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Therapeutic Hypercapnia Is Not Protective in the *in vivo* Surfactant-Depleted Rabbit Lung

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ABSTRACT

Permissive hypercapnia because of reduced tidal volume is associated with improved survival in lung injury, whereas therapeutic hypercapnia—deliberate elevation of arterial P_{CO_2}—protects against *in vivo* reperfusion injury and injury produced by severe lung stretch. No published studies to date have examined the effects of CO_2 on *in vivo* models of neonatal lung injury. We used an established *in vivo* rabbit model of surfactant depletion to investigate whether therapeutic hypercapnia would improve oxygenation and protect against ventilator-induced lung injury. Animals were randomized to injurious (tidal volume, 12 mL/kg; positive end-expiratory pressure, 0 cm H_2O) or protective ventilatory strategy (tidal volume, 5 mL/kg; positive end-expiratory pressure, 12.5 cm H_2O), and to receive either control conditions or therapeutic hypercapnia (fraction of inspired CO_2, 0.12). Oxygenation (alveolar-arterial O_2 difference, arterial P_{O_2}), lung injury (alveolar-capillary protein leak, impairment of static compliance), and selected bronchoalveolar lavage and plasma cytokines (IL-8, growth-related oncogene, monocyte chemoattractant protein-1, and tumor necrosis factor-α) were measured. Injurious ventilation resulted in a large alveolar-arterial O_2 gradient, elevated peak airway pressure, increased protein leak, and impaired lung compliance. Therapeutic hypercapnia did not affect any of these outcomes. Tumor necrosis factor-α was not increased by mechanical stretch in any of the groups. Therapeutic hypercapnia abolished the stretch-induced increase in bronchoalveolar lavage monocyte chemoattractant protein-1, but did not affect any of the other mediators studied. Therapeutic hypercapnia may attenuate the impairment in oxygenation and inhibit certain cytokines. Because hypercapnia inhibits certain cytokines but does not alter lung injury, the pathogenic role of these cytokines in lung injury is questionable. **(*Pediatr Res* 55: 42–49, 2004)**

Abbreviations

A-aO_2, alveolar-arterial O_2 gradient
BAL, bronchoalveolar lavage
CON_{inj}, normocapnia, injurious ventilation
CON_{pro}, normocapnia, protective ventilation
CPAP, continuous positive airway pressure
ΔPaw, delta airway pressure
ETT, endotracheal tube
Fico_2, fraction of inspired carbon dioxide
Fio_2, fraction of inspired oxygen
GRO, growth-related oncogene
MCP-1, monocyte chemoattractant protein-1
Paco_2, partial pressure of arterial carbon dioxide
Pao_2, partial pressure of arterial oxygen
PEEP, positive end-expiratory pressure
TH_{inj}, therapeutic hypercapnia, injurious ventilation
TH_{pro}, therapeutic hypercapnia, protective ventilation
TNF-α, tumor necrosis factor-α
VALI, ventilator-associated lung injury
V_t, tidal volume

Mechanical ventilation is central to neonatal and pediatric critical care. Limiting V_t during mechanical ventilation in the setting of acute lung injury results in several important effects, including elevation of Pa_{CO_2} (1, 2), alteration of lung inflammatory events (3), decreased duration of mechanical ventilation (4), and improved survival (5). Although the associations among these effects are clear, the mechanistic relationships are uncertain. Lung stretch is associated with a complex series of pulmonary and systemic events, including neutrophil recruitment (6) and release of inflammatory prostanoids (7, 8) and cytokines (9–11) as well as morphologic injury (6, 12).

Alterations in CO_2 tension may be important in this paradigm for several reasons (13). First, changes in Pa_{CO_2} are

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inextricably linked to tidal stretch, such that reduced stretch increases $Paco_2$ and increased stretch lowers $Paco_2$. The impact of reduced V_t on $Paco_2$ can be abrogated by inversely adjusting ventilatory frequency to maintain overall alveolar ventilation. Nonetheless, some residual elevation of $Paco_2$ is usual (2, 3, 14), resulting in the original term "permissive hypercapnia" (2). Indeed, this clinical approach, originally reported in a case series by Wung *et al.* (1), has been extended in a randomized controlled trial to neonates with hypoxemic respiratory failure (4), with improvement in short-term respiratory outcome. In any case, when stretch is altered in patients, so too is $Paco_2$. Second, alteration of $Paco_2$ has important effects on gas exchange (15, 16) because hypocapnia worsens oxygenation whereas hypercapnia may enhance tissue oxygenation. Third, elevated CO_2 can exert direct antiinflammatory effects in lung injury (17–19). Antioxidant effects of hypercapnia or acidosis have also been demonstrated in cell culture (20), and because many of these inflammatory processes may be operational in stretch-induced injury, their modulation by CO_2 suggests that elevation of $Paco_2$ could have protective effects in VALI. Fourth, deliberate elevation of CO_2—"therapeutic hypercapnia"—protects against primary and secondary ischemia-reperfusion-induced lung injury (17–19), and protective effects have been reported for systemic ischemic injury (21). In addition, two recent studies reported that hypercapnic acidosis protects against stretch-induced lung injury *ex vivo* (22) and *in vivo* (23). However, the clinical role of CO_2 alterations in neonatal practice is unclear (24, 25), and the efficacy of hypercapnic acidosis in *in vivo* models of lung injury associated with surfactant-depletion lung injury is not known.

We used an established *in vivo* rabbit model (saline lung lavage) of surfactant depletion and ventilator-induced lung injury, and used clinically relevant V_t. Our primary aim was to investigate whether therapeutic hypercapnia would improve oxygenation and protect against ventilator-induced lung injury.

METHODS

Male New Zealand White rabbits (Charles River Inc., Quebec, Canada), 2.5 to 3.0 kg, were used in all experiments. All experimental work conformed to the guidelines of the Canadian Council for Animal Care, and was approved by the Animal Care Committee at the Research Institute, Hospital for Sick Children.

Anesthesia and surgical dissection. Premedication was with ketamine (85 mg/kg, intramuscularly). Anesthesia was induced with pentobarbital sodium (15 to 25 mg/kg, i.v.). Incremental bolus doses of pentobarbital (5 mg/kg) were administered as required. Sterile technique was used during all manipulations. A tracheotomy was performed, and a 4-mm (internal diameter) ETT was inserted to a depth of 1 cm and secured in place. Pancuronium (1 mg, i.v.) was administered after depth of anesthesia was confirmed by absence of response to paw compression. Pilot experiments established that anesthetized animals did not exhibit escape behavior in the absence of neuromuscular blockade. The lungs were ventilated using a small animal ventilator (model 683; Harvard Apparatus, MA, U.S.A.) with Fio_2 1.0, rate 22 breaths/min, V_t 9 mL/kg, and 1 cm H_2O PEEP. Under aseptic conditions the right carotid artery was cannulated for arterial pressure measurement and blood sampling. Anesthesia was maintained with pentobarbital ($3–6\ mg\cdot kg^{-1}\cdot h^{-1}$) and muscle relaxation with pancuronium ($0.1–0.2\ mg\cdot kg^{-1}\cdot h^{-1}$), and lactated Ringer's crystalloid ($10–20\ mL\cdot kg^{-1}\cdot h^{-1}$) was administered by continuous infusion into bilateral marginal ear vein cannulas. Body temperature was maintained ($39.0° \pm 1.0°C$) by use of a heating pack, and confirmed with an indwelling rectal temperature probe.

Saline lavage. Surfactant depletion was induced by lavaging the lungs with warmed sterile saline 0.9% (15 mL/kg, temperature 37°C), using a modification of previously described techniques (26, 27). Saline was infused into the trachea from a height of 30 cm. The abdomen and chest were massaged for 30 s to assist intrapulmonary distribution. The effluent was then drained by gravity. After the first lavage, ventilation was recommenced but with PEEP set to 5 cm H_2O with the other ventilatory variables remaining as before. This lavage procedure was repeated until Pao_2 was less than 100 mm Hg. After this target Pao_2 was achieved, ventilation was continued for 30 min, and Pao_2 was measured again. If the Pao_2 had increased to greater than 100 mm Hg by the end of this period, additional lavage was performed.

Lung recruitment. In rabbits in which the Pao_2 was sustained at less than 100 mm Hg at 30 min after lavage, a recruitment maneuver was performed. CPAP at 25–30 cm H_2O was administered for 30 s. After this, the ETT was clamped and reconnected to the ventilator circuit with PEEP set at 12.5 cm H_2O. The ETT was unclamped during expiration, and ventilation was continued for 10 min. Pao_2 measurement was repeated, and recruitment was considered successful when Pao_2 was greater than 200 mm Hg. If Pao_2 was less than 200 mm Hg, the recruitment maneuver was repeated. If at this stage Pao_2 was less than 200 mm Hg, the animal was excluded from further study.

Group allocation. Animals were then randomly allocated to one of four treatment groups, defined by the type of ventilation strategy (protective *versus* injurious) and $Fico_2$, as follows:

- Normocapnia with injurious ventilation (CON_{inj}: $Fico_2$ 0.00; Fio_2 0.75; balance N_2)
- Therapeutic hypercapnia with injurious ventilation (TH_{inj}: $Fico_2$ 0.12; Fio_2 0.75; balance N_2)
- Normocapnia with protective ventilation (CON_{pro}: $Fico_2$ 0.00; Fio_2 0.75; balance N_2)
- Therapeutic hypercapnia with protective ventilation (TH_{pro}: $Fico_2$ 0.12; Fio_2 0.75; balance N_2)

In addition, Evans blue dye (10 mg/kg, i.v.) was administered after randomization.

Ventilatory strategy. The injurious ventilatory strategy consisted of: V_t 12 mL/kg, PEEP 0 cm H_2O, and a rate of 19 breaths/min. The protective ventilatory strategy consisted of: V_t 5 mL/kg, with PEEP 12.5 cm H_2O and rate 52 breaths/min. Ventilation was continued for 2.5 h (150 min), at which time the animals were killed by exsanguination under anesthesia.

Physiologic variables. Systemic mean arterial pressure, peak airway pressures, and rectal temperature were recorded from a standard monitor (Spacelabs Monitor model 90303B;

Spacelabs Medical Products Ltd., Mississauga, Ontario, Canada) at baseline and every 30 min after randomization.

Blood sampling protocol. Arterial blood samples were measured for pH, P_{CO_2}, and P_{O_2}, using an ABL-300 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Lung mechanics. In all experiments, static inflation compliance was measured at baseline and at completion of the study. Static inflation compliance was determined by injection of incremental 5-mL volumes of 100% O_2 and measurement of pressure attained 4 s after each injection until a total volume of 60 mL was injected. Airway pressure was recorded using a pressure transducer (MP45; Validyne Engineering Corporation, Northridge, CA, U.S.A.) connected to the side port of the ETT adapter, and recorded by an online PC IBM-compatible computer (IPC, Pentium microprocessor, 75 Hz, MS DOS 6.0), using analog-to-digital conversion at a sampling rate of 250 Hz (DT2802A; Data Translocation, Marlboro, MA, U.S.A.) and the software package ANADAT/LABDAT (McGill University, Montreal, Quebec, Canada). An inspiratory pressure-volume curve was then constructed.

Bronchoalveolar lavage. Alveolar-capillary protein permeability was performed using a modification of previously described methodology (28, 29). At the end of the experiment, sternotomy was performed, and the lungs excised and lavaged with 20 mL of saline (0.9%, at 37°C), and 3 mL of BAL fluid was collected. This fluid was divided into two 1.5-mL aliquots and centrifuged. One aliquot was then snap-frozen in liquid nitrogen and stored at −70°C for subsequent cytokine analysis. In the second, the concentration of Evans blue dye in the supernatant was then determined spectrophotometrically by measurement of its absorbance at 620 nm.

Cytokine assay. Analysis of serum and BAL fluid IL-8, GRO, MCP-1, and TNF-α was carried out in duplicate and in a blinded fashion. All were measured with previously described rabbit specific immunoassays (30, 31).

Statistical analysis. Baseline variables and lung permeability were compared with one-way ANOVA. Serial variables were compared using 2-way repeated-measures ANOVA, followed, if significant, by one-way ANOVA within groups over time or among groups at each time. Student-Newman-Keuls or Tukey's tests were used for *post hoc* comparisons. Significance was set at $p < 0.05$. A-aO_2 gradient was calculated using the following formula (32):

$$P(A\text{-}a)O_2 = (([R \times PiO_2] + PiCO_2\text{-}PaCO_2 \times [1\text{-}(1\text{-}R) \times FiO_2])/(R + [1\text{-}R] \times FiCO_2))\text{-}(PaO_2)$$

Results are expressed as mean ± SEM. Data were analyzed using SigmaStat (Version 2.0; Jandel Corporation, San Rafael, CA, U.S.A.).

RESULTS

Seven animals failed baseline exclusion criteria, and were not randomized. After *in vivo* surfactant depletion, 28 animals were randomized to one of the following groups:

- Normocapnia with injurious ventilation (high V_t, no PEEP; CON_{inj}; $n = 8$)
- Therapeutic hypercapnia with injurious ventilation (high V_t, no PEEP; TH_{inj}; $n = 8$)
- Normocapnia with protective ventilation (low V_t, PEEP; CON_{pro}; $n = 6$)
- Therapeutic hypercapnia with protective ventilation (low V_t, PEEP; TH_{pro}; $n = 6$)

Baseline variables. The baseline variables (animal weight, temperature, airway pressure, Hb, and base excess) were comparable in all four experimental groups.

Mortality. Two animals in the TH_{inj} group died near the end of the protocol; data from these animals up to the time of death were recorded and used in the analysis.

Arterial Pco_2 and acid base. $Paco_2$ and arterial pH were comparable in all groups at baseline (Fig. 1). After surfactant depletion, randomization, and alteration of $Fico_2$ and ventilatory strategy, $Paco_2$ became most elevated in TH_{inj} (Fig. 1*A*). The final rank order of $Paco_2$ was $TH_{inj} > TH_{pro} > CON_{inj} > CON_{pro}$ ($p < 0.05$; Fig. 1*A*). Arterial pH decreased in all groups except CON_{pro} after institution of the experimental protocol, with the final rank order of pH $TH_{inj} < CON_{inj} < TH_{pro} < CON_{pro}$ ($p < 0.05$; Fig. 1*B*). There were no differences in plasma HCO_3^- among the groups at baseline. Through the experiment, HCO_3^- decreased in CON_{inj}, remained unchanged in TH_{inj}, and increased in both protective ventilation (CON_{pro} and TH_{pro}) groups (data not shown). These changes likely represent a combination of buffering response (renal retention of bicarbonate with hypercapnia) and lactic acidosis in the injury groups.

Oxygenation. There was no difference in baseline Pao_2 among groups (Table 1). After randomization and alteration of Fio_2 (from 1.00 to 0.75) and ventilatory strategy, Pao_2 fell significantly in all groups, but was significantly higher in CON_{pro} and TH_{pro} compared with CON_{inj} and TH_{inj}. Pao_2 remained stable thereafter within all groups for the remainder of the experiment. Pao_2 was higher in TH_{pro} *versus* CON_{pro} at 30 min (Table 1), indicating an effect of hypercapnia that is independent of the ventilation strategy. The rank order of Pao_2 at 150 min was $CON_{inj} = TH_{inj} < CON_{pro} = TH_{pro}$ ($p < 0.05$; Table 1). The A-aO_2 gradient was comparable at baseline in all four groups (Fig. 2*A*). At the end of the experiment (150 min), the rank order of the A-aO_2 gradient was $CON_{inj} = TH_{inj} < CON_{pro} = TH_{pro}$ ($p < 0.05$; Fig. 2*A*).

Lung permeability. Injurious ventilation worsened alveolar-capillary protein permeability as measured by BAL Evans blue dye concentration, compared with protective ventilation (Fig. 2*B*), producing a rank order of microvascular leakage of $CON_{inj} = TH_{inj} > CON_{pro} = TH_{pro}$ ($p < 0.05$). Thus, lung permeability was not altered by CO_2 tension.

Pulmonary mechanics. Peak airway pressure (Paw) was comparable in all groups at baseline. The ΔPaw ($Paw_{final} - Paw_{30\ min}$) was significantly greater in the two injurious ventilation groups (CON_{inj}, TH_{inj}) *versus* the two protective groups (CON_{pro}, TH_{pro}; $p < 0.05$; Table 1), demonstrating a significant impact of ventilatory strategy but no effect of therapeutic hypercapnia. Static inspiratory compliance was comparable in all groups at baseline (Fig. 3*A*). Static inspiratory compliance decreased significantly over the course of the experiment in both injurious ventilation groups (CON_{inj}, TH_{inj}), but remained

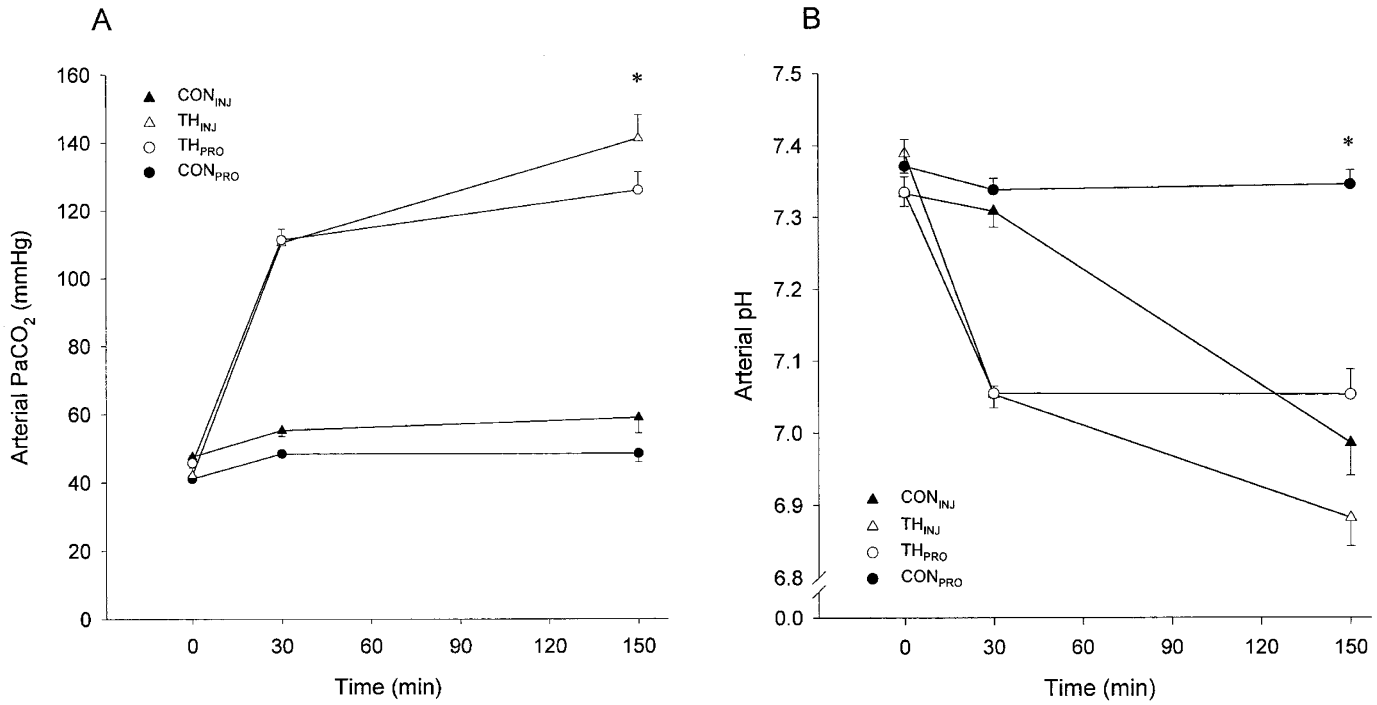

Figure 1. *A,* rank order of final $Paco_2$ was $TH_{inj} > TH_{pro} > CON_{inj} > CON_{pro}$, $*p < 0.05$. *B,* rank order of final pH was $TH_{inj} < CON_{inj} < TH_{pro} < CON_{pro}$, $*p < 0.05$.

Table 1. *Oxygenation and airway pressure*

	CON_{INJ}	TH_{INJ}	CON_{PRO}	TH_{PRO}
Pao_2 (mm Hg)				
Baseline	521.4 ± 12.0	525.2 ± 7.1	497.8 ± 27.9	537.9 ± 13.8
30 min	29.7 ± 0.7*	45.0 ± 2.4*	282.4 ± 32.7*	361.7 ± 24.2*
150 min	34.1 ± 1.7*	38.2 ± 1.6*	282.4 ± 24.9*	325.4 ± 40.2*
Delta peak airway pressure (cm H_2O)	2.4 ± 0.4†	3.4 ± 0.5†	0.3 ± 0.2†	0.5 ± 0.3†

Data are mean ± SEM. Delta peak airway pressure is the difference in peak airway pressure (final minus baseline).
* $CON_{PRO} = TH_{PRO} > CON_{INJ} = TH_{INJ}$; $p < 0.05$.
† $CON_{INJ} = TH_{INJ} > CON_{PRO} = TH_{PRO}$; $p < 0.05$.

unchanged throughout the experiment in both protective ventilation groups (CON_{pro}, TH_{pro}; Fig. 3*B*). Thus, the rank order of final static compliance was $CON_{inj} = TH_{inj} < CON_{pro} = TH_{pro}$ ($p < 0.05$), demonstrating an absence of effect of CO_2 tension on final static lung compliance.

Cytokine measurements. Cytokine measurements were obtained from a limited number of animals ($n = 6$ CON_{inj}, TH_{inj}; $n = 4$ CON_{pro}, TH_{pro}). No TNF-α was detected in the BAL from any samples from any of the groups, and there were only minimal levels of TNF-α in the plasma after injurious ventilation (Table 2). After injurious ventilation, therapeutic hypercapnia was associated with significantly lower BAL MCP-1 levels compared with CON_{inj} and comparable to levels seen with protective ventilation (TH_{pro}, CON_{pro}; Fig. 4*A*). Plasma MCP-1 (Fig. 4*B*) levels were significantly elevated in all groups. Mean levels of these cytokines were lower in both therapeutic hypercapnic groups (TH_{inj}, TH_{pro}) compared with CON_{inj}, although this was not statistically significant. Plasma IL-8 levels increased significantly in all groups during the course of the experiment (Table 2). Plasma GRO levels were significantly elevated compared with baseline in CON_{inj}, and were not significantly elevated in other groups (Table 2). Finally, there were no among-group differences in BAL GRO levels (Table 2).

DISCUSSION

The current study demonstrates that therapeutic hypercapnia did not protect against VALI in terms of oxygenation, vascular permeability, or lung mechanics in an *in vivo* surfactant-depletion model.

Ventilation-induced lung injury. The model used in the current study has been used since the 1980s (6, 33), and is well characterized. The histologic injury in this model reflects the deterioration in oxygenation, as well as the impairment of compliance and microvascular leak (6, 33). The model is useful because the high V_t-low PEEP approach combines the effects of tidal stretch, as well as atelectasis, and thus may be a better model of clinical VALI than pure extreme stretch models given that extreme V_t are no longer used.

CO_2 tension and adult respiratory distress syndrome. Limiting V_t during mechanical ventilation in the setting of acute lung injury

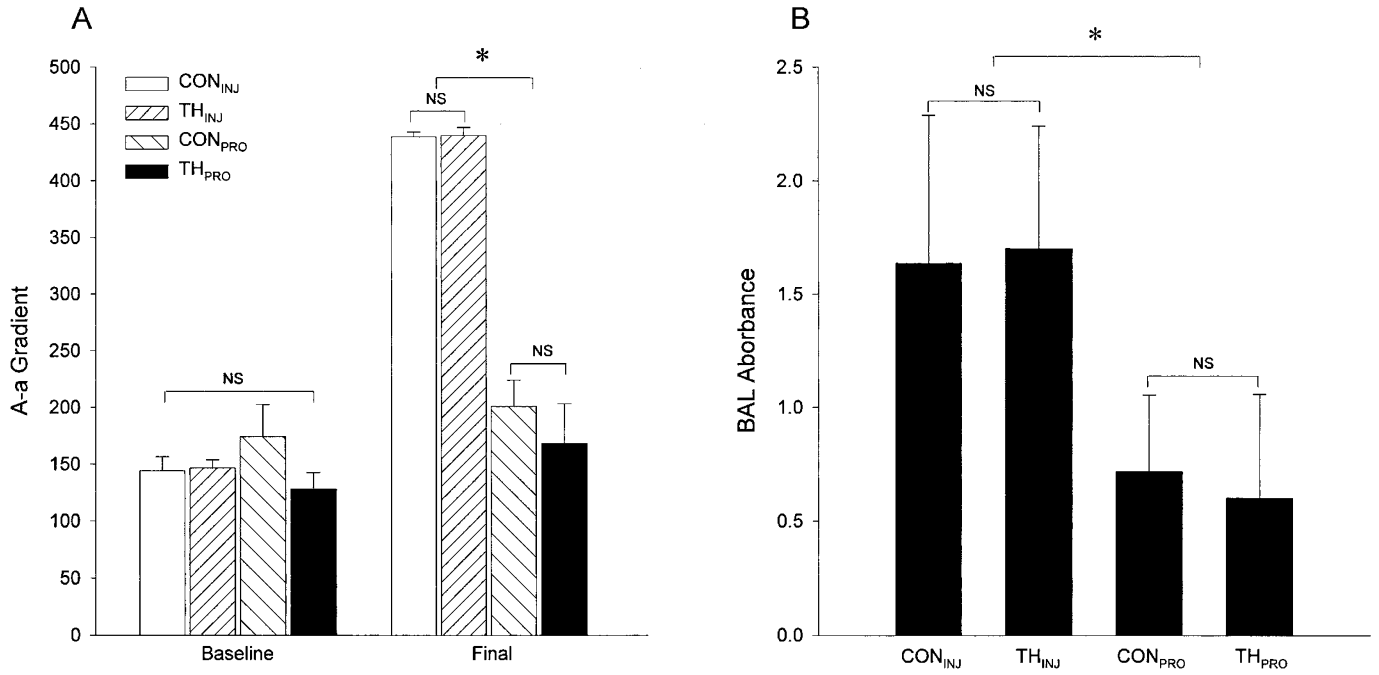

Figure 2. *A,* baseline values for A-aO_2 were similar among groups. The final A-aO_2 was greater in both injurious (TH_{inj}, CON_{inj}) *vs* both protective (CON_{pro}, TH_{pro}) groups (*$p < 0.05$). *B,* represents BAL absorbance of Evans blue dye, reflecting microvascular leak. The rank order of final values was $TH_{inj} = CON_{inj} > CON_{pro} = TH_{pro}$ (*$p < 0.05$).

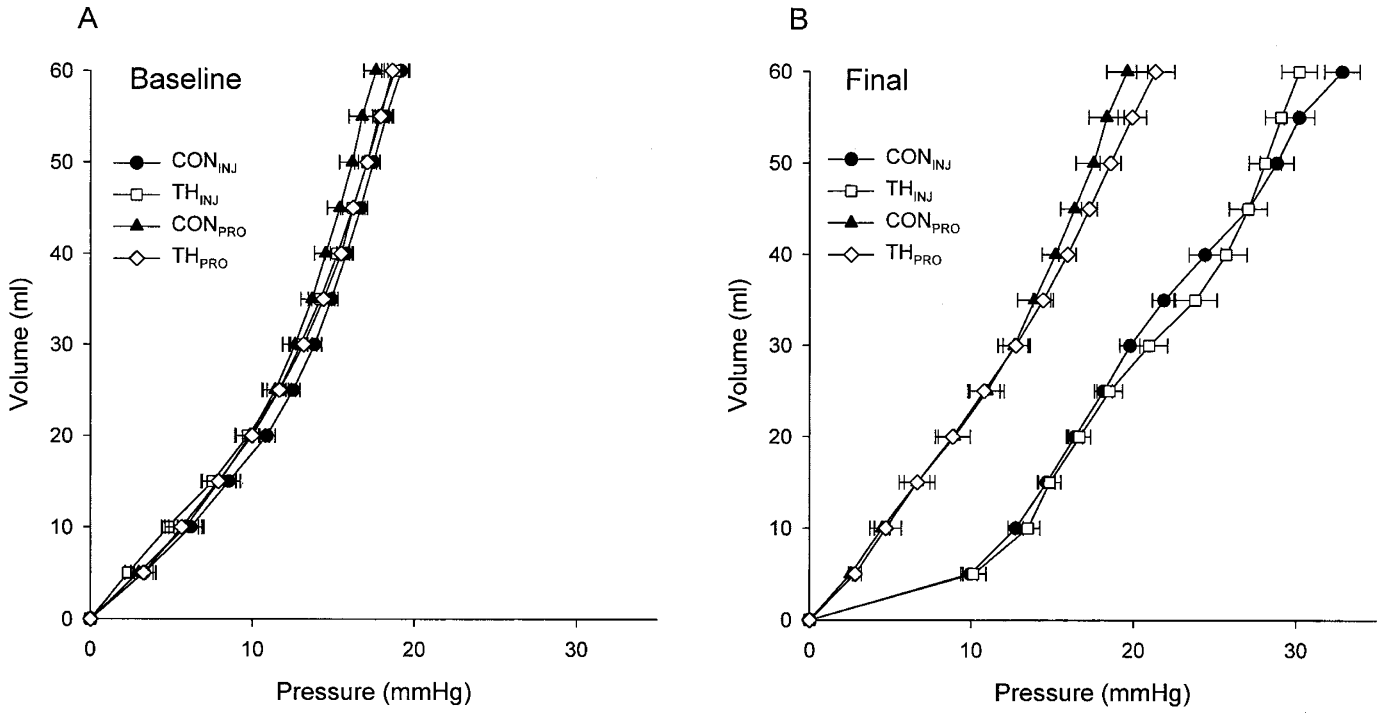

Figure 3. *A,* static inflation compliance was comparable among all groups at baseline. *B,* rank order of final static compliance was $TH_{inj} = CON_{inj} < CON_{pro} = TH_{pro}$ (*$p < 0.05$).

results in elevation of $Paco_2$ (2). This clinical approach, originally reported in a case series by Wung *et al.* (1), has been extended in a randomized controlled trial to neonates with hypoxemic respiratory failure (4). CO_2 has been traditionally considered to play a passive role in the improved outcome observed with protective ventilatory strategies, and was thus termed permissive hypercapnia (2). However, deliberate elevation of CO_2—therapeutic hypercapnia—directly attenuated acute lung injury after free radical generation (19), as well as both primary and secondary ischemia-reperfusion–induced acute lung injury (17–19). In the clinical context, the original reports of deliberate addition of inspired CO_2 were in patients with posttraumatic lung injury (34), in whom the

Table 2. *BAL and plasma cytokine levels*

	CON_{INJ}	TH_{INJ}	CON_{PRO}	TH_{PRO}
BAL concentration (ng/mL)				
IL-8	5.30 ± 6.40	2.80 ± 2.40	5.10 ± 1.50	5.70 ± 1.20
GRO	0.92 ± 0.26	0.93 ± 0.12	0.82 ± 0.13	0.76 ± 0.04
TNF-α	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Plasma concentration (ng/mL)				
TNF-α				
Baseline	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
30 min	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
150 min	0.50 ± 0.80	0.50 ± 1.20	0.00 ± 0.00	0.00 ± 0.00
IL-8				
Baseline	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
30 min	0.03 ± 0.03	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00
150 min	0.75 ± 0.43†	0.44 ± 0.24†	0.89 ± 0.35†	1.05 ± 0.44†
GRO				
Baseline	0.23 ± 0.08	0.20 ± 0.09	0.26 ± 0.08	0.43 ± 0.17
30 min	0.47 ± 0.15	0.25 ± 0.11	0.43 ± 0.15	0.34 ± 0.18
150 min	0.69 ± 0.08*	0.55 ± 0.13	0.63 ± 0.09	0.37 ± 0.07

Plasma GRO concentration increased as a function of time in the CON_{INJ} group (* $p < 0.05$), but not in any other groups. Plasma IL-8 increased with time in all groups († $p < 0.05$), with no among-group differences. Data are mean ± SEM.

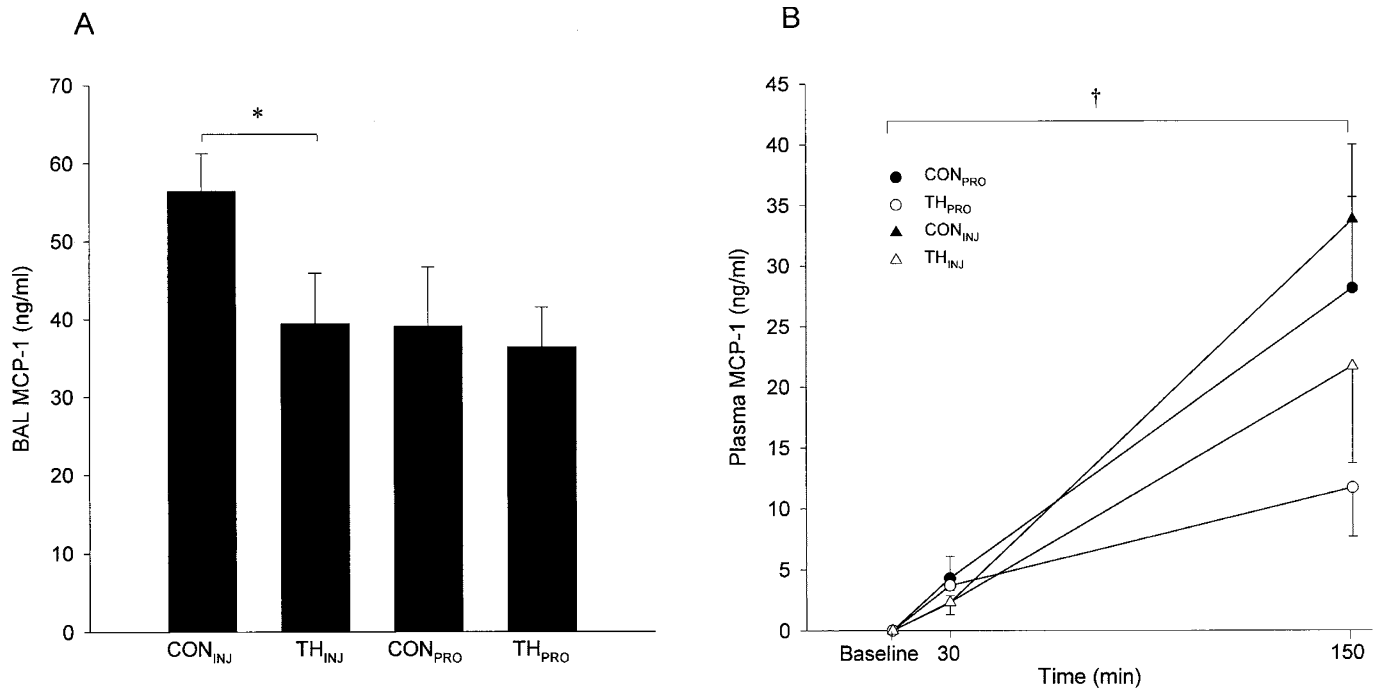

Figure 4. *A,* BAL MCP-1 concentration was less in TH_{inj} *vs* CON_{inj}, *$p < 0.05$; values were similar in the other groups. *B,* plasma MCP-1 increased with time in all groups, †$p < 0.05$, with no among-group differences.

elevated CO_2 appeared to improve cardiac output and oxygenation.

CO_2 and stretch-induced injury. Many of the inflammatory processes described above play a pivotal role in the pathogenesis of VALI. This has led to the suggestion that therapeutic hypercapnia could have protective effects in VALI, a contention supported by recent studies that reported hypercapnic acidosis protects against lung injury induced by severe lung stretch, in both *ex vivo* (22) and *in vivo* (23) models. However, the data in the current study demonstrate that therapeutic hypercapnia does not attenuate indices of lung damage, such as lung permeability and lung compliance in this *in vivo* model of VALI.

Several important experimental differences exist that may help reconcile the contrasting results. The experimental model used by Broccard *et al.* (22) was an *ex vivo* isolated perfused lung preparation, which allows a unique opportunity to measure specific variables (*e.g.* capillary filtration coefficient, microvascular hydrostatic pressure), but which is devoid of the effects of important circulating blood components. The study by Sinclair *et al.* demonstrated protective effects of elevated CO_2, but important differences include the fact that the V_t was extreme (25 mL/kg), the Fio_2 was high (100% inspired O_2), and surfactant depletion was not performed (23); these issues may restrict interpretation. Thus, differences between the current data and other data in the literature may reflect a variety of

model characteristics, including saline lavage, lung volume derecruitment, extremes of V_t, and differences in Fio_2 or Pao_2. Finally, the model used in the current study is prone to development of atelectasis (26, 27), and it is possible that whereas CO_2 may attenuate injury caused by tidal stretch—*i.e.* large V_t (22, 23)—CO_2 may be less effective in ameliorating injury caused by atelectasis. This may be important with the increasingly recognized spectrum of atelectasis-associated lung injury (35).

CO_2 and oxygenation. Alteration of CO_2 tension has important effects on gas exchange (16). In a canine oleic acid–induced lung injury model, hypocapnia worsened oxygenation (15), whereas in a bovine pneumonia model, hypercapnia improved Pao_2 (36). When the complete formula (32) for calculation of A-aO_2 gradient was used with the current data, there was no effect of CO_2. However, the complexity of *in vivo* models is such that important interactions, such as the obligate effect of increased cardiac output on intrapulmonary shunt (37), must be taken into account. The lower systemic arterial blood pressure observed in the CON_{inj} group may reflect the complex nature of heart-lung interactions.

Cytokines and VALI. An evolving body of evidence points to a link between high-stretch ventilation and the release of cytokines, such as TNF-α (8–10, 38, 39) and IL-1β (9, 10, 39). These findings are supported by clinical data demonstrating that high V_t is associated with elevated levels of BAL (3) and plasma IL-6 (3, 5) in patients with adult respiratory distress syndrome. However, three separate *in vivo* studies (40–42) have not been able to demonstrate stretch-induced production of TNF-α, challenging the contention that TNF-α is central to the pathogenesis of VALI. Our findings demonstrate no release of TNF-α into the BAL fluid, and minimal TNF-α release into the plasma with injurious ventilation. These findings cast further doubt on the likelihood of a central pathogenic role for this cytokine in VALI.

A number of studies have reported elevation of IL-8 (or of its functional analog, macrophage inflammatory protein-2) in a variety of different experimental models (40, 43, 44). In the current study, IL-8 is increased to a similar degree in all groups; however, the degree of injury is quite different between protective *versus* injurious ventilatory strategies. Taken together, these data suggest that although IL-8 is released in this model—and other models (40, 43, 44)—the cytokine is unlikely to be uniformly pathogenic in the development of VALI.

Therapeutic hypercapnia and cytokines. Although we detected minimal—or zero—levels of TNF-α resulting from high V_t in the current study, the issue with MCP-1 and GRO is less clear. However, the fact that therapeutic hypercapnia attenuated BAL MCP-1 levels and may have decreased plasma GRO, but did not attenuate the development of severe lung injury in this model, suggests that these cytokines may not be pathogenic in the current model, and casts doubt on an obligatory pathogenic role for these cytokines in VALI. Finally, these findings raise the possibility that in other disease processes in which a pathogenic role for cytokines has been clearly established (*e.g.* sepsis), therapeutic hypercapnia may, by suppressing cytokine production, provide some protection against injury.

Limitations of current findings. There are several issues that limit extrapolation to other contexts. The systemic organ effects of therapeutic hypercapnia were not examined in the current experiments. It is possible that therapeutic hypercapnia-induced attenuation of cytokines may translate to improved systemic organ function; however, the possibility of increased mortality with therapeutic hypercapnia does not support this. It is possible that significant species—or model—variability exists, *e.g.* distribution of pulmonary xanthine oxidase content (45) or species susceptibility to capillary stress failure (46), that could limit application of the findings. However, the current model, or modifications thereof, has been widely studied and has played a major part in the evolution of the literature leading to the clinical recognition of VALI (6, 38, 47). In addition, although designed to represent surfactant depletion as observed in neonates, it is important to note that the animals used in the current study were all adult animals. Finally, the duration of effect may be important, because like virtually all *in vivo* experimental models, the current model uses experimental intervals that are orders of magnitude shorter than the duration of almost any relevant clinical illness. It is possible that with increased duration of injury, different results may have been observed.

CONCLUSIONS

The recognition that lower V_t—without substantial differences in $Paco_2$—improves patient outcome from adult respiratory distress syndrome demonstrates the potential that exists to institute protective ventilatory strategies without resorting to significant hypercapnia. This may be critical, because there could be additional beneficial effects of hypercapnia. However, the opposite might be true, and management with what is now accepted permissive hypercapnia might counter the (now-proven) benefits of lessened lung stretch. This work demonstrates that deliberate hypercapnia does not appear to modulate the severity of ventilation-induced lung injury after surfactant depletion. In addition, we were unable to confirm a consistent effect of stretch on release of cytokines. These findings cast doubt on the potential for therapeutic hypercapnia to attenuate VALI, particularly in the context of atelectasis and severe hypoxemia. Additional experimental work will be needed before clinical testing of therapeutic hypercapnia on outcome can be undertaken.

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