EXPERIMENTAL

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Effects of different tidal volumes in pulmonary and extrapulmonary lung injury with or without intraabdominal hypertension

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Abstract *Purpose:* We hypothe-
sized that: (1) intraabdominal
hypertension increases pulmonary
inflammatory and fibrogenic respon-
ses in acute lung injury (ALI); (2) in
the presence of intraabdominal
hypertension, higher tidal volume
reduces lung damage in extrapulmo-
nary ALI, but not in pulmonary ALI.
Methods: Wistar rats were
randomly allocated to receive *Esche-
richia coli* lipopolysaccharide
intratracheally (pulmonary ALI) or
intraperitoneally (extrapulmonary
ALI). After 24 h, animals were ran-
domized into subgroups without or
with intraabdominal hypertension
(15 mmHg) and ventilated with
positive end expiratory pressure =
5 cmH$_2$O and tidal volume of 6 or
10 ml/kg during 1 h. Lung and chest
wall mechanics, arterial blood gases,
lung and distal organ histology, and
interleukin (IL)-1β, IL-6, caspase-3
and type III procollagen (PCIII)
mRNA expressions in lung tissue
were analyzed. *Results:* With intra-
abdominal hypertension, (1) chest-
wall static elastance increased, and
PCIII, IL-1β, IL-6, and caspase-3
expressions were more pronounced
than in animals with normal intraab-
dominal pressure in both ALI groups;
(2) in extrapulmonary ALI, higher
tidal volume was associated with
decreased atelectasis, and lower IL-6
and caspase-3 expressions; (3) in
pulmonary ALI, higher tidal volume
led to higher IL-6 expression; and (4)
in pulmonary ALI, liver, kidney, and
villi cell apoptosis was increased, but
not affected by tidal volume.
Conclusions: Intraabdominal
hypertension increased inflammation
and fibrogenesis in the lung indepen-
dent of ALI etiology. In
extrapulmonary ALI associated with
intraabdominal hypertension, higher
tidal volume improved lung mor-
phometry with lower inflammation in
lung tissue. Conversely, in pulmonary
ALI associated with intraabdominal
hypertension, higher tidal volume
increased IL-6 expression.

Keywords Acute lung injury ·
Intra-abdominal hypertension ·
Cytokines · Type III procollagen ·
Apoptosis

Introduction

Intraabdominal hypertension may be associated with pulmonary and extrapulmonary acute lung injury and acute respiratory distress syndrome (ALI/ARDS) [1], compressing the thoracic cavity, increasing pleural pressure [2, 3], and causing alveolar edema [2] and atelectasis [3]. Low tidal volume (V_T) ventilation and airway plateau pressure below 30 cmH$_2$O have been shown to reduce ventilator-associated lung injury (VALI) and mortality [4] in a mixed population of ALI/ARDS patients. However, in the presence of intraabdominal hypertension, low V_T may promote alveolar derecruitment, depending on the degree of inspiratory transpulmonary pressure [5].

Such effects could be more pronounced in extrapulmonary ALI, which is characterized by less consolidation and higher potential of recruitment [1, 6–8]. Therefore, in extrapulmonary ALI associated with intraabdominal hypertension, high V_T may lead to increased inspiratory transpulmonary pressure and better recruitment, reducing the risk of VALI, while protective ventilation may further increase atelectasis and worsen lung damage. Furthermore, VALI may lead to activation of inflammatory mediators and increased damage to peripheral organs [9].

In the present study, we hypothesized that (1) intraabdominal hypertension increases pulmonary inflammatory and fibrogenic responses in ALI; and (2) in the presence of intraabdominal hypertension, higher V_T may reduce VALI in extrapulmonary, but not in pulmonary ALI. We compared the effects of protective (6 ml/kg) and higher V_T (10 ml/kg) on arterial blood gases, respiratory system, lung and chest wall mechanics, lung histology, inflammatory and fibrogenic mediator mRNA expression, and apoptosis in lung and distal organs in experimental pulmonary and extrapulmonary ALI with or without intraabdominal hypertension.

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 Health Sciences Center Ethics Committee at the Federal University of Rio de Janeiro, Brazil. Forty-eight Wistar rats (300–350 g) were randomly assigned to two groups. Animals received *Escherichia coli* lipopolysaccharide [O55:B5] either intratracheally (200 µg) (pulmonary ALI, ALIp; $n = 24$) or intraperitoneally (1,000 µg) (extrapulmonary ALI, ALIexp; $n = 24$), suspended in saline solution with total volumes equal to 20 and 1,000 µl, respectively. These doses of *E. coli* lipopolysaccharide yielded a similar 1.5-fold increase in lung static elastance in ALIp and ALIexp groups. Twenty-four hours after ALI induction, rats were sedated (diazepam 5 mg intraperitoneally), anesthetized (thiopental sodium 20 mg/kg intraperitoneally), and tracheotomized. A polyethylene catheter (PE-50) was introduced into the carotid artery for blood sampling and monitoring of mean arterial pressure. Animals were paralyzed (vecuronium bromide 2 mg/kg, intravenously) and mechanically ventilated (Servo-i, MAQUET, Sweden) in volume-controlled ventilation with: $V_T = 6$ ml/kg, respiratory rate (RR) = 80 breaths/min, inspiratory-to-expiratory ratio = 1:2, fraction of inspired oxygen (FiO$_2$) = 1.0, and positive end-expiratory pressure (PEEP) = 0 cmH$_2$O (ZEEP) during 5 min. Blood (300 µl) was drawn into a heparinized syringe to determine arterial oxygen partial pressure (PaO$_2$), arterial carbon dioxide partial pressure (PaCO$_2$), and arterial pH (i-STAT, Abbott Laboratories, IL, USA) (Baseline-ZEEP). Mechanical ventilation was set as follows: RR = 80 breaths/min, PEEP = 5 cmH$_2$O, and FiO$_2$ = 0.4. Each subgroup was randomized to ventilation with V_T of 6 or 10 ml/kg ($n = 12$/group).

Respiratory system, lung, and chest wall mechanics were measured (Baseline). In each group, the animals were further randomized, and intraabdominal hypertension (15 mmHg) was induced by laparotomy and placement of cotton dressings or not (normal intraabdominal pressure) ($n = 6$/group). Intraabdominal hypertension was maintained at this level throughout the experiment. Manipulation of the normal intraabdominal pressure group was identical, except for the use of dressings. Mechanical properties of the respiratory system, lung, and chest wall were measured again immediately after intraabdominal hypertension induction or sham surgery (After-surgery), and after 1 h ventilation (End). Following this step, FiO$_2$ was set at 1.0. After 5 min, arterial blood gases were analyzed at PEEP = 5 cmH$_2$O. Animals were then euthanized. IL-6, IL-1β, caspase-3, and PCIII mRNA expressions were measured in lung tissue. A schematic flow chart of study design and the timeline representation of the procedure are shown in the electronic supplementary material (Figs. ESM1, ESM2).

Data acquisition and processing

Airflow and tracheal pressure were continuously recorded using LabVIEW® software (National Instruments, Austin, TX, USA). Tidal volume was obtained by integration of airflow. Changes in esophageal pressure, which reflect chest wall pressure, were measured. Transpulmonary pressures were calculated during inspiration and expiration as the difference between airway and esophageal pressures. Respiratory system, lung, and chest wall static

elastance were measured by the end-inflation occlusion technique [8, 10]. Furthermore, respiratory system plateau pressure, delta transpulmonary pressure (difference between end-inspiratory and end-expiratory transpulmonary pressure), and delta chest wall pressure were computed.

Lung and distal organ histology

Light microscopy

At the end of the experiments (End-PEEP), lungs, liver, kidneys, and small intestine were removed, fixed (3% buffered formaldehyde), paraffin embedded, and stained with hematoxylin-eosin. Fraction areas of the lung occupied by hyperinflated structures, and collapsed and normal lung areas were determined by the point-counting technique [11] across ten random, non-coincident microscopic fields [8, 12, 13].

Transmission electron microscopy

Three slices measuring $2 \times 2 \times 2$ mm were cut from three different segments of the left lung and diaphragm, and fixed for electron microscopy (JEOL 1010 Transmission Electron Microscope, Tokyo, Japan). Each electron microscopy image (15/animal) was analyzed for damage to the alveolar capillary membrane, type II pneumocyte, and endothelial cells. Pathological findings were graded according to a 5-point semi-quantitative severity-based scoring system as: $0 =$ normal lung parenchyma, $1 = 1–25\%$, $2 = 26–50\%$, $3 = 51–75\%$, and $4 = 76–100\%$ of examined tissue [8, 10, 14].

Apoptosis assays

Apoptotic cells of lung, kidney, liver, and small intestine villi were quantified using terminal deoxynucleotidyl transferase biotin-dUTP nick end Labeling (TUNEL) assay in a blinded fashion by two pathologists. A 5-point semi-quantitative severity-based scoring system was used to assess apoptosis: $0 =$ normal lung parenchyma; $1 = 1–25\%$; $2 = 26–50\%$; $3 = 51–75\%$; $4 = 76–100\%$ of examined tissue [13, 15].

IL-6, IL-1β, caspase-3, and PCIII mRNA expressions

Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed to measure the relative levels of expression of interleukin (IL)-6, IL-1β, caspase-3, and type III procollagen genes in ALIp and ALIexp [10]. For ALIp, each set of experiments contained: ALIp-nIAP-V_T6, ALIp-nIAP-V_T10, ALIp-IAH-V_T6, and ALIp-IAH-V_T10. Each gene was studied in triplicate for each animal. For the set of ALIp, ALIp-nIAP-V_T6 was taken as 1. The entire set of experiments was performed four times, one for each of four animals in each group ($n = 4$). The same was performed for ALIexp (ALIexp-nIAP-V_T6, ALIexp-nIAP-V_T10, ALIexp-IAH-V_T6, and ALIexp-IAH-V_T10), and again ALIexp-nIAP-V_T6 was taken as 1. For each sample, the expression of each gene was normalized to housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression using the $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct = Ct$, reference gene-Ct, target gene.

Statistical analysis

In each ALI group, the effects of different V_T with or without intraabdominal hypertension were assessed with two-way ANOVA or two-way ANOVA on ranks followed by Tukey's test. Parametric data are expressed as mean $\pm$ SD, while non-parametric data are expressed as median (interquartile range). All tests were performed using the Sigma Stat 3.1 statistical software package (Jandel Corporation, San Rafael, CA, USA). Significance was established at $p < 0.05$.

Results

Intraabdominal hypertension yielded greater chest wall static elastance compared to normal intraabdominal pressure, with no significant changes in lung and respiratory system static elastance (Table ESM1; Fig. ESM5). All ALI animals showed alveolar collapse (Figs. 1, 2), cytoplasmatic degeneration of type II pneumocytes (PII), endothelial injury, and alveolar capillary damage (Table 1; Fig. ESM6). In ALI groups, intraabdominal hypertension and V_T did not affect lung cell apoptosis (Table 2). IL-6, IL-1β, PCIII, and caspase-3 mRNA expressions were more elevated in intraabdominal hypertension than in normal intraabdominal pressure, independent of ALI etiology (Fig. 3).

Extrapulmonary ALI

In ALIexp, intraabdominal hypertension resulted in higher alveolar collapse at V_T6 (Figs. 1, 2). The volume fraction of alveolar collapse was lower with V_T10 compared to V_T6 (Fig. 2). Endothelial cell injury was more prevalent in ALIexp than ALIp (Table 1). Alveolar capillary membrane and endothelial cell damages were not affected by either intraabdominal hypertension or V_T (Table 1, Fig. ESM6). V_T10 reduced IL-6 and caspase-3,

Fig. 1 Photomicrographs of lung parenchyma stained with hematoxylin-eosin in pulmonary (p) and extrapulmonary (exp) acute lung injury (ALI) associated with intraabdominal hypertension (IAH) or not (nIAP), ventilated with a tidal volume of 6 ml/kg (V_T6) or 10 ml/kg (V_T10). Photomicrographs are representative of data obtained from lung sections derived from six animals and were taken at an original magnification of ×200. Note the presence of alveolar collapse (*arrows*) and interstitial edema (*asterisk*)
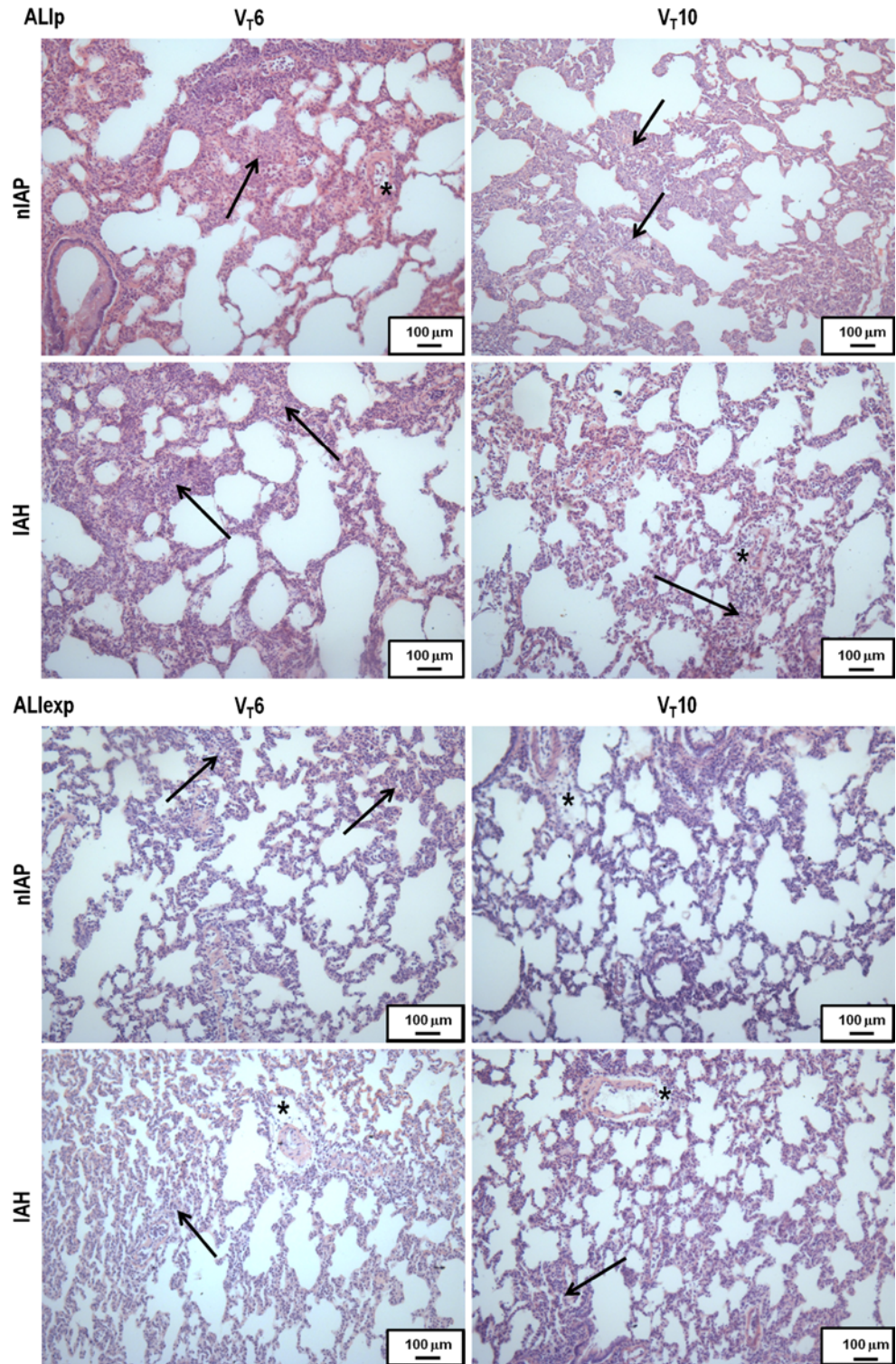
but not IL-1β and PCIII mRNA expressions (Fig. 3) in the presence of intraabdominal hypertension. Conversely, with normal intraabdominal pressure, V_T did not alter IL-6, IL-1β, PCIII, and caspase-3 mRNA expressions.

Pulmonary ALI

In ALIp, neither intraabdominal hypertension nor V_T affected the volume fraction of collapsed alveoli

Fig. 2 The volume fraction of the lung occupied by normal pulmonary areas, collapsed alveoli, and hyperinflated structures in pulmonary (p) and extrapulmonary (exp) acute lung injury (ALI), associated with intraabdominal hypertension (IAH) or not (nIAP), ventilated with tidal volume of 6 ml/kg (V_T6) or 10 ml/kg (V_T10). *Each bar* represents the mean ± SD of six rats in each group. †Significantly different from nIAP ($p < 0.05$). ‡Significantly different from V_T6 ($p < 0.05$)
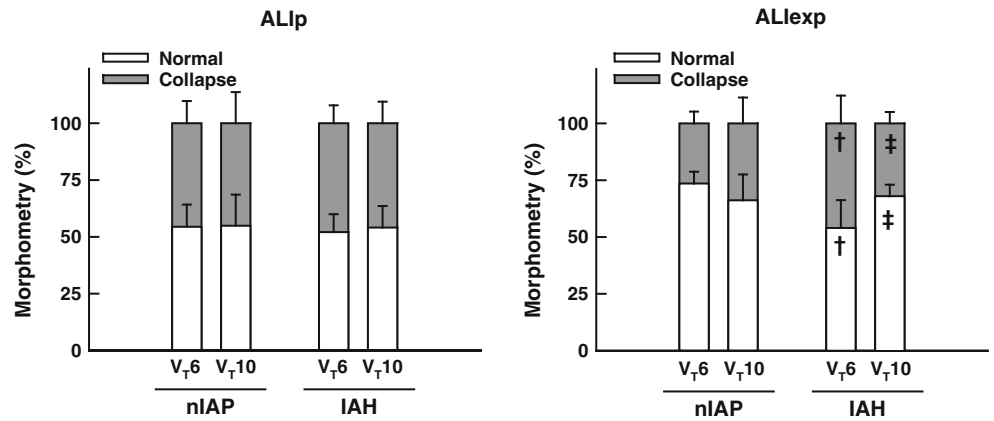

Table 1 Semi-quantitative analysis of lung electron microscopy

Groups	Lung		
	Alveolar capillary membrane	Type II pneumocyte	Endothelial cells
ALIp			
nIAP			
V_T6	2 (2–3)	3 (2.75–3)	2 (1.75–2.25)
V_T10	4 (3–4)[a]	4 (3–4)	2 (1.75–2.25)
IAH			
V_T6	4 (3.75–4)[b]	3 (2.75–4)	2 (2–3)
V_T10	4 (3.75–4)	4 (4–4)	3 (3–3.25)[b]
ALIexp			
nIAP			
V_T6	2 (2–2.25)	2 (1.75–2)	3 (3–3.25)
V_T10	1 (1–2)	2 (1–2)	3 (3–4)
IAH			
V_T6	2 (2–3)	2 (2–2.25)	3 (3–3.25)
V_T10	2 (1–2)	1 (1–2)[a]	3 (3–3.25)

Values are medians (25–75th‰) of five animals in each group. A 5-point semiquantitative severity-based scoring system was used. These pathological findings were graded as: 0 = normal lung parenchyma; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of examined tissue

ALIp pulmonary acute lung injury, *ALIexp* extrapulmonary acute lung injury, *nIAP* normal intra-abdominal pressure, *IAH* intraabdominal hypertension, V_T6 tidal volume of 6 ml/kg, V_T10 tidal volume of 10 ml/kg

[a] Significantly different from V_T6 ($p < 0.05$)
[b] Significantly different from nIAP ($p < 0.05$)

Table 2 Cell apoptosis in lung and distal organs

Groups	Lung	Liver	Kidney	Villi
ALIp				
nIAP				
V_T6	3 (3–3.25)	2 (2–2.25)	2 (2–2.25)	2 (1.75–2.25)
V_T10	3 (3–4)	2 (1.75–2)	2 (2–2.25)	2 (1.75–2)
IAH				
V_T6	3 (3–4)	4 (3–4)[a]	3 (3–4)[a]	4 (3–4)[a]
V_T10	3 (3–4)	3 (2.75–3.25)[a]	3 (3–3.25)[a]	3 (3–4)[a]
ALIexp				
nIAP				
V_T6	2 (2–2.25)	3 (3–4)	3 (3–4)	4 (3–4)
V_T10	2 (2–2.25)	4 (2.75–4)	3 (3–4)	3 (2.75–4)
IAH				
V_T6	3 (2–3.25)	4 (3–4)	4 (4–4)	4 (3.75–4)
V_T10	2 (1.75–2.25)	4 (3–4)	3 (3–4)	4 (3.75–4)

Values are medians (25–75th‰) of five animals in each group. A 5-point semiquantitative severity-based scoring system was used. The apoptotic findings were graded as: 0 = normal lung parenchyma; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of examined tissue

ALIp pulmonary acute lung injury, *ALIexp* extrapulmonary acute lung injury, *nIAP* normal intra-abdominal pressure, *IAH* intraabdominal hypertension, V_T6 tidal volume of 6 ml/kg, V_T10 tidal volume of 10 ml/kg

[a] Significantly different from nIAP ($p < 0.05$)

(Figs. 1, 2). Epithelial cell damage was more pronounced in ALIp than ALIexp. Alveolar capillary membrane injury was more intense at V_T6, and the degree of endothelial cell damage was greater at V_T10. The degree of cell apoptosis in liver, kidney, and villi was higher with intraabdominal hypertension compared to normal intraabdominal pressure, independent of V_T (Table 2). In the presence of intraabdominal hypertension, IL-6 mRNA expression was higher in V_T10 compared to V_T6, but no significant differences were observed in IL-1β, PCIII, and caspase-3 mRNA expressions (Fig. 3). In the presence of normal intraabdominal pressure, V_T10 led to higher IL-6

and caspase-3 mRNA expressions compared to V_T6, while IL-1β and PCIII mRNA expressions were not affected by V_T (Fig. 3).

Discussion

In the present study, we investigated the effects of intraabdominal hypertension as well as the impact of protective (6 ml/kg) and higher (10 ml/kg) V_T in experimental pulmonary and extrapulmonary ALI with or without intraabdominal hypertension. In the presence of intraabdominal hypertension, we found that: (1) chest wall static elastance, IL-6, IL-1β, PCIII, and caspase-3

Fig. 3 Real-time polymerase chain reaction analysis of interleukin (IL)-6, IL1-β, caspase-3, and type III procollagen (PCIII) mRNA expressions in lung tissue of animals with pulmonary (p) and extrapulmonary (exp) acute lung injury (ALI) associated with intraabdominal hypertension (IAH) or not (nIAP), ventilated with a tidal volume of 6 ml/kg (V_T6) or 10 ml/kg (V_T10). *Each bar* represents the mean $\pm$ SD of four rats in each group. Data are normalized to GAPDH expression. The *y* axis represents fold changes in mRNA expression compared with the level detected in ALIp-nIAP-V_T6 (for ALIp) and ALIexp-nIAP-V_T6 (for ALIexp), which were considered as 1. †Significantly different from nIAP ($p < 0.05$). ‡Significantly different from V_T6 ($p < 0.05$)
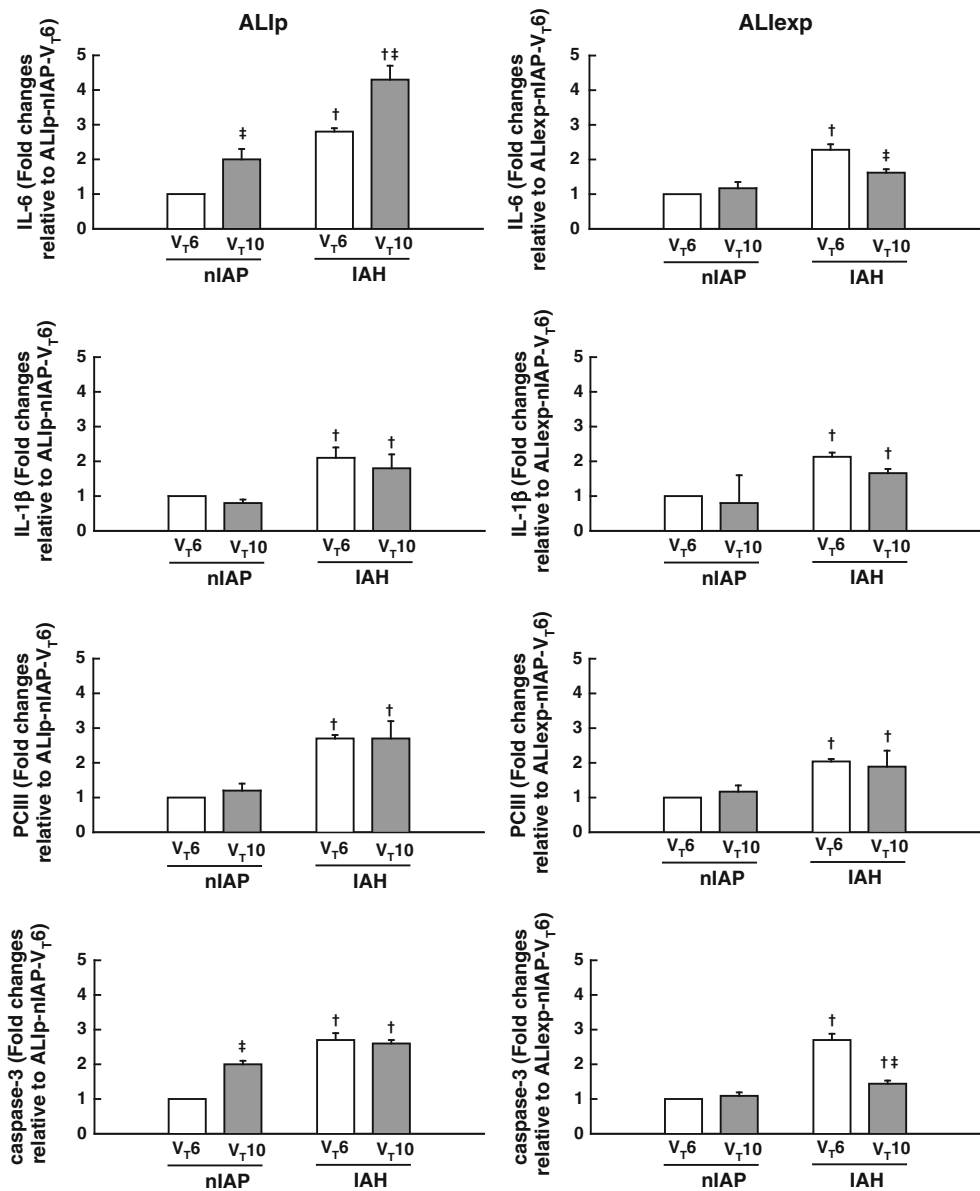
mRNA expressions in lung tissue were more pronounced as compared to normal intraabdominal pressure in both ALI groups; (2) in ALIexp, higher V_T (10 ml/kg) was associated with decreased atelectasis and lower IL-6 and caspase-3 mRNA expressions in lung tissue; (3) in ALIp, higher V_T led to higher IL-6 mRNA expression in lung tissue; (4) the degree of liver, kidney, and villi cell apoptosis was increased in ALIp, but not affected by V_T.

To assess the effects of intraabdominal hypertension on ALIp and ALIexp, we chose models with similar deterioration in lung mechanics (Table ESM1) and oxygenation (Table ESM2). Intraabdominal hypertension was induced by inserting cotton dressings into the abdominal cavity until intraabdominal pressure of 15 mmHg was achieved, because, as previously described, intraperitoneal fluid infusion has the potential to be absorbed and therefore interfere with the pathophysiological response to intraabdominal hypertension [16], while the use of air inflation with CO_2 may represent an additional physiologic variable related to the body's response to intraabdominal hypertension [17]. The advantage of our intraabdominal hypertension model was the maintenance of high intraabdominal pressure and the absence of any side effects related to the gas inflated into the peritoneum. Although these animals may be ventilated with different combinations of V_T and PEEP, we used $V_T = 6$ ml/kg and 10 ml/kg, which have been considered to be in a safe range [18, 19], associated with PEEP = 5 cmH$_2$O. Additionally, in the presence of intraabdominal hypertension, the increase in PEEP may lead to negative hemodynamic effects, as well

as fluid and/or vasoactive drug requirements to stabilize hemodynamics. IL-6, IL-1β, and caspase-3 mRNA expressions in lung were analyzed because of their role as mediators in the pathogenesis of ventilator induced lung injury [20]. The expression of PCIII mRNA was evaluated in lung tissue because it is the first collagen to be remodeled in the development/course of lung fibrogenesis [21], and also an early marker of lung parenchyma remodeling [13, 14, 22].

The impact of intraabdominal hypertension on acute lung injury

As previously reported, intraabdominal hypertension causes an increase in chest wall static elastance [2, 23–25]. In our study, during ventilation with V_T6, intraabdominal hypertension did not affect lung static elastance in either ALI group. Lung static elastance was not correlated to lung morphology. This may be explained by a greater alveolar collapse and the presence of adhesive atelectasis and consolidation in ALIp [8]. In short, the extent and characteristics of atelectasis at baseline in ALIp may have limited the effects of intraabdominal hypertension, preventing further alveolar collapse. Conversely, intraabdominal hypertension resulted in increased alveolar collapse mainly in ALIexp ventilated with V_T6.

Intraabdominal hypertension increased mRNA expression of IL-6, IL-1β, PCIII, and caspase-3 independent of ALI etiology. Intraabdominal hypertension is known to release pro-inflammatory cytokines, leading to neutrophil infiltration [26, 27]. However, the effects of intraabdominal hypertension on the fibrogenic process as well as caspase-3 have not been investigated. The increase in PCIII may be related to the increase in inflammatory mediators. Although caspase-3 expression was more elevated in intraabdominal hypertension, no significant changes were observed in the degree of apoptosis measured by TUNEL or electron microscopy. This could be explained by the fact that caspase-3 expression was evaluated at RNA instead of the protein level.

Our data suggest that the inflammatory response might not be related to the presence of alveolar collapse, but rather associated with alveolar-capillary barrier damage in ALIp with intraabdominal hypertension (Table 1). Therefore, the type of ALI seems to be deeply related to the type of effect exerted by intraabdominal hypertension on lung morphology and biochemical response.

The effects of ALI associated with intraabdominal hypertension on inflammatory response might be ascribed to changes in regional chest wall mechanics, which may yield different local transpulmonary pressures [1] resulting in inhomogeneous distribution of ventilation and/or re-opening and closing of atelectatic lung regions, with different stress and strain; increase in thoracic blood volume [28]; reduction in lymphatic drainage [29]; and

development of systemic inflammation from the abdomen, affecting the lung [27]. Furthermore, in ALIexp, a greater inflammatory response from the abdomen could be expected in the presence of intraabdominal hypertension.

V_T6 versus V_T10 in pulmonary and extrapulmonary ALI with intraabdominal hypertension

A V_T of 6 ml/kg has been recommended during protective lung ventilation to improve survival in patients with different clinical risk factors for ALI/ARDS [18, 30]. A V_T of 10 ml/kg has been found not to significantly affect outcome as compared to 6 ml/kg [19]. However, no study so far has evaluated the response to different V_T in ALIp and ALIexp associated with intraabdominal hypertension.

In the present study, V_T setting influenced respiratory function and lung injury depending on ALI etiology. In ALIp with or without intraabdominal hypertension, V_T did not significantly affect the amount of alveolar collapse. However, the use of V_T10 increased lung injury, which may be related to the presence of alveolar consolidation and/or sticky alveolar collapse, likely requiring higher inspiratory transpulmonary pressures to reopen. These data are in line with previous works showing lung injury at higher V_T [31–33] and peak inspiratory pressure [34, 35].

In ALIexp associated with intraabdominal hypertension, V_T6 increased while V_T10 decreased atelectasis. The reduction in atelectasis led to oxygenation improvement and may be related to higher end-inspiratory transpulmonary pressure at V_T10 and different characteristics of alveolar collapse. Higher V_T led to a reduction in inflammatory mediators in the lung tissue.

Our data suggest that even if higher end-inspiratory transpulmonary pressure were reached with V_T10, the reduction in alveolar collapse would play a major role in minimizing lung injury in the presence of intraabdominal hypertension. Similar effects have been reported with other ALI models [31, 36]. Although it has been accepted that lung injury correlates with variation in transpulmonary pressure [37], under certain conditions this may not be valid. In this context, in the presence of intraabdominal hypertension, the higher transpulmonary pressure between end-inspiration and end-expiration at V_T10 compared to V_T6 was not associated with increased lung injury.

In the present study, we were not able to measure strain, i.e., the ratio between tidal volume and aerated lung volume at end-expiration. However, the percentage of aerated areas may be regarded as a surrogate of aerated lung volume. Since V_T did not change aeration in ALIp associated with intraabdominal hypertension, we may hypothesize that V_T10 was associated with increased strain of lung parenchyma. Conversely, in ALIexp associated

with intraabdominal hypertension, V_T10 promoted an increase in aerated lung volume, suggesting that strain decreased or remained unaltered.

At V_T6, intraabdominal hypertension increased peripheral organ damage mainly in ALIp, perhaps because of the greater initial peripheral organ damage in ALIexp. This is expected as a result of endotoxin release in the blood. Moreover, intraabdominal hypertension may evolve to abdominal compartment syndrome. Increased intraabdominal pressure reduces blood flow to intraabdominal organs [38], with consequent apoptosis of enterocytes [39] and hepatocytes [40]. However, independent of V_T, liver, kidney, and villi cell apoptosis were increased with intraabdominal hypertension.

results could have been obtained at higher PEEP levels and/or if recruitment maneuvers were applied; (5) respiratory rate was kept constant, since its increase has been reported to be associated with the release of inflammatory mediators [12]; (6) inflammatory mediators, apoptosis, and fibrogenetic response were measured in lung tissue but not in the blood; (7) anti-inflammatory responses were not evaluated; and (8) since the observation time was relatively short (1 h mechanical ventilation), the expression of mediators was quantified using RT-PCR instead of ELISA. In this line, it is well known that 1 h is sufficient time to produce changes in mRNA expression, but not to significantly change the level of protein [8, 10, 12–14, 22].

Limitations

This study has several limitations: (1) we used specific models of ALIp and ALIexp induced by intratracheal and intraperitoneal endotoxin injection. Our results may not be extrapolated to other ALI models in small or large animals; (2) we did not assess possible long-term effects of V_T and intraabdominal hypertension; (3) a specific level of intraabdominal hypertension (15 mmHg) and V_T (6 vs. 10 ml/kg) were used; (4) PEEP was not individually titrated [2, 13]; rather, a fixed PEEP level (5 cmH$_2$O) was applied. It is therefore possible that the observed increase in atelectasis and changes in biochemical parameters might reflect the inability of the combination between PEEP and V_Ts to open the lung or the failure of PEEP to keep the alveoli open at the present levels of intraabdominal hypertension. We cannot rule out that different

Conclusions

Intraabdominal hypertension increased inflammation and fibrogenesis in the lung independent of ALI etiology. With intraabdominal hypertension, V_T10 improved lung morphometry with lower inflammation in lung tissue compared to V_T6 in ALIexp; conversely, it increased IL-6 expression in ALIp. Thus, V_T10 is one possible strategy to be proposed in the presence of intraabdominal hypertension, but other strategies deserve further investigation.

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