ORIGINAL ARTICLE

Addition of 5% CO_2 to Inspiratory Gas Prevents Lung Injury in an Experimental Model of Pulmonary Artery Ligation

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Abstract

Rationale: Unilateral ligation of the pulmonary artery may induce lung injury through multiple mechanisms, which might be dampened by inhaled CO_2.

Objectives: This study aims to characterize bilateral lung injury owing to unilateral ligation of the pulmonary artery in healthy swine undergoing controlled mechanical ventilation and its prevention by 5% CO_2 inhalation and to investigate relevant pathophysiological mechanisms.

Methods: Sixteen healthy pigs were allocated to surgical ligation of the left pulmonary artery (ligation group), seven to surgical ligation of the left pulmonary artery and inhalation of 5% CO_2 (ligation + $F_{I_{CO_2}}$ 5%), and six to no intervention (no ligation). Then, all animals received mechanical ventilation with V_T 10 ml/kg, positive end-expiratory pressure 5 cm H_2O, respiratory rate 25 breaths/min, and $F_{I_{O_2}}$ 50% ($\pm F_{I_{CO_2}}$ 5%) for 48 hours or until development of severe lung injury.

Measurements and Main Results: Histological, physiological, and quantitative computed tomography scan data were compared between groups to characterize lung injury. Electrical impedance tomography and immunohistochemistry analysis were performed in a subset of animals to explore mechanisms of injury. Animals from the ligation group developed bilateral lung injury as assessed by significantly higher histological score, larger increase in lung weight, poorer oxygenation, and worse respiratory mechanics compared with the ligation + $F_{I_{CO_2}}$ 5% group. In the ligation group, the right lung received a larger fraction of V_T and inflammation was more represented, whereas CO_2 dampened both processes.

Conclusions: Mechanical ventilation induces bilateral lung injury within 48 hours in healthy pigs undergoing left pulmonary artery ligation. Inhalation of 5% CO_2 prevents injury, likely through decreased stress to the right lung and antiinflammatory effects.

Keywords: VILI; pulmonary perfusion; CO_2 inhalation; therapeutic hypercapnia

At a Glance Commentary

Scientific Knowledge on the Subject: Unilateral pulmonary artery ligation may trigger detrimental mechanisms leading to lung injury. The addition of CO_2 to inspiratory gas may dampen such mechanisms and prevent injury.

What This Study Adds to the Field: In a large animal model, ligation of the left pulmonary artery leads to inhomogeneous ventilation and excessive inflammation, yielding bilateral ventilator-induced lung injury within 48 hours. Inhaled CO_2 prevents V_T redistribution and activation of inflammation, preserving both lungs from injury.

(Received in original form January 17, 2021; accepted in final form July 12, 2021)

Supported by institutional funding of the Department of Anesthesia, Critical Care and Emergency, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Ricerca corrente 2019) and by a grant from the Italian Ministry of Health, Rome, Italy, Ricerca Finalizzata 2016 (project GR-2016-02362428).

Am J Respir Crit Care Med Vol 204, Iss 8, pp 933–942, Oct 15, 2021

Originally Published in Press as DOI: 10.1164/rccm.202101-0122OC on July 12, 2021
Internet address: www.atsjournals.org

Previous studies started exploring interruption of pulmonary blood flow as a pivot determinant of ventilation-induced lung injury (VILI). Edmunds and colleagues described awake spontaneously breathing dogs developing lung hemorrhage and edema 5 days after ligation of the left pulmonary artery (1). Kolobow and colleagues showed massive pulmonary infarction in awake spontaneously breathing lambs undergoing complete cardiopulmonary bypass (no circulation in the pulmonary arterial tree) (2). Nonperfused lung units are also typically found in acute respiratory distress syndrome (ARDS), their amount being associated with severity. Greene and colleagues described increased mortality in patients with ARDS and radiologic signs of pulmonary vascular occlusion compared with patients with ARDS with normal angiography (3). More recently, interest in these phenomena was renewed by large clinical studies describing bedside measures of dead space fraction and ventilatory ratio as predictors of ARDS severity and mortality (4–6).

Previous experimental data suggested potential mechanisms causing lung injury in the presence of ventilated nonperfused units, potentially subjected to hypocapnia. Laffey and colleagues conducted a study on isolated lungs of rabbits and proved that hypocapnia was associated with increased inflammatory microvascular permeability (7). Subsequent research on the short-term effects of regional block of pulmonary blood flow with preserved ventilation showed local inflammation, decreased surfactant activity (8–10), and sudden reduction of local compliance with redistribution of V_T, potentially causing hyperventilation of the remaining perfused units (8, 11).

Enrichment by CO_2 of inspiratory gas might be a specific intervention to dampen these detrimental effects and prevent lung injury, as demonstrated in spontaneously breathing animals with perfusion interruption (1, 2), in animal models of VILI induced by large V_T and LPS (12, 13), and in *in vivo* models of acute lung injury after ischemia–reperfusion injury (14). Short-term studies suggested that inhaled CO_2 might be protective through reduced inflammation (13, 14) and prevention of regional loss of compliance, halting imbalances of V_T distribution (9).

In the present study, we aimed to describe whether controlled mechanical ventilation induces VILI in a long-term model of intubated sedated healthy swine undergoing unilateral pulmonary artery ligation. In addition, we investigated whether VILI is prevented by the addition of 5% CO_2 to inspiratory gas, and we performed an explorative analysis on the mechanisms of lung protection by CO_2.

If proved true, our results might add the following to previous literature on models of unilateral ligation of the pulmonary artery (1, 8): the role of controlled ventilation versus spontaneous breathing, a description of injury to the contralateral perfused lung through regional hyperventilation, a detailed physiological characterization of lung injury, and an exploratory analysis of the mechanisms underlying long-term lung protection by CO_2.

Some of the results of these studies have been previously reported in the form of abstracts (15, 16).

Methods

The study was approved by the Italian Ministry of Health (protocol no. 543/2018-PR) and conducted according to the European Directive 2010/63/EU on the protection of animals used for scientific purposes and Italian legislative decree 26/2014. Approval by the Institutional Animal Care Committee was obtained before starting the experiments.

Anesthesia and Animal Preparation

Twenty-nine healthy female pigs (34 ± 6 kg) were sedated to perform surgical tracheostomy. *See* online supplement for details on animal preparation, anesthesia, instrumentation, and protocols followed for fluids, hemodynamic management, and prevention of infections and deep vein thrombosis.

Study Protocol

After tracheostomy and until the end of the experiment (48 hours or development of severe lung injury), all animals were ventilated in the prone position on volume-controlled mode with V_T of 10 ml/kg, respiratory rate of 25 breaths/min, positive end-expiratory pressure (PEEP) of 5 cm H_2O, and $F_{I_{O_2}}$ of 0.5. Fixed low PEEP was considered the best compromise to prevent atelectasis and limit protection from lung injury in healthy animals. Similarly, $F_{I_{O_2}}$ was selected to balance the risk of desaturation versus reabsorption atelectasis.

Pigs were assigned to the following groups: surgical left pulmonary artery ligation (ligation group, $n = 16$), left pulmonary artery ligation and addition of 5% CO_2 to inspired gas (ligation + $F_{I_{CO_2}}$ 5% group, $n = 7$), or mechanical ventilation with no intervention (no-ligation group, $n = 6$).

See online supplement for details on study protocol and surgical procedure of the left pulmonary artery ligation.

In the ligation + $F_{I_{CO_2}}$ 5% group, right after the ligation procedure, inspired gases were switched to a mixture of 50% O_2, 5% CO_2, and 45% N_2 administered by dedicated

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Author Contributions: I.M. and E. Scotti carried out the experiments, analyzed the results, and wrote the manuscript; E. Spinelli conceived, planned, and carried out the experiments, interpreted the results, and wrote the manuscript; A.M., L.M., and Y.-M.W. carried out the experiments and collected the results; G.C. processed experimental data; O.B. and M.B. helped in the implementation of the experiments; F.R. collected and processed biological samples and analyzed the results; T.L., C.L., L.R., S.G., and A.Z. provided critical feedback and helped shape the research, analysis, and manuscript; S.F., V.V., and G.L. performed the histological analysis, contributed to the interpretation of the results, and provided critical feedback in the writing of the manuscript; A.P. conceived of and supervised the project and contributed to the writing of the manuscript; T.M. conceived of, planned, and carried out the experiments, interpreted the results, took the lead in the writing of the manuscript, and directed the project; and all authors discussed the results and contributed to the final manuscript.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

tanks (Linde Medicale) used as the only source of gas for the ventilator.

Study Measures

In every study group, data from respiratory mechanics, hemodynamics, arterial and mixed venous blood gas analysis, and quantitative computed tomographic (CT) scans were collected after 2, 12, 24, 36, and 48 hours from the end of the ligation procedure for the ligation and ligation + FI_{CO_2} 5% groups or instrumentation for the no-ligation group. Quantitative CT scan analysis (Lightspeed, General Electric) was processed offline as previously described (11) with commercially available software (Maluna 3.17): for each CT scan, lung masks were manually determined by two experienced researchers. CT windows were iteratively modified to exclude areas with partial volume effect, chest wall, mediastinum, and pleural effusions. A CT scan was not performed in four animals in the ligation group because of technical reasons.

In four animals from the ligation group and seven from the ligation + FI_{CO_2} group, monitoring by electrical impedance tomography (EIT) allowed for the measurement of V_T distending right versus left lung and right and left respiratory system compliances (from 2 hours after start [T2] to the end of the experiment) (17). Details on study measures are provided in the online supplement.

Euthanasia, Autopsy, Histology, and Immunohistochemistry

The animals continued the protocol until 48 hours or development of severe lung injury. Then, the animals were killed and underwent autopsy for collection of histological samples. Histological score (range 0–30) of the lungs was calculated from six samples per animal stored in formaldehyde (three for each side). Ten main histological alterations were evaluated, as previously described (18). Samples for the wet to dry calculation were collected and processed.

In four randomly chosen animals from each group, samples underwent quantitative immunohistochemistry analysis to identify the percentage of cells positive for MPO (myeloperoxidase, for neutrophils), IBA-1 (ionized calcium-binding adaptor 1, for macrophages), CD3 (cluster of differentiation 3, for T lymphocytes), and CD20 (for B lymphocytes) (19). Details of sample staining and analysis are provided in the online supplement.

Statistical Analysis

Histological score of the lungs was the primary endpoint of the study. Sample size was similar to previous animal studies on the same topic (1, 2, 11, 20–22). However, we performed an exploratory power analysis, and we assumed a physiologically relevant difference in our primary endpoint of 10, with an SD of 4.5, as observed in previous publications using the same score to detect lung injury. To obtain power of 0.9 with α of 0.05, the minimum sample size resulted $n = 6$ per group. An imbalance in study numerosity, with a larger sample size for the ligation group, was sought to limit variability, which was hard to predict given the novelty of the model.

Data are shown as mean $\pm$ SD or median (interquartile range), unless otherwise indicated. Comparisons between histological and physiological variables and quantitative CT scan in the three study groups at the end of the experiment were performed by one-way ANOVA or Kruskal-Wallis test for normally and nonnormally distributed variables. A Holm-Sidak test or Dunn's test was applied for *post hoc* analysis with an adjustment of P value to allow for multiple comparisons using the Benjamini, Krieger, and Yekutieli procedure, as appropriate. Within the ligation group, differences in histological score and quantitative CT scan data between right and left lung were analyzed by a paired t test (normally distributed variables) or Wilcoxon test (nonnormally distributed variables). Differences between groups along the study of physiological and EIT variables were analyzed using generalized estimating equation models to account for repeated measures in time (longitudinal data) with possible unobserved time points. The model included group and time as main independent factors and a group-by-time interaction. Statistical significance was defined by a P or q value <0.05. Analyses were performed using GraphPad Prism (version 9.00).

Results

Study Groups

Before the start of the experiment, there were no differences in respiratory mechanics, gas exchange, hemodynamics, and lung weight between animals subsequently allocated to the three groups (*see* Table E1 in the online supplement).

All animals in the no-ligation group, all in the ligation + FI_{CO_2} 5% group, and 14 out of 16 in the ligation group completed the 48 hours of study. Two pigs in the ligation group developed severe lung injury earlier and were killed at 24 and 30 hours, respectively.

VILI in the Study Groups

At the end of the experiment, microscopic analysis showed that animals in the ligation group developed bilateral lung injury with elevated histological scores (Figure 1A). The addition of 5% FI_{CO_2} protected the lungs of animals in the ligation + FI_{CO_2} 5% group from lung injury: at the end of the experiment, the histological score in this group was very low and comparable to the no-ligation group (Figure 1A). Figure 2 shows representative microscopic images from the three study groups. Table E2 reports values for each item from the histological score: all were higher in the ligation group, with larger differences in the presence of inflammatory cells.

A CT scan analysis confirmed the development of lung edema only in the ligation group. At the end of the experiment, the increase in lung weight was larger in the ligation group compared with the other two groups (Figure 1B). Similarly, the fraction of collapsed nonaerated lung tissue was significantly higher in the ligation group compared with both the ligation + FI_{CO_2} 5% and no-ligation groups (Figure 1C).

At the end of the experiment, lung injury was characterized by extremely decreased compliance of the respiratory system in the ligation group (Figure 1D), whereas the ligation + FI_{CO_2} 5% and no-ligation groups maintained relatively normal higher compliance values (Figure 1D). Consequently, plateau and driving inspiratory pressures were significantly higher in the ligation group compared with both the ligation + FI_{CO_2} 5% and no-ligation groups (Table 1). Of notice, chest wall compliance remained similar between study groups, and derangements in respiratory system compliance were attributable to alterations in lung compliance (Table 1).

At the end of the experiment, the Pa_{O_2}/FI_{O_2} ratio was significantly lower in the ligation group, whereas oxygenation was only slightly impaired in the other two groups (Figure 1E). As expected, animals in the ligation + FI_{CO_2} 5% group were characterized by higher Pa_{CO_2} and lower pH

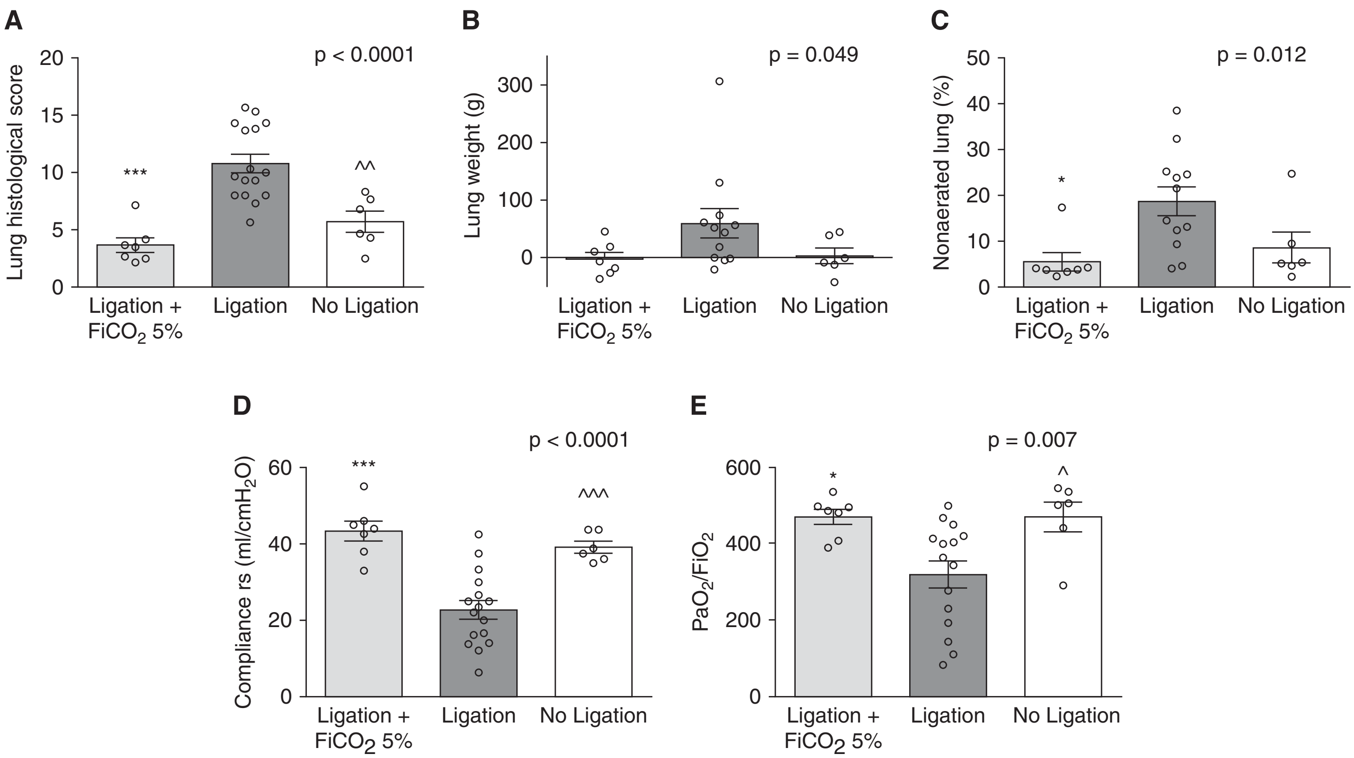

Figure 1. Severity of histological, computed tomographic scan, and physiological alterations at the end of the experiment. (*A*) The histological score of lungs from each study group. The score had 10 components that were ranked between 0 and 3 and summed within apical, medial, and basal samples from each lung. Then, scores from the six samples were averaged to obtain the total lung histological score for each animal (range 0–30). (*B*) Quantitative computed tomographic scan analysis showing change in lung weights from the baseline (after instrumentation and before surgical ligation of the left pulmonary artery) to the end of the experiment (48 hours or at development of severe lung injury) (Δlung weight) in each study group. (*C*) Proportion of nonaerated lung tissue in each study group. (*D* and *E*) Compliance of the respiratory system (rs) (*D*) and Pa_{O_2}/FI_{O_2} (*E*) at the end of the experiment in each study group. Data are expressed as scatter dot plot with mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by *post hoc* Holm-Sidak test (*A*, *C*, *D*, and *E*) or Kruskal-Wallis test (*B*). *P* values in the graph refer to ANOVA/Kruskal-Wallis *P* value. $^*P < 0.05$ and $^{***}P < 0.001$, ligation + FI_{CO_2} 5% versus ligation group. ^$P < 0.05$, ^^$P < 0.01$, and ^^^$P < 0.01$, no-ligation versus ligation group.

compared with both the ligation and no-ligation groups (Table 1).

Ligation of the left pulmonary artery led to higher mean and systolic pulmonary arterial pressure in the ligation group compared with the other two groups, with increased, although not significantly, pulmonary vascular resistance only in the ligation group (Table 1).

In the ligation group, at the end of the experiment, macroscopic qualitative examination showed increased lung size with diffuse edema and regional collapse of the right lung, whereas the left hypoperfused lung was smaller, darker, and congested (Figure E1). Lungs from the ligation + FI_{CO_2} 5% and no-ligation groups were, conversely, pink, with no sign of collapse, edema, or congestion (Figure E1).

Finally, at the end of the experiment, in the ligation + FI_{CO_2} 5% group, the wet to dry ratio was significantly lower if compared with both the ligation and no-ligation groups (4.8 [4.7–5.0] vs. 5.5 [5.4–5.8] vs. 5.1 [4.7–5.7]; Kruskal-Wallis test *P* value: 0.002; *post hoc* Dunn's test: $P < 0.01$, ligation + FI_{CO_2} 5% vs. ligation).

Mechanisms of Lung Protection by CO_2

EIT analysis showed significantly higher V_T fraction reaching the right nonligated lung in the ligation group as compared with the ligation + FI_{CO_2} 5% group (Figure 3A, Figure E2, and Table E3) from T2 to T36, whereas distribution of ventilation was more homogenous at T48 (Figure 3A, Figure E2, and Table E3). Larger V_T reaching the right lung in the ligation group was likely due to lower compliance of the left side in comparison to the ligation + FI_{CO_2} group (Figure 3B). Then, at T48, compliance of the right side declined in the ligation group (Figure 3C), becoming more similar to the left side (Figure 3B) and redistributing V_T more evenly (Figure 3A and Table E3).

The immunohistochemical analysis showed that, globally, inflammation was prevented by the addition of CO_2 after ligation (Figures 4A–4D and Figures E3A–E3D). In particular, the innate immunity population MPO-positive neutrophils (Figures 4A and 4B) and IBA-1–positive macrophages (Figures 4C and 4D) were significantly decreased in the ligation + FI_{CO_2} group compared with ligation or no ligation sets. Similarly, mature B-lymphocyte infiltrates were marginally decreased in the ligation + FI_{CO_2} group up to the control levels (Figures E3C and E3D), whereas the CD3-positive T-cell subpopulation was modestly affected (Figures E3A and E3B).

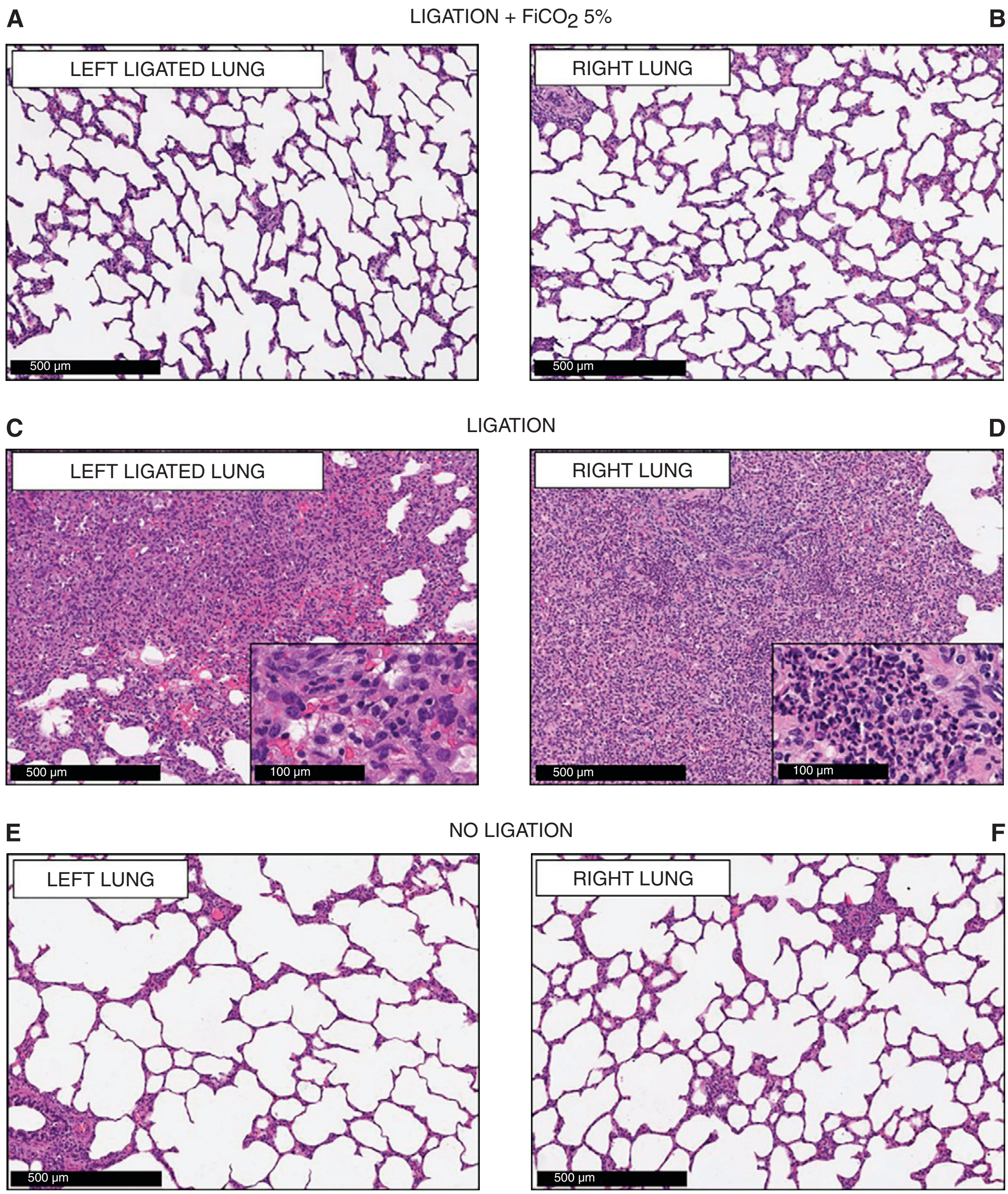

Figure 2. Microscopic appearance of the lungs at the end of the experiment. (*A–D*) Representative microphotographs of the left ligated and right nonligated lungs from the ligation + $F_{I_{CO_2}}$ 5% group (*A* and *B*) and ligation group (*C* and *D*). (*C*) The left lung of the ligation group shows a marked inflammatory infiltrate composed primarily of lymphocytes and macrophages, with vascular congestion and hemorrhage. (*D*) The right lung of the ligation group shows a patchy acute inflammatory infiltrate composed primarily of neutrophils (hematoxylin and eosin [H&E]). Notably, the lungs of the ligation + $F_{I_{CO_2}}$ 5% group (*A* and *B*) showed no inflammatory changes (H&E). (*E* and *F*) In the lower panels, representative microphotographs of the lungs from the no ligation group with no significant histological alterations (H&E). Scale bars: main panels, 500 μm; insets, 100 μm.

Differences between Left Ligated and Right Nonligated Lung in the Ligation Group

The histological score did not differ between right and left ligated lung for the global value (Figure 5A). However, an analysis of the single items composing the score showed higher neutrophil infiltration and interstitial lymphocyte proliferation for the right nonligated lung and more extensive alveolar hemorrhage for the left ligated lung (Table E4).

The increase in lung weight (lung edema) happened only in the right nonligated lung (Figure 5B). Increased lung weight likely determined the larger fraction of nonaerated lung tissue in the right lung compared with the left ligated lung (Figure 5C).

Table 1. Physiological Characteristics of the Study Groups at the End of the Experiment

	Ligation + $F_{I_{CO_2}}$ 5% (*n* = 7)	Ligation (*n* = 16)	No Ligation (*n* = 6)	*P* Value*
Respiratory mechanics				
Peak pressure, cm H_2O	20 ± 3	33 ± 12†‡	19 ± 2	**0.004**
Plateau pressure, cm H_2O	14 ± 1.5	23 ± 8.2†‡	14 ± 0.8	**0.003**
Mean airway pressure, cm H_2O	9 ± 0.9	12 ± 2.8†‡	9 ± 0.8	**0.007**
Driving pressure, cm H_2O	9 ± 1.2	18 ± 8.2§‖	9 ± 0.8	**0.002**
Respiratory system compliance, ml/cm H_2O	38 ± 6	23 ± 10¶**	39 ± 4	**<0.001**
Lung compliance, ml/cm H_2O	83 (50–102)	32 (17–50)‖	70 (54–88)	**0.004**
Chest wall compliance, ml/cm H_2O	80 ± 14	82 ± 20	102 ± 31	0.154
Arterial blood gas analyses data				
$Pa_{O_2}/F_{I_{O_2}}$	478 ± 96	320 ± 137†‡	471 ± 95	**0.009**
Pa_{CO_2}, mm Hg	63 (57–66)	33 (31–40)‖	32 (30–36)‖	**0.001**
pH	7.42 (7.40–7.45)	7.49 (7.45–7.51)	7.53 (7.51–7.55)‖	**0.002**
HCO_3^-, mmol/L	37.8 ± 2.4	27.3 ± 3.0**	28.4 ± 2.2**	**<0.001**
Capnography				
$P_{ET_{CO_2}}$, mm Hg	67 (63–71)	30 (26–34)‖	29 (27–35)‖	**0.001**
Hemodynamics				
Systolic arterial pressure, mm Hg	107 ± 10	119 ± 12	122 ± 18	0.103
Diastolic arterial pressure, mm Hg	63 ± 13	84 ± 17	88 ± 26	**0.037**
Mean arterial pressure, mm Hg	86 ± 13	101 ± 13	100 ± 16	**0.050**
Systolic pulmonary artery pressure, mm Hg	31 ± 4	35 ± 9†	25 ± 3	**0.012**
Diastolic pulmonary artery pressure, mm Hg	15 ± 4	19 ± 8	15 ± 2	0.222
Mean pulmonary artery pressure, mm Hg	20 ± 2	27 ± 7	23 ± 3	**0.047**
Pulmonary vascular resistance, $dyn \cdot s \cdot cm^{-5}$	255 (230–291)	307 (226–398)	280 (237–331)	0.389
Wedge pressure, mm Hg	8 ± 1	10 ± 3	8 ± 2	0.434
Cardiac output, L/min	5.1 ± 1.1	4.4 ± 1.2	3.6 ± 0.5	0.082
Heart rate, beats/min	97 (80–103)	96 (78–107)	80 (72–85)	0.169
Fluid balance, ml	67 ± 458	57 ± 474	−71 ± 1,622	0.946

Definition of abbreviation: $P_{ET_{CO_2}}$ = end-tidal CO_2 pressure.
Data are expressed as mean ± SD (normally distributed values) or median (quartiles) (nonnormally distributed values). Significant *P* values are shown in bold.
*Comparisons are obtained with one-way ANOVA or Kruskal-Wallis test for normally and nonnormally distributed values, respectively, followed by Holm-Sidak or Dunn multiple comparison tests as appropriate.
†$P < 0.05$ versus no-ligation group.
‡$P < 0.05$ versus ligation + $F_{I_{CO_2}}$ 5% group.
§$P < 0.01$ versus no-ligation group.
‖$P < 0.01$ versus ligation + $F_{I_{CO_2}}$ 5% group.
¶$P < 0.001$ versus no-ligation group.
**$P < 0.001$ versus ligation + $F_{I_{CO_2}}$ 5% group.

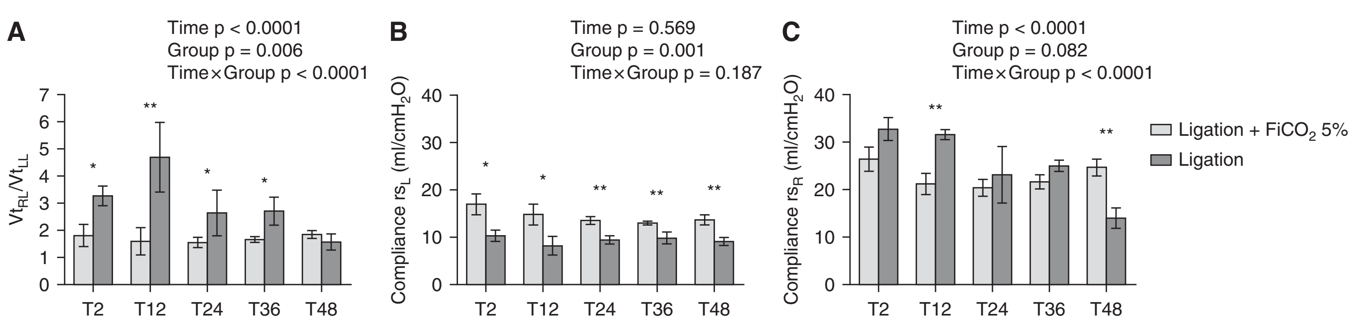

Figure 3. Ventilation distribution and regional respiratory system compliance along the experiments. (*A*) The ratio between VT distending the right and the left lung along the study time points shows significant imbalance in the ligation group, which was prevented by inhalation of CO_2. (*B* and *C*) Respiratory system compliance for left (*B*) and right (*C*) respiratory hemisystem. In the ligation group, left compliance was lower than the ligation + $F_{I_{CO_2}}$ group and remained stable over time, whereas in the right side of the ligation group, it declined along the study; $F_{I_{CO_2}}$ maintained both compliances stable. Data are expressed as mean ± SEM. Statistical analysis was performed using generalized estimating equation models to account for repeated measures in time (longitudinal data); the model included group and time as main independent factors and group-by-time interaction. *$P < 0.05$ and **$P < 0.01$, ligation + $F_{I_{CO_2}}$ 5% versus ligation group. Compliance rs_L = respiratory system compliance for left respiratory hemisystem; Compliance rs_R = respiratory system compliance for right respiratory hemisystem; $V_{T_{RL}}/V_{T_{LL}}$ = the ratio between VT distending the right and the left lung.

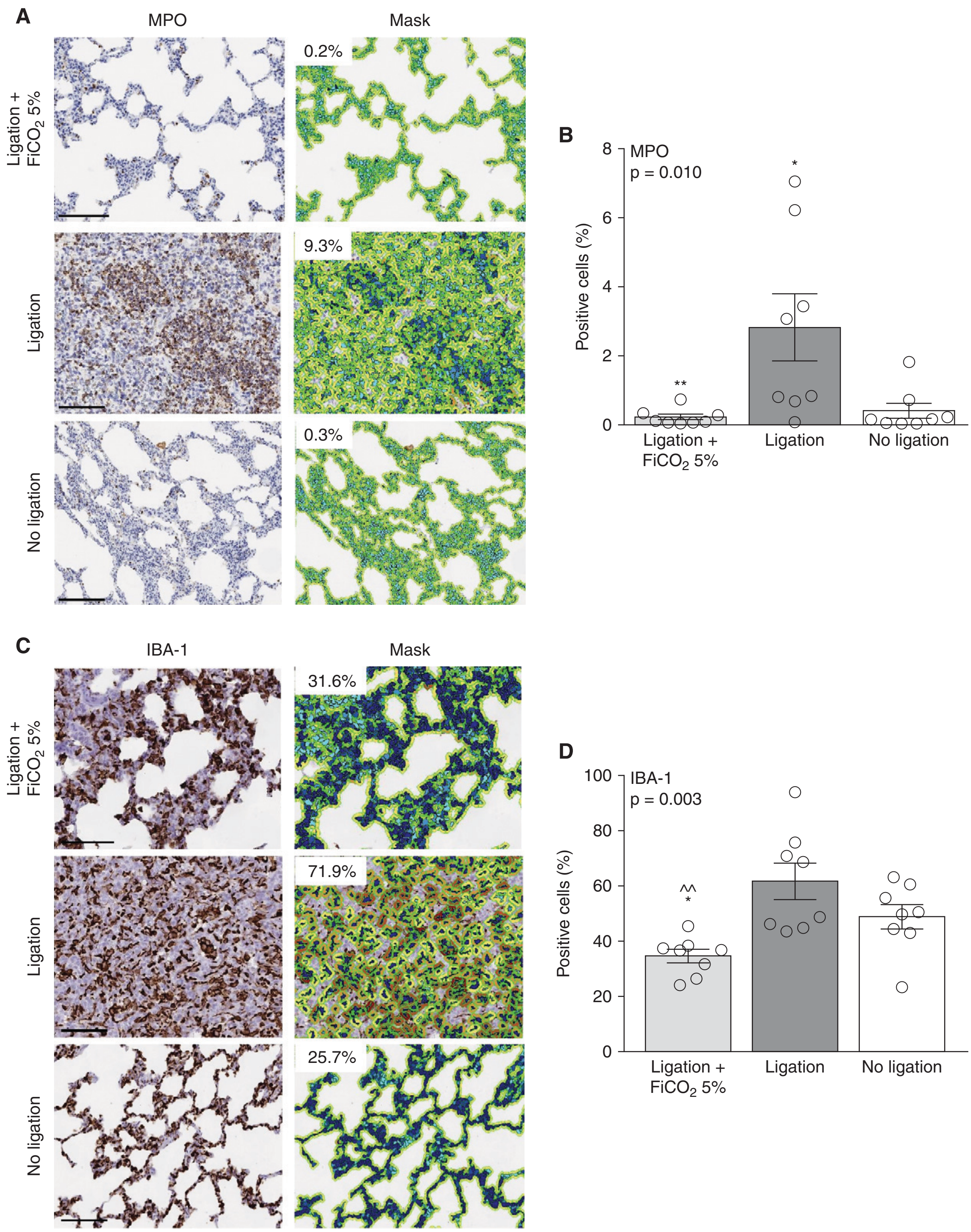

Figure 4. Characterization of the lung immune cell infiltrates in the different groups by immunohistochemistry. (*A* and *C*) Representative image of the MPO (myeloperoxidase)-positive (*A*) or IBA-1 (ionized calcium-binding adaptor 1)–positive (*C*) infiltrates in the lungs of pigs from the three different groups of treatment with the corresponding digital quantification (mask). Images show representative samples from the most affected side. Scale bars, 100 μm. (*B* and *D*) Quantification of the MPO-positive (*B*) or IBA-1–positive (*D*) cells in the different groups. Each circle is a sample ($n = 8$ per group); bars, mean ± SEM. (*B*) *$q = 0.010$, no ligation versus ligation; **$q = 0.008$, ligation versus ligation + $F_{I_{CO_2}}$ 5%. (*D*) ^^$q = 0.001$, no ligation versus ligation + $F_{I_{CO_2}}$ 5%; *$q = 0.014$, no ligation versus ligation + $F_{I_{CO_2}}$ 5%. *P* values from Kruskal-Wallis tests are reported in the graphs; *q* values are false discovery rate–adjusted *P* values from Dunn's *post hoc* test according to the Benjamini, Krieger, and Yekutieli method.

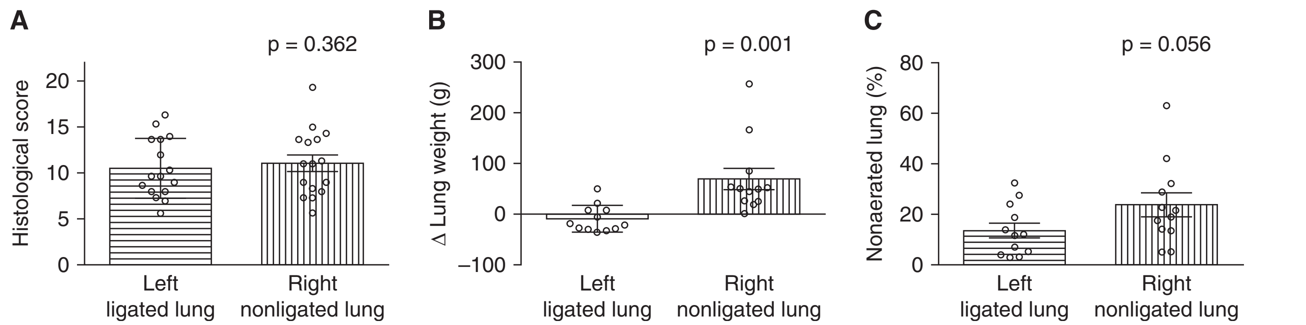

Figure 5. Differences between left ligated and right nonligated lung in the ligation group. (*A*) Histological score of each lung in the ligation group at the end of the experiment. (*B*) Quantitative computed tomographic scan analysis showing change in lung weight of each lung in the ligation group from the baseline (after instrumentation and before surgical ligation of the left pulmonary artery) to the end of the experiment (48 h or at development of severe lung injury) (Δlung weight). (*C*) Proportion of nonaerated lung tissue of each lung in the ligation group at the end of the experiment. Data are expressed as scatter dot plot with mean ± SEM. Statistical analysis was performed by a paired *t* test, and *P* values are reported in the graph.

Evolution of Physiological Parameters along the Study Time

To better understand the time needed to generate differences between the three groups, we analyzed values along the course of the experiment of physiological parameters. Most parameters remained stable until the T24–T36 interval, suggesting that lung injury developed nonlinearly and mostly in the second half of the experiment (Table E3).

Discussion

Surgical ligation of the left pulmonary artery induces bilateral lung injury within 48 hours in healthy pigs undergoing controlled mechanical ventilation; excessive ventilation to the nonligated lung and acute inflammation may have a key role in the development of injury. The addition of 5% CO_2 to inspiratory gas protects the lungs by effectively preventing these detrimental mechanisms.

In this study, bilateral VILI after unilateral pulmonary artery ligation was characterized by multiple physiological derangements, including histopathological alterations indicating development of diffuse lung injury, increased lung weight and alveolar consolidations, impaired respiratory mechanics, and poor oxygenation.

Wasted ventilation of nonperfused regions induces reduction of the local compliance with diversion of VT to perfused regions (11, 22, 23). The decrease of compliance may be due to local activation of cell apoptosis, triggering inflammation and depletion of surfactant in the left ligated lung, as previously demonstrated (8, 24), and to the alteration in vascular permeability and edema affecting the right nonligated lung (22, 25). Our data confirmed that relative hyperventilation (of the right lung) and inflammation (affecting both lungs) characterized the ligation group and likely contributed to the development of bilateral lung injury (Figure 6). In the ligation group, compliance of the left side was lower than in the ligation + $F_{I_{CO_2}}$ group, and this may have induced hyperventilation in the right side. When compliance of the right side became similar to the left side in the ligation group (i.e., at T48), VT distribution became more homogenous. This biphasic phenomenon (hyperventilation owing to the difference in left vs. right compliance in the ligation group followed by more homogenous ventilation when compliances became more similar)

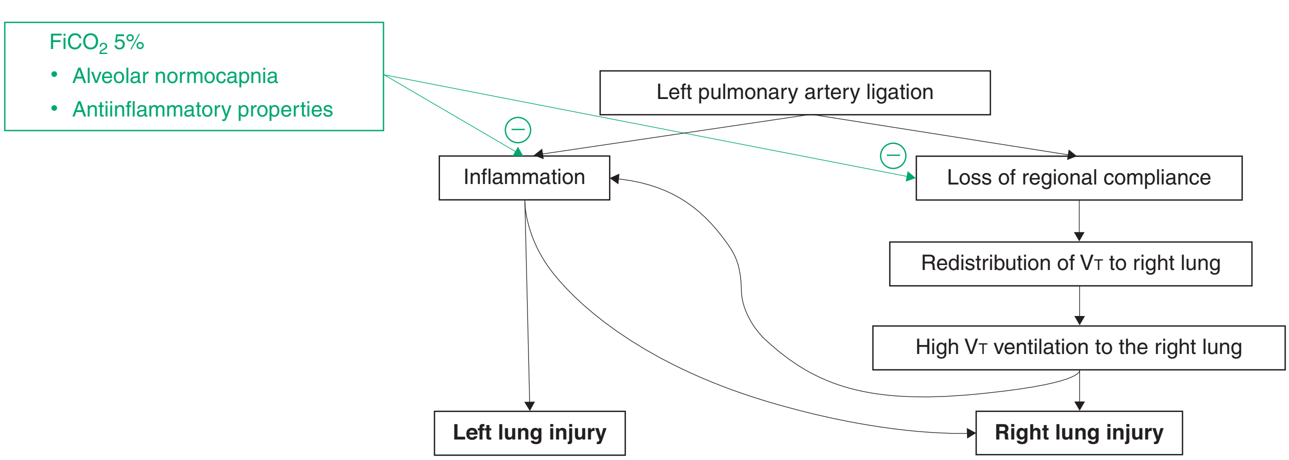

Figure 6. Pathophysiological mechanisms inducing bilateral lung injury and protective effects of inhaled CO_2. Left pulmonary artery ligation induces bilateral lung injury through multiple processes (black pathway). The addition of 5% CO_2 to inspiratory gases guarantees alveolar normocapnia and exerts antiinflammatory properties, blocking critical processes and ultimately preventing injury (green pathways).

may suggest that both high regional VT and local inflammation played a role in the development of right lung injury (Figure 6). Lymphocytes, macrophages, and neutrophils were highly represented in both lungs with significant expression of inflammatory markers.

The addition of FI_{CO_2} 5% might dampen such mechanisms, as shown by pilot experimental data (1, 8, 12–14). We described that 5% CO_2 inhalation prevents inhomogeneous distribution of VT and recruitment and activation of inflammatory cells. This probably halted the development of bilateral lung injury after unilateral ligation of the pulmonary artery: animals in the ligation + FI_{CO_2} 5% group were characterized by low histological injury and did not develop lung edema or lung collapse; inhaled CO_2 preserved respiratory mechanics and oxygenation, too.

CO_2 administration through inspiratory gas might have dampened detrimental mechanisms, leading to VILI by correction of alveolar hypocapnia and/or by induction of systemic hypercapnia. Indeed, CO_2 inhalation avoids the deleterious consequences of alveolar hypocapnic alkalosis (1, 2, 12–14, 26, 27), hampering pneumoconstriction and surfactant depletion. In our experiment, this might have determined more homogenous distribution of VT. On the other hand, systemic hypercapnia exerts antiinflammatory properties (28–31), and these might have led to the lower inflammatory reaction observed in our model. Differential ventilation with administration of inhaled CO_2 only to the right nonligated lung (obtaining systemic hypercapnia without correction of alveolar hypocapnia) could prove if the protective effects that we observed should be ascribed to systemic hypercapnia.

Our results show that, despite histological scores as high as the left ligated lung, only the right nonligated lung in the ligation group developed edema, which was very likely caused by the diversion of VT rather than by increased regional blood flow (24). Indeed, if the main mechanism had been the redistribution of blood flow, the ligation + FI_{CO_2} 5% group would have shown the same increase in weight of the right nonligated lung. Previous studies indicate that VILI induced by large VT in healthy lungs is characterized by edema and collapse (18), as observed in the right nonligated lung from the ligation group. This data resemblance reinforces the hypothesis that the inhomogeneous distribution of VT may have induced VILI (11, 22, 23, 32).

Carbon dioxide is a potent vasoconstrictor of the pulmonary circulation (31), but, at the end of the experiment, mean pulmonary artery pressure was lower in the ligation + FI_{CO_2} 5% group compared with the ligation group. These findings may suggest that the major determinant of pulmonary artery pressure level was lung injury rather than CO_2.

Our experimental protocol offers unique insights in comparison to previous studies on pulmonary hypoperfusion during spontaneous breathing (1, 2) or in isolated lungs (7–9). The application of controlled mechanical ventilation with more contemporary settings and use of advanced respiratory monitoring and immunohistochemistry technique yielded novel specific observations. After unilateral ligation of the pulmonary artery, lung injury develops during controlled mechanical ventilation; the contralateral perfused lung is not spared; multiple physiological derangements of gas exchange, respiratory mechanics, lung weight, regional compliance, and inflammatory reaction characterize injury; and, finally, lung protection by inhaled CO_2 may be mediated by more homogeneous VT distribution and dampening of inflammation.

Our findings may have clinical implications for intubated critically ill patients with increased dead space (33–36). Mechanical ventilation might trigger the abovementioned detrimental mechanisms (loss of regional compliance, tidal redistribution, and inflammation) with the potential of worsening lung injury. The role of hypercapnic acidosis and/or inhaled CO_2 in preventing this type of VILI remains to be determined.

Limitations

This study has limitations. First, mechanical ventilation settings were somehow arbitrarily chosen and remained fixed for the study duration. Different settings (e.g., higher PEEP, lower FI_{O_2}, and different frequency and mode for recruitment maneuvers) and/or adaptation of ventilation to the evolving lung injury (e.g., reduction of VT to maintain protective driving pressure) might have yielded different results. Second, Pa_{CO_2} of 30–35 mm Hg in the ligation group, in adjunction to the metabolic alkalosis that characterizes pigs of this size (37), resulted in significantly higher pH values in the ligation group than the ligation + FI_{CO_2} group (Table E3). However, data on the effects of alkalotic pH on mechanisms of lung injury (e.g., inflammation) are currently lacking (38). Third, we only performed surgical ligation of the left artery, and we do not know if ligation of the right one would have led to the same results. Fourth, animals from the ligation + FI_{CO_2} group were not randomized with those from other groups for technical reasons. Nevertheless, physiology was comparable before the start of the experiment (*see* Table E1). Finally, we were not able to dissect the relative contribution of local effects of inhaled CO_2 versus systemic benefits of hypercapnia.

Conclusions

Our study shows that healthy pigs undergoing surgical ligation of the left pulmonary artery develop bilateral lung injury within 48 hours of controlled mechanical ventilation. The addition of 5% CO_2 to inspiratory gas prevents the onset of bilateral VILI through reduced VT, reaching the nonligated lung and modulation of inflammation. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Samanta Oldoni for the logistical support in the animal laboratory.

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