0031-3998/03/5303-0468
PEDIATRIC RESEARCH
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Vol. 53, No. 3, 2003
Printed in U.S.A.

Effects of High P_{CO_2} on Ventilated Preterm Lamb Lungs

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ABSTRACT

High P_{CO_2} levels attenuate reperfusion injury and ventilation-induced injury in isolated and perfused lungs. We asked whether premature lambs could tolerate 6 h of ventilation with a P_{CO_2} >80 mm Hg and whether the high P_{CO_2} modulated the ventilator-induced injury. Preterm surfactant-treated lambs were ventilated for 30 min with a high tidal volume (V_T) to induce lung injury. The lambs then were ventilated for 5.5 h with a V_T of 6–9 mL/kg to achieve a P_{CO_2} of 40–50 mm Hg in the control group. CO_2 was added to the ventilator circuit of a high P_{CO_2} group to maintain an average P_{CO_2} of 95 ± 5 mm Hg. The high P_{CO_2} lambs had heart rates, blood pressures, plasma cortisol values, and oxygenation equivalent to the control lambs. The lungs of the high P_{CO_2} group had significantly higher gas volumes and had less lung injury by histopathology. Indicators of inflammation (white blood cells, hydrogen peroxide production, and IL-1β and IL-8 cytokine mRNA expression in cells from the alveolar wash) qualitatively indicated less injury in the high P_{CO_2} group, although the differences were not significant. Preterm lambs tolerated a very high P_{CO_2} without physiologic compromise for 6 h. The high P_{CO_2} may attenuate ventilator-induced lung injury in the preterm. **(*Pediatr Res* 53: 468–472, 2003)**

Abbreviations
BPD, bronchopulmonary dysplasia
V_T, tidal volume
PIP, peak inspiratory pressure
PEEP, positive end-expiratory pressure

BPD in preterm infants is highly associated with the use of mechanical ventilation and supplemental oxygen (1, 2). Efforts to decrease the incidence of BPD have included attempts to avoid mechanical ventilation with the use of continuous positive airway pressure (3), different techniques for mechanical ventilation such as high-frequency oscillatory ventilation (4), and lower V_T ventilation resulting in higher P_{CO_2} values (5). In the mature lung, ventilator-mediated injury increases if the lung is inadequately inflated at end-expiration or overinflated at end-inspiration (6, 7). Attempts to decrease lung distention by decreasing V_T will result in increases in P_{CO_2}, referred to as "permissive hypercapnia" (8). Laffey *et al.* (9) recently extended this concept to "therapeutic hypercapnia" by demonstrating that reperfusion injury of the lung can be prevented if the injury and reperfusion occur in the presence of a P_{CO_2} of 80 mm Hg. This work demonstrates that high P_{CO_2} can protect the lung from reperfusion injury, most likely *via* attenuation of free radical damage. This effect has also been reported for reperfusion injury of other organ systems, such as the brain and heart in adult and newborn animal models (10, 11). Broccard *et al.* (12) recently reported that high P_{CO_2} also minimized ventilator-induced lung injury in isolated and perfused lungs. However, a protective effect of high P_{CO_2} values has not been demonstrated for ventilator-induced injury *in vivo* in animals or for the preterm lung. Therefore, we ventilated surfactant-treated preterm lambs to achieve normal P_{CO_2} values or equivalently ventilated other lambs with supplemental CO_2 to evaluate the tolerance of the preterm to high P_{CO_2} and the effect of the high P_{CO_2} on indicators of lung injury.

METHODS

Delivery and ventilation of lambs. The protocol was approved by the Animal Care Use Committee of the Cincinnati Children's Hospital Research Foundation. Anesthetized pregnant Suffolk ewes at 130–132 d gestational age (term = 150 d) were delivered by cesarean section as previously described (13). Each lamb was intubated, fetal lung fluid was aspirated, and 100 mg/kg of a surfactant containing human recombinant surfactant protein C and phospholipids (Venticute, Byk Gulden, Konstanz, Germany) was administered before the first breath (14). This surfactant has been shown to be as effective as natural surfactants and does not independently induce inflammation (15). Each lamb was randomized to a high P_{CO_2} group or a control group. The lambs were ventilated in a

Received March 26, 2002; accepted September 13, 2002.
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Supported by grant DH-12714 from the National Institute of Child Health and Human Development.

DOI: 10.1203/01.PDR.0000049463.76133.8F

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pressure-control mode with pressure-limited, time-cycled ventilators (16). The high P_{CO_2} group had supplemental CO_2 bled into the ventilator circuit to maintain an arterial P_{CO_2} of >80 mm Hg. The control group was ventilated to a P_{CO_2} of 40–50 mm Hg. The initial ventilator settings were as follows: 40 breaths per minutes, target V_T of 12–15 mL/kg with a pressure limit of 40 cm H_2O, a PEEP of 4 cm H_2O, an inspiratory time of 0.6 s, and a fraction of inspired oxygen (F_{IO_2}) of 100%. V_T were monitored continuously (16, 17). The high V_T was maintained for 30 min to produce a mild lung injury. The V_T was then lowered to 6–9 mL/kg for 5.5 h with a maximum allowable PIP of 35 cm H_2O. The F_{IO_2} was adjusted to maintain a P_{O_2} of 100–150 mm Hg. Cord blood and arterial blood were analyzed for white blood cell numbers and differential counts and plasma cortisol levels (16). All animals were sedated with intramuscular injections of ketamine and acepromazine to suppress any spontaneous respirations and no neuromuscular blockade was used. At 6 h, the lambs were deeply anesthetized with pentobarbital (25 mg/kg i.v.), ventilated briefly with 100% oxygen, and the airway was occluded to permit oxygen absorption.

Analyses. The deflation limb of the pressure-volume curve was measured (17). The left lung was used for an alveolar wash (18). The washes were pooled and saved for saturated phosphatidylcholine (Sat PC) analysis (19), total protein (20), cell counts and differentials, and hydrogen peroxide (H_2O_2) assay (21). The right upper lobe was inflation fixed in 10% formalin at 30 cm H_2O pressure (22). Total RNA was isolated from lung tissue and cell pellets from alveolar wash (22). IL1-β, IL-4, IL-6, IL-8, and tumor necrosis factor-α mRNA were quantified using ribonuclease protection assays with the ribosomal mRNA L32 as a reference RNA (22). Flow cytometry was performed to assess the percentage of cells that were apoptotic using propidium iodide/FITC staining (BD PharMingen, San Diego, CA, U.S.A.) (23). Cells were also incubated with MAb (Serotec, Oxford, UK) against CD11b (αM subunit of integrin CR3), CD14 (receptor for complex of lipopolysaccharide binding protein), and CD44 (proteoglycan link protein) and assayed by flow cytometry (23). The right upper lobe was airway inflation fixed with formalin at a pressure of 30 cm H_2O. The amount of inflammation in the right upper lobe was graded in a blinded fashion on three 5-μm sections for each animal (24).

Data analysis. Student's two-tailed unpaired *t* tests were used to compare the two groups. Significance was accepted at $p < 0.05$.

RESULTS

Description of lambs. One lamb randomized to the high P_{CO_2} group was excluded because it had a congenital diaphragmatic hernia and could not be ventilated. The remaining eight control and six high P_{CO_2} animals had similar birth weights, cord pH, and P_{CO_2} levels at delivery (Table 1). Blood pressures and heart rates for the animals with the high P_{CO_2} levels were similar to controls at 3 and 6 h. There were no differences in the peripheral white blood cell counts at 0, 3, and 6 h between high P_{CO_2} and control groups. Cortisol values also were similar at 0, 3, and 6 h.

Respiratory outcomes. Although the target V_T during the first 30 min of ventilation was 12–15 mL/kg, the average V_T was 10.8 ± 0.7 mL/kg for the control group and 12.1 ± 0.3 mL/kg for the high P_{CO_2} group because most of the animals were at the maximum allowable PIP of 40 cm H_2O (Figs. 1, *A* and *B*). This trend toward a V_T difference would bias the results

Table 1. *Physiologic variables of control and high P_{CO_2} groups at delivery, 3 h and 6 h of ventilation*

	Control	High P_{CO_2}
No.	8	6
Birth weight (kg)	3.30 ± 0.25	3.58 ± 0.36
Cord blood		
pH	7.22 ± 0.05	7.28 ± 0.05
P_{CO_2} (mm Hg)	56 ± 5	48 ± 3
Total WBC (× 10^3 cells/μL)	2.9 ± 0.6	3.5 ± 0.6
Neutrophils (number of cells/μL)	298 ± 76	408 ± 73
Cortisol (μg/dL)	4.6 ± 0.9	5.8 ± 1.3
At 3 h		
Total WBC (× 10^3 cells/μL)	1.2 ± 0.2	1.8 ± 0.3
Neutrophils (number of cells/μL)	474 ± 62	557 ± 92
Cortisol (μg/dL)	4.0 ± 0.9	4.6 ± 0.7
Blood pressure (mm Hg)		
Systolic	54 ± 1	54 ± 1
Diastolic	32 ± 1	36 ± 2
Heart rate (bpm)	132 ± 9	147 ± 11
At 6 h		
Total WBC (× 10^3 cells/μl)	1.5 ± 0.2	1.5 ± 0.3
Neutrophils (number of cells/μl)	759 ± 119	685 ± 195
Cortisol (μg/dl)	4.1 ± 0.6	5.4 ± 0.8
Blood pressure (mm Hg)		
Systolic	58 ± 2	55 ± 1
Diastolic	33 ± 2	33 ± 1
Heart rate (bpm)	132 ± 13	139 ± 10

WBC, white blood cells; bpm, beats per minute. Values are means ± SE.

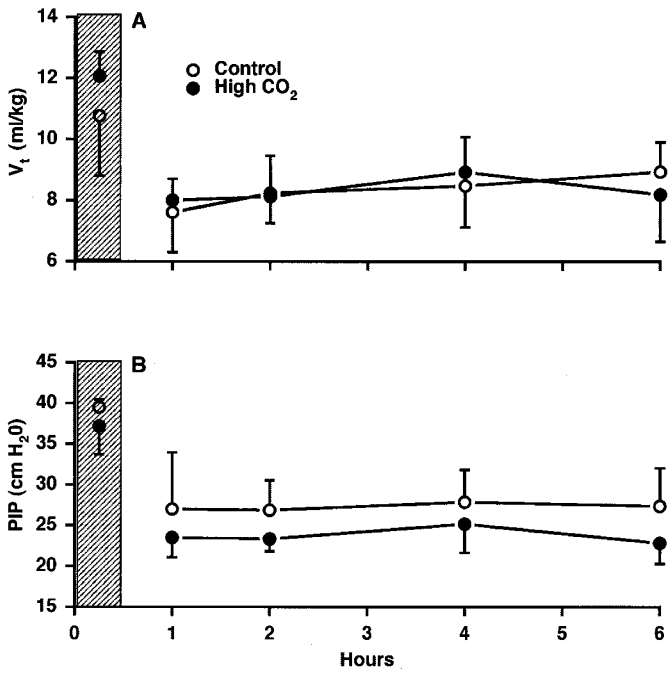

Figure 1. Sequential measurements of tidal volume (V_T) and peak inspiratory pressure (PIP) during ventilation in premature lambs. The *hatched area* indicates the first 30 min of high V_T to induce lung injury. (*A*) V_T was similar in both groups throughout the period of ventilation. (*B*) The PIP used to achieve the similar V_T for both groups was not different. Values are means ± SE.

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toward the null hypothesis. The V_T was decreased to the target range of 6–9 mL/kg after 30 min. There were no significant differences between the groups for V_T or PIP over the first 30 min or for the subsequent 5.5 h of ventilation.

The P_{CO_2} values of the high P_{CO_2} group were maintained at >80 mm Hg using between 0.125 and 1.5 L/min supplemental CO_2 in the ventilator circuit (Fig. 2*A*). The average P_{CO_2} over the 6 h of ventilation was 95 ± 5 mm Hg for the high P_{CO_2} group and was maintained between 40 and 50 mm Hg for the control group by study design. The pH reflected the P_{CO_2} difference throughout the experiment (Fig. 2*B*). The base deficit for the control group averaged −2.5 ± 1.3 and for the high P_{CO_2} group averaged −6.4 ± 1.3, values that were not different. Oxygenation, as reflected by the a/A ratio, was not different between groups throughout the experiment (Fig. 2*C*). The compliance for the control group averaged 0.35 ± 0.03 mL/cm H_2O/kg and, for the high P_{CO_2} group, averaged 0.42 ± 0.04 mL/cmH_2O/kg ($p = 0.17$) (Fig. 3*A*). The volumes measured by the deflation pressure volume curves of the lungs were higher for the high P_{CO_2} group than for the control animals (Fig. 3*B*).

Indicators of lung inflammation. The alveolar wash from the high P_{CO_2} group had a lower total protein, but the difference was not statistically significant (Table 2). Both the total white blood cell counts and the neutrophil counts in the alveolar washes tended to be lower in the high P_{CO_2} group. The hydrogen peroxide production by the cells in the alveolar wash for the high P_{CO_2} group was half of the control. There were no differences in mean fluorescence units for CD11b, CD14, or CD44 expression on cells in the alveolar wash between groups. There was also no difference between the percentage of apoptotic cells per kilogram for the two groups.

Lung injury scores from the histopathology are shown in Table 2. There was no difference for the lung tissue between the control and high P_{CO_2} groups. There was a significant decrease in the score for inflammatory cells in the air spaces for the high P_{CO_2} group compared with the control. Neither group demonstrated severe edema, hemorrhage, or atelectasis.

The levels of cytokine mRNA for IL-1β, IL-4, IL-6, IL-8, and tumor necrosis factor-α from lung tissue were evaluated for the two groups and were not different (Table 3). The cytokine mRNA levels for IL-1β and IL-8 from the cells in the alveolar wash were lower for the high P_{CO_2} group than the control group although the differences were not significant (Table 3). IL-6 mRNA was not detected in cells from the alveolar wash for either group of animals.

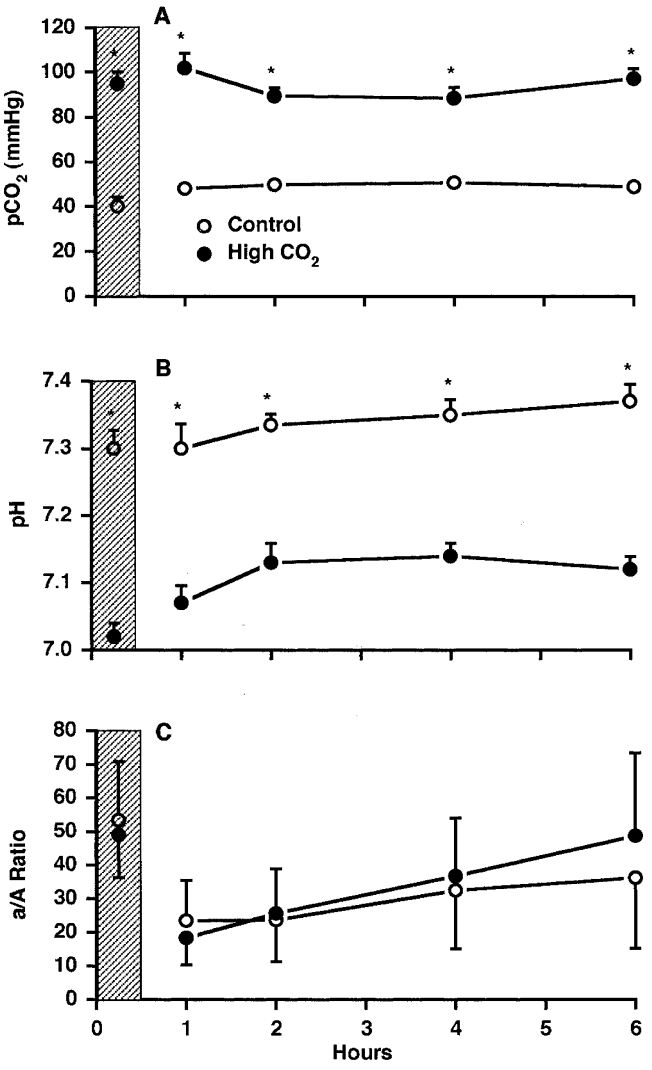

Figure 2. Sequential measurements of P_{CO_2}, pH, and a/A ratio during the ventilation of the preterm lambs. (*A*) P_{CO_2} was higher in the high P_{CO_2} group by study design. (*B*) The pH was reflective of the P_{CO_2} difference throughout the ventilation period. (*C*) Oxygenation, as evaluated by the a/A ratio, was not different between the two groups throughout the ventilation period. The a/A ratio was calculated as $Pao_2/(Pio_2 - Paco_2) = Pao_2/[Fio_2 (P_B - PH_2O) - Paco_2]$. *$p < 0.05$ *versus* the control group. Values are means ± SE.

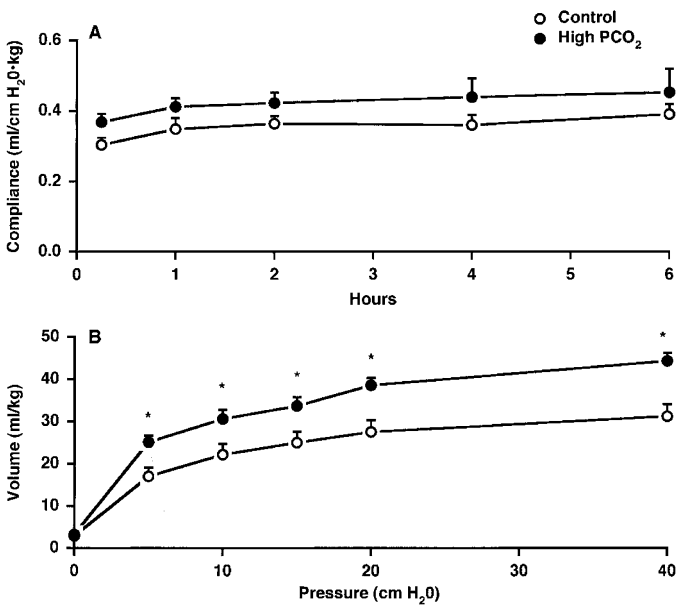

Figure 3. Compliance and pressure-volume curves. (*A*) Sequential measurements of compliance throughout the ventilation period. There were no differences in compliance between the groups. (*B*) Deflation limbs of the pressure volume curves. After 6 h of ventilation there was a significant difference between the high P_{CO_2} group and the control group. *$p < 0.05$ *versus* the control group. Values are means ± SE.

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Table 2. *Markers of lung injury in alveolar wash and by histopathology*

	Control	High P_{CO_2}	*p* Value
Values for alveolar wash			
Protein (mg/kg)	76 ± 10	62 ± 6	0.29
Total WBC ($\times 10^6$ cells/kg)	3.2 ± 0.5	2.1 ± 0.4	0.13
Neutrophils ($\times 10^6$ cells/kg)	2.0 ± 0.4	1.0 ± 0.3	0.10
H_2O_2 (μmol/kg)	601 ± 143	357 ± 68	0.19
Sat PC (μmol/kg)	29.8 ± 4.9	30.8 ± 8.2	0.12
CD 11b (mean fluorescence units)	460 ± 89	292 ± 108	0.25
CD 14 (mean fluorescence units)	14 ± 9	11 ± 9	0.84
CD 44 (mean fluorescence units)	79 ± 22	62 ± 43	0.70
Apoptosis (% of cells)	8.0 ± 2.6	8.9 ± 1.8	0.81
Injury score by histopathology*			
Lung tissue	1.9 ± 0.3	1.6 ± 0.3	0.46
Air space	1.6 ± 0.3	0.7 ± 0.3	0.007

WBC, white blood cells.

* The injury score was graded as 0 (no inflammatory cells); 1 (a few cells); 2 (a moderate cell infiltration), and 3 (large numbers of inflammatory cells in airspaces and tissue). Average scores for airspaces and tissue were calculated for each animal.

Table 3. *Messenger RNA expression in lung tissue and cells from alveolar wash*

	High P_{CO_2} Relative to Control
Lung tissue	
IL-1β	2.00 ± 0.34
IL-4	0.78 ± 0.22
IL-6	0.84 ± 0.12
IL-8	1.22 ± 0.24
TNF-α	0.86 ± 0.28
Alveolar cells	
IL-1β	0.50 ± 0.12
IL-6	Not detectable
IL-8	0.59 ± 0.13

Control values are normalized to L32 mRNA and given a value of 1.0. Values are means ± SE.

DISCUSSION

This study was designed to test the effects of a high P_{CO_2} in a premature animal model of ventilator-induced lung injury. Our endpoints were the assessment of the physiologic tolerance of a high P_{CO_2} and alterations in lung function or inflammation. We found no physiologic instability as reflected by blood pressure or heart rate with the high P_{CO_2}. We did find an overall trend toward decreased inflammation and lung injury. The mean deflation pressure-volume curve for the high P_{CO_2} animals retained more gas volume and the inflammation score for the airspace was less in the high P_{CO_2} group. The other indicators of inflammation and injury (total protein in the alveolar washes, white blood cells in alveolar washes, hydrogen peroxide production by alveolar cells, and proinflammatory cytokine expression by alveolar cells) were qualitatively lower in the high P_{CO_2} group.

We chose the preterm lamb for these studies because we could evaluate both physiology and multiple indicators of inflammation and injury in this large animal model. We have characterized the proinflammatory response to mechanical ventilation of preterm lambs (16). For this study, we avoided a severe lung injury by treating with surfactant, but we initially used a high V_T and high pressures for 30 min to cause a mild injury in both groups of animals. The subsequent ventilation was designed to cause minimal further injury by using low V_T and avoiding hyperventilation of the low P_{CO_2} group (17). By matching the ventilation using V_T to adjust pressures, both groups of animals were exposed to the same amount of mechanical ventilation under virtually identical conditions except for the supplemental CO_2 received by the high P_{CO_2} group. Therefore, we were able to successfully isolate the P_{CO_2} as the single variable between the two groups of animals. We did not attempt to correct the respiratory acidosis, because pH correction prevented the beneficial effects of high P_{CO_2} in the lung reperfusion injury model (25).

Permissive hypercapnia is being evaluated as a way to avoid the barotrauma required to normalize P_{CO_2} values in ventilated adults with severe lung injury (26, 27). Low P_{CO_2} values have been associated with an increased risk of BPD in ventilated preterm infants (28), but high P_{CO_2} values increase cerebral blood flow and could be a risk factor for intraventricular hemorrhage in the preterm infant (29). Nevertheless, several small trials have randomized ventilated preterm infants to lower *versus* higher P_{CO_2} targets and report trends consistent with some protection from BPD in the higher P_{CO_2} groups (5, 30). Although many preterm infants with BPD will have chronic elevations of P_{CO_2} into the 50–60 mm Hg range and a few will maintain higher P_{CO_2} levels, there is no clinical information about the safety of high P_{CO_2} values soon after delivery and early in the course of respiratory failure in the preterm. This experiment was designed to be an extreme test of the ability of the preterm to cope with very high P_{CO_2} levels.

The adult human or animal tolerates the respiratory acidosis associated with an acute increase in P_{CO_2} by increasing catecholamines and maintaining cardiac output (31). These preterm lambs, with P_{CO_2} values that averaged 95 mm Hg, had no changes in heart rate or blood pressure and cortisol levels did not change over 6 h. We did not measure catecholamines, although they would be expected to be very high after preterm delivery (32). A limitation of this study is that we did not evaluate cardiac output or other organ system function. In a previous study evaluating low V_T for the initiation of mechanical ventilation in preterm lambs, lambs with P_{CO_2} values of about 90 mm Hg in the first 30 min of life had a 1.5-fold increase in cardiac output and a 5-fold increase in cerebral blood flow relative to lambs with normal P_{CO_2} values (13).

Another concern about high P_{CO_2} levels in the immediate newborn period is that the high pulmonary artery pressure characteristic of the fetal lung would be maintained after birth in the high P_{CO_2}-low pH environment (33). Low pH and high P_{CO_2} values have been reported to interfere with the normal vasodilatation of the pulmonary vasculature that occurs after birth (34). Although we did not measure pulmonary artery pressures, oxygenation was not different in these surfactant-treated lambs. Overall postnatal adaptation was essentially equivalent despite the very high P_{CO_2} values.

Our second question was to ask whether "therapeutic hypercapnia" would decrease lung injury. High P_{CO_2} levels decrease multiple indicators of lung injury after ischemia-reperfusion injury (9), and correction of the respiratory acidosis with bicarbonate abrogates the protective effect of the high P_{CO_2}

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(35). High P_{CO_2} in the range of 55 mm Hg also protects the newborn rat brain from injury after unilateral carotid ligation and hypoxia (36). The high P_{CO_2} protects the brain by increasing perfusion and maintaining high energy metabolite levels. High P_{CO_2} also can protect the isolated heart from reperfusion injury (11). The common theme is that the oxidant-mediated ischemia-reperfusion injury cascade is blunted by high P_{CO_2} values. High P_{CO_2} values also can protect the isolated and perfused rabbit lung from ventilator-induced lung injury (12). However, CO_2 effects on ventilator-mediated lung injury in the whole animal have not been reported. We evaluated a number of the variables that have been associated with lung injury in ventilated adult and preterm animals (16, 37). We were able to show a statistically significant difference between the pressure-volume curves of the two groups and the inflammatory cell infiltration in the airspaces. It is interesting that we could show a difference in the infiltration of inflammatory cells into the airspaces, but not into the lung tissue. One possibility is a small difference in cell number is more apparent in the airspaces than in lung tissue sections. Another consideration is the physiologic action of the high CO_2 level: does it exert a systemic effect decreasing inflammatory signaling and cell migration, or does it act locally, disabling the inflammatory cells already found in the lungs? With the group size that we used, we did not demonstrate definitively that the high P_{CO_2} levels protected the lungs from the inflammatory mediators that appear with the initiation of mechanical ventilation of the preterm lung. However, the directional changes of all the variables that we measured favored the high P_{CO_2} group.

Recent clinical reports of the benefits of a more conservative approach to the management of the preterm lung emphasize avoiding intubation in the delivery room and the use of continuous positive airway pressure to avoid mechanical ventilation (38). A result of such an approach is that the infant will have higher P_{CO_2} values (39). Our results demonstrate no measured adverse effects of extremely high P_{CO_2} levels. However, this study does not address any potential benefits of the levels of elevated P_{CO_2} that occur clinically, nor does it address a number of potential injuries that might be caused by elevated P_{CO_2} values in the preterm.

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