (0–0)	2.5 (2–3)	0 (0–0)	2.5 (1.5–3.5)	0 (0–0)	2 (1.5–2.5)	0 (0–0)	3 (2.5–3.5)	0 (0–0)	2 (1.5–2.5)

Values are median (25 percentile–75 percentile) of four animals in each group. The apoptotic findings were graded as negative = 0, slight = 1, moderate = 2, high = 3 and severe = 4 in ten non-coincident microscopic fields (×400 magnification). A mean score was then calculated (0 = normal lung parenchyma; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of structures altered). *NR* non-recruited, RM-CPAP: a 40-s inflation to a peak airway pressure of 40 cmH_2O, S180/40: 180 sighs/h and plateau pressure of 40 cmH_2O, S10/40: 10 sighs/h and plateau pressure of 40 cmH_2O, and S10/20: 10 sighs/h and a plateau pressure of 20 cmH_2O. †Significantly different from NR group ($P < 0.05$). ‡Significantly different from RM-CPAP ($P < 0.05$). #Significantly different from S180/40 ($P < 0.05$). **All data from ALI groups were significantly different from CTRL ($P < 0.05$)

Reduction of frequency during sigh

Higher respiratory frequencies may induce lung injury by both elevating the magnitude of shear stress from more rapid inflations (thereby exceeding the failure limit and reducing the number of cycles required for failure) and more rapidly achieving the total number of cycles required for failure [37, 38]. Additionally, the increase in respiratory frequency has been reported to be associated with the release of inflammatory mediators [39]. Therefore, lower sigh frequency resulted in less lung epithelial and endothelial damage, leading to reduced PCIII expression and lung epithelial cell apoptosis. We speculate that there is a sigh frequency threshold beyond which the intrinsic reparative properties of the lung epithelium are overwhelmed. Clearly, the optimal sigh frequency may be different in healthy and ALI animals/patients.

We also observed a reduction in kidney epithelial cell apoptosis that could be related to the attenuation of the overall inflammatory process [17]. On the other hand, we cannot rule out the possible improvement of regional perfusion during reduced sigh frequency, leading to better oxygen delivery to peripheral organs.

Reduction of plateau pressure during sigh

The use of a reduced inspiratory Pplat of 20 cmH_2O was based on some pilot studies which showed that PCIII mRNA expression remained higher in S10/40 than NR-ALI animals. This level of Pplat is approximately double the mean Pplat achieved during conventional tidal volume in this experimental ALI model. We found that the association of reduced sigh frequency and Pplat (S10/20) worsened oxygenation, lung mechanics and histology with no significant modification in epithelial cell apoptosis of the lung and distal organs, and PCIII mRNA expression. Thus, although lower Pplat during sigh reduced the tensile stress, it was unable to open the collapsed alveoli resulting in shear stress while maintaining higher PCIII mRNA expression [12].

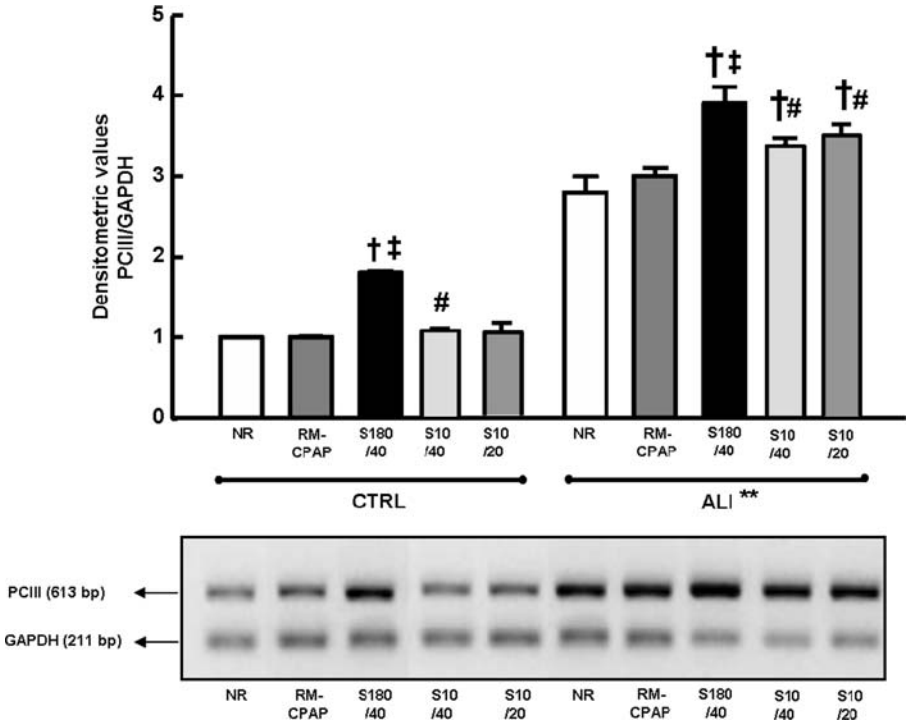

Fig. 3 Relative expression of type III procollagen mRNA (PCIII) obtained by amplification of PCIII and glyceraldehydes-3-phosphate-dehydrogenase (GAPDH) by semi-quantitative reverse-transcription and polymerase chain reaction (RT-PCR) of rat lung tissue in control (*CTRL*) and acute lung injury (*ALI*) groups. CTRL and ALI groups were randomized as follows: (a) non-recruited (NR), (b) a 40-s inflation to a peak airway pressure of 40 cmH_2O (RM-CPAP), (c) 180 sighs/h and plateau pressure of 40 cmH_2O (S180/40), (d) 10 sighs/h and plateau pressure of 40 cmH_2O (S10/40), and (e) 10 sighs/h and a plateau pressure of 20 cmH_2O (S10/20). Values are mean ± SEM ($n = 4$) of the ratio between the densitometric values of PCIII and GAPDH bands obtained in RT-PCR experiments. †Significantly different from NR group ($P < 0.05$). ‡Significantly different from RM-CPAP group ($P < 0.05$). #Significantly different from S180/40 ($P < 0.05$). §Significantly different from S10/40 ($P < 0.05$). **All data from ALI groups were significantly different from CTRL ($P < 0.05$)

Limitation of the study

The current study has several limitations: (1) we used a specific experimental model of moderate ALI induced by paraquat. Thus, we do not know if similar results can be obtained in other experimental models of ALI, in larger animals, with different degrees of lung injury, amount of recruitable tissue or consolidation; (2) the short duration of the experiment, just 1 h, which hinders assessment of possible long term effects of RMs; (3) different types of RMs have been proposed, with periodic increase in Pplat, PEEP or both [9, 17]. However, in the present study, we only evaluated sigh performed by periodic changes in Pplat at different frequencies. We cannot exclude that sigh frequencies lower than 10/h (S10/40) but higher than 1/h (RM-CPAP), may further improve respiratory function minimizing lung injury. Additionally, the reduction of sigh frequency under the same inspiratory plateau pressure reduced lung injury; (4) PEEP was not individually titrated. A fixed level of 5 cmH_2O PEEP was applied to avoid the possible bias due to the interaction between different PEEP levels and RMs. Thus, we cannot exclude that different results could have been obtained at higher PEEP levels; (5) only PCIII mRNA expression was measured, therefore the impact of RMs on different inflammatory mediators was not evaluated; and (6) although mean arterial pressure remains stable and at adequate levels during the experiments, the association between reduced regional perfusion induced by RMs and distal organ damage cannot be ruled out.

Conclusion

This study, under the present experimental conditions, demonstrates that sigh at 40 cmH_2O Pplat was effective at opening collapsed alveoli, improving oxygenation and lung mechanics independent of the frequency. The reduction in sigh frequency led to a better lung morphofunctional and molecular profile. However, the combination of lower sigh frequency and inspiratory plateau pressure worsened lung

function and histology, with no further protective effects on lung and distal organs. The best method of recruitment manoeuvre remains uncertain and the optimal inspiratory plateau pressure, duration and periodicity need to be elucidated.

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