ORIGINAL ARTICLE

Near-Apneic Ventilation Decreases Lung Injury and Fibroproliferation in an Acute Respiratory Distress Syndrome Model with Extracorporeal Membrane Oxygenation

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Abstract

Rationale: There is wide variability in mechanical ventilation settings during extracorporeal membrane oxygenation (ECMO) in patients with acute respiratory distress syndrome. Although lung rest is recommended to prevent further injury, there is no evidence to support it.

Objectives: To determine whether near-apneic ventilation decreases lung injury in a pig model of acute respiratory distress syndrome supported with ECMO.

Methods: Pigs (26–36 kg; $n = 24$) were anesthetized and connected to mechanical ventilation. In 18 animals lung injury was induced by a double-hit consisting of repeated saline lavages followed by 2 hours of injurious ventilation. Then, animals were connected to high-flow venovenous ECMO, and randomized into three groups: *1*) nonprotective (positive end-expiratory pressure [PEEP], 5 cm H_2O; V_T, 10 ml/kg; respiratory rate, 20 bpm), *2*) conventional-protective (PEEP, 10 cm H_2O; V_T, 6 ml/kg; respiratory rate, 20 bpm), and *3*) near-apneic (PEEP, 10 cm H_2O; driving pressure, 10 cm H_2O; respiratory rate, 5 bpm). Six other pigs were used as sham. All groups were maintained during the 24-hour study period.

Measurements and Main Results: Minute ventilation and mechanical power were lower in the near-apneic group, but no differences were observed in oxygenation or compliance. Lung histology revealed less injury in the near-apneic group. Extensive immunohistochemical staining for myofibroblasts and procollagen III was observed in the nonprotective group, with the near-apneic group exhibiting the least alterations. Near-apneic group showed significantly less matrix metalloproteinase-2 and -9 activity. Histologic lung injury and fibroproliferation scores were positively correlated with driving pressure and mechanical power.

Conclusions: In an acute respiratory distress syndrome model supported with ECMO, near-apneic ventilation decreased histologic lung injury and matrix metalloproteinase activity, and prevented the expression of myofibroblast markers.

Keywords: acute respiratory distress syndrome; extracorporeal membrane oxygenation; ventilator-induced lung injury; mechanical ventilation; myofibroblast

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At a Glance Commentary

Scientific Knowledge on the Subject: Approaches to mechanical ventilation during extracorporeal membrane oxygenation in acute respiratory distress syndrome are widely variable. Although lung rest strategies have been proposed, there is scarce evidence to support a recommendation. This is a highly relevant issue because the final impact of extracorporeal membrane oxygenation may depend on the possibility of promoting resolution of lung injury.

What This Study Adds to the Field: In this study, in a 24-hour experimental model of severe acute respiratory distress syndrome supported with extracorporeal membrane oxygenation, we compared the use of near-apneic ventilation with a nonprotective and with a conventional protective ventilatory strategy. Near-apneic ventilation decreased lung injury compared with the other strategies. In addition, an early fibroproliferative response characterized by extensive staining for myofibroblasts and procollagen III, and increased activity of matrix metalloproteinase-2 and -9, was observed in the lungs of the group ventilated with a nonprotective strategy. This response was more consistently decreased by near-apneic ventilation than by a conventional protective ventilation.

Extracorporeal membrane oxygenation (ECMO) is increasingly being used to treat patients with severe acute respiratory distress syndrome (ARDS) and refractory hypoxemia (1). Along with improving oxygenation, ECMO enables more protective ventilation by decreasing the intensity of mechanical stimulus on lung tissue.

The concept of resting the lungs with the aid of extracorporeal lung support was first proposed by Gattinoni and colleagues (2) who applied in a noncontrolled series of ARDS patients a low-frequency ventilation consisting of respiratory rate (RR) less than 5 breaths/min (bpm), with a positive end-expiratory pressure (PEEP) level ranging from 15 to 25 cm H_2O, and peak inspiratory pressures less than 35–45 cm H_2O, combined with extracorporeal CO_2 removal. They observed a higher survival than expected (2). However, later Morris and colleagues (3) were unable to demonstrate an advantage of this strategy in a randomized controlled trial. Interestingly, these studies were performed decades ago, well before the development of current understanding of the mechanisms of ventilator-induced lung injury (VILI).

After the landmark studies that have defined the essentials of protective ventilation for ARDS (4–6), recommendations for lung rest during ECMO have also evolved targeting lower driving pressures, but still promoting low RRs. Such a strategy, based mainly on expert opinion, is recommended by the Extracorporeal Life Support Organization (7), and was applied in the CESAR trial to patients connected to ECMO (8). However, recent observational studies and surveys indicate a huge variability in the approach to mechanical ventilation during ECMO for ARDS, and although most physicians support the rationale of resting the lungs (9), most patients are ventilated with rather conventional ventilatory settings (9, 10). The need for better evidence in this issue has been recently highlighted (11, 12). This factor may be relevant concerning the impact of ECMO on ARDS outcomes. In severe ARDS the remaining aerated lung is usually very small and therefore, conventional protective ventilation with V_T of 6 ml/kg may still constitute an excessive mechanical load that can promote further lung injury and even an irreversible fibroproliferative response, counteracting the potential benefits of ECMO.

We hypothesized that in severe acute lung injury the use of near-apneic ventilation, consisting of very low levels of V_T, driving pressure, and RR, may prevent further damage by minimizing energy transfer to the lungs. The goal of this study was to determine whether a near-apneic ventilatory strategy decreases lung injury and early fibroproliferation, compared with a conventional protective ventilatory strategy, in a severe ARDS model supported with ECMO. Some of the results of this study have been previously reported in the form of an abstract (13).

Methods

Additional details are available in the online supplement. Domestic pigs (28.6 ± 0.4 kg) were treated following recommended guidelines (14). The Institutional Animal Ethics Committee approved the study (Protocol 12-029).

Interventions and Study Groups

Figure 1 summarizes study design.

1. Preparation: Anesthetic protocol, monitoring, and fluid therapy have been previously described (15). Initially, animals were ventilated using volume-controlled ventilation with V_T 10 ml/kg, RR 16–18 bpm, inspiratory/expiratory time ratio (I/E) 1:2, and PEEP 5 cm H_2O (baseline settings). $F_{I_{O_2}}$ was kept at 1.0 during the whole study. After baseline measurements animals were randomly allocated to sham ($n = 6$) or lung injury ($n = 18$).
2. Induction of lung injury: repeated lung lavages (30 ml/kg warm 0.9% saline solution intratracheally) were performed until $Pa_{O_2}/F_{I_{O_2}}$ was less than 250 mm Hg, followed by 2 hours of injurious ventilation (pressure-controlled ventilation with PEEP 0 cm H_2O, inspiratory pressure 40 cm H_2O, RR 10 bpm, and I/E 1:1). In parallel, a 23F catheter bicaval dual-lumen cannula (Avalon ELITE) was placed through the jugular vein as previously described (15). Thereafter, ventilation was returned to baseline settings for 10 minutes, time 0 (T_0) measurements were performed, and ECMO started targeting a blood flow greater than 60 ml/kg/min. Sweep gas flow ($F_{I_{O_2}}$ 1.0) was initially set 1:1 to blood flow and then titrated to keep Pa_{CO_2} at 40 ± 10 mm Hg. At T_0 animals with lung injury were randomly allocated to three groups ($n = 6$ per group): nonprotective, conventional protective, and near-apneic.
   Sham animals received neither lung injury nor ECMO. Instead, they underwent a 3-hour stabilization period before performing T_0 measurements.
3. Study period: After T_0 measurements animals underwent a 24-hour study period during which they were ventilated as follows:
   - Sham and nonprotective groups: volume-controlled ventilation with

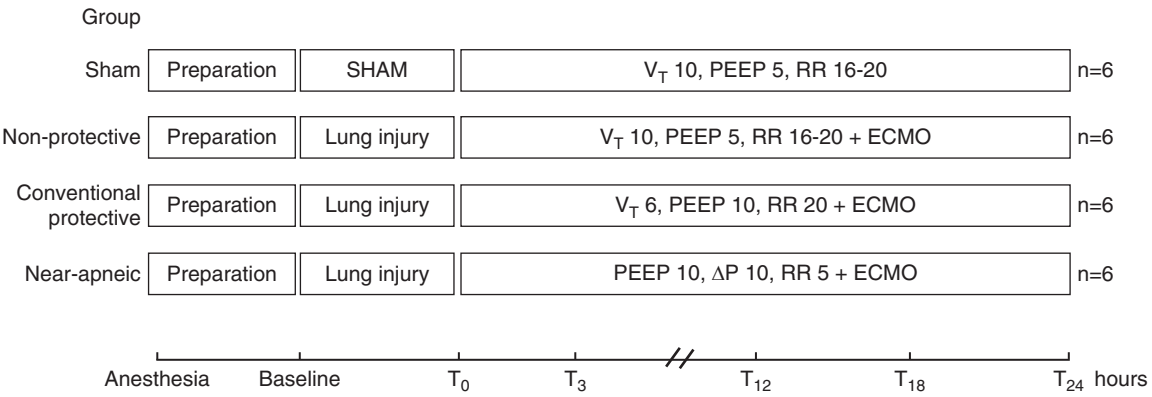

Figure 1. Study design and timeline. Preparation corresponds to anesthesia and invasive monitoring, which took 1–1.5 hours. Lung injury corresponds to the induction of lung injury by two hits: repeated saline lavages (1–1.5 h) followed by 2 hours of injurious ventilation. T_0 to T_{24} corresponds to the study period, during which each group received a specific ventilatory strategy. VT in ml/kg. ΔP = driving pressure in cm H_2O; ECMO = extracorporeal membrane oxygenation; PEEP = positive end-expiratory pressure in cm H_2O; RR = respiratory rate in breaths/min.

VT 10 ml/kg, PEEP 5 cm H_2O, RR as baseline, I/E 1:2

- Conventional protective group: volume-controlled ventilation with VT 6 ml/kg, PEEP 10 cm H_2O, RR 20 bpm, I/E 1:2
- Near-apneic group: pressure-controlled ventilation, PEEP 10 cm H_2O, driving pressure 10 cm H_2O, RR 5 bpm, I/E 1:1.

Data Recording and Tissue Analysis

Respiratory and hemodynamic data were registered at baseline, T_0, and at 3, 12, and 24 hours of the study period (T_3, T_{12}, and T_{24}). At T_{24}, animals were killed, and the lungs were extracted for histology and other tissue analysis.

Semiquantitative histologic scores ranging from 0 (normal) to 3 (severe alteration) were used to evaluate acute lung injury (hematoxylin and eosin), and the presence of α-SMA (α-smooth muscle actin) and procollagen-3 proteins (immunohistochemistry). Real-time PCR was used to measure α-SMA, collagens I and III, and TGF-β1 (transforming growth factor-β1) mRNA levels. MMP-2 (matrix metalloproteinase-2) and MMP-9 activities were measured using zymography. TGF-β1 protein levels were analyzed by ELISA.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7. Longitudinal data was analyzed using repeated measures two-way ANOVA, followed by Tukey multiple comparisons test (16). Single time point data were compared using one-way ANOVA. Linear regression analysis was also performed. Statistical significance was set at P less than 0.05, and data are expressed as mean ± SEM.

Results

Respiratory, Hemodynamic, and Biochemical Variables

Lung injury led to severe hypoxemia and decreased compliance, without differences between the three injured groups at T_0 (Table 1). Once connected to ECMO oxygenation improved rapidly to Pa_{O_2}/FI_{O_2} levels greater than 60 mm Hg as a result of extracorporeal gas exchange, but later on Pa_{O_2}/FI_{O_2} continued to increase throughout the study period reaching Pa_{O_2}/FI_{O_2} levels greater than 200 mm Hg at T_{24}, without differences among groups. In contrast, compliance remained low at T_{24} in the three groups with lung injury.

During the study period minute ventilation remained unchanged in the nonprotective group, but decreased 30–40% in the conventional protective group, and 10–20 times in the near-apneic group (Table 1). Despite these large differences in minute ventilation, Pa_{CO_2} and pH were not different among groups because of the compensatory modifications made in the sweep gas flow of the ECMO circuit. Other ECMO parameters were similar among groups (*see* Table E1 in the online supplement).

As a result of the different ventilatory strategies applied to the three groups with lung injury, large differences were observed in two determinants of VILI: driving pressures and mechanical power. Driving pressures remained between 21 and 24 cm H_2O in the nonprotective group, 14–15 cm H_2O in the conventional protective group, and 9–10 cm H_2O in the near-apneic group (Figure 2A). Likewise, mechanical power ranged from 11 to 13 J/min in the nonprotective group, 7–8 J/min in the conventional protective group, and 0.4–0.5 J/min in the near-apneic group, which represents a difference of 10- to 20-fold compared with the other groups (Figure 2B).

The three injured groups exhibited pulmonary hypertension at T_0, which improved during the study period. Because of hypotension not responding to fluid loading, noradrenaline infusion was required in the three groups with lung injury, with increasing doses throughout the study, and without differences among groups (Table 2).

Analysis of blood biochemical data revealed mild renal and liver dysfunction in the nonprotective group, as indicated by an increase in creatinine and aspartate transaminase plasma levels (*see* Table E2).

Markers of Acute Lung Injury and Early Fibroproliferative Response

Injured animals presented variable degrees of diffuse alveolar damage (*see* Figure E1). The severity of injury was lowest in the near-apneic group, as evidenced by less alveolar disruption, neutrophil infiltration, and hemorrhage (*see* Table E3), and a lower lung injury score than nonprotective and conventional protective groups (Figure 3). In terms of lung water content, all injured

Table 1. Respiratory Variables

Variable	Group			
	Sham	Nonprotective	Conventional Protective	Near-Apneic
Pa_{O_2}/FI_{O_2}, mm Hg				
Baseline	356 ± 83	353 ± 23	442 ± 23	384 ± 59
T_0	372 ± 24	78 ± 22*†	55 ± 10*†	56 ± 8*†
T_3	348 ± 20	148 ± 20*	168 ± 19*‡	101 ± 18*
T_{12}	388 ± 33	233 ± 34*‡	300 ± 26	237 ± 28*‡
T_{24}	375 ± 28	259 ± 25*‡	256 ± 35	300 ± 31*‡
Pa_{CO_2}, mm Hg				
Baseline	37 ± 2	42 ± 2	35 ± 2	42 ± 3
T_0	37 ± 2	36 ± 3	30 ± 2	38 ± 4
T_3	41 ± 1	35 ± 1	38 ± 3‡	45 ± 5
T_{12}	44 ± 3	34 ± 0	45 ± 2‡§	45 ± 4‡§
T_{24}	37 ± 3	36 ± 2	41 ± 3‡	45 ± 6
$P\bar{v}_{O_2}$, mm Hg				
T_0	n.d.	44 ± 6	28 ± 2	30 ± 1
T_{24}	n.d.	85 ± 8‡	92 ± 10‡	78 ± 4‡
Respiratory rate, breaths/min				
Baseline	18 ± 0	20 ± 1	20 ± 1	19 ± 1
T_0	18 ± 0	19 ± 1	18 ± 1	19 ± 1
T_3	18 ± 0	20 ± 0	19 ± 1	5 ± 0*‡§‖
T_{12}	19 ± 1	20 ± 0	20 ± 0‡	5 ± 0*‡§‖
T_{24}	20 ± 0	20 ± 0	20 ± 0‡	5 ± 0*‡§‖
V_T, ml/kg				
Baseline	10.1 ± 0.1	10.2 ± 0.1	9.8 ± 0.3	9.8 ± 0.4
T_0	10.1 ± 0.1	10.2 ± 0.1	9.8 ± 0.3	9.8 ± 0.4
T_3	10.4 ± 0.3	10.1 ± 0.4	5.9 ± 0.3*‡§	2.0 ± 0.2*‡§‖
T_{12}	10.2 ± 0.3	9.3 ± 0.4‡	5.9 ± 0.3*‡§	2.1 ± 0.1*‡§‖
T_{24}	10.1 ± 0.3	10.1 ± 0.1	6.0 ± 0.2*‡§	2.1 ± 0.2*‡§‖
Minute ventilation, L/min				
Baseline	5.2 ± 0.2	5.9 ± 0.3	5.4 ± 0.1	5.2 ± 0.3
T_0	5.4 ± 0.3	5.4 ± 0.2	5.1 ± 0.2	5.3 ± 0.3
T_3	5.4 ± 0.2	5.8 ± 0.2	3.2 ± 0.1*‡§	0.3 ± 0.0*‡§‖
T_{12}	5.4 ± 0.3	5.3 ± 0.2	3.3 ± 0.1*‡§	0.3 ± 0.0*‡§‖
T_{24}	5.5 ± 0.3	5.8 ± 0.1	3.4 ± 0.0*‡§	0.3 ± 0.0*‡§‖
Plateau pressure, cm H_2O				
Baseline	13 ± 0	14 ± 1	14 ± 0	14 ± 0
T_0	13 ± 0	22 ± 1*†	21 ± 1*†	24 ± 1*§
T_3	13 ± 1	29 ± 1*‡	25 ± 2*‡	20 ± 0*†‡‖
T_{12}	14 ± 1	28 ± 1*‡	25 ± 2*‡	20 ± 0*†‡‖
T_{24}	15 ± 1	26 ± 1*‡	24 ± 2*‡	19 ± 0*†‡‖
PEEP, cm H_2O				
Baseline	5 ± 0	5 ± 0	5 ± 0	5 ± 0
T_0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
T_3	5 ± 0	5 ± 0	10 ± 0*‡§	10 ± 0*‡§
T_{12}	5 ± 0	5 ± 0	10 ± 0*‡§	10 ± 0*‡§
T_{24}	5 ± 0	5 ± 0	10 ± 0*‡§	10 ± 0*‡§
RS static compliance, ml/cm H_2O				
Baseline	35 ± 2	35 ± 3	31 ± 2	32 ± 1
T_0	37 ± 2	17 ± 1*†	19 ± 2*†	15 ± 1*†
T_3	36 ± 5	13 ± 0.6*	12 ± 2*	7 ± 1*‡
T_{12}	35 ± 4	12 ± 0.4*	12 ± 1*‡	7 ± 1*‡
T_{24}	32 ± 4	15 ± 1*	13 ± 1*	7 ± 2*‡

Definition of abbreviations: n.d. = not determined; PEEP = positive end-expiratory pressure; RS = respiratory system; T = time in hours.
*$P < 0.05$ compared with sham.
†$P < 0.05$ for T_0 compared with baseline.
‡$P < 0.05$ compared with T_0.
§$P < 0.05$ compared with nonprotective.
‖$P < 0.05$ compared with conventional protective. All time points were compared with T_0.

groups had significantly higher wet–dry weight ratios compared with the sham group, but no differences were observed among them (Figure 4).

Immunohistochemistry staining for α-SMA was increased in the nonprotective and conventional protective groups compared with sham, but not in the near-apneic group (Figure 5). Procollagen III and TGF-β1 were increased only in the nonprotective group (*see* Figures E2 and E3). MMP-2 and -9 activities were

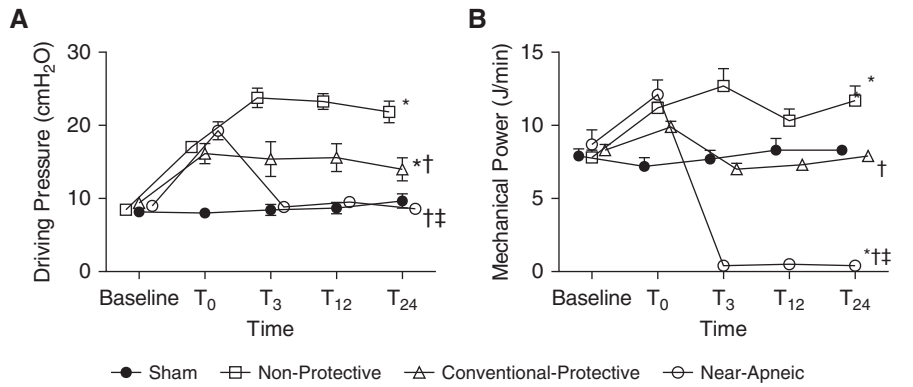

Figure 2. Determinants of ventilator-induced lung injury. (*A*) Driving pressure, calculated as plateau pressure – positive end-expiratory pressure, at different time points for each study group. (*B*) Mechanical power, calculated according to Gattinoni and colleagues (17), at different time points for each study group. $^{*}P < 0.05$ compared with sham from T_3 to T_{24}. $^{\dagger}P < 0.05$ compared with nonprotective from T_3 to T_{24}. $^{\ddagger}P < 0.05$ compared with conventional protective from T_3 to T_{24}. Only statistical differences between groups are marked.

increased in the three injured groups, but the near-apneic group had significantly lower levels of activity than nonprotective and conventional protective groups (Figure 6).

There was an increase in lung tissue mRNA expression of α-SMA (>10-fold) and collagen III (>1,000-fold) expression in all injured groups compared with sham, but not in collagen I, nor in TGF-β1. No differences among the three injured groups were observed (Table 3).

Interestingly, when considering the data of each injured animal individually, we found a positive correlation of histologic injury and myofibroblast and procollagen III scores with both driving pressure and mechanical power (*see* Figure E4).

Discussion

The main result of the present study is that in a model of severe ARDS supported with ECMO, 24 hours of nonprotective ventilation induces severe lung injury and an early fibroproliferative response, which is more consistently prevented by applying near-apneic ventilation than by just providing conventional protective ventilation.

We designed an experimental study in a model of acute lung injury in pigs supported with ECMO to compare near-apneic ventilation with a conventional protective

Table 2. Hemodynamic Variables

Variable	Group			
	Sham	Nonprotective	Conventional Protective	Near-Apneic
Heart rate, beats/min				
Baseline	95 ± 6	65 ± 9	71 ± 5	82 ± 5
T_0	79 ± 5	91 ± 7	74 ± 8	96 ± 6
T_3	68 ± 8	124 ± 5*†	130 ± 16*†	130 ± 11*†
T_{12}	98 ± 4	121 ± 5*†	124 ± 6*†	122 ± 7*†
T_{24}	97 ± 2	110 ± 5	111 ± 9†	120 ± 3†
MAP, mm Hg				
Baseline	100 ± 6	102 ± 7	85 ± 9	88 ± 5
T_0	102 ± 4	103 ± 8	76 ± 2*	87 ± 4
T_3	92 ± 5	78 ± 6†	75 ± 6	73 ± 3
T_{12}	83 ± 7	73 ± 3†	77 ± 5	69 ± 2
T_{24}	72 ± 4†	77 ± 6†	69 ± 4	67 ± 4
mPAP, mm Hg				
Baseline	21 ± 1	22 ± 1	18 ± 1	18 ± 1
T_0	21 ± 1‡	39 ± 1*‡	27 ± 2*‡§	41 ± 4*‡‖
T_3	20 ± 1	28 ± 1*†	26 ± 1*	36 ± 2*†
T_{12}	21 ± 2	19 ± 0†	24 ± 1	24 ± 2†
T_{24}	19 ± 2	16 ± 1†	19 ± 2†	20 ± 2†
Norepinephrine dose, μg/kg/min				
Baseline	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
T_0	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.05 ± 0.03
T_3	0.00 ± 0.00	0.12 ± 0.01*†	0.12 ± 0.04*†	0.10 ± 0.03*
T_{12}	0.00 ± 0.00	0.13 ± 0.01*†	0.16 ± 0.03*†	0.15 ± 0.03*†
T_{24}	0.00 ± 0.00	0.24 ± 0.02*†	0.22 ± 0.02*†	0.31 ± 0.04*†‖
Cumulative fluids, L				
T_{24}	1.6 ± 1.4	3.0 ± 0.3*	3.0 ± 0.4*	3.0 ± 0.6*

Definition of abbreviations: MAP = mean systemic arterial pressure; mPAP = mean pulmonary artery pressure; T = time in hours.
$^{*}P < 0.05$ compared with sham.
$^{\dagger}P < 0.05$ compared with T_0.
$^{\ddagger}P < 0.05$ for T_0 compared with baseline.
$^{\S}P < 0.05$ compared with nonprotective.
$^{\|}P < 0.05$ compared with conventional protective. All time points were compared with T_0.

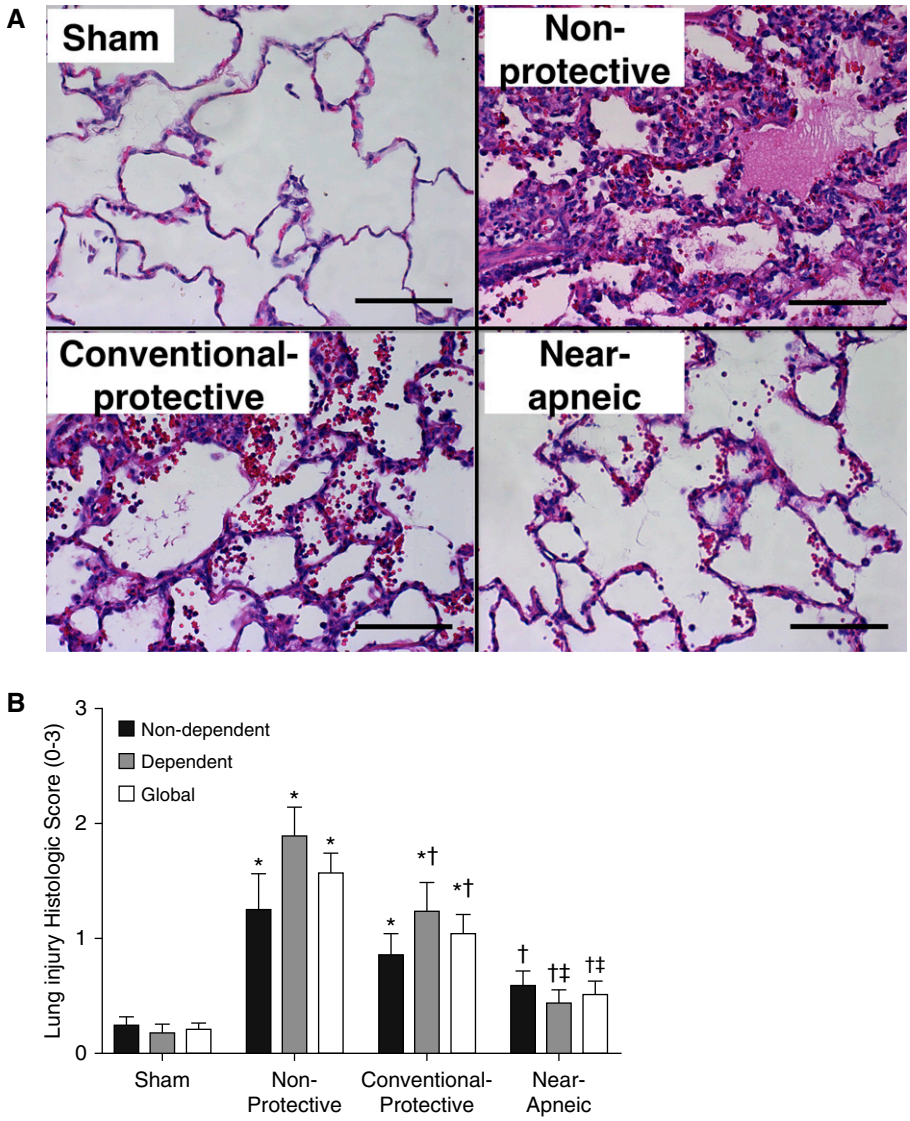

Figure 3. Histologic assessment of lung injury. (*A*) Representative images of lung histology for each study group (hematoxylin and eosin, scale bar = 100 μm). Images from nonprotective and conventional protective groups presented diffuse alveolar damage with alveolar edema, hemorrhage, hyaline membranes, and inflammatory cells in the interstitium and alveolar spaces. (*B*) Quantitative score for lung injury (from 0 = normal to 3 = maximal alteration), calculated by averaging the scores for alveolar disruption, neutrophil infiltration, and hemorrhage, for dependent and nondependent areas of the right lung, and the global score (mean of scores for dependent and nondependent areas). $^{*}P < 0.05$ compared with sham. $^{\dagger}P < 0.05$ for differences with nonprotective. $^{\ddagger}P < 0.05$ for differences with conventional protective.

ventilatory strategy, in its ability to modulate lung injury. In addition, we included a group ventilated with a nonprotective ventilatory strategy, as a positive control to confirm whether the model was sensitive to the influence of the ventilatory strategy. The design was characterized by high severity of lung injury, to reproduce the clinical context in which ECMO is applied in ARDS patients; a high flow venovenous ECMO, to allow for effective lung rest; and a prolonged timeframe, to provide enough time for differences to manifest. In a previous report we described how this ARDS model is highly lethal without ECMO because of severe hypoxemia, particularly in the first hours (15).

Ventilatory Strategies and Acute Lung Injury

Near-apneic ventilation significantly decreased histologic lung injury compared with both the nonprotective and the conventional protective ventilatory strategies. The three ventilatory strategies applied were associated to marked differences in driving pressures, but also in mechanical power. When looking at individual data, driving pressure and mechanical power were positively correlated with lung injury scores. These two variables have been proposed as predictors of VILI (5, 17). Although driving pressure reflects the relation between applied V_T and compliance, mechanical power is a relatively new concept in VILI, aimed at unifying different ventilator parameters into one single, energy input concept, which is influenced not only by V_T, driving pressure and PEEP, but also by flow and RR. Compared with nonprotective and conventional protective ventilatory strategies, near-apneic ventilation decreased driving pressure by 60% and 40%, respectively. However, the reduction in terms of mechanical power was around 10 times. In the study by Cressoni and colleagues (18) a 12 J/min threshold was established as enough energy to induce whole-lung edema in pigs. However, those pigs initially had normal lungs. The fact that in our study there was a positive correlation between mechanical power and lung injury, and that animals in the conventional protective group showed significantly more injury (despite having a mean mechanical power of 7.6 J/min) than those in the near-apneic group, suggests that mechanical power may be an important factor in the progression of lung damage during severe ARDS and that safe thresholds may depend on the baseline status of the affected lungs.

Interestingly, although the model was characterized by a marked increase in wet–dry weight ratio, this variable was not modulated by the ventilator strategy applied. We speculate that resolution of lung edema may require a longer time frame. Tagami and colleagues (19) showed that decreases in extravascular lung water in ARDS patients that survived were only evident after 48 hours of evolution.

The very low RR contributed significantly to the marked decrease in mechanical power observed in the near-apneic group. Previous experimental studies have shown that decreasing RR may prevent VILI (20, 21). Furthermore, lung rest strategies proposed for ARDS patients on ECMO consistently include RRs of

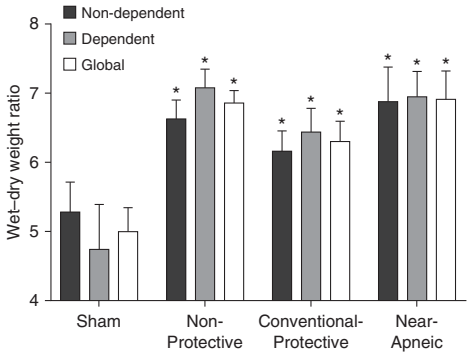

Figure 4. Wet–dry weight ratio of dependent and nondependent areas of the left lung. Global columns correspond to the average of the dependent and nondependent areas. All injured groups showed a significant increase in their lung water content compared with sham but no differences were detected among them. *$P < 0.05$ compared with sham.

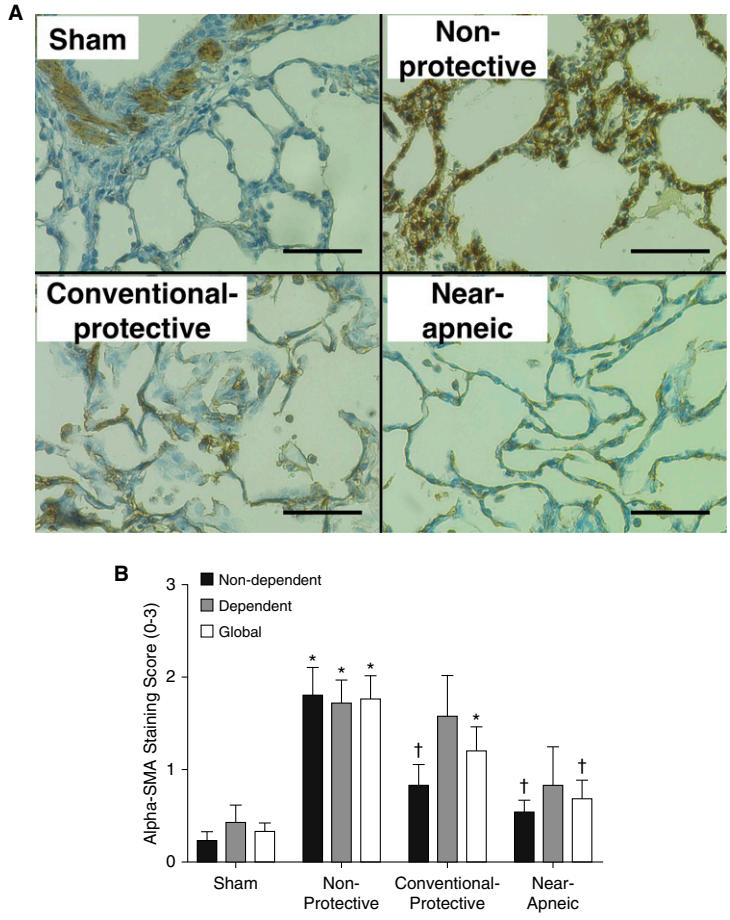

Figure 5. Immunohistochemical staining of α-SMA (α-smooth muscle actin) protein as a surrogate for myofibroblasts in lung tissue preparations. (*A*) Representative images for each study group. Brown staining shows a positive reaction for α-SMA (scale bar = 50 μm). In the image corresponding to a sham animal, α-SMA staining is limited to the bronchial wall, which usually has a smooth muscle layer. In contrast, the animal from the nonprotective group has extensive staining on its alveolar walls, whereas the animal from the conventional protective group exhibits a moderate staining. (*B*) Quantitative score for myofibroblast staining (from 0 = no staining to 3 = maximal staining) for dependent and nondependent areas of the right lung, and the global score (mean of scores for dependent and nondependent areas). Staining in bronchial and vascular walls was not considered for scoring. *$P < 0.05$ compared with sham. †$P < 0.05$ for differences with nonprotective.

5–10 bpm (7, 8). Accordingly, we decided to set RR at 5 bpm in the near-apneic group. In contrast, more conservative approaches to mechanical ventilation during ECMO usually apply RRs rather similar to those applied during conventional protective ventilation for ARDS. Schmidt and colleagues (10) reported that RRs applied the first day after connection to ECMO in 168 ARDS patients ranged from 10 to 25 bpm. Similarly, in the recently published EOLIA trial, after connection to ECMO patients were ventilated with a mean RR of 23 bpm (22). Therefore, to reflect this strategy we set RR at 20 bpm in the conventional protective group. However, from the present study we cannot define the relative contribution of decreasing RR versus decreasing V_T, in the benefits observed in the near-apneic group.

In a study using a post-pneumonectomy ARDS model in pigs, Iglesias and colleagues (23) showed that near-apneic ventilation associated to extracorporeal CO_2 removal decreased lung injury, compared with a conventional protective ventilatory strategy. In that model standard protective ventilation with V_T 6 ml/kg was associated to driving pressures greater than 20 cm H_2O, which decreased to less than 5 cm H_2O in the near-apneic strategy (23). In a randomized controlled trial in 79 patients with moderate to severe ARDS, Bein and colleagues (24) studied the effect of decreasing V_T to 3 ml/kg with the aid of extracorporeal CO_2 removal, compared with conventional treatment with V_T of 6 ml/kg. In the treated arm driving pressure could be decreased from 13 to 8 cm H_2O, and there was a decrease in serum IL-6 concentrations in plasma, but there were no differences in any clinical outcome. In this study patients ventilated with conventional treatment were not exposed to high driving pressures, which may explain the negative result of the trial. In fact, a *post hoc* analysis showed that in the subgroup of patients with more severe ARDS there was a significant increase in ventilator-free days in the treated group (24). Unfortunately, mechanical power calculations cannot be extracted from the available data of these studies. Nevertheless, our data suggest that the rationale for setting mechanical ventilation during ECMO should consider mechanical predictors of VILI, such as driving pressure and mechanical power.

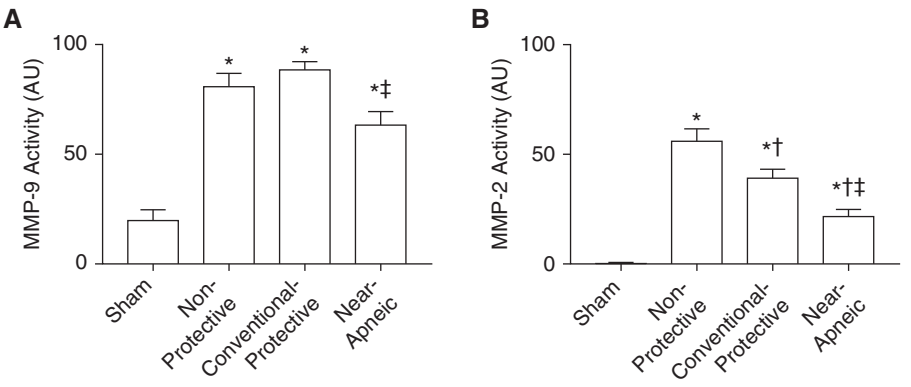

Figure 6. MMP-9 (matrix metalloproteinase-9) and MMP-2 activities in lung tissue. Quantitative analysis of gelatin zymography performed in homogenates of the left lung is shown. Data are expressed in arbitrary units of the 90-kD band, corresponding to the activity of MMP-9 (left), and of the sum of the 68- and 72-kD bands, corresponding to the activity of MMP-2 (right). *$P < 0.05$ compared with sham. †$P < 0.05$ for differences with nonprotective. ‡$P < 0.05$ for differences with conventional protective. AU = arbitrary units.

A recent observational study in patients with severe ARDS supported by ECMO showed that maintaining low driving pressures during the first days is associated with lower mortality (25).

Early Fibroproliferative Response

In our 24-hour model we found consistent evidence of an early fibroproliferative response, as indicated by the strong presence of myofibroblasts and procollagen III in alveolar walls, increased concentrations of TGF-β1 in lung tissue, increased mRNA expression of collagen III and α-SMA, and increased activity of MMP-2 and -9. Moreover, these markers of fibroproliferation were modulated by the ventilatory strategy applied, with near-apneic ventilation exhibiting the least alterations. Other studies had previously observed evidence of early fibroproliferation in acute lung injury models (26).

Several authors have implicated VILI in the etiology of ARDS-associated fibrosis (27, 28). Although the incidence of fibrosis seems to have dropped with the implementation of protective ventilation (29), recent studies suggest that it still represents a potential and serious complication of ARDS (30). Bhattacharya and Matthay (27) suggested that ECMO support might be helpful to rest the lungs to facilitate lung repair by using low to very low V_T. Physiologic lung repair is a delicate process that can turn into pathologic and lead to irreversible fibrosis in ARDS patients by different stimuli (28). Pathologic extracellular matrix formation, including inhibition of myofibroblast apoptosis and increased synthesis of procollagen 3, have been described in the presence of abnormally high mechanotransduction (31), which in the face of highly inhomogenous ARDS lungs, may occur even when using conventional low V_T ventilation. In fact, although in our study the staining for myofibroblasts and procollagen 3 was particularly high in the nonprotective group, we observed a positive and significant correlation of myofibroblast and procollagen 3 scores with both driving pressure and mechanical power. MMPs have also been shown to be involved in the pathogenesis of ARDS and VILI (32, 33), and near-apneic ventilation decreased MMP activity compared with the other strategies. These observations suggest that decreasing strain and energy applied to lungs of ARDS patients, by combining ECMO with near-apneic ventilation, may help prevent a fibroproliferative phenotype.

It is important to acknowledge that the early fibroproliferative response observed could represent either a normal repair process after lung injury, or an abnormal fibroproliferation that ultimately ends in fibrosis. Future studies evaluating the long-term impact of this early fibroproliferative response are warranted. Persistence of excessive mechanotransduction is key in perpetuating pathologic fibroproliferation (31), which may explain the lower fibroproliferation observed in the near-apneic group.

Table 3. Expression of Genes Involved in Fibroproliferation

Variable	Group		
	Nonprotective	Conventional Protective	Near-Apneic
Procollagen I	1.07 ± 0.46	1.08 ± 0.39	0.99 ± 0.59
Procollagen III	1,936 ± 0.83*	1,651 ± 0.70*	1,838 ± 0.6*
α-SMA	10.40 ± 0.60*	11.85 ± 0.27*	14.13 ± 0.38*
TGF-β1	0.33 ± 0.66	0.34 ± 0.36	0.37 ± 0.58

Definition of abbreviations: SMA = smooth muscle actin; TGF = transforming growth factor.
The $2^{-\Delta\Delta CT}$ values are shown as an estimate of the relative fold expression (in relation to sham) of the mRNA in tissue homogenates obtained from the left lung.
*$P < 0.05$ compared with sham. No differences were observed among nonprotective, conventional protective, and near-apneic groups.

Limitations

Our study has several limitations. The model applied reproduces the main clinical features of ARDS, but may differ from human ARDS in several pathophysiologic aspects. Another limitation is that we used an $F_{I_{O_2}}$ of 1.0 throughout the study. Although this deviates from clinical practice and may contribute to lung injury, in pilot experiments we realized that many animals were unable to keep Pa_{O_2} greater than 60 mm Hg, despite maximal ECMO support, unless a high $F_{I_{O_2}}$ was applied for mechanical ventilation. Pigs have a higher metabolism than humans and this may explain why extracorporeal O_2 transfer alone was insufficient to match metabolic O_2 consumption. In addition, we did not want to modify $F_{I_{O_2}}$ throughout the study to avoid new covariates that could influence final results. But of course, an $F_{I_{O_2}}$ of 1.0 should not be part of a lung rest strategy during ECMO in patients. Finally, because the different strategies applied differed in

several ventilatory parameters, we cannot define the relative contribution of each parameter to the results observed.

Clinical Implications

The results of this study highlight the relevance of optimizing mechanical ventilation in ARDS patients connected to ECMO. In the most severe cases, such as those patients with very low compliance, such optimization may require decreasing the intensity of mechanical ventilation well beyond conventional ventilatory settings, to prevent further lung injury. However, caution is needed in extrapolating these results to clinical practice, because this approach may conflict with other relevant goals, such as decreasing sedatives or allowing spontaneous breathing efforts. Therefore, controlled clinical studies are required to determine the impact of near-apneic ventilation on clinically relevant outcomes.

Conclusions

In an experimental model of severe ARDS supported with ECMO, near-apneic ventilation induced less histologic lung injury and MMP activity than nonprotective and conventional protective ventilatory strategies. In addition, near-apneic ventilation prevented the expression of myofibroblast markers, which was observed in the groups ventilated with nonprotective and conventional protective strategies. ■

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

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