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On the crucial ventilatory setting adjustment from two- to one-lung ventilation

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ABSTRACT

Lung mechanics, histology, oxygenation and type-III procollagen (PCIII) mRNA were studied aiming to evaluate the need to readjust ventilatory pattern when going from two- to one-lung ventilation (OLV). Wistar rats were assigned to three groups: the left lung was not ventilated while the right lung received: (1) tidal volume (V_T) = 5 ml/kg and positive end-expiratory pressure (PEEP) = 2 cm H_2O (V5P2), (2) V_T = 10 ml/kg and PEEP = 2 cm H_2O (V10P2), and (3) V_T = 5 ml/kg and PEEP = 5 cm H_2O (V5P5). At 1-h ventilation, V5P2 showed hypoxemia, alveolar collapse and impaired lung function. Higher PEEP minimized these changes and prevented hypoxemia. Although high V_T prevented hypoxemia and maintained a higher specific compliance than V5P2, a morphologically inhomogeneous parenchyma and higher PCIII expression resulted. In conclusion, the association of low V_T and an adequate PEEP level could be useful to maintain arterial oxygenation without inducing a possible inflammatory/remodeling response.

1. Introduction

One-lung ventilation (OLV) can be used to isolate a lung or to facilitate ventilatory management in patients undergoing thoracic surgery. In these procedures, one lung is mechanically ventilated while the other remains occluded, resulting in impaired gas exchange (Watanabe et al., 2000; Ishikawa and Lohser, 2011). In order to prevent hypoxemia, guidelines recommended for years the use of a high tidal volume (V_T) (Brodsky and Fitzmaurice, 2001; Gal, 2006). Indeed, the same tidal volume initially delivered to both lungs is given to the ventilated one during OLV (Unzueta et al., 2007; Pardos et al., 2009). However, high V_T injured isolated perfused rabbit lung, which was prevented by the application of low V_T and positive end-expiratory pressure (PEEP) during OLV (Gama de Abreu et al., 2003). In accordance with their findings, recent studies suggest the use of low tidal volume (4–6 ml/kg), routine PEEP, and permissive hypercapnia (Lohser, 2008; Ishikawa and Lohser, 2011) to prevent ventilator induced-lung injury. However, some authors still apply high V_T (8–10 ml/kg) during thoracic surgery (Unzueta et al., 2007; Pardos et al., 2009), even though protective OLV has been increasingly recommended (Michelet et al., 2006; Kilpatrick and Slinger, 2010; Montes et al., 2010).

To better elucidate the controversial issues related to V_T and PEEP during OLV, taking into consideration the practice of applying to one lung the same tidal volume previously delivered to two lungs, the current study analyzed lung mechanics, histology, end-expiratory lung volume (EELV), oxygenation, and type-III procollagen mRNA expression in rats, aiming to determine whether different ventilatory settings can induce tissue remodeling during OLV even in the face of adequate oxygenation.

2. Materials and methods

This study was approved by the Ethics Committee on the Use of Animals, Health Sciences Centre, Federal University of Rio de Janeiro (Protocol No. IBCCF 019). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, USA, and according to the Helsinki

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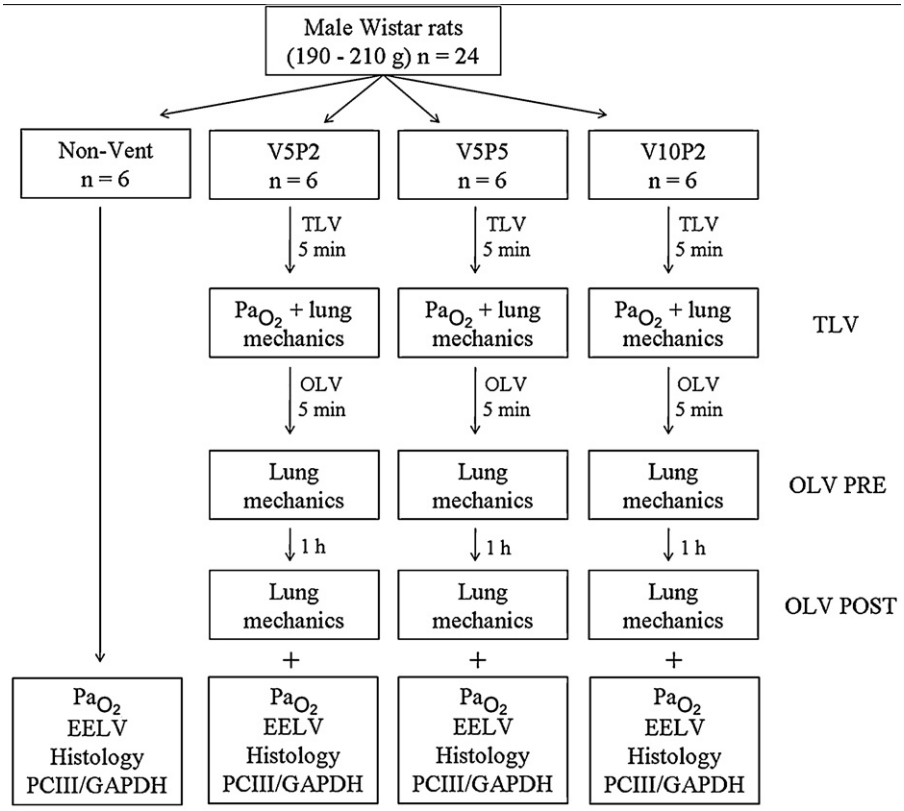

Fig. 1. Experimental protocol. In V5P2 and V10P2 groups the right lungs were ventilated with V_T = 5 or 10 ml/kg, respectively, and positive end-expiratory pressure (PEEP) of 2 cm H_2O; in V5P5 group the right lung was ventilated with V_T = 5 ml/kg and PEEP = 5 cm H_2O (in both instances the left lung was collapsed). In Non-Vent group animals did not undergo mechanical ventilation. TLV, two-lung ventilation; OLV, one-lung ventilation; OLV PRE, after stabilization of OLV; OLV POST, 1 h of OLV; Pa_{O_2}, partial arterial oxygen pressure; EELV, end-expiratory lung volume; PCIII/GAPDH, relative expression of type-III procollagen (PCIII) mRNA obtained by amplification of PCIII and glyceraldehydes-3-phosfate-dehydrogenase (GAPDH) by semi-quantitative RT-PCR.

convention for the use and care of animals. Experimental study was carried in a research laboratory.

2.1. Animal preparation

Eighteen normal male Wistar rats (190–210 g) were randomly divided into three groups: the left lung was not ventilated while the right lung received: (1) V_T = 5 ml/kg and PEEP = 2 cm H_2O (V5P2), (2) V_T = 10 ml/kg and PEEP = 2 cm H_2O (V10P2), and (3) V_T = 5 ml/kg and PEEP = 5 cm H_2O (V5P5). In V5P2 and V10P2 groups, physiological PEEP (2 cm H_2O) was applied to avoid lung collapse (open-chest animals, see below). Another 6 rats (Non-Vent group) did not undergo mechanical ventilation, i.e., the animals were euthanized and the lungs were removed at end-expiratory lung volume.

The animals were sedated (diazepam, 5 mg i.p.), anesthetized (pentobarbital sodium, 20 mg/kg i.p.), paralyzed (gallamine triethyliodide, 2 mg/kg i.v.) and tracheotomized in the supine position on a surgical table. Thereafter, a constant flow ventilator provided artificial ventilation (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) with an inspired oxygen fraction of 0.21.

The physiological PEEP level was determined as follows: before the pleural space was opened, the airways were occluded at end expiration. After pleural incision, the increase in airway pressure corresponds to the elastic recoil pressure of the lung at relaxation volume. Thereafter, the same pressure was applied to the lung, 2 cm H_2O on the average (Saldiva et al., 1992), except in V5P5 group that received 5 cm H_2O of PEEP. The anterior chest wall was then surgically removed.

An arterial cannula was inserted into the femoral artery for the determination of arterial partial pressure of oxygen (Pa_{O_2}) (AVL Biomedical Instruments, Roswell, GA, USA). Pa_{O_2} was measured at the beginning of the experiment and at the end of 1-h OLV (Fig. 1).

2.2. Experimental protocol and mechanical parameters

The experimental protocol is depicted in Fig. 1. Two-lung volume-controlled ventilation was first established. After stabilization of the mechanical parameters under two-lung ventilation, the tracheal cannula was further introduced into the right main stem bronchus in order to exclude the left lung from ventilation.

As seen in Fig. 1, pulmonary mechanics were measured in three occasions: immediately after stabilization of two-lung ventilation (TLV), immediately after stabilization of one-lung ventilation (OLV PRE) and 1 h after the second measurement (OLV POST).

Pulmonary mechanics were measured by the end-inflation occlusion method (Bates et al., 1985). In an open-chest preparation tracheal pressure reflects transpulmonary pressure. Driving pressure [difference between plateau pressure (Pplat) and PEEP], viscoelastic/inhomogeneous pressure (ΔP2) and static compliance (Cst) were measured. Cst was corrected by end-expiratory lung volume (EELV) in order to obtain specific compliance (Csp), enabling the comparison between one- and two-lung ventilation. Pulmonary mechanics were measured 10 times in each animal in each occasion.

All data were analyzed using ANADAT data analysis software (RHT InfoData, Montreal, QC, Canada).

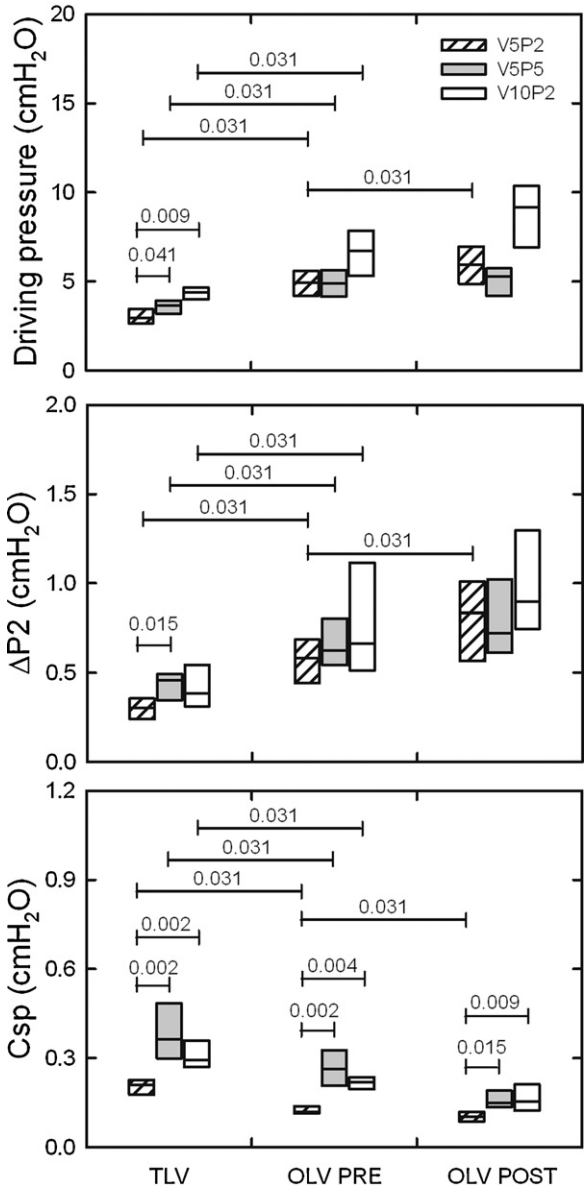

Fig. 2. Driving pressure, pressure spent to overcome viscoelastic/inhomogeneous mechanical components (ΔP2), and specific compliance (Csp) measured after stabilization of two-lung ventilation (TLV), after stabilization of one-lung ventilation (OLV PRE) and at the end (OLV POST) of 1-h ventilation. In V5P2 and V10P2 groups the right lungs were ventilated with V_T = 5 or 10 ml/kg, respectively, and positive end-expiratory pressure (PEEP) of 2 cm H_2O; in V5P5 group the right lung was ventilated with V_T = 5 ml/kg and PEEP = 5 cm H_2O (in both instances the left lung was collapsed). Data are presented as median (straight lines inside the boxes) and the 1st to 3rd quartiles) of six animals in each group. *P* values depicted above horizontal bars.

2.3. Lung histology

A laparotomy was performed immediately after the determination of lung mechanics, and heparin (1000 IU) was intravenously injected (abdominal vena cava). The trachea (Non-Vent group) or the right main stem bronchus (V5P2, V5P5, and V10P2 groups) was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. The lungs (Non-Vent) or the right lung (V5P2, V5P5, and V10P2 groups) were removed and weighed. End-expiratory lung volume (EELV) was determined by volume displacement (Scherle, 1970).

To perform the morphometrical study, the middle lobe of the right lung was isolated at EELV, quick-frozen by immersion in liquid nitrogen, and fixed with Carnoy's solution (ethanol:chloroform:acetic acid, 70:20:10) at −70 °C. After 24-h, ethanol concentrations were progressively increased (70, 80, 90 and 100%, respectively, 1 h at each solution, at −20 °C). The lungs were then kept in 100% ethanol for 24 h at 4 °C (Nagase et al., 1996). After fixation, tissue blocks were embedded in paraffin and 4-μm thick slices were cut and mounted. Slides were stained with hematoxylin–eosin. Morphometric analysis was done with an integrating eyepiece with a coherent system made of a 100-point grid consisting of 50 lines, coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). The volume fraction of collapsed and normal pulmonary areas and the fraction of the lung occupied by large-volume gas-exchanging air spaces (wider than 120 μm) were determined by the point-counting technique (Gundersen et al., 1988; Weibel, 1990) at a magnification of 200× across 10 random, non-coincident microscopic fields. Points falling on collapsed, normal or hyperinflated alveoli were counted and divided by the total number of points hitting alveoli in each microscopic field. Polymorpho- (PMN) and mononuclear (MN) cells were counted at 1000× magnification, and divided by the total number of points falling on tissue area in each microscopic field. Thus, data are reported as the fractional area of pulmonary tissue.

2.4. Type-III procollagen mRNA expression

Lung parenchyma strips (3 mm × 3 mm × 10 mm) were longitudinally cut from right lungs. Pleural tissue was removed, and the strips were stored in liquid nitrogen for analysis of type-III procollagen (PCIII) mRNA expression. Total RNA was isolated from the frozen lung tissue (Chomczynsky and Sacchi, 1987). The relative expression of type-III procollagen mRNA (PCIII mRNA) was obtained by semi-quantitative reverse-transcription and polymerase chain reaction (RT-PCR). In the PCIII mRNA detection by RT-PCR, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as internal positive control. The semi-quantitative method of RT-PCR, used to quantify the PCIII mRNA expression in the experimental rat lung, was validated in preliminary experiments (Garcia et al., 2004; Farias et al., 2005). All reactions included a negative control RT (-). The identity of the amplification was confirmed by determination of the molecular size on agarose gel electrophoresis with 100 bp DNA molecular markers (Gibco BRL, Grand Island, NY, USA).

2.5. Statistical analysis

SigmaPlot 11 software package (SYSTAT, Chicago, IL, USA) was used. To evaluate the consequences of mechanical ventilation, ventilated groups were compared to Non-Vent. In order to analyze the effects of PEEP during OLV with low V_T, comparisons between V5P2 and V5P5 were done, while the effects of high V_T during OLV with physiological PEEP were assessed by comparisons between V5P2 and V10P2. The normality of the data (Kolmogorov–Smirnov test with Lilliefors' correction) and the homogeneity of variances (Levene median test) were tested. When both conditions were satisfied one-way ANOVA test followed by Dunnett's test and Student *t*-test were used. In the negative case, Kruskal–Wallis one-way analysis of variance on ranks followed by Dunn's test and Mann–Whitney Rank Sum Test were applied. Wilcoxon Signed Rank Test was used to compare mechanical parameters between TLV and OLV PRE and between OLV PRE and OLV POST. In all instances the significance level was set at 5%.

Table 1
Lung histology and arterial oxygenation.

	Non-Vent	V5P2	V5P5	V10P2
Alveolar collapse (%)	0.9 (0.4–7.8)	33.3 (30.9–35.3)*	18.5 (9.3–23.8)**	16.1 (12.4–18.7)**
Large volume spaces (%)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	34.3 (27.7–38.2)*,**
Total cells (%)	31.4 (28.8–32.0)	45.5 (44.4–48.4)*	45.6 (41.4–48.9)*	43.5 (40.4–45.6)
PMN (%)	2.3 (2.2–2.6)	30.2 (28.7–33.4)*	23.0 (20.4–24.2)**	32.7 (29.6–35.5)*
Pa_{O_2} (mmHg)	94.0 ± 1.5	78.5 ± 1.7*	95.5 ± 3.3**	94.2 ± 2.2**

Data are expressed as median (1st to 3rd quartiles) of 10 random, non-coincident fields per animal ($n = 6$), or mean ± SEM of six animals in each group (two samples per rat). All measurements in ventilated animals were done at the end of the 1-h ventilation period ensuing ventilator protocol adjustment. In V5P2 and V10P2 groups the right lungs were ventilated with V_T = 5 or 10 ml/kg, respectively, and positive end-expiratory pressure (PEEP) of 2 cm H_2O (the left lungs were collapsed). In V5P5 group the right lung was ventilated with V_T = 5 ml/kg and PEEP = 5 cm H_2O (the left lung was collapsed). In Non-Vent group, animals did not undergo mechanical ventilation. PMN, polymorphonuclear cells; Pa_{O_2}, arterial oxygen partial pressure. In all instances $\alpha = 5\%$.

* Significantly different from Non-Vent.

** Significantly different from V5P2.

3. Results

After stabilization of two-lung ventilation (TLV), V5P5 showed higher mechanical parameters (driving and viscoelastic pressures, and specific compliance) than V5P2 while V10P2 displayed greater driving pressure and Csp than V5P2. Csp was higher in V5P5 and V10P2 than in V5P2 right after one-lung ventilation (OLV PRE) and at 1 h (OLV POST, Fig. 2).

In the three groups OLV worsened all mechanical parameters in relation to TLV. Additionally, 1-h OLV (OLV POST, Fig. 2) deteriorated the mechanical parameters in relation to OLV PRE in V5P2.

With the exception of V5P5, end-expiratory lung volume (EELV) was lower in all groups compared to Non-Vent rats. EELV was higher in V5P5 than in V5P2. EELV did not differ between V5P2 and V10P2. The median EELV (1st to 3rd quartiles) measured in Non-Vent, V5P2, V5P5 and V10P2 groups amounted to 1.57 (1.25–1.73), 0.63 (0.53–0.72), 0.77 (0.68–0.93) and 0.65 (0.57–0.87) ml, respectively.

The fractional area of alveolar collapse was higher in V5P2 than in Non-Vent, V5P5 and V10P2 groups. V10P2 animals presented an inhomogeneous lung parenchyma characterized by a higher fraction area of the lung occupied by large-volume gas-exchanging air spaces than Non-Vent and V5P2 (Table 1). Total cell content was higher in V5P2 and V5P5, while the percentage of PMN was higher in V5P2 and V10P2 than in Non-Vent group. The amount of PMN cell was smaller in V5P5 than in V5P2 (Table 1).

At the beginning of the study all animals presented normal arterial oxygenation. The mean Pa_{O_2} (±SEM) of all groups was 94.0 ± 3.3 mmHg. At the end of 1-h ventilation with 5 ml/kg V_T (V5P2) hypoxemia was established, which was avoided by 5 cm H_2O PEEP (V5P5). High tidal volume (10 ml/kg) did not cause hypoxemia (Table 1).

PCIII mRNA expression was increased only when one-lung ventilation with high tidal volume (10 ml/kg, V10P2 group) was used (Fig. 4).

4. Discussion

In relation to low V_T associated with physiological PEEP, OLV with higher PEEP or V_T prevented deterioration of lung mechanics and alveolar collapse and maintained arterial blood gas oxygenation at the end of 1-h ventilation. We also demonstrated that in the face of normal Pa_{O_2} and stable Csp, high V_T and physiological PEEP induced alveolar hyperinflation and expressed PCIII mRNA in lung homogenate.

In order to analyze the effects of OLV, the animals underwent 1-h volume-controlled ventilation (VCV). Although pressure-controlled ventilation (PCV) allows a more homogeneous distribution of lung ventilation (Prella et al., 2002) and also reduces peak airway pressure (Unzueta et al., 2007) when compared to VCV, the evidence on the benefit of PCV for arterial oxygenation during OLV remains contradictory (Tugrul et al., 1997; Unzueta et al., 2007; Pardos et al., 2009). In a recent study, protective OLV with PCV instead of VCV did not improve oxygenation in patients with normal pulmonary function, although PCV was associated with lower peak airway pressure (Montes et al., 2010). In this context, we used VCV as the ventilatory model.

As seen in Fig. 2, the increment in PEEP (V5P5) or V_T (V10P2) increased driving pressure and Csp in relation to V5P2 soon after stabilization of TLV. Under TLV and V5, tidal volume is distributed between both lungs, each receiving a low volume (approximately 2.5 ml/kg), resulting in a smaller driving pressure in V5P2 than in V5P5 (higher PEEP) and V10P2 (higher tidal volume). In addition, both PEEP (V5P5) and V_T (V10P2) increments yielded higher compliances than V5P2, despite increased driving pressure, since normal rats were used. As previously observed, static and dynamic compliance increased during mechanical ventilation with V_T 5–15 ml/kg at zero end-expiratory pressure as well as with the increment of PEEP up to 6 cm H_2O, in patients with acute lung failure (Suter et al., 1978).

Immediately after stabilization of OLV (OLV PRE) each group presented a worse mechanical profile than during TLV. As expected, the increase in pulmonary volume resulting from the change from TLV to OLV elevated driving pressure in all groups. This transition would increase peak and plateau pressures (PEEP included), as previously demonstrated in pigs (Michelet et al., 2005) and humans undergoing thoracic surgery (Schilling et al., 2005). At the end of 1-h OLV (OLV POST) in V5P2 mechanics worsened in relation to OLV PRE, possibly as a result of distal airway/airspaces closure (Mead and Collier, 1959). On the other hand, during OLV mechanical parameters remained unaltered within groups due to either higher PEEP (V5P5) or V_T (V10P2). V5P5 and V10P2 showed higher Csp than V5P2 both at OLV PRE and OLV POST (Fig. 2). PEEP improves compliance by increasing functional residual capacity due to the recruitment of collapsed air spaces, while tidal volume alters compliance by changing the end-inspiratory point of tidal ventilation on the pressure–volume curve (Suter et al., 1978).

Specific compliance and ΔP2 deterioration in V5P2 could be attributed to an increase in stiffness of lung tissue due to alveolar collapse (D'Angelo et al., 2002), resulting in lung inhomogeneity (Rocco et al., 2001). A 5-cm H_2O PEEP was enough to prevent alveolar collapse and a fall in EELV even with low V_T OLV (Fig. 3, Table 1). It is well documented that the use of PEEP during mechanical ventilation reduces alveolar collapse by providing resistance to expiration, and may increase EELV, as evidenced in normal lungs (Lohser, 2008). On the other hand, a 10 ml/kg- V_T increased ΔP2 immediately after the transition from TLV to OLV (Fig. 2). The resulting hyperinflation (Fig. 3, Table 1) could stiffen lung tissue and increase tissue resistance owing to viscoelastic mechanical properties (Kochi et al., 1988; Similowski et al., 1989).

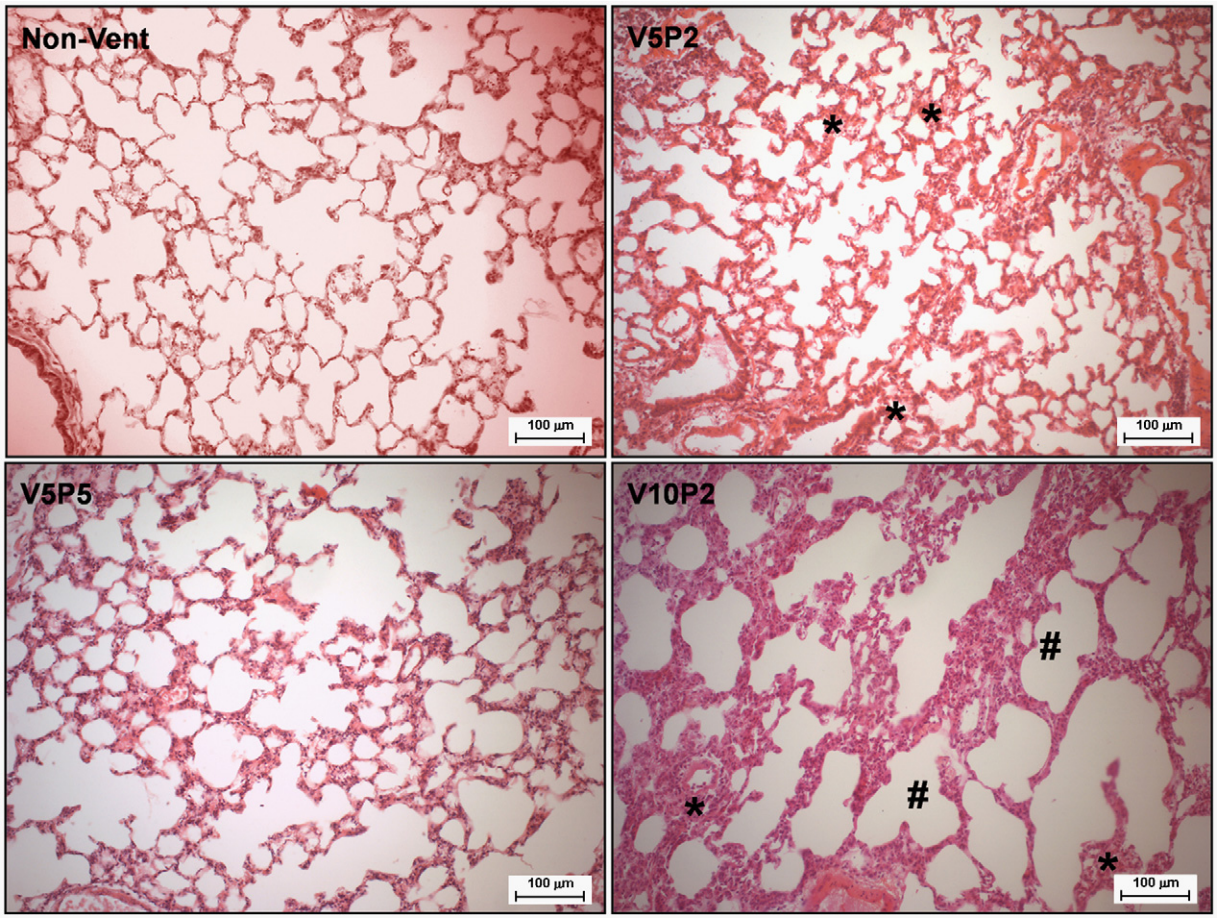

Fig. 3. Photomicrographs of lung parenchyma stained with hematoxylin–eosin. In V5P2 and V10P2 groups the right lungs were ventilated with V_T = 5 or 10 ml/kg, respectively, and positive end-expiratory pressure (PEEP) of 2 cm H_2O; in V5P5 group the right lung was ventilated with V_T = 5 ml/kg and PEEP = 5 cm H_2O (in both instances the left lung was collapsed). In Non-Vent group, animals did not undergo mechanical ventilation. (*) Alveolar collapse. (#) Hyperinflation. Scale Bars = 100 µm.

Although we did not compare the deterioration seen in OLV and that in a control group continued for an hour on TLV, Prost et al. (2007) found no mechanical difference in control rats ventilated (TLV) for 3 h with low V_T and PEEP (similar to our V5P5 group), but at the end of a 3-h high-volume mechanical ventilation their animals' peak airway pressure increased and compliance fell. The difference between theirs and our results (V10P2) may result from our shorter experiment (1 h) and somewhat smaller V_T. Additionally, in line with De Carvalho et al. (2007) we disclosed an early triggering of type-III procollagen mRNA expression (see below) in the latter animals.

Some mechanical ventilation conditions produce or worsen lung injury. During the initial stage of ventilator-induced lung injury (VILI) proinflammatory cytokines are released (Copland et al., 2003), triggering infiltration of PMN leukocytes into the alveoli (Dreyfuss and Saumon, 1998). However, the exact time profile of PMN recruitment into the lung during VILI and its underlying physiological mechanisms remain poorly understood. Tekinbas et al. (2007) observed time-dependent inflammatory cell infiltration during OLV in both collapsed and contralateral lungs. In addition, Musch et al. (2007) demonstrated inflammatory cell activation by positron emission tomography in VILI lungs even when gas exchange, respiratory compliance, and lung histology were still preserved. In the present study a 1-h OLV sufficed to increase the amount of PMN in the lung parenchyma in V5P2 and V10P2 in relation to Non-Vent, whereas a 5-cm H_2O PEEP avoided such recruitment. Possibly during V5P2 shear forces triggered the inflammatory response owing to the cyclic closing and reopening of airspaces at low lung volumes (Gattinoni et al., 2003), while V10P2 led to the same outcome because of an excessive volume being delivered to one lung (Schilling et al., 2005). V5P5 avoided the phenomenon both because of the slightly higher EELV and the conservative tidal volume.

One-hour of V5P2 OLV led to hypoxemia (Table 1). The application of a higher V_T or PEEP was enough to prevent this alteration. Higher volume may promote end-inspiratory alveolar recruitment and PEEP could have expanded collapsed alveoli (Lohser, 2008). In this context higher volume or PEEP promoted a better ventilation–perfusion matching. In accordance with our findings, Michelet et al. (2005) demonstrated an improvement in oxygenation with increasing PEEP, during OLV with 7 ml/kg V_T and 0.4 Fi_{O_2} in healthy lungs. However, these authors did not examine the effects of this protective strategy on tissue damage. It should be stressed that very frequently only oxygenation (Watanabe et al., 2000) or oxygenation and lung mechanics (Michelet et al., 2005; Unzueta et al., 2007; Pardos et al., 2009) are taken into account to evaluate the status of the respiratory system during OLV. Finally, according to our results, even in the face of a regular Pa_{O_2} lung morphology was altered and tissue damage produced (Fig. 3, Table 1).

Ventilation at both very high and low volumes can lead to VILI (Frank et al., 2002). When connective tissue and parenchymal cells are exposed to high mechanical load, an adaptation process to tensile stress can start. Once extracellular matrix provides pulmonary structural mechanical support, it can be altered in response to mechanical stress (Parker et al., 1997). Collagen represents one of these structural proteins and the stimulus to its synthesis can

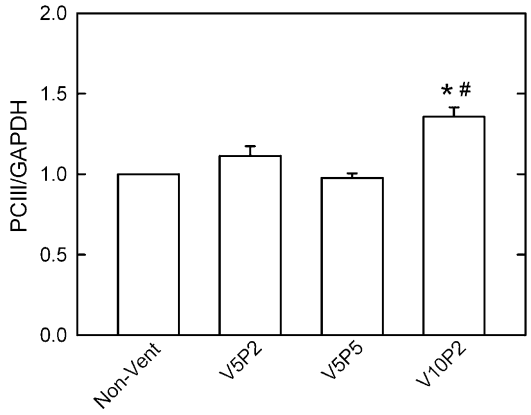

Fig. 4. Relative expression of type-III procollagen (PCIII) mRNA, obtained by amplification of PCIII and glyceraldehydes-3-phosfate-dehydrogenase (GAPDH) by semi-quantitative RT-PCR of rat lung tissue in different situations. In V5P2 and V10P2 groups the right lungs were ventilated with V_T = 5 or 10 ml/kg, respectively, and positive end-expiratory pressure (PEEP) of 2 cm H_2O; in V5P5 group the right lung was ventilated with V_T = 5 ml/kg and PEEP = 5 cm H_2O (in both instances the left lung was collapsed). In Non-Vent group, animals did not undergo mechanical ventilation. Values are mean + SEM (n = 3) of the ratio between the densitometric values of PCIII and GAPDH bands obtained in RT-PCR experiments. (*) Significantly different from Non-Vent; (#) Significantly different from V5P2. In all instances α = 5%.

be pinpointed by the expression of PCIII mRNA expression (Raghu et al., 1985). Thus, we used PCIII mRNA as a marker of tissue damage since type-III procollagen is one of the first molecules to be synthesized during the lung fibrotic process (Raghu et al., 1985). Indeed, PCIII mRNA was significantly higher in V10P2 group at the end of OLV (Fig. 4). The early response of PCIII mRNA is in line with previous two-lung ventilation studies (Garcia et al., 2004; Farias et al., 2005; De Carvalho et al., 2007). According to De Carvalho et al. (2007), overdistension due to mechanical ventilation with high V_T leads to an early response of the extracellular matrix, resulting in a significantly increase of PCIII mRNA expression. Interestingly, the extra pressure added to the respiratory system by the 3 cm H_2O difference in PEEP (from V5P2 to V5P5) increased lung volume by 0.62 ml at the beginning of OLV and by 0.35 ml at the end of OLV (calculated considering Csp at each instance, as depicted in Fig. 2, and EELV to calculate compliance, and, then delta volume), whereas the change in lung volume due to the extra gas volume added to the system from V5P2 to V10P2 was about 1 ml (= 5 ml/kg BW × 200 g BW). To our knowledge, no study has examined procollagen type-III expression during OLV.

Under the translational point of view, it should be stressed that in the present study both hemithoraces were open to the atmosphere, since the animals were in the supine position, as sometimes used in median sternotomy (Asaph et al., 2000). In this context, our results suggest that the use of high or low tidal volume without PEEP should be avoided during OLV applied in the face of median sternotomy, and perhaps under other sorts of thoracotomy as well.

The authors acknowledge limitations in the current study. First, we used only one ventilation mode (VCV). It would be interesting to compare the present results with those in PCV ventilation mode. Second, hemodynamic parameters were not controlled. PEEP may interfere with vascular pressure and cardiac output. Third, OLV lasted just 1 h and, thus, we cannot exclude the possibility that longer ventilation time with low tidal volume (5 ml/kg), independently of PEEP level, could increase PCIII mRNA expression. Fourth, PCIII mRNA represents an indicator of PCIII synthesis, which may not happen after all. Fifth, our blood gas measurement was restricted to Pa_{O_2}. The monitoring of pH and Pa_{CO_2} could have added important missing information. Sixth, we did not analyze the atelectatic lung.

In conclusion, considering that tidal volumes calculated on the basis of two healthy lungs are twice as great in their impact when delivered to a single lung, our results suggest that a high tidal volume that would be appropriate to two-lung ventilation should be avoided when changing into OLV. In addition, the use of 5 cm H_2O PEEP associated with a protective tidal volume could be useful to maintain arterial oxygenation without inducing a possible inflammatory/remodeling response.

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