Hypercapnic Acidosis Is Protective in an *In Vivo* Model of Ventilator-induced Lung Injury

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To investigate whether hypercapnic acidosis protects against ventilator-induced lung injury (VILI) *in vivo*, we subjected 12 anesthetized, paralyzed rabbits to high tidal volume ventilation (25 cc/kg) at 32 breaths per minute and zero positive end-expiratory pressure for 4 hours. Each rabbit was randomized to receive either an $F_{I_{CO_2}}$ to achieve eucapnia ($Pa_{CO_2} \sim 40$ mm Hg; n = *6*) or hypercapnic acidosis (Pa_{CO_2} 80–100 mm Hg; n = *6*). Injury was assessed by measuring differences between the two groups' respiratory mechanics, gas exchange, wet:dry weight, bronchoalveolar lavage fluid protein concentration and cell count, and injury score. The eucapnic group showed significantly higher plateau pressures (27.0 ± 2.5 versus 20.9 ± 3.0; p = 0.016), change in Pa_{O_2} (165.2 ± 19.4 versus 77.3 ± 87.9 mm Hg; p = 0.02), wet:dry weight (9.7 ± 2.3 versus 6.6 ± 1.8; p = 0.04), bronchoalveolar lavage protein concentration (1,350 ± 228 versus 656 ± 511 μg/ml; p = 0.03), cell count ($6.86 \times 10^5 \pm 0.18 \times 10^5$ versus $2.84 \times 10^5 \pm 0.28 \times 10^5$ nucleated cells/ml; p = 0.021), and injury score (7.0 ± 3.3 versus 0.7 ± 0.9; p < 0.0001). We conclude that hypercapnic acidosis is protective against VILI in this model.

Keywords: respiratory acidosis; hypercapnia; mechanical ventilation; acute lung injury; rabbits

Research efforts in the acute respiratory distress syndrome (ARDS) have recently focused on attempts to improve outcome by limiting potential new or worsening lung injury caused by adverse mechanical ventilation strategies. The ARDS Network study (1) has shown improved clinical outcomes using "low stretch" ventilation strategies that limit tidal volumes and inflation pressures and presumably reduce ventilator-induced lung injury (VILI). Low tidal volume ventilation is frequently associated with a concomitant respiratory acidosis that, rather than being a side effect to be corrected or tolerated, has been suggested by some to be potentially beneficial.

Hypercapnic acidosis has a multitude of effects at the systemic, end organ, cellular, and molecular level. These effects impact blood flow and tissue oxygenation (2, 3), ventilation–perfusion heterogeneity (4, 5), surfactant production (6), cytoskeletal regulation of vascular permeability (7), gene expression (8, 9), and nitric oxide (NO) production (10–13). In addition, hypercapnic acidosis attenuates inflammation (14, 15) and reduces injury due to ischemia-reperfusion in the heart, liver, and lung (16–20). Recently, Broccard and colleagues (12) demonstrated the protective effects of hypercapnic acidosis in an isolated, perfused lung model of VILI. It is not known whether these same protective effects against VILI occur in an intact organism.

The present study was designed to determine whether hypercapnic acidosis modulates the development of VILI in an intact rabbit model of mechanical ventilation-induced lung injury.

METHODS

All methods were approved by the University of Washington Animal Care Committee in accordance with National Institutes of Health guidelines (*see also* the expanded Methods section in the online data supplement).

Animal Preparation

New Zealand white rabbits (~ 3.0 kg) were anesthetized with intramuscular ketamine (30 mg/kg) and xylazine (7.5 mg/kg) followed by a maintenance infusion (ketamine 0.05mg/kg/minute; xylazine (0.003 mg/kg/minute) and intravenous pancuronium (0.15–0.2 mg/kg) every 30 minutes.

Ventilation via tracheostomy was initiated with $V_T \sim 8$ cc/kg, PEEP = 5 cm H_2O, respiratory rate to maintain Pa_{CO_2} of ~ 40 mm Hg and $F_{I_{O_2}} = 0.5$ for 30 minutes and baseline data collected.

Lung Injury Protocol

Animals were randomized to eucapnia ($Pa_{CO_2} \sim 40$ mm Hg; *n = 6*) or hypercapnic acidosis (Pa_{CO_2} of 80–100 mm Hg; *n = 6*) and ventilated supine with V_T = 25cc/kg via pressure control mode (inspiratory time 0.5; PEEP = 0 cm H_2O) for 4 hours (Servo 900C; Semens-Elema, Stockholm, Sweden). Respiratory rate was 32 breaths per minute to ensure an equal number of potential insults per animal and achieve significant injury within the allotted time without developing intrinsic PEEP. Inspiratory pressure was adjusted to maintain target tidal volume. The high minute ventilation required an $F_{I_{CO_2}} \sim 4$–5% and $F_{I_{CO_2}} \sim 12\%$ to maintain eucapnia and hypercapnia, respectively. Normal saline was given at 10 cc/kg/hour and 10 cc/kg boluses as needed to support blood pressure. $F_{I_{O_2}} = 0.5$ was maintained throughout the protocol.

Physiologic Measurement

Data were recorded using PowerLab data acquisition software (AD-Instruments, Castle Hill, New South Wales, Australia).

Blood pressures, heart rates, thermodilution cardiac outputs, right ventricular pressures, and airway pressures were recorded at baseline, initiation of test conditions, and 1-hour intervals thereafter as previously described (21). Inspired and exhaled CO_2 were measured by mass spectrometer (Perkin-Elmer, Pomona, CA). Temperature corrected arterial blood gases (ABL 5; Radiometer, Copenhagen, Denmark) were measured at the same intervals.

Bronchoalveolar Lavage

At the end of the experiment, animals were exsanguinated and the heart and lungs removed. A randomly selected left or right lung was lavaged as previously described (22). A 1.0-ml aliquot was used for cell counts. The remaining fluid was spun at 200 × *g* and the cell free supernatant stored at −70°C. A 1.0-ml aliquot was used to measure total protein concentration (BCA; Pierce, Rockford, IL).

Histology

From the unlavaged lung, a 0.9-cm^3 core sample was extracted from the visually estimated center, fixed in 10% buffered formalin, sectioned,

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stained, and scored by a pathologist blinded to experimental conditions. Samples were assigned an injury score in each of four categories (interstitial edema, alveolar edema, neutrophil infiltration, and hemorrhage) based on severity (0 = not present, 4 = severe and present throughout).

Gravimetric Analysis

An additional three core samples were removed and frozen. Remaining lung tissue was weighed before (WW) and after drying (DW) to calculate wet to dry weight ratios (WW:DW). These data were not corrected for residual intrapulmonary blood.

Statistics

The mean values of measurements made only once during the protocol were compared using unpaired *t* tests. Measurements made more than once per animal were compared using repeated measures ANOVA with Tukey/Kramer *post hoc* analysis for within-group comparisons. Significance was set at $p < 0.05$. All values are reported as mean ± SD.

RESULTS

Twelve rabbits (six in each group) survived the 4-hour ventilation protocol and are included in the following results and analyses. Four rabbits were randomized, but died before the 2-hour mark and were excluded from the study. Two animals died from progressive hypotension and at necropsy had evidence of bacterial pneumonia (one from each group). These were the first two animals in the series. In response to this, all subsequent animals were specific pathogen–free (SPF) and had white blood cell counts measured on the day before and day of each experiment. Animals with peripheral blood white blood cell counts > 6,000 were to be excluded. No animals were excluded based on these criteria. Two additional animals (one from each group) died of pneumothorax as evidenced by obvious pneumoperitoneum and observed air leak(s) when the chests were opened. Pneumothorax was excluded in the other animals by the absence of air leaks or air in the thoracic or abdominal cavities when the chests were opened. No data from the four animals that died before completing the protocol was included in the analysis below.

Baseline Characteristics

The two groups did not differ in their baseline physiologic characteristics as summarized in Table E1 in the online data supplement. Table 1 summarizes the physiologic measurements made in the two study groups during the 4-hour ventilation protocol. Baseline data obtained after 1 hour of stabilization are compared with measurements made at the end of the 4-hour protocol.

Hemodynamic Data

The heart rates and vascular pressures were similar between the eucapnic and hypercapnic groups during high tidal volume ventilation. The only difference was a higher cardiac output in the hypercapnic group at hour 1, which was not seen in subsequent measurements. No other significant differences in hemodynamic response were observed.

Gas Exchange and Mechanics

Tidal volume, normalized to body weight, did not differ between the two groups (24.9 ± 2.2 versus 24.9 ± 3.3). FI_{CO_2} (4.9 ± 0.3% eucapnic group versus 11.9 ± 1.6% hypercapnic group) was sufficient to maintain the target Pa_{CO_2} in both groups. Figures E1 and E2 in the online data supplement demonstrate the differences in pH and Pa_{CO_2}, respectively, between the eucapnic and hypercapnic groups. Whereas Pa_{CO_2} remained stable in both groups throughout the study period, pH progressively dropped in both groups, due to metabolic acidosis, but the hypercapnic group had a significantly lower pH at every time point. Arterial P_{O_2} was similar between the two groups initially. Pa_{O_2} at 4 hours was significantly lower in the eucapnic group compared with the hypercapnic animals (Figure 1).

Inspiratory hold or plateau airway pressure was similar in both groups at the start of the ventilation protocol, then decreased over the first hour in both groups to a similar level. From hours 1–4, the eucapnic group exhibited a steady increase in plateau pressure, whereas pressures remained stable and no different from those measured at 1 hour in the hypercapnic group (Figure 2).

Indices of Lung Injury

Wet to dry weight ratios (WW:DW), bronchoalveolar lavage (BAL) fluid protein concentration, and histology score were used to estimate degree of lung injury. WW:DW was significantly greater in the eucapnic compared with the hypercapnic group (9.7 ± 2.3 versus 6.6 ± 1.8, respectively; $p = 0.043$). In ad-

TABLE 1. CHARACTERISTICS AT THE BEGINNING AND END OF STUDY PERIOD

	Eucapnic Group		Hypercapnic Group	
Variables	Hour 1	Hour 4	Hour 1	Hour 4
Heart rate, min^{-1}	208 ± 13	249 ± 39	217 ± 34	244 ± 27
Arterial pressure *systolic,* mm Hg	64.5 ± 14.4	63.0 ± 13.4	57.0 ± 5.3	63.8 ± 7.9
Arterial pressure *diastolic,* mm Hg	37.0 ± 14.9	35.3 ± 11.8	31.5 ± 7.2	34.5 ± 9.1
Arterial pressure *mean,* mm Hg	46.2 ± 13.9	44.6 ± 12.3	40.0 ± 5.7	44.3 ± 8.3
RVP *mean,* mm Hg	9.1 ± 1.3	11.2 ± 1.3*	8.3 ± 1.9	9.1 ± 1.7
Cardiac output, L/min	0.22 ± 0.04	0.23 ± 0.09	0.26 ± 0.07†	0.24 ± 0.06
Tidal volume, cc/kg	24.9 ± 2.2	24.9 ± 2.2	24.9 ± 3.3	24.9 ± 3.3
Airway pressure *peak,* cm H_2O	27.0 ± 3.5	33.2 ± 3.8*†	26.5 ± 2.4	27.3 ± 3.5
Airway pressure *plateau,* cm H_2O	22.0 ± 3.1	27.0 ± 2.5*†	20.4 ± 2.3	20.9 ± 3.0
pH	7.45 ± 0.05†	7.14 ± 0.13*†	7.17 ± 0.07	6.99 ± 0.07*
Pa_{O_2}, mm Hg	316 ± 18	143 ± 30*†	321 ± 11	246 ± 75*
Pa_{CO_2}, mm Hg	40.7 ± 3.4†	41.3 ± 1.1†	85.2 ± 6.2	94.3 ± 5.9*

* $p < 0.05$ compared with 1-hour value.
† $p < 0.05$ compared with hypercapnic group.

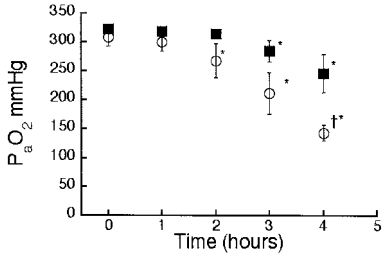

Figure 1. Arterial partial pressure of oxygen as it varies over time between the two study groups. *Solid squares* = hypercapnic group; *open circles* = eucapnic group. *$p < 0.05$ compared with time zero value within the same group. †$p < 0.05$ compared with hypercapnic group at same time point.

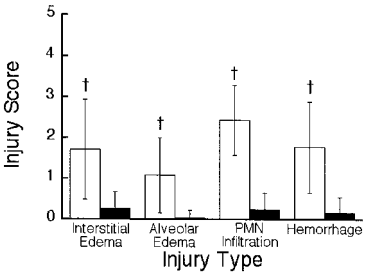

Figure 3. All four types of injury are reduced in the hypercapnic group, particularly PMN infiltration and hemorrhage. *Black bars* = hypercapnic group; *white bars* = eucapnic group. †$p < 0.05$.

dition, the eucapnic group also had significantly higher BAL fluid protein concentrations compared with the hypercapnic animals (1350 ± 228 μg/ml versus 656 ± 511 μg/ml, respectively; $p = 0.029$) (*see* Figure E3 in the online supplement).

Major differences were also seen in histologic damage between the two groups. Significant differences are seen in all four categories of injury examined, especially PMN infiltration and hemorrhage (Figure 3). These differences are illustrated in Figures 4A and 4B, which show representative photomicrographs from eucapnic (*A*) and hypercapnic (*B*) animals. There was a marked increased in edema, hemorrhage, and inflammatory cell infiltrate in the eucapnic ventilated specimen.

BAL Fluid Cell Counts

BAL fluid from the eucapnic group was more cellular than that from the hypercapnic group by 2.4-fold ($6.86 \times 10^5 \pm 0.18 \times 10^5$ versus $2.84 \times 10^5 \pm 0.28 \times 10^5$ nucleated cells/ml, respectively; $p = 0.021$). Not only were the total cell counts higher but the differential counts of the observed nucleated cells were markedly different between the study groups. The group subjected to hypercapnic acidosis had fewer total cells, mostly comprised of alveolar macrophages, with few inflammatory cells present (70% macrophages, 30% neutrophils). By contrast, the much more cellular fluid from the eucapnic group contained a significantly higher percentage of neutrophils, in excess of all other nucleated cells, including alveolar macrophages (74% neutrophils, 26% macrophages; $p = 0.001$) (Figure 5).

DISCUSSION

The principle finding of this study is that hypercapnic acidosis decreased the severity of VILI in this *in vivo* model. Given that both groups were ventilated in identical fashion (i.e., 25 cc/kg tidal volumes, 32 respirations/minute, 4-hour time period), this suggests that hypercapnic acidosis has an effect that is independent of tidal volume. The two groups were physiologically very similar throughout the study. Hemodynamic effects, resulting from the altered acid-base status, are therefore unlikely to account for the observed differences in degree of lung injury. Based on the marked inflammatory cell response in the BAL fluid of the more injured eucapnic group, our data support the hypothesis that hypercapnic acidosis may reduce the severity of lung injury by attenuating the inflammatory response induced by lung overdistension and/or repetitive airway collapse and expansion (RACE).

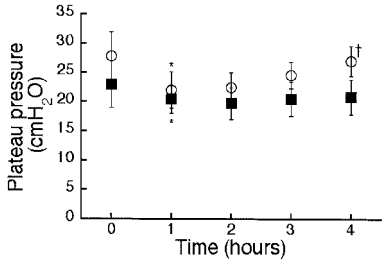

Figure 2. Airway plateau (i.e., inspiratory hold) pressure as it varies over time between the two study groups. *Solid squares* = hypercapnic group; *circles* = eucapnic group. *$p < 0.05$ compared with time zero value within the same group. †$p < 0.05$ compared with hypercapnic group at same time point.

The Experimental Model

To test the effect of hypercapnic acidosis on VILI, we must be able to reproducibly cause lung injury with the ventilation protocol. We have described this model previously (23), and similar high tidal volume models have been used by others to induce VILI (24–27).

This model is limited in several important ways that prevent its direct extrapolation to other models or to patients with respiratory failure. First, no uninjured control animals were included in the analysis. We chose not to include uninjured controls due to previous analysis of uninjured animals in our laboratory and the differences seen in preliminary comparisons between the study groups. In a series of uninjured controls, WW:DW is significantly less than both groups in this study (WW:DW = 3.9 ± 0.4) and injury scores are similar to those seen in the hypercapnic group (interstitial edema = 0.2 ± 0.2; alveolar edema = 0.04 ± 0.02; PMN infiltration = 0.3 ± 0.2; and hemorrhage = 0.05 ± 0.03; n = 6 [unpublished data]).

In addition, the experimental protocol was limited to 4 hours of mechanical ventilation, which may be insufficient time to document prevention versus delay in the development of VILI.

The large tidal volumes used are clearly not clinically applicable, especially in light of the advantageous outcomes recently observed with low tidal volume ventilation in ARDS (1). It is also unlikely that a patient with acute lung injury (ALI) would be ventilated without the benefit of positive end-expiratory pressure. In addition, most patients with ARDS or ALI have another risk factor (i.e., sepsis, trauma, aspiration, etc.) for lung injury besides mechanical ventilation.

We chose this large tidal volume model of pure mechanical ventilation–induced lung injury for several reasons. First, it is a stable and reproducible model of lung injury. Second, it allows isolation of a single variable (hypercapnic acidosis) and its impact on the development of lung injury without clouding the results with other potential variables (i.e., saline lavage, oleic acid, LPS, PEEP level, etc.). Last, the model allows comparison to prior observations made in an *ex vivo* lung preparation, an even less easily generalized model than this intact preparation.

Physiologic Comparisons

The two groups did not differ significantly in any of the measured parameters during the baseline ventilation period (*see* Table E1 in the online data supplement).

Hemodynamics

Hemodynamic parameters did not differ significantly between the two groups. The only observed difference was an initially higher cardiac output in the hypercapnic animals, which was not seen on subsequent measurements. An initial hypercapnia-

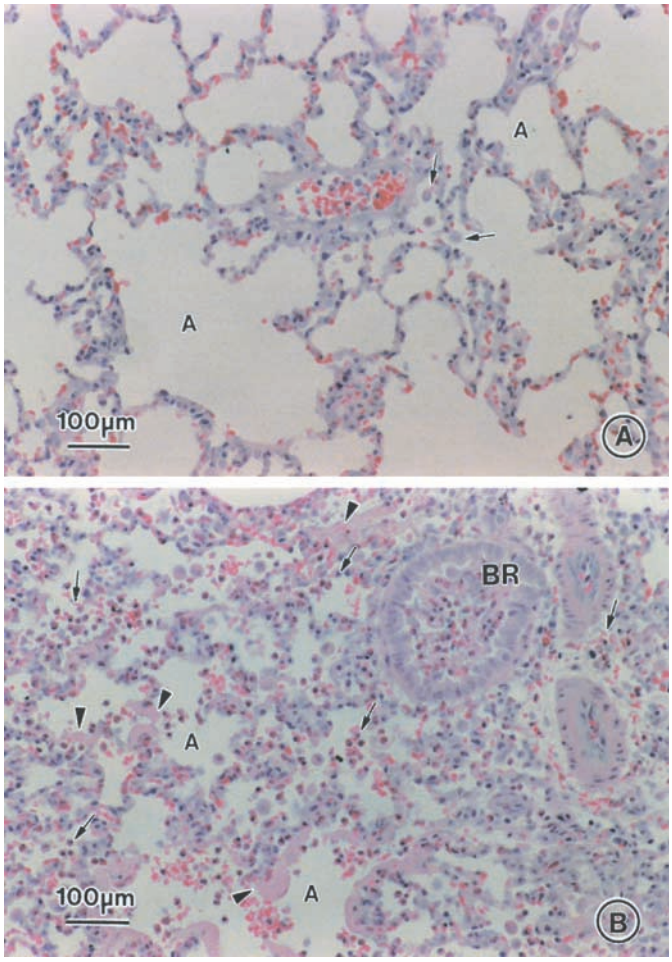

Figure 4. (*A*) A representative micrograph of lung tissue from a hypercapnic animal. The alveoli are free of edema and cellular infiltrate (A), normal parenchymal architecture is maintained, and only a small number of macrophages are present in the alveoli (*arrow*). (*B*) A comparable micrograph from the eucapnic group. There is marked cellular infiltration in the interstitium and alveoli (*arrows*), which also fills the lumen of a bronchiole (BR). Alveolar and interstitial edema, as well as hyaline membrane formation (*arrowheads*) are also present. (Hematoxylin and Eosin stained; ×120 magnification; scale indicates 100 μm.)

induced vasodilatory effect and/or increased heart rate due to neuroadrenal stimulation (these effects may wane over several hours) likely account for this. It is doubtful that this had any meaningful impact on the severity of VILI. This was surprising given that others have noted marked hemodynamic instability with hypercapnic acidosis in a rabbit model of saline lavage-induced injury (28). These differences in hemodynamic response are most likely due to the higher positive end-expiratory pressure (PEEP) (14.3 ± 0.48 versus 0.0 cm H_2O) and mean airway pressures compared with the present study. Hickling and colleagues (29) observed that even more extreme hypercapnic acidosis (Pa_{CO_2} 150–250 mm Hg) can be tolerated in rabbits without significant hemodynamic compromise.

Respiratory Mechanics and Gas Exchange

Both groups had significantly higher peak and plateau airway pressures at the start of the ventilation protocol than at the 1-hour mark (Figure 2). This is likely due to an increased recruitment volume upon initiation of high tidal volume ventilation, which has also been observed in ARDS patients ventilated with 10 cc/kg versus 6 cc/kg tidal volumes (30). Airway

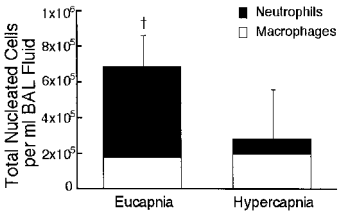

Figure 5. BAL fluid total and differential cell counts. Eucapnic animals had significantly more nucleated cells per ml, most of which were neutrophils, whereas fewer cells, predominately macrophages, were found in the hypercapnic group. †$p < 0.05$.

pressures then remained stable through hour 2, after which significant increases are seen in the eucapnic group. Likewise, Pa_{O_2} was comparable between groups at baseline and through the first hour of ventilation (Figure 1). Subsequently, a steady decline in oxygenation was seen in the eucapnic animals, which correlated well with the rise in airway pressures, as well as with other indices of injury. An identical ventilation strategy was used for all subjects, which precludes differences in PEEP or atelectasis as viable explanations for the observed differences in gas exchange. The progressive worsening over time also favors increasing severity of injury as the most likely cause of these differences.

Despite a stable Pa_{CO_2} level, pH in both groups declined gradually over time. This was due to a metabolic acidosis, as evidenced by a negative base excess in both groups at the end of the experiment (eucapnic BE = −13.6 ± 6.3 versus hypercapnic BE = −11.2 ± 3.9; p = 0.14). This metabolic acidosis could have several etiologies, most likely a lactic acidosis due to local or global hypoperfusion. Hyperchloremic metabolic acidosis from normal saline infusion is another potential contributing factor (31).

Indices of Lung Injury

We assessed the severity of lung injury by several different measures, including respiratory mechanics, gravimetric analysis (WW:DW), BAL protein concentration, as a surrogate indicator of altered permeability, and histology. These measures, taken individually, are limited. However, the significant differences observed in all these indices between the two groups give us confidence that the ventilation protocol we used did cause significant lung injury and that hypercapnic acidosis produced a real reduction in its severity.

BAL Fluid Analysis

The right lung of the rabbit is significantly larger than the left, therefore, our choice to randomly alternate the lung to be lavaged rather than using the same side each time is potentially problematic. Fortunately, we used an elimination randomization scheme such that the same number of right and left lungs were lavaged in each study group. We also recorded the volume of lavage return for each animal. Interestingly, this volume was not significantly different between left and right lungs in either group (right = 64.3 cc versus left = 63.2 cc from 75 cc total lavage volume, p = 0.45). This does not, however, preclude error in the interpretation of the protein and cell concentration data if it is analyzed as pooled means. To address this, we also reported the data in the online supplement (Figures E3 and E4) indicating the study group, side lavaged, and lavage return volume. We found no significant differences between protein concentrations or cell counts in the right versus left lungs within either study group, whereas significant differences persisted between groups, regardless of side lavaged.

Possible Protective Mechanisms of Hypercapnic Acidosis

The data presented here show that hypercapnic acidosis reduces the severity of VILI in this *in vivo* model. Although this

study was not designed to determine the mechanisms by which this protection is rendered, several possible mechanisms can be hypothesized based on known effects of hypercapnic acidosis.

Nitric Oxide

There are documented interactions between nitric oxide (NO) production and CO_2 that may be important in the setting of lung injury. Reduction in NO production has been observed in the presence of hypercapnia (10), and its levels correlated well with severity of lung injury in an isolated, perfused rabbit model (12). Indeed, increased NO synthesis has recently been linked to lung stretch (11, 13), a probable culprit in VILI and in chronic metabolic acidosis, which was also associated with lung injury, in rats (32). There are, however, conflicting reports regarding levels of NO and their potential effect on lung injury. In some cases, it appears protective, in others injurious. One explanation is that NO effects differ depending on the physiologic state (33). The present study did not address NO production.

Antiinflammatory Effect

The marked reduction in severity of lung injury in the hypercapnic group was associated with a reduction in BAL fluid cell count, primarily due to a decreased number of acute inflammatory cells. Hypercapnic acidosis may itself have an antiinflammatory effect. Early studies of VILI by Kolobow and colleagues demonstrated that hyperventilation of normal lungs with high airway pressures produced significant lung injury and death (34). However, if sufficient inhaled CO_2 was given to achieve normocapnia, injury was less severe and death was delayed. *In vitro*, acidosis suppresses tumor necrosis factor-α release by lipopolysaccharide-stimulated rabbit alveolar macrophages (14). Acidosis also blunts expression of intercellular adhesion molecule-1 and E-selectin, which are important for neutrophil–endothelial cell adhesion and may impair neutrophil diapedesis (15). Subsequent animal studies have shown reduced neutrophil counts in saline-lavaged animals ventilated with permissive hypercapnia compared with controls (29).

Mechanical ventilation has been shown to have significant effects on levels of inflammatory cytokines and soluble mediators in the lung. Lung injury due to mechanical ventilation in a saline-lavaged rabbit model was markedly attenuated by granulocyte depletion (35). In animal models of VILI, both high distending volumes and cyclical airway closure and reopening have been associated with increases in lung neutrophil accumulation and activation (36, 37) as well as increased lavage-fluid levels of inflammatory mediators (platelet activating factor, tumor necrosis factor-α, interleukin-1β, interleukin-6, and interleukin-8) (38–40). A recent study documented significantly greater production of the granulocyte recruiting chemokine interleukin-8 from human type II pneumocytes (A549) when subjected to cycles of larger versus smaller stretch (41). A similar phenomenon may result from comparable stresses on the lung during mechanical ventilation with an injurious ventilation strategy.

Conversely, increases in inflammatory mediator production/liberation have been recently questioned in both intact and saline-lavaged models (42). In addition, the recent *ex vivo* perfused rabbit lung study in which hypercapnia was protective against VILI used a perfusate of only 3% blood, nearly devoid of inflammatory cells. The relatively short time course of the current experiments may also be insufficient to see the impact of inflammation on severity of injury. Further investigation is needed to clarify if modulation of the inflammatory response by hypercapnia plays a significant role.

Cytoskeleton and Barrier Function

Disruption of the semipermeable barrier formed by the endothelial cells lining the pulmonary vasculature occurs during acute lung injury and ARDS and results in the movement of fluid, macromolecules, and inflammatory cells into the interstitium and airspaces. Loss of barrier integrity is due to an imbalance between cell–cell adhesive forces and competing cellular contractile forces. The key event in most models of barrier dysfunction is the increase in cellular contractile forces induced by increased phosphorylation of myosin light chains catalyzed by Ca^{2+}/calmodulin-dependent myosin light chain kinase (43). This reaction is agonized by various stimuli including mechanical stretch, a likely mediator of VILI. Data documenting decreased VILI after inhibition of myosin light chain kinase supports this hypothesis (44).

Hypercapnia may decrease the critical phosphorylation of myosin light chains by reducing intracellular Ca^2 available to bind with calmodulin, resulting in reduced phosphoryation of myosin light chain kinase, reduced cellular contractile forces, maintenance of barrier function, and less injury (45).

Conclusion

Our results confirm the recent data by Broccard and colleagues (12) that hypercapnic acidosis confers a protective effect against VILI and further extends their results from an isolated, perfused lung preparation to an intact living model. It further adds to prior studies by Laffey and colleagues, which showed attenuation of ischemia–reperfusion lung injury with hypercapnia (18). The demonstration of this protective effect in an intact animal model further supports the potential of hypercapnic acidosis as an adjunct therapeutic strategy to prevent ongoing lung injury, rather than merely a tolerated side effect of low-stretch ventilation.

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