

Effect of Positive End-Expiratory Pressure After Porcine Unilateral Left Lung Transplant

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Abstract

Objectives: To evaluate the effects of 2 different levels of positive end-expiratory pressure on pigs who had unilateral lung transplants.

Materials and Methods: A left lung transplant was performed in 12 pigs. The animals were randomized into 2 groups based on positive end-expiratory pressure: group 1 (5 cm H₂O) and group 2 (10 cm H₂O). Hemodynamics, gas exchange, and respiratory mechanics were measured before and after surgery. Cytokines, oxidative stress, and histologic scores were assessed in the lung tissue of each pig.

Results: Pigs in group 2 exhibited a significantly higher mean heart rate ($P = .006$), static compliance ($P = .001$), lower mean arterial pressure ($P = .003$), and airway resistance ($P = .001$) than did pigs in group 1. There were no postoperative differences between the groups in concentrations of thiobarbituric acid reactive substances, superoxide dismutase, and interleukin 8. At the end of the observation period, pigs in group 2 had higher

levels of thiobarbituric acid reactive substances ($P = .001$) and interleukin 8 ($P = .05$), and pigs in group 1 had higher levels of superoxide dismutase ($P = .05$) than they did at baseline.

Conclusions: After unilateral lung transplant, higher positive end-expiratory pressure was associated with improved respiratory mechanics, a negative effect on hemodynamics, a stronger inflammatory response, and increased production of reactive oxygen species, but no effect on gas exchange.

Key words: Mechanical ventilation, Pulmonary, Hemodynamic, Inflammation

Introduction

Lung transplant is a life-saving procedure for patients with end-stage lung disease. However, approximately 15% to 25% transplanted patients may have severe postoperative complications related to the graft.¹ These events are the consequence of primary graft dysfunction, which is the most important cause of early morbidity and mortality after lung transplant.¹ The syndrome of severe hypoxemia, lung edema, and diffuse pulmonary radiographic opacities may result from acute lung rejection, infection, or ischemia-reperfusion injury.² Survivors of primary graft dysfunction may require prolonged mechanical ventilatory support and longer hospital stay, and they may have lower lung function.³

Mechanical ventilation may increase the extent of damage to transplanted lungs, worsen pre-existing lung injury, and trigger a release of proinflammatory cytokines.⁴⁻⁷ Therefore, to reduce the potential adverse effects of mechanical ventilation, protective

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strategies have been proposed that limit tidal volume, plateau pressure, and positive airway pressure.^{8,9} Positive end-expiratory pressure (PEEP) is commonly used in the intensive care unit in critically ill patients and allows for alveolar recruitment by preventing end-expiratory bronchiolar collapse, reducing pulmonary edema, and improving gas exchange. However, increased PEEP levels may excessively distend noninjured lung areas and decrease cardiac output.¹⁰

Most studies of mechanical ventilation have focused on patients with acute respiratory distress syndrome and animal models of lung injury. Higher PEEP levels are not suitable for all patients with lung injury, and the optimal PEEP level must be determined individually.⁹ Primary graft dysfunction caused by ischemia-reperfusion injury has similar clinical and pathophysiologic features as acute respiratory distress syndrome, and ventilatory strategies that are effective in patients with acute respiratory distress syndrome might be applicable to critically ill patients after lung transplant.¹ However, this issue is controversial, and there are few studies about early postoperative mechanical ventilation and its potential effect on lung graft performance.⁹

We hypothesized that higher PEEP levels (10 cm H₂O) could improve postoperative gas oxygenation and lung graft performance after lung transplant. We studied the effects of 2 levels of PEEP on lung grafts with ischemia-reperfusion injury using a unilateral lung transplant animal model.

Materials and Methods

Pigs (approximate weight, 30 kg; large white breed) were obtained and used as organ donors (n=12 pigs) and recipients (n=12 pigs). The local Institutional Animal Care Committee and the Ethical and Research Committee approved the protocols used in this study. All animals received humane care in compliance with guidelines published by the United States National Institutes of Health.¹¹

Donors

All pigs had general anesthesia induced with ketamine 10% (15 mg/kg IM) and midazolam (0.8 mg/kg IM) followed by propofol (2 to 4 mg/kg IV) and fentanyl citrate (2-5 µg/kg IV). After orotracheal intubation, mechanical ventilation was started with 100% oxygen and a pressure-controlled

ventilatory mode (Nikkei, Takaoka, Brazil). The parameters were adjusted to maintain tidal volume 8 mL/kg, respiratory rate 20 breaths/minute, inspiratory:expiratory ratio 1:2, and PEEP 5 cm H₂O. Anesthesia was maintained by the continuous infusion of fentanyl citrate (50 µg/kg/h) and propofol (11-15 mg/kg/h). Muscle relaxation was achieved with atracurium (0.4 mg/kg/h).

Donor lung recovery was performed as previously described.¹² Briefly, after a median sternotomy, the great vessels were dissected and the animal was heparinized (5 mg/kg; 300 IU/kg IV) via the pulmonary artery. A perfusion cannula was placed into the left atrium, and the aorta and vena cava were occluded. Retrograde perfusion was performed with low potassium dextran solution (50 mL/kg) at 4°C, with maximum pressure of 35 mm Hg. Ventilation was continued throughout the perfusion period. Neither vasodilators nor vasoactive drugs were used. The heart-lung block was excised with both lungs inflated in an end-inspiratory state and stored at 4°C for 16 hours.

Recipients

The same anesthetic and ventilatory protocol for donor pigs was used in recipient animals, except that recipients received inhaled isoflurane (1.0% to 1.5%) instead of propofol for anesthesia maintenance. After intubation, a carotid catheter was inserted to measure mean arterial pressure (MAP). A Swan-Ganz catheter (Edwards, Irvine, CA, USA) was positioned in the main pulmonary artery via the external jugular vein for pulmonary artery pressure (PAP) measurement. Intermittent bladder catheterization was performed for urinary output. After intraoperative stabilization for 15 minutes, baseline measurements (defined as measurements before lung excision and transplant) were performed for arterial blood sampling (PaO₂ and PaCO₂), hemodynamic parameters (heart rate, MAP, and PAP), and respiratory measurements (static compliance and airway resistance).

A left pneumonectomy was performed as previously described.⁸ The heart-lung block was dissected, a biopsy of the right upper lobe was done (initial time), and the left donor lung was implanted as previously described.¹² After the pulmonary artery clamp was released, the graft was ventilated with the same settings used for the baseline measurements. A left upper lobe lung biopsy was

performed before chest wall closure (after transplant time).

The recipients were randomized after stabilization using the following settings: mechanical ventilation with PEEP 5 cm H₂O (group 1; n=6 pigs) and 10 cm H₂O (group 2; n=6 pigs), keeping all other ventilatory parameters stable. Pigs in both groups were maintained under anesthesia for 210 minutes using the same anesthetic technique, and vasoactive drugs were used when necessary. The respiratory rate was adjusted to maintain PaCO₂ < 55 mm Hg. Data for gas exchange, respiratory mechanics, and hemodynamics were obtained after transplant and 120 and 210 minutes after completion of the procedure.

After observation (240 minutes), the sternum was opened, biopsies of the left lung were obtained, and the animals were killed by exsanguination. The lung biopsy specimens were divided into 2 groups for storage in either liquid nitrogen at -80°C or 10% formalin.

Histologic analyses

Specimens were fixed in 10% formalin and embedded in paraffin. Sections (thickness, 4 μm) were stained with hematoxylin-eosin and labeled with a numeric code. A pathologist who was blinded to both the experimental protocol and the region of sampling performed quantitative analysis using a light microscope. Each sample was examined under both low- and high-power fields. At least 4 sections were obtained from each block, and 20 fields were randomly selected and analyzed from each section.¹³ The severity of lesions was assessed with using a histology score that was based on 6 parameters: intra-alveolar edema, hyaline membrane formation, hemorrhage, recruitment of granulocytes into the air spaces, focal alveolar collapse or consolidation, and epithelial desquamation or necrosis of the airways or alveoli. Each parameter was evaluated semi-quantitatively (0, absent; 1, mild; 2, moderate; 3, prominent). The percentage of the involved area of each histologic specimen was estimated (0% to 100%) to quantify the histologic changes.

Assays

Concentration of thiobarbituric acid reactive substances (TBARS) was determined with a spectrophotometric method. The tissue samples were placed in assay tubes and added to trichloroacetic

acid (10%; 0.75 mL), lung homogenate (0.25 mL), thiobarbituric acid (0.67%; 0.5 mL), and distilled water (25 mL). Each tube was agitated, heated to 100°C, and cooled in ice, and then n-butyl alcohol (1.5 mL) was added. Subsequently, each tube was agitated in a vortex apparatus (Biomatic, Porto Alegre, RS, Brazil) for 45 seconds and centrifuged for 10 minutes at 3000 rpm (1110g). The stained tissue was removed and read in a spectrophotometer (CARY 3E UV-Visible Spectrophotometer; Varian, Palo Alto, CA) at 535 nm. The concentration of TBARS was expressed as nmol/mg protein.¹⁴

Superoxide dismutase (SOD) activity level was determined using a pulse radiolytic method that was based on the autoxidation of epinephrine, as previously described.¹⁵

Interleukin 8 (IL-8) analysis was done from lung tissue. After samples were thawed, a microplaque of 96 holes was sensitized with IL-8 monoclonal antibody. These plaques were then treated with homogenized lung (100 μL, dilution 1:2), positive and negative controls 100 μL and recombinant IL-8 100 μL at a concentration determined by the standard curve established by the manufacturer (Creative Biomart, New York, NY). Then, polyclonal conjugated anti-IL-8 marked with peroxidase (100 μL was added to the plaques for 3-hour incubation at room temperature). The plaque was washed 4 times with detergent solution, and hydrogen peroxide (0.02%) and tetramethylbenzene (2%) were added. The reaction was interrupted after 30 minutes using sulfuric acid (1 M). Color intensity was measured as optic density in an automatic enzyme-linked immunosorbent assay reader (Titertek Multiskan, Stockholm, Sweden) at 450 nm. The IL-8 concentrations in the homogenized lung were calculated using a standard curve.

Statistical analyses

The data from the experiments were coded, recorded, and analyzed using statistical software (SPSS: An IBM Company, version 10.0.1, IBM Corporation, Armonk, New York, USA). Differences between groups were compared with an analysis of variance for parametric data and Kruskal-Wallis test for nonparametric data. When the analysis of variance revealed a significant difference, the post hoc multiple comparisons procedure (Tukey test) was used to evaluate the differences between groups. Comparison of the histologic scores was done with

t test. The general linear models repeated measures test was used to compare differences between groups of related dependent variables for which more than 1 measurement was obtained throughout the observation period. In each test, the data were expressed as the means \pm standard error. Statistical significance was defined by $P < .05$.

Results

Ten pigs (83%) that had a lung transplant (5 pigs from group 1 and 5 pigs from group 2) survived the observation period. The cause of death in 2 pigs was hypotension that was refractory to vasopressor drugs. There were no significant differences between the 2 groups in body weight, total ischemic time, anastomosis time, total dose of anesthetic or vasoactive drugs, volume of electrolyte solutions administered, or urinary output (data not shown). Ephedrine was used only in group 2 because of persistent hypotension after transplant.

Mean heart rate was significantly higher in group 2 than it was in group 1 during the observation

period ($P = .001$), but group 1 had higher mean MAP than did group 2 throughout the experiments ($P = .03$) (Table 1). There were no significant differences between the groups regarding mean PAP, PaO₂, and PaCO₂. Mean static compliance was significantly higher ($P = .02$) and airway resistance was significantly lower in group 2 than it was in group 1 ($P = .006$) (Table 1).

There were no differences between the 2 groups in mean concentration of TBARS, SOD, or IL-8 during the observation period (Table 2). However, compared with baseline, there was a significant increase in TBARS in group 2 upon reperfusion immediately after transplant ($P < .001$) and at the end of the observation period ($P < .05$). In group 1, SOD concentration was significantly higher immediately after transplant and at the end of the observation period than it was at baseline ($P < .05$). In group 2, the IL-8 concentration was significantly higher at the end of the observation period than it was initially after transplant ($P < .05$).

At the end of the observation period, both groups had histologic changes characteristic of acute lung

Table 1. Effect of Positive End-Expiratory Pressure on Hemodynamics, Gas Exchange, and Respiratory Mechanics After Left Lung Transplant in Pigs*

Variable	Baseline [†]			0 Minutes			120 Minutes			After Transplant		
	5	10	$P \leq \ddagger$	5	10	$P \leq \ddagger$	5	10	$P \leq \ddagger$	5	10	$P \leq \ddagger$
Hemodynamics												
PEEP (cm H ₂ O)	5	10		5	10		5	10		5	10	
HR (beats/min)	89 \pm 9	90 \pm 9	NS	129 \pm 10	146 \pm 12	.04	148 \pm 12	195 \pm 12	.0001	144 \pm 10	196 \pm 13	.001
MAP (mm Hg)	99 \pm 20	82 \pm 21	NS	90 \pm 14	70 \pm 18	.04	71 \pm 18	70 \pm 20	NS	68 \pm 17	54 \pm 17	NS
Gas exchange												
PaO ₂ (cm H ₂ O)	266 \pm 32	333 \pm 31	.004	230 \pm 29	188 \pm 20	.02	212 \pm 25	241 \pm 21	NS	199 \pm 19	233 \pm 19	.01
PaCO ₂ (cm H ₂ O)	41 \pm 10	34 \pm 6	NS	52 \pm 9	33 \pm 9	.003	52 \pm 9	54 \pm 11	NS	54 \pm 11	50 \pm 11	NS
Respiratory mechanics												
Cst (cm H ₂ O.l ⁻¹ .s)	24 \pm 6	31 \pm 6	NS	18 \pm 4	23 \pm 2	.03	17 \pm 2	24 \pm 2	.002	17 \pm 3	24 \pm 6	.03
Rest (cm H ₂ O.l ⁻¹ .s)	8.6 \pm 0.8	7 \pm 2	NS	11 \pm 2	8.1 \pm 0.9	.009	11 \pm 1	7 \pm 1	.006	11 \pm 3	8 \pm 1	NS

Abbreviations: Cst, static compliance; HR, heart rate; MAP, mean arterial pressure; PaCO₂, partial pressure of arterial carbon dioxide; PaO₂, partial pressure of arterial oxygen; PAP, pulmonary artery pressure; PEEP, positive end-expiratory pressure; Rest, airway resistance

*Data reported as means \pm SE

[†]Baseline: intraoperative, before pneumonectomy

[‡]NS, not significant ($P > .05$)

Table 2. Effect of Positive End-Expiratory Pressure on Inflammatory and Histologic Assays After Left Lung Transplant in Pigs*

Variable	Baseline [†]			After Transplant [†]			After Observation Period [†]		
	5	10	$P \leq \ddagger$	5	10	$P \leq \ddagger$	5	10	$P \leq \ddagger$
PEEP (cm H ₂ O)	5	10		5	10		5	10	
TBARS assay (nmol/mg protein)	0.146 \pm 0.04	0.053 \pm 0.01	.03	0.509 \pm 0.14	0.235 \pm 0.10	.003	0.544 \pm 0.15	0.421 \pm 0.06	.09
SOD (U/mg protein)	6.81 \pm 0.42	7.88 \pm 0.79	.01	9.70 \pm 0.36	8.83 \pm 0.52	.007	9.07 \pm 0.45	8.83 \pm 0.91	.57
IL-8 (pg/L)	1750.8 \pm 985.8	1312.8 \pm 264.7	.31	2142.9 \pm 402.9	2172.1 \pm 565.4	.92	2428.2 \pm 653.4	3001.1 \pm 667.4	.16
HIS	2 \pm 1.2	2.4 \pm 1.1	.56	2.6 \pm 0.5	3.2 \pm 1.1	.25	5 \pm 2.9	4.6 \pm 1.8	.77

Abbreviations: HIS, histology score; IL-8; interleukin 8; PEEP, positive end-expiratory pressure; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances

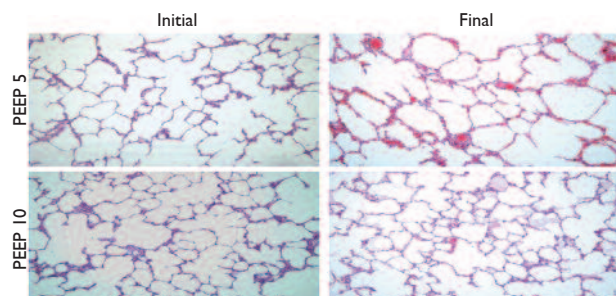
*Data reported as means \pm SE

[†]Baseline: intraoperative, before pneumonectomy; After Transplant, before closing the chest; After Observation Period, 210 minutes after lung transplant

[‡]NS, not significant ($P > .05$)

injury, but the difference between groups was not significant (Table 2 and Figure 1).

Figure 1. Histologic Appearance of Lung Before and After Left Lung Transplant in Pigs With Different Levels of Positive End-Expiratory Pressure (PEEP)



PEEP 5 = 5 cm H₂O; PEEP 10 = 10 cm H₂O
Hematoxylin-eosin, original magnification ×200

Discussion

There have been few studies involving mechanical ventilation in experimental models of lung transplant that tried to improve graft performance using protective ventilatory strategies or different ventilatory modes.^{8,16} In the present study, we used 2 different levels of PEEP after unilateral lung transplant in pigs and demonstrated that mechanical ventilation with higher PEEP levels (10 cm H₂O) using a pressure-controlled mode increased lung compliance and reduced airway resistance (Table 1).

Higher PEEP was not associated with better oxygenation in pigs after unilateral lung transplant (Table 1). This observation is in contrast with studies that showed that higher PEEP levels were associated with better gas exchange and survival in patients with acute respiratory distress syndrome.^{9,10}

The higher heart rate observed in group 2 animals (PEEP, 10 cm H₂O) may have been caused by the high doses of vasopressor drugs (ephedrine) used in these animals because of persistent hypotension after transplant. The higher PEEP levels may have affected hemodynamics and caused increased intrathoracic pressure and decreased cardiac output, as described by others.^{17,18}

The present ventilatory findings were consistent with those from previous studies that evaluated the effects of increasing levels of PEEP at constant tidal volume, which resulted in higher compliance and lower resistance.¹⁶ Experimental and clinical studies have shown that protective ventilatory strategies and

open-lung strategies may affect the progression of lung injury.¹⁹⁻²²

The present histologic findings included severe acute lung injury in both groups after transplant and at the end of the observation period. This suggests that these lungs were less suitable to alveolar recruitment because of severe acute lung injury associated with the long cold ischemic period.

The increase in TBARS concentration in both groups after transplant suggested that there was activation of free radical oxygen species that can be deleterious to lung performance. These free radicals may have been a result of the ischemia-reperfusion injury itself, with no relation to the change in PEEP levels. However, the increase in TBARS concentration in group 2 throughout the observation period compared with baseline suggested a deleterious effect associated with higher levels of PEEP. Although we did not observe any difference in SOD expression after transplant in either group, the group with lower PEEP level (5 cm H₂O) showed a significant increase in SOD activity after transplant compared with baseline.

There were no differences in the measured inflammatory response between the 2 groups after transplant. Nevertheless, the higher concentration of IL-8 in group 2 corroborated previous findings that there may be a small but consistent increase in the inflammatory response at higher PEEP levels, which may aggravate lung function and potentiate alveolar injury.¹² High IL-8 levels in transplanted lungs may be associated with clinical impairment after lung transplant and a higher incidence of early severe ischemia-reperfusion injury.²³⁻²⁵

The present model of lung transplant produced severe lung injury, which could be associated with the long cold ischemic time. This limitation led us to use a short observation period. The severity of injury may have obscured differences between pigs having different PEEP levels. A shorter cold ischemic time may have produced less injury and potentially may have better demonstrated the effects of ventilation on the transplanted lungs.

In summary, the present study of unilateral lung transplant in pigs showed that a higher PEEP (10 cm H₂O) was associated with better respiratory mechanics, a negative effect on hemodynamics, but no benefits for gas exchange, inflammation, or oxidative stress. Future studies involving different ischemic times and alternative methods of protective

lung ventilation may determine the optimal ventilatory strategy after lung transplant.

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