Biological Impact of Transpulmonary Driving Pressure in Experimental Acute Respiratory Distress Syndrome

Cynthia S. Samary, Ph.D., Raquel S. Santos, M.Sc., Ph.D., Cíntia L. Santos, Ph.D., Nathane S. Felix, M.S., Maira Bentes, R.T., Thiago Barboza, M.D., Vera L. Capelozzi, M.D., Ph.D., Marcelo M. Morales, M.D., Ph.D., Cristiane S. N. B. Garcia, Ph.D., Sergio A. L. Souza, Ph.D., John J. Marini, M.D., Marcelo Gama de Abreu, M.D., Ph.D., Pedro L. Silva, Ph.D., Paolo Pelosi, M.D., F.E.R.S., Patricia R. M. Rocco, M.D., Ph.D.

ABSTRACT

Background: Ventilator-induced lung injury has been attributed to the interaction of several factors: tidal volume (V_T), positive end-expiratory pressure (PEEP), transpulmonary driving pressure (difference between transpulmonary pressure at end-inspiration and end-expiration, $\Delta P,L$), and respiratory system plateau pressure (Pplat,rs).

Methods: Forty-eight Wistar rats received *Escherichia coli* lipopolysaccharide intratracheally. After 24 h, animals were randomized into combinations of V_T and PEEP, yielding three different $\Delta P,L$ levels: $\Delta P,L_{LOW}$ (V_T = 6 ml/kg, PEEP = 3 cm H_2O); $\Delta P,L_{MEAN}$ (V_T = 13 ml/kg, PEEP = 3 cm H_2O or V_T = 6 ml/kg, PEEP = 9.5 cm H_2O); and $\Delta P,L_{HIGH}$ (V_T = 22 ml/kg, PEEP = 3 cm H_2O or V_T = 6 ml/kg, PEEP = 11 cm H_2O). In other groups, at low V_T, PEEP was adjusted to obtain a Pplat,rs similar to that achieved with $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$ at high V_T.

Results: At $\Delta P,L_{LOW}$, expressions of interleukin (IL)-6, receptor for advanced glycation end products (RAGE), and amphiregulin were reduced, despite morphometric evidence of alveolar collapse. At $\Delta P,L_{HIGH}$ (V_T = 6 ml/kg and PEEP = 11 cm H_2O), lungs were fully open and IL-6 and RAGE were reduced compared with $\Delta P,L_{MEAN}$ (27.4 ± 12.9 *vs.* 41.6 ± 14.1 and 0.6 ± 0.2 *vs.* 1.4 ± 0.3, respectively), despite increased hyperinflation and amphiregulin expression. At $\Delta P,L_{MEAN}$ (V_T = 6 ml/kg and PEEP = 9.5 cm H_2O), when PEEP was not high enough to keep lungs open, IL-6, RAGE, and amphiregulin expression increased compared with $\Delta P,L_{LOW}$ (41.6 ± 14.1 *vs.* 9.0 ± 9.8, 1.4 ± 0.3 *vs.* 0.6 ± 0.2, and 6.7 ± 0.8 *vs.* 2.2 ± 1.0, respectively). At Pplat,rs similar to that achieved with $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$, higher V_T and lower PEEP reduced IL-6 and RAGE expression.

Conclusion: In the acute respiratory distress syndrome model used in this experiment, two strategies minimized ventilator-induced lung injury: (1) low V_T and PEEP, yielding low $\Delta P,L$ and Pplat,rs; and (2) low V_T associated with a PEEP level sufficient to keep the lungs open. **(Anesthesiology 2015; 123:423-33)**

ACUTE respiratory distress syndrome (ARDS) is a severe inflammatory condition characterized by heterogeneous pulmonary injury with both normal and diseased areas throughout the lung.[1] Patients with ARDS require mechanical ventilation (MV), which improves gas exchange while minimizing harm to already injured tissue. The selection of adequate tidal volume (V_T), positive end-expiratory pressure (PEEP), respiratory system plateau pressure (Pplat,rs), and transpulmonary driving pressure ($\Delta P,L$; difference between transpulmonary pressure at end-inspiration and at end-expiration) settings is crucial to reducing ventilator-induced lung injury (VILI).[2–5]

A lung-protective strategy (V_T ≤6 ml/kg predicted body weight and plateau pressures ≤30 cm H_2O) and adequate levels of PEEP have been proposed to reduce VILI and mortality

What We Already Know about This Topic

- Recent retrospective analysis of clinical acute respiratory distress syndrome trials suggested that driving pressure was an important factor associated with mortality.

What This Article Tells Us That Is New

- Different combinations of tidal volume and positive end-expiratory pressure (PEEP) were used to create a range of driving pressures in a rat model of acute respiratory distress syndrome due to tracheal instillation of endotoxin for 24 h. Low transpulmonary driving pressure was associated with alveolar collapse and high driving pressure was associated with hyperinflation. The combination of a tidal volume of 6 ml/kg predicted body weight and the lowest PEEP and driving pressure to maintain oxygenation in a normal range minimized ventilator-induced lung injury even in the presence of alveolar collapse.

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Submitted for publication September 14, 2014. Accepted for publication March 20, 2015. From the Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.L.S.); Radiology Department, National Center of Structural Biology and Image, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (T.B., S.A.L.S.); Department of Pathology, School of Medicine, University of São Paulo, São Paulo, Brazil (V.L.C.); Laboratory of Cellular and Molecular Physiology, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (M.M.M.); Rio

in patients with ARDS.[2] Controversy remains regarding optimal PEEP, Pplat,rs, and driving pressures across the respiratory system and lungs. The use of lower V_T and lower PEEP may be associated with more homogeneous ventilation of aerated lung and maintenance of alveolar collapse, reducing inflation shear stress.[3,4] However, MV with lower V_T and higher PEEP may: (1) not be enough to keep the alveoli open at end-expiration, resulting in less atelectasis compared with lower PEEP but creating lung regions with alveolar instability and repeated opening and closing of lung units; (2) be able to open and keep open most of the collapsed alveoli, thus resulting in less recruitment and derecruitment, but potentially leading to hyperinflation of aerated lungs; and (3) increase Pplat,rs and ΔP,L, predisposing to VILI. However, thus far, no study has compared the effects of combinations of different V_T and PEEP settings resulting in different fixed levels of ΔP,L and Pplat,rs on lung morphology and biological markers of VILI in experimental ARDS.

We hypothesized in intratracheal endotoxin-induced ARDS that (1) low V_T associated with lower PEEP ($\Delta P,L_{LOW}$) would minimize VILI because areas of alveolar collapse would remain unaltered, thus avoiding cyclic recruitment/derecruitment of distal lung units, whereas in normal lung regions, no hyperinflation would occur, thus reducing end-inspiratory stress and lung inflammation; (2) at the same level of ΔP,L, low V_T combined with a level of PEEP not enough to open the lungs might result in VILI; and (3) the combination of low V_T with higher PEEP, when associated with higher Pplat,rs and ΔP,L, would increase lung damage. For this purpose, we investigated the effects of different fixed levels of ΔP,L or Pplat,rs induced by different combinations of V_T and PEEP on lung morphology and biological markers in experimental ARDS.

Materials and Methods

This study was approved by the Federal University of Rio de Janeiro Health Science Center Research Ethics Committee (Rio de Janeiro, Brazil). All animals received humane care in compliance with the National Society for Medical Research "Principles of Laboratory Animal Care" and the U.S. National Academy of Sciences "Guide for the Care and Use of Laboratory Animals" (Washington, D.C.).

Animal Preparation and Experimental Protocol

Forty-eight adult male Wistar rats (weight 342 ± 13 g) were assigned to ARDS induction by intratracheal instillation of *Escherichia coli* lipopolysaccharide [O55:B5] (200 μg suspended in 20 μl saline solution).[6] Twenty-four hours after ARDS induction, rats were anesthetized (ketamine 75 mg/kg and xylazine 2.5 mg/kg intraperitoneally) and tracheotomized. Six rats with ARDS were not mechanically ventilated (nonventilated group) and were used for molecular biology analysis. A polyethylene catheter (PE-50) was introduced into the carotid artery for blood sampling and monitoring of mean arterial pressure (MAP). Changes in esophageal pressure (Pes) were measured with a water-filled catheter (PE205) with side holes at the tip connected to a differential pressure transducer (UT-PL-400; SCIREQ, Canada). The catheter was passed into the stomach and slowly returned into the esophagus; proper positioning was assessed using the "occlusion test."[7] MAP was continuously recorded (LifeWindow 6000V Networked Multi-Parameter Veterinary Monitor; Digicare Animal Health, USA). The tail vein was punctured for continuous infusion of Ringer's lactate solution (10 ml kg^{-1} h^{-1}). Gelafundin® (B. Braun, Germany) was administered (titrated in 0.5-ml increments) to keep MAP greater than 60 mmHg. Muscle paralysis was achieved by administration of pancuronium (0.4 mg intramuscularly). Animals were then mechanically ventilated (Servo-i; MAQUET, Sweden) in volume-controlled mode with V_T = 6 ml/kg, minute ventilation = 160 ml/min, inspiratory-to-expiratory ratio = 1:2, fraction of inspired oxygen (F_{IO_2}) = 1.0, and PEEP = 3 cm H_2O for 5 min. Arterial blood (300 μl) was drawn into a heparinized syringe to determine arterial oxygen partial pressure (Pa_{O_2}), arterial carbon dioxide partial pressure (Pa_{CO_2}), and arterial pH (pHa) (ABL80 FLEX; Radiometer, Denmark) (BASELINE). After blood gas analysis, F_{IO_2} was reduced to 0.4 to prevent possible iatrogenic effects, and lung mechanics were assessed. Rats were then assigned to the following groups: (1) according to ΔP,L: the first group was ventilated with V_T = 6 ml/kg and PEEP = 3 cm H_2O ($\Delta P,L_{LOW}$ = 7.5 cm H_2O); the second, with a V_T that generated sufficient ΔP,L to keep animals alive during 1 h ($\Delta P,L_{HIGH}$ = 12 cm H_2O, V_T = 22 ml/kg, PEEP = 3 cm H_2O); the third, with a V_T that generated a $\Delta P,L_{MEAN}$ = ($\Delta P,L_{LOW}$ + $\Delta P,L_{HIGH}$)/2 = 10 cm H_2O (V_T = 13 ml/kg, PEEP = 3 cm H_2O); the fourth, with a V_T = 6 ml/kg and PEEP adjusted to reach the $\Delta P,L_{HIGH}$ (V_T = 6 ml/kg, PEEP = 11 cm H_2O); and the fifth group was ventilated with a V_T = 6 ml/kg and a PEEP adjusted to reach $\Delta P,L_{MEAN}$ (V_T = 6 ml/kg, PEEP = 9.5 cm H_2O); and (2) according to Pplat,rs: V_T = 6 ml/kg was applied and PEEP was adjusted to achieve Pplat,rs similar to that observed in the mean and high V_T groups (14 and 17 cm H_2O), in which V_T was 13 and 22 ml/kg, respectively (fig. 1). After this step, animals were ventilated for 1 h, after which F_{IO_2} was set at 1.0 for 5 min, and arterial blood gases were analyzed (END). Animals were killed using sodium thiopental injection (60 mg/kg) and lungs were extracted for histological and molecular biology analysis.

de Janeiro Federal Institute of Education, Science and Technology, Rio de Janeiro, Brazil (C.S.N.B.G.); University of Minnesota, Minneapolis/Regions Hospital, Pulmonary and Critical Care Medicine, St. Paul, Minnesota (J.J.M.); Pulmonary Engineering Group, Department of Anesthesiology and Intensive Care Therapy, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany (M.G.d.A.); and IRCCS AOU San Martino-IST, Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy (P.P.).

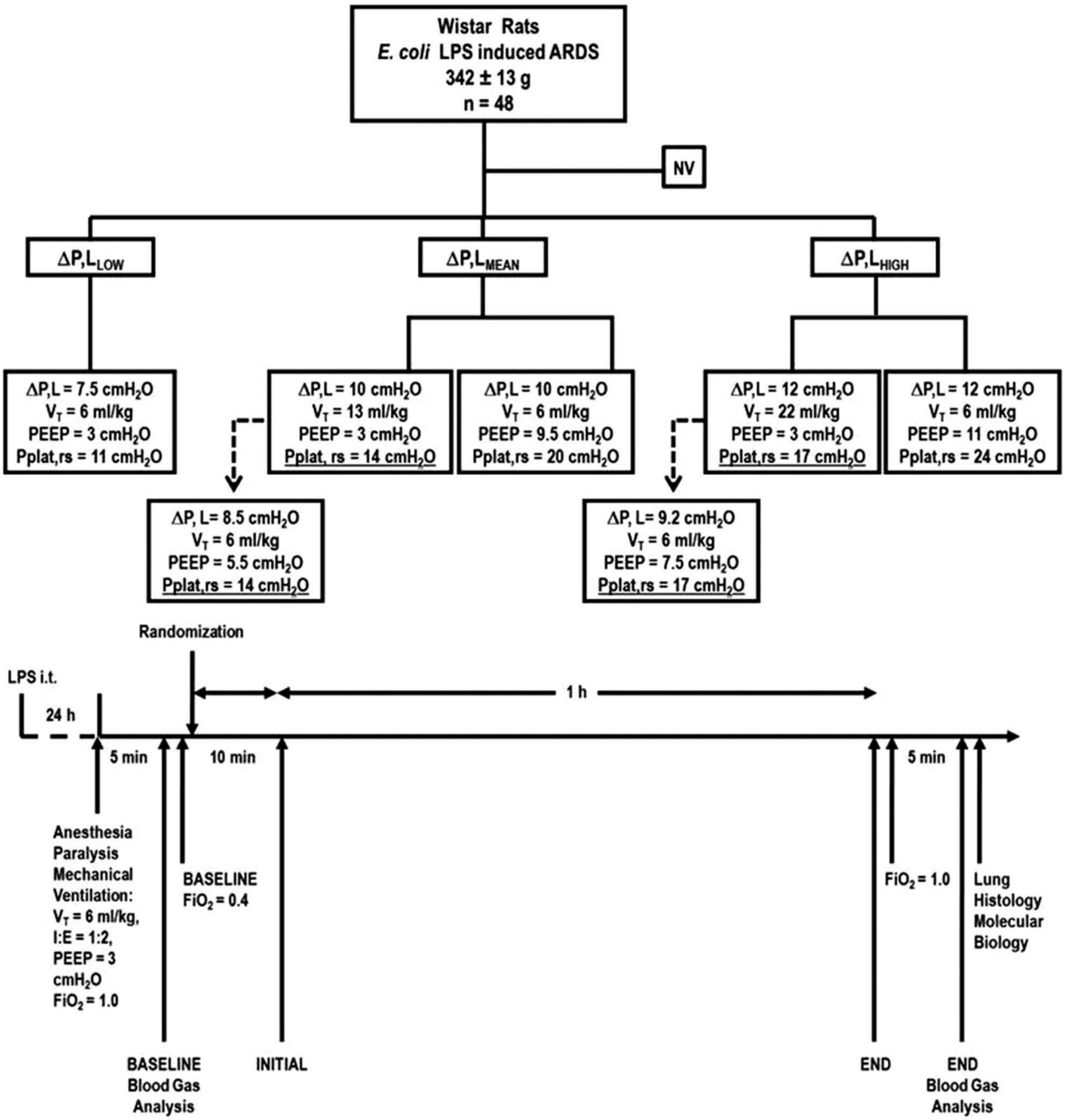


Fig. 1. Schematic flowchart of study design (*top*) and timeline of the procedure (*bottom*). *Dashed lines* represent additional groups in which tidal volume (V_T) = 6 ml/kg was applied and positive end-expiratory pressure (PEEP) adjusted to similar respiratory system plateau pressure (Pplat,rs) achieved when $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$ were associated with high V_T (13 ml/kg [Pplat,rs = 14 cm H_2O] and 22 ml/kg [Pplat,rs = 17 cm H_2O]). ARDS = acute respiratory distress syndrome; F_{IO_2} = inspiratory fraction of oxygen; I:E = inspiratory-to-expiratory ratio; i.t. = intratracheally; LPS = *Escherichia coli* lipopolysaccharide; NV = nonventilated group; RR = respiratory rate; ΔP,L = transpulmonary driving pressure.

Data Acquisition and Processing

Airflow, V_T, and tracheal and esophageal pressures were measured.[6,8] Transpulmonary pressure was calculated as the difference between the pressure in the alveoli and the pressure in the pleural cavity and can be used to estimate lung stress (transpulmonary pressure at end-inspiration), whereas ΔP,L was the difference between the transpulmonary pressure during end-inspiration and end-expiration.[6,9] Respiratory system static elastance (Est,rs) was calculated as the difference between Pplat,rs and PEEP divided by the V_T. Signals were filtered (200 Hz), amplified by a 4-channel conditioner (SC-24; SCIREQ), sampled at 200 Hz with a 12-bit analog-to-digital converter (NI-USB-6008; National Instruments, USA), and continuously recorded throughout the experiments.

Light Microscopy

Laparotomy was performed immediately after blood sampling at END, and heparin (1,000 IU) was injected into the tail vein. The trachea was clamped at end-expiration. Lungs were removed *en bloc* with end-expiratory volume. The left lung was frozen in liquid nitrogen and submerged in Carnoy solution. Four-micrometer-thick slices were longitudinally cut from the left lung and stained with hematoxylin–eosin. Lung morphometric analysis was performed using an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines of known length coupled to a conventional light microscope (Olympus BX51; Olympus Latin America, Brazil). Both dorsal and ventral areas of the lungs were analyzed. The volume fractions of the lung occupied by collapsed alveoli, normal pulmonary areas, or hyperinflated structures (alveolar ducts, alveolar sacs, or alveoli; maximal chord length in air >120 μm) were determined by the point-counting technique at a magnification of ×200 across 10 random, noncoincident microscopic fields.[10]

Transmission Electron Microscopy

Three slices (2 × 2 × 2 mm) were cut from three different segments of the left lung and fixed for electron microscopy. On each lung electron microscopy image (20 fields per animal), damage to alveolar capillary membrane, type II epithelial and endothelial cells, and degree of interstitial edema were graded on a five-point, semiquantitative, severity-based scoring system as follows: 0 = normal lung parenchyma, 1 to 4 = changes in 1 to 25%, 26 to 50%, 51 to 75%, and 76 to 100% of examined tissue, respectively.[6] Lung morphometry and electron microscopy analysis were performed in a blinded manner (V.L.C. and N.S.F.).

Expressions of Interleukin-6, Amphiregulin, Type 3 Procollagen, and Receptor for Advanced Glycation End Products

Quantitative real-time reverse-transcription polymerase chain reaction was performed to measure the expression of interleukin (IL)-6, type III procollagen (PCIII), receptor for advanced glycation end products (RAGE), and amphiregulin in lung tissue. Central slices of right lung were cut, collected in cryotubes, quick-frozen by immersion in liquid nitrogen, and stored at −80°C. Total RNA was extracted from frozen tissues using SV total RNA Isolation System (Promega Corporation, USA) following the manufacturer's recommendations. RNA concentration was measured by spectrophotometry in a Nanodrop ND-1000 system (Thermo Fisher Scientific, USA). First-strand complementary DNA was synthesized from total RNA using the GoTaq® 2-STEP RT qPCR (reverse transcription-quantitative polymerase chain reaction) System (Promega Corporation). The primers used are shown in Supplemental Digital Content 1, http://links.lww.com/ALN/B149. Relative mRNA levels were measured with a SYBR green detection system using ABI 7500 real-time PCR (polymerase chain reaction) (Applied Biosystems, USA). Samples were measured in triplicate. Relative gene expression was calculated as a ratio of the average gene expression levels compared with the reference gene (acidic ribosomal phosphoprotein P0 [36B4])[11] and expressed as fold change relative to nonventilated animals.

Statistical Analysis

Each variable was tested for normality using the Kolmogorov–Smirnov test. Data are presented as mean ± SD unless otherwise specified. Comparisons among ventilatory parameters, lung functional data and morphometry, and molecular biology data were performed using one-way ANOVA with Bonferroni *post hoc* test among groups with the same V_T. A *t* test with Bonferroni correction was used to compare groups with the same ΔP,L. Comparisons among results of semiquantitative analysis of electron microscopy were performed by one-way ANOVA on ranks with Bonferroni *post hoc* test. Pearson correlations of ΔP,L, Pplat,rs, alveolar hyperinflation and collapse, and expression of biological markers were calculated. Multiple linear regression analysis was done between independent (ΔP,L, Pplat,rs, V_T, and PEEP) and dependent (IL-6, RAGE, amphiregulin, PCIII, alveolar collapse, and hyperinflation) variables. Sample size calculation was based on pilot studies and our past experience with the ventilator strategies in small animals.[8] All tests were performed in GraphPad Prism v5.00 (GraphPad Software, USA). Significance was established at *P* value less than 0.05. All ventilatory variables associated with ΔP,L and Pplat,rs (*i.e.*, V_T and PEEP) were shown because these parameters exhibit collinearity at different levels.

Results

The survival rate was 100% in all groups during the investigation. Forty-eight animals were used, with 6 animals allocated to each group, including the nonventilated group.

Ventilatory parameters, Est,rs, and arterial blood gases at BASELINE (PEEP = 3 cm H_2O) (see table 1, Supplemental Digital Content 2, http://links.lww.com/ALN/B150) showed similar functional impairment among groups before modification of ventilator settings, suggesting a similar degree of lung damage. ΔP,L, Est,rs, V_T, PEEP, Pplat,rs, and respiratory rate did not differ from INITIAL to END in any group. MAP remained greater than 60 mmHg throughout the experiments (see table 2, Supplemental Digital Content 2, http://links.lww.com/ALN/B150).

Arterial blood gases at END with different ventilator settings are shown in table 1. At similar fixed levels of ΔP,L and Pplat,rs, oxygenation did not differ with changes in V_T and PEEP. At low V_T, higher PEEP led to a progressive improvement in oxygenation. $Paco_2$ markedly increased with V_T = 6 ml/kg and PEEP = 11 cm H_2O compared with V_T = 6 ml/kg and PEEP = 5.5 cm H_2O and declined with V_T = 22 ml/kg and PEEP = 3 cm H_2O compared with V_T = 6 ml/kg and PEEP = 11 cm H_2O.

The highest degree of alveolar collapse was observed at $\Delta P,L_{LOW}$, and the most hyperinflation at $\Delta P,L_{HIGH}$ (fig. 2).

Table 1. Arterial Blood Gases at END

	NV	$\Delta P,L_{LOW}$		$\Delta P,L_{MEAN}$			$\Delta P,L_{HIGH}$	
$\Delta P,L$ (cm H_2O)	—	7.5	8.5	10	10	9.2	12	12
V_T (ml/kg)	—	6	6	13	6	6	22	6
PEEP (cm H_2O)	—	3	5.5	3	9.5	7.5	3	11
Pplat,rs (cm H_2O)	—	11	14	14	20	17	17	24
pHa	7.27 ± 0.06	7.30 ± 0.50	7.30 ± 0.01	7.39 ± 0.10	7.21 ± 0.10*†	7.30 ± 0.10‡	7.47 ± 0.10	7.19 ± 0.10*§
$Paco_2$ (mmHg)	50 ± 9.9	59 ± 8	44 ± 8	47 ± 9	71 ± 16*	44 ± 9‡	34 ± 5	73 ± 18*§
Pao_2 (mmHg)	142 ± 59.8	302 ± 83	368 ± 110	468 ± 84	511 ± 44‖	385 ± 126	498 ± 79	543 ± 16*‖

Values are expressed as mean ± SD of six animals per group. One-way ANOVA followed by Bonferroni *post hoc* test. Dashed lines represent Pplat,rs similar to $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$ at high V_T (13 ml/kg [Pplat,rs = 14 cm H_2O] and 22 ml/kg [Pplat,rs = 17 cm H_2O]). For this purpose, V_T was kept low (6 ml/kg) and PEEP was adjusted for the level of Pplat,rs. Gas exchange was evaluated at FIO_2 = 1.0.

* *vs*. V_T6-PEEP5.5; † *vs*. V_T13-PEEP3; ‡ *vs*. V_T6-PEEP9.5; § *vs*. V_T22-PEEP3; ‖ *vs*. V_T6-PEEP3.

NV = nonventilated group; $Paco_2$ = arterial carbon dioxide partial pressure; Pao_2 = arterial oxygen partial pressure; PEEP = positive end-expiratory pressure; pHa = arterial pH; Pplat,rs = respiratory system plateau pressure; V_T = tidal volume; $\Delta P,L$ = transpulmonary driving pressure.

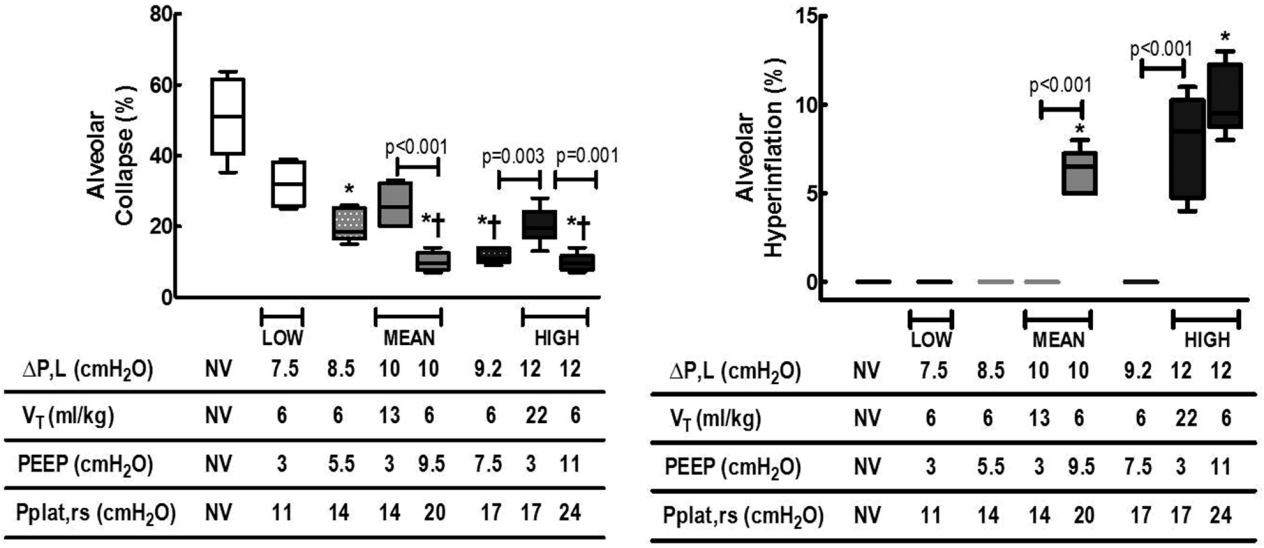
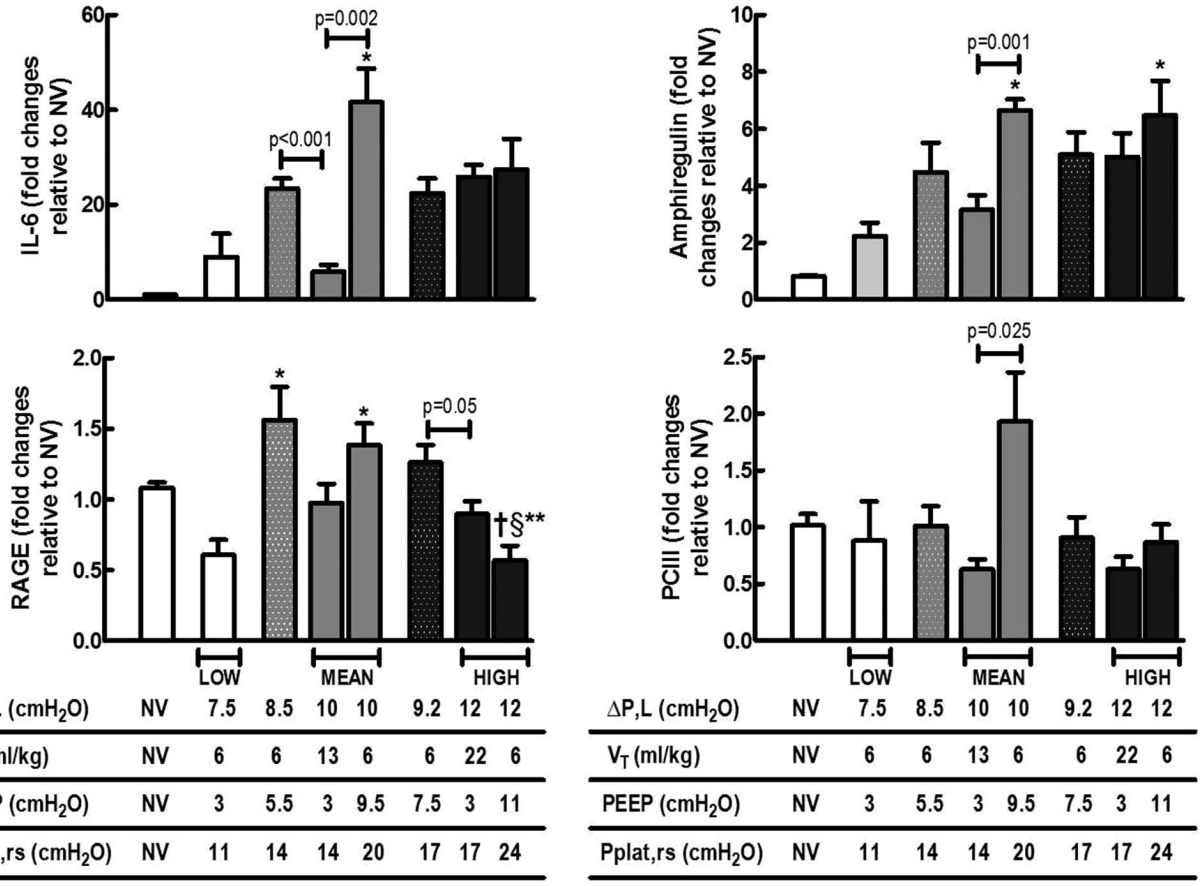


Fig. 2. Lung morphometry. Box plot of the fractional area of alveolar collapse and hyperinflation. All values were computed in 10 random, noncoincident fields of view per rat. *Boxes* show the interquartile (25 to 75%) range, *whiskers* encompass the range (minimum–maximum), and *horizontal lines* represent median values of six animals per group. Student *t* test followed by Bonferroni correction was performed between groups with similar fixed levels of $\Delta P,L$ and Pplat,rs. One-way ANOVA followed by Bonferroni *post hoc* test was used among the groups with similar V_T. * *Versus* V_T6-PEEP3; † *versus* V_T6-PEEP5.5 ($P < 0.05$). NV = nonventilated group; PEEP = positive end-expiratory pressure; Pplat,rs = respiratory system plateau pressure; V_T = tidal volume; $\Delta P,L$ = transpulmonary driving pressure.

At $\Delta P,L_{MEAN}$, alveolar collapse was lower with V_T = 6 ml/kg and PEEP = 9.5 cm H_2O compared with V_T = 13 ml/kg and PEEP = 3 cm H_2O; however, hyperinflation was present with the former strategy. At $\Delta P,L_{HIGH}$, the extent of alveolar collapse was lower with V_T = 6 ml/kg and PEEP = 11 cm H_2O than with V_T = 22 ml/kg and PEEP = 3 cm H_2O, but both strategies produced hyperinflation. At Pplat,rs = 17 cm H_2O, ventilation with V_T = 6 ml/kg and PEEP = 7.5 cm H_2O was associated with less alveolar collapse and hyperinflation compared with V_T = 22 ml/kg and PEEP = 3 cm H_2O. For V_T = 6 ml/kg, the increase in PEEP levels reduced alveolar collapse while increasing hyperinflation.

All animals showed cytoplasmic degeneration of type I and II epithelial and endothelial cells as well as alveolar-capillary damage. At similar levels of $\Delta P,L$ and Pplat,rs, regardless of V_T and PEEP, no significant differences were observed in damage to the alveolar-capillary membrane, type I and II epithelial cells, or degree of interstitial edema. At V_T = 6 ml/kg, ventilation with the highest PEEP to open the lung (PEEP = 11 cm H_2O) led to less injury to the alveolar capillary membrane and type I and II epithelial cells compared with PEEP = 7.5 cm H_2O and PEEP = 9.5 cm H_2O, and less interstitial edema than PEEP = 9.5 cm H_2O (see table 3, Supplemental Digital Content 2, http://links.lww.com/ALN/B150, and fig. 1, Supplemental Digital Content 3, http://links.lww.com/ALN/B151).

Gene expression of biological markers associated with inflammation (IL-6), damage inflicted to type I epithelial cells (RAGE), pulmonary stretch (amphiregulin), and fibrogenesis (PCIII) is shown in figure 3. IL-6, RAGE,



$\Delta P,L$ (cmH_2O)	NV	7.5	8.5	10	10	9.2	12	12
V_T (ml/kg)	NV	6	6	13	6	6	22	6
PEEP (cmH_2O)	NV	3	5.5	3	9.5	7.5	3	11
Pplat,rs (cmH_2O)	NV	11	14	14	20	17	17	24

$\Delta P,L$ (cmH_2O)	NV	7.5	8.5	10	10	9.2	12	12
V_T (ml/kg)	NV	6	6	13	6	6	22	6
PEEP (cmH_2O)	NV	3	5.5	3	9.5	7.5	3	11
Pplat,rs (cmH_2O)	NV	11	14	14	20	17	17	24

Fig. 3. Expression of biological markers. Real-time polymerase chain reaction analysis of biological markers associated with inflammation (interleukin [IL]-6), alveolar overdistension (amphiregulin), damage inflicted upon alveolar type I epithelial cells (receptor for advanced glycation end products [RAGE]), and fibrogenesis (type III procollagen [PCIII]). Relative gene expression was calculated as a ratio of the average gene expression levels compared with the reference gene (*36B4*) and expressed as fold change relative to nonventilated (NV) animals. Values expressed as mean ± SD of six animals per group. Student *t* test followed by Bonferroni correction was performed between groups with similar fixed levels of $\Delta P,L$ and Pplat,rs. One-way ANOVA followed by Bonferroni *post hoc* test. * *Versus* V_T6-PEEP3; † *versus* V_T6-PEEP5.5; § *versus* V_T6-PEEP9.5 ($P < 0.05$). PEEP = positive end-expiratory pressure; Pplat,rs = respiratory system plateau pressure; V_T = tidal volume; $\Delta P,L$ = transpulmonary driving pressure.

and amphiregulin expressions were lower in $\Delta P,L_{LOW}$ (V_T = 6 ml/kg and PEEP = 3 cm H_2O) compared with $\Delta P,L_{MEAN}$ (V_T = 6 ml/kg and PEEP = 9.5 cm H_2O). $\Delta P,L_{HIGH}$ (V_T = 6 ml/kg and PEEP = 11 cm H_2O) was associated with less RAGE expression than in all other groups ventilated with V_T = 6 ml/kg, except $\Delta P,L_{LOW}$. At $\Delta P,L_{MEAN}$, IL-6, amphiregulin, and PCIII expressions were higher with V_T = 6 ml/kg and PEEP = 9.5 cm H_2O than with V_T = 13 ml/kg and PEEP = 3 cm H_2O. At $\Delta P,L_{HIGH}$, different combinations of V_T and PEEP did not affect these biological markers. At Pplat,rs = 14 cm H_2O, IL-6 expression was higher with V_T = 6 ml/kg and PEEP = 5.5 cm H_2O than with V_T = 13 ml/kg and PEEP = 3 cm H_2O. At Pplat,rs = 17 cm H_2O, RAGE expression was higher with V_T = 6 ml/kg and PEEP = 7.5 cm H_2O than with V_T = 22 ml/kg and PEEP = 3 cm H_2O. IL-6, RAGE, and amphiregulin expressions were higher with V_T = 6 ml/kg and PEEP = 9.5 than with V_T = 6 ml/kg and PEEP = 3 cm H_2O. Ventilation with V_T = 6 ml/kg and PEEP = 11 cm H_2O reduced the expression of RAGE compared with V_T = 6 ml/kg and PEEP = 9.5 cm H_2O although amphiregulin expression remained high.

Correlation analyses of mechanical, biological, and morphological data among all groups are shown in table 4, Supplemental Digital Content 2, http://links.lww.com/ALN/B150. $\Delta P,L$ correlated with alveolar hyperinflation and alveolar collapse. Pplat,rs correlated positively with IL-6 and amphiregulin expressions and alveolar hyperinflation and negatively with alveolar collapse. PEEP correlated positively with IL-6, amphiregulin, and PCIII expressions and alveolar hyperinflation and negatively with alveolar collapse. V_T did not present significant correlations with IL-6, RAGE, amphiregulin, or PCIII expressions, alveolar hyperinflation, or alveolar collapse (see table 4, Supplemental Digital Content 2, http://links.lww.com/ALN/B150). At V_T = 6 ml/kg, $\Delta P,L$ and Pplat,rs correlated positively

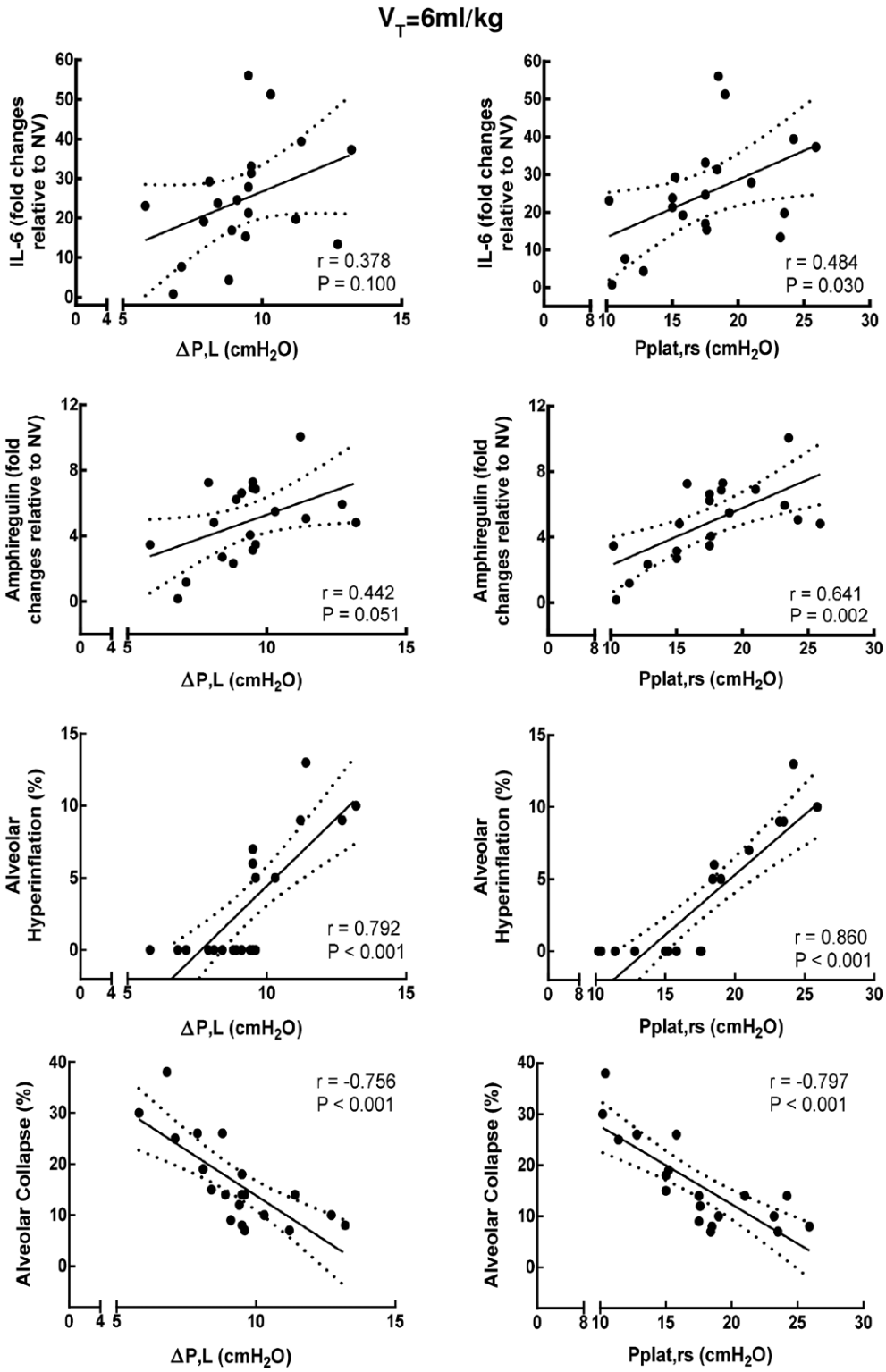


Fig. 4. Pearson correlations of transpulmonary driving pressure (ΔP,L) and respiratory system plateau pressure (Pplat,rs) with interleukin (IL)-6 and amphiregulin expressions and alveolar collapse and hyperinflation at fixed tidal volume (V_T) = 6 ml/kg. The *r* value represents the correlation coefficient, and *P*, the respective *P* value. Statistical significance was accepted at $P < 0.05$. NV = nonventilated group.

with IL-6 and amphiregulin expressions, as well as with alveolar hyperinflation, and negatively with alveolar collapse (fig. 4).

The following variables were found to be predictors of IL-6, amphiregulin, and PCIII expressions, respectively: (1) V_T (coefficient = 2.646, $P = 0.005$) and PEEP

(coefficient = 8.977, $P = 0.002$); (2) V_T (coefficient = 0.393, $P = 0.005$); and (3) V_T (coefficient = 0.0975, $P = 0.015$) and PEEP (coefficient = 0.496, $P < 0.001$).

Discussion

In the model of ARDS used in this study, we found that (1) $\Delta P,L_{LOW}$ led to alveolar collapse, preventing cyclic recruitment/derecruitment of distal lung units, and to no hyperinflation, thus resulting in no end-inspiratory stress and lung inflammation; (2) at fixed $\Delta P,L_{MEAN}$, low V_T combined with a PEEP level not enough to keep lungs fully open at end-expiration reduced alveolar collapse but increased hyperinflation, consequently increasing lung inflammation and fibrogenesis, probably due to alveolar instability; (3) at fixed $\Delta P,L_{HIGH}$, low V_T with higher PEEP led to alveolar hyperinflation as measured by amphiregulin expression. However, no further lung inflammation and fibrogenesis possibly associated with reduced cyclic recruitment/derecruitment of distal lung units were detected. The latter findings contradict our previous third hypothesis. After multiple linear regression analyses, V_T was an independent predictor of biological markers of inflammation, lung cell stretch, and fibrogenesis. PEEP was associated with IL-6 and PCIII expression. $\Delta P,L$ and Pplat,rs seem to have similar effects on the evolution of injury and inflammation in this ARDS model.

Different mechanical factors may promote VILI, such as V_T, PEEP, Pplat,rs, and $\Delta P,L$. Previous studies found these variables to interact, precluding dissociation of their individual contributions to VILI.[8,12–16] In the ARDS model used herein, endotoxin was intratracheally instilled[6,17] as a first hit to induce lung inflammation. After 24 h, animals exhibited impaired lung mechanics, atelectasis, damage to epithelium and alveolar-capillary membrane, interstitial edema, and increased IL-6 expression.[5] Animals were then randomized to receive different MV parameters as a second hit.[18,19] After ventilatory strategies, VILI was defined as histological and mechanical alterations present plus an increase in at least one of the following mediators: IL-6, amphiregulin, RAGE, and PCIII.

To the best of our knowledge, this is the first study describing the individual contributions of V_T and PEEP at each level of $\Delta P,L$ and Pplat,rs on lung morphology and molecular biology in experimental ARDS. Furthermore, unlike in previous studies, the effects of ventilator strategies settings were investigated 24 h after the insult (when the morphofunctional and biological changes of ARDS were already present), and an esophageal catheter was used to measure the driving pressure of the lung.

Airway pressure is influenced by chest wall properties and respiratory muscle activity, whereas the transpulmonary pressure at end-inspiration and end-expiration ($\Delta P,L$) enables estimation of the actual distending pressure of lungs, unencumbered by chest wall and patient effort on recorded airway pressures.[9] The driving pressure is a function not only of V_T but also of PEEP. In fact, different levels of PEEP are associated with possible changes in lung compliance. For this reason, a given driving pressure can be achieved at higher V_T with lower PEEP as well as at lower V_T with higher PEEP. Those conditions have been addressed in the current study.

We measured mRNA expression of IL-6 as a surrogate of inflammation during VILI.[20] PCIII expression was evaluated because it is a marker of lung fibrogenesis,[21] and RAGE expression reflects alveolar type I cell injury.[22] Amphiregulin expression is positively modulated by hyperinflation, activates chemokines, cytokines, and adhesion molecules,[23–25] and represents a novel candidate gene in VILI.[24,26]

Our data suggest that the increase in $\Delta P,L$ induced by higher PEEP or V_T promotes VILI. The finding that $\Delta P,L_{LOW}$ (V_T = 6 ml/kg, PEEP = 3 cm H_2O) reduced VILI when compared with $\Delta P,L_{HIGH}$ (V_T = 6 ml/kg, PEEP = 11 cm H_2O) may be explained by the maintenance of areas of alveolar collapse (avoiding cyclic recruitment–derecruitment) without hyperinflation, thus minimizing lung inflammation, type I epithelial cell damage, and pulmonary mechanical stress. This is consistent with the concept of "lung rest" or "permissive atelectasis" for lung protection, which has been shown in animals[4,27,28] and humans[29] to reduce alveolar damage in aerated and atelectatic lung regions. It is worth noting that $\Delta P,L_{MEAN}$ with V_T = 6 ml/kg and PEEP = 9.5 cm H_2O was associated with the highest increase in markers of inflammation, epithelial cell damage, lung cell stretch, and fibrogenesis. We hypothesize that if PEEP is not high enough to keep the lungs open at end-expiration, alveolar instability may occur, with cyclic recruitment and derecruitment of lung units and increased shear stress in the unstable zone. At V_T = 6 ml/kg and PEEP = 11 cm H_2O, alveolar collapse and markers of epithelial cell damage were reduced, suggesting decreased strain and regional dynamic stress. By using low V_T but high PEEP, the lungs are kept tonically inflated above their functional residual capacity and thus exposed to an additional static strain.[16] $Paco_2$ also increased, probably due to the associated increase in dead space.

The increased $\Delta P,L$ resulting from different PEEP levels at fixed V_T = 6 ml/kg yielded less alveolar collapse but higher Est,rs. The lack of correlation between alveolar recruitment and changes in Est,rs has been previously reported[29,30] and might be attributed to the prevalence of hyperinflation instead of alveolar reopening with higher PEEP. Our data indicate that during ventilation with V_T = 6 ml/kg, the level of PEEP titrated according to the lowest $\Delta P,L$ and, consequently, the best elastance, may be associated with less activation of biological markers and hyperinflation, as previously shown in experimental and human studies.[3,31,32]

The increase in $\Delta P,L$ induced by changes in V_T (from 6 to 22 ml/kg) at fixed low PEEP (3 cm H_2O) yielded increased alveolar collapse and hyperinflation. However, the increase in $\Delta P,L$ obtained by using higher V_T rather than higher PEEP led to increased IL-6 expression, but no change in amphiregulin, suggesting that the cellular response to inflammation was more pronounced than stretch in the presence of greater strain and dynamic stress.

At fixed $\Delta P,L_{MEAN}$, but not at $\Delta P,L_{HIGH}$, low V_T and high PEEP compared with high V_T and low PEEP resulted in increased IL-6, amphiregulin, and PCIII expressions. We hypothesize that there could be a threshold of $\Delta P,L$ above which differences in biomarkers of cellular activation are no longer observed by different combinations of V_T and PEEP.

Injured lungs have markedly different sizes and variable Pplat,rs. Therefore, the use of high V_T may result in greater lung damage compared with low V_T.[33] In our study, we observed that, compared with high V_T with low PEEP, low V_T with high PEEP increased IL-6 expression at fixed Pplat,rs = 14 cm H_2O and RAGE expression at Pplat,rs = 17 cm H_2O. The mechanotransduction response to Pplat,rs seems to differ from that induced by $\Delta P,L$. This may be due to the different limits of end-inspiratory and end-expiratory stress/strain for each combination of V_T and PEEP. In short, the relative contributions of dynamic and static stress/strain to VILI depend on Pplat,rs.

Multiple linear regressions showed that all ventilator settings investigated herein led to increased biological markers of inflammation, type I alveolar epithelial cell damage, cell stretch, and fibrogenesis. Taken together, these observations suggest that the best approach for protective MV may be to reduce V_T, PEEP, $\Delta P,L$, and Pplat,rs. Statistically, the most important ventilator variable associated with VILI was V_T, which is in line with experimental[27] and human studies[33,34] in ARDS.

Possible Clinical Implications

Our data suggest that ventilation with V_T = 6 ml/kg combined with the lowest PEEP and $\Delta P,L$ to keep oxygenation within a safe range can minimize VILI. Doing so is in accordance with the hypothesis that these ventilator settings may protect the collapsed distal lung units from excessive strain, while avoiding overstretching and inflammation in normal lung regions. This strategy is also known as "permissive atelectasis." However, if life-threatening hypoxemia with low protective V_T occurs, a defensible compromise would be to set the least PEEP needed to achieve and sustain alveolar recruitment.

In a lung exposed to a first hit (endotoxin), 1 h of MV is enough to modulate different genes associated with VILI depending on the ventilatory strategy (second hit). This is contrary to the behavior observed in healthy lungs subjected to different ventilatory strategies. Therefore, preexisting lung alterations (edema, atelectasis, or pneumonia) make the diseased lung much more susceptible to mechanical injury.[19]

Limitations

First, ARDS was induced by intratracheal administration of endotoxin, and our results can be extended neither to other ARDS models with different degrees of severity nor to human ARDS. Second, the PEEP levels used in the current study, while often used in rats, may not be directly extrapolated to the clinical setting; nevertheless, theoretical analyses have shown that PEEP levels in rats could be equivalent to double those in humans, according to the estimated transpulmonary pressure.[35] Therefore, the range of PEEP levels used in the current study resembles that used in mechanically ventilated critical care patients (6 to 22 cm H_2O). Third, we decided to forgo recruitment maneuvers, to avoid possible confounding effects concerning different biological impacts on lung tissue,[6,36,37] but cannot rule out that such maneuvers might have further improved lung function and affected different biomarkers.[38] However, the highest PEEP levels used in the current study were able to keep alveolar units open, as demonstrated both by morphometric data and by biological markers. This study was based on a proof of concept, not on the best ventilatory strategy. Fourth, mediators were measured in lung tissue, but not in blood. Fifth, acidosis may modulate the inflammatory process,[39] but its potential effects were not evaluated. Sixth, the high level of variability in physiological data within each group may account for the lack of between-group differences at similar driving or plateau pressures. Finally, the observation time was relatively short as compared with previous studies (1 *vs.* 2 to 6 h), precluding changes in protein levels of all biological markers analyzed. Furthermore, to keep animals with endotoxin-induced ARDS alive for 4 to 6 h, greater amounts of fluid and/or inotropes would be required, which could interfere with gene expression.[40]

Conclusions

In experimental endotoxin-induced ARDS, a ventilation strategy combining low V_T (6 ml/kg) and low PEEP that resulted in low $\Delta P,L$ and Pplat,rs mitigated VILI, despite allowing alveolar collapse. Furthermore, V_T = 6 ml/kg combined with PEEP at the highest level to open the lungs reduced inflammation and epithelial cell damage, despite allowing hyperinflation. In short, V_T = 6 ml/kg with PEEP levels not high enough to keep lungs open can cause alveolar instability with subsequent VILI.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Rocco: Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. prmrocco@gmail.com.

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