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Hypercapnic Acidosis Attenuates Lung Injury Induced by Established Bacterial Pneumonia

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Background: **Hypercapnic acidosis protects against lung injury after ischemia–reperfusion, endotoxin-induced and ventilation-induced lung injury. The effects of hypercapnic acidosis in the setting of established pulmonary sepsis are not known. The authors investigated whether hypercapnic acidosis—induced by adding carbon dioxide to inspired gas—would be beneficial or deleterious in established *Escherichia coli* pneumonia in an *in vivo* model, in the presence and absence of antibiotic therapy.**

Methods: **Adult male Sprague-Dawley rats were anesthetized and ventilated. In the first set of experiments, rats were anesthetized, *E. coli* (5–6.4 × 10^9/ml colony-forming units) was instilled intratracheally, and the animals were allowed to recover. After 6 h, during which time a severe pneumonia developed, they were reanesthetized and randomly assigned to normocapnia (fraction of inspired carbon dioxide [F_{ICO_2}] = 0.00, n = 10) or hypercapnic acidosis (F_{ICO_2} = 0.05, n = 10). The second set of experiments was performed in a manner identical to that of series 1, but all rats (n = 10 per group) were given intravenous ceftriaxone (30 mg/kg) at randomization. All animals received normocapnia or hypercapnic acidosis for 6 h, and the severity of lung injury was assessed.**

Results: **In the absence of antibiotic therapy, hypercapnic acidosis reduced the pneumonia-induced increase in peak airway pressure and the decrease in static lung compliance compared with control conditions. In the presence of antibiotic therapy, which substantially reduced lung bacterial counts, hypercapnic acidosis significantly attenuated the extent of pneumonia-induced histologic injury.**

Conclusions: **Hypercapnic acidosis reduced the magnitude of the lung injury induced by established *E. coli* pneumonia.**

IN patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), "protective" ventilatory strategies improve outcome.[1,2] These ventilatory strategies reduce tidal and minute ventilation, and the resultant hypercapnic acidosis (HCA) is permitted to realize the benefits of low lung stretch. However, HCA exerts multiple important effects—potentially beneficial and deleterious—on multiple biologic pathways.[3,4] Deliberately induced HCA—by means of added inspired carbon dioxide—is protective in multiple models of lung injury, including ALI induced by free radicals,[5] pulmonary[6] and systemic ischemia–reperfusion,[7] pulmonary endotoxin instillation,[8] and excessive lung stretch.[9,10] Of importance, HCA may have improved outcome in patients who received high stretch mechanical ventilation in the Acute Respiratory Distress Syndrome Network (ARDSnet) tidal volume study.[11]

The safety of HCA in the setting of live bacterial infection is a significant concern.[12,13] ALI and ARDS develop most commonly in the context of severe sepsis,[14–19] particularly infection with gram-negative bacilli[20–22] such as *Escherichia coli*.[23] We have previously demonstrated that HCA does not increase the severity of evolving bacterial pneumonia–induced ALI.[24] However, in the setting of established bacterial infection, such as pneumonia, the antiinflammatory effects of HCA may result in the impairment of the host response to an invading pathogen, permitting greater bacterial proliferation, and ultimately worsening lung injury.[12,13]

Given these issues, we wished to test the hypothesis that HCA would worsen ALI induced by an established *E. coli* bacterial pneumonia in the absence of effective antibiotic therapy. We further hypothesized that this deleterious effect of HCA would be abrogated in the presence of effective antibiotic therapy.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 9A.

This article is accompanied by an Editorial View. Please see: Michelet P: Hypercapnic acidosis: How far? ANESTHESIOLOGY 2008; 109:771–2.

Materials and Methods

Specific pathogen–free adult male Sprague-Dawley rats (300–400 g) were used in all experiments. The experimental model was based on that previously reported by our group, with several modifications.[8,24] All work was approved by the Research Ethics Committee of the National University of Ireland, Galway, Ireland, and conducted under license from the Department of Health, Dublin, Ireland.

Experimental Model

The *E. coli* used in these experiments was of serotype O9 K30 H10 and was supplied by the National Collection of Type Cultures, Central Public Health Laboratory, Lon-

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Table 1. Extent of Lung Injury Developed 6 h after Intrapulmonary *Escherichia coli* Instillation in Comparison with Uninjured Control Animals

Variable	Control, n = 8	*E. coli* Pneumonia, n = 8
Animal weight, g	345 ± 34	353 ± 21
pH	7.49 ± 0.03	7.42 ± 0.01*
Arterial carbon dioxide tension, mmHg	32 ± 4	37 ± 3
Arterial oxygen tension, mmHg	157 ± 6	114 ± 25*
Alveolar–arterial oxygen gradient, mmHg, final (F_{IO_2} = 0.3)	16 ± 6	53 ± 24*
Peak airway pressure, mmHg	4.1 ± 0.8	5.5 ± 0.7*
Static lung compliance, ml/mmHg	0.9 ± 0.1	0.5 ± 0.1*
Mean arterial blood pressure, mmHg	138 ± 19	134 ± 29
Serum bicarbonate, mM	26.5 ± 1.5	24.6 ± 1.6*
Base excess	1.4 ± 1.9	−0.6 ± 2.0

Data are expressed as mean ± SD. Final data were collected upon completion of the experimental protocol.

* Significantly different from control ($P < 0.05$, *t* test).

F_{IO_2} = fraction of inspired oxygen.

don, England. The *E. coli* were stored on preservative beads (Protect, Lancashire, England) at −80°C. The preparation of the bacterial inoculum has been described in detail previously.[24] In brief, beads were placed in 3-ml vials of peptone water (Cruinn Diagnostics, Dublin, Ireland) and incubated at 37°C for 24 h to allow bacterial concentrations to reach a plateau. The bacterial suspension was then centrifuged, washed in phosphate-buffered saline, recentrifuged, and finally resuspended in phosphate-buffered saline to produce the inoculum. The bacterial load in each inoculum was determined by plating serial dilutions on agar plates.

A preliminary series of experiments was performed to determine the bacterial load of intratracheal *E. coli* required to produce an established lung injury over a 6-h period. These experiments demonstrated that the intratracheal instillation of an inoculum of 2.5–7.5 × 10^9 colonies of *E. coli* suspended in 0.3 ml phosphate-buffered saline produced a severe established ALI, compared with noninoculated controls within this time frame (table 1).

Animals were anesthetized by inhalational induction with isoflurane and an intraperitoneal injection of 40 mg/kg ketamine (Pfizer, Kent, United Kingdom). After confirmation of depth of anesthesia by paw clamp, laryngoscopy was performed (Welch Allyn Otoscope®; Buckinghamshire, United Kingdom), and the animals were intubated with a size 16 intravenous catheter (BD Insyte®; Becton Dickinson Ltd., Oxford, United Kingdom). The inoculum of *E. coli* bacteria, suspended in 0.3 ml phosphate-buffered saline, was delivered intratracheally. The animals were then allowed to recover and were returned to their cages.

Six hours after bacterial instillation, at which stage a significant lung injury had been demonstrated to develop in pilot studies, the animals were reanesthetized with intraperitoneal 80 mg/kg ketamine and 8 mg/kg xylazine (Vétoquinol, Dublin, Ireland). After confirming depth of anesthesia by absence of response to paw compression, intravenous access was gained *via* the dorsal penile vein, and anesthesia was maintained with repeated intravenous boluses of Saffan® (0.9% alfaxalone and 0.3% alfadolone acetate; Schering Plough, Welwyn Garden City, United Kingdom). After this, a tracheostomy tube (1-mm ID) was inserted and secured, and intraarterial access (22- or 24-gauge cannulae; Becton Dickinson, Franklin Lakes, NJ) was sited in the carotid artery. Sterile technique was used during all manipulations. After confirmation of the absence of a hemodynamic response to paw clamp, 0.5 mg cisatracurium besylate (GlaxoSmithKline, Dublin, Ireland) was administered intravenously to achieve muscle relaxation, and the lungs were mechanically ventilated (model 683; Harvard Apparatus, Holliston, MA) at a respiratory rate of 80/min, with a tidal volume of 6 ml/kg and positive end-expiratory pressure of 2 cm H_2O. To minimize lung derecruitment, a recruitment maneuver consisting of positive end-expiratory pressure at 15 cm H_2O for 20 breaths was applied every 15 min throughout the protocol.

After assessment of baseline variables, rats were randomly allocated to receive either normocapnia (fraction of inspired carbon dioxide [F_{ICO_2}] = 0.00; fraction of inspired oxygen [F_{IO_2}] = 0.30; fraction of inspired nitrogen [F_{IN_2}] = 0.70) or HCA (F_{ICO_2} = 0.05; F_{IO_2} = 0.30; F_{IN_2} = 0.65) and were then subjected to mechanical ventilation for 6 h. An F_{ICO_2} of 0.05, which has been demonstrated to produce optimal lung protection without the cardiovascular instability seen at higher F_{ICO_2}, was used.[7]

Depth of anesthesia was assessed every 15 min by monitoring the cardiovascular response to paw clamp, and repeated doses of 0.5 mg cisatracurium besylate were administered at regular intervals throughout the protocol to ensure muscle relaxation. Body temperature was maintained at 36°–37.5°C using a thermostatically controlled blanket system (Harvard Apparatus) and confirmed with an indwelling rectal temperature probe. If at any stage during the protocol the mean arterial blood pressure decreased below 40 mmHg for more than 15 min, the experiment was terminated. When animals fulfilled these termination criteria, the measurements obtained at the end of the previous scheduled hourly interval were taken as final measurements. Heparin (400 U/kg) was administered intravenously, and the animals were then killed by exsanguination.

Experimental Protocol

Series 1 was designed to investigate the effects of HCA in established bacterial pneumonia–induced ALI. After anesthesia and dissection, and commencement on mechanical ventilation, animals were randomly assigned to normocapnia (n = 10) or HCA (n = 10) and ventilated for 6 h.

Series 2 was designed to investigate the effects of HCA on established pneumonia-induced ALI in the presence of effective antibiotic therapy. All animals received 30 mg/kg ceftriaxone (Roche Products Ltd., Welwyn Garden City, United Kingdom) intravenously immediately before randomization to normocapnia or HCA. In all other respects, the protocol was identical to that in series 1. The groups were designated as (1) normocapnia (n = 10) and (2) HCA (n = 10).

Measurements

Systemic arterial blood pressure, peak airway pressure, and body temperature were measured after animals were cannulated and mechanically ventilated. After 20 min, an arterial blood sample was drawn for blood gas measurement (ABL500; Radiometer, Copenhagen, Denmark). Lung compliance was assessed by measuring static inflation pressure developed in response to the injection of 5 ml air in 1-ml increments. These measurements were repeated at hourly intervals throughout the protocol.

After completion of the 6-h ventilation protocol or when termination criteria were fulfilled, heparin (400 U/kg) was administered intravenously, the animals were exsanguinated during anesthesia, and the heart–lung block was dissected. Blood was centrifuged, and the plasma was snap frozen for similar analysis.

Immediately postmortem, the heart–lung block was dissected from the thorax, and bronchoalveolar lavage (BAL) was performed. BAL was performed by intratracheal instillation of three aliquots (5 ml each) of normal saline and collection of the returned fluid by free drainage. Total leukocyte numbers per milliliter in the BAL fluid were counted, and differential cell counts were performed after staining with Hema-Gurr Rapid Staining set for Hematology (BDH Laboratory Supply, Poole, United Kingdom). Samples of BAL fluid were centrifuged, and the supernatant was snap frozen in liquid nitrogen and stored at −80°C for further analysis. The concentration of bacteria in the BAL fluid was determined by plating serial dilutions on blood agar plates and performing a colony count 24 h later. The concentration of tumor necrosis factor (TNF) α and interleukin (IL) 6 in the BAL was determined using a quantitative sandwich enzyme-linked immunosorbent assay (R&D Systems, Abingdon, United Kingdom) as previously described.[25,26]

The left lung was isolated and fixed for morphometric examination as previously described.[8,24,27] Briefly, the pulmonary artery was cannulated, the left atrium was incised, and the pulmonary circulation was perfused with normal saline at a constant hydrostatic pressure of 35 cm H_2O until the left atrial effluent was clear of blood. The left lung was then inflated through the tracheal catheter using paraformaldehyde (4% wt/vol) in phosphate-buffered saline (300 mOsmol) at a pressure of 25 cm H_2O. Paraformaldehyde was then instilled through the pulmonary artery catheter at a pressure of 62.5 cm H_2O. The left atrium was then tied off to prevent pulmonary venous inflow into the atrium, creating a constant distending pressure across the pulmonary vasculature, and maximally distending the pulmonary vessels. After 30 min, the pulmonary artery and trachea were ligated, and the lung was stored in paraformaldehyde for 24 h and then embedded in paraffin wax.[27]

The extent of histologic lung damage was determined using quantitative stereologic techniques as previously described.[28] Briefly, the vertical axis of each left lung was identified, and the lung was cut perpendicular to this axis into 4-mm-thick slices with a sharp blade beginning at a position chosen by random number within the first slice. These tissue slices were then embedded in paraffin and sections (7 μm) from each slice mounted on slides and stained with hematoxylin and eosin. An image of each complete lung section was captured as previously described.[7] A point-counting grid was superimposed on the image of each section to estimate its area, and the number of randomly chosen visual fields sampled from any section was proportionate to its area. Each field was examined under light microscopy (× 10 objective; Leica, Laboratory Instruments, Wetzlar, Germany). The images were acquired as described and then imported into Stereology Toolbox (Morphometrix, Davis, CA) at a final magnification of × 60. The intraacinar tissue was defined as all tissues within the gas exchange portion of the lung, *i.e.*, respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli, including blood vessels contained within their walls. The intraacinar airspace was defined as all airspaces within the lumen of respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. The volume fractions of intraacinar tissue and intraacinar airspace were then determined by the use of a point-counting grid as previously described.[28,29]

Statistical Analysis

The distribution of all data was tested for normality using the Kolmogorov–Smirnov test. Results are expressed as mean and SD if normally distributed, and as median and interquartile range if nonnormally distributed. Data that were obtained at multiple time points throughout the experiment, such as arterial oxygen and carbon dioxide tension and pH and airway pressures, were analyzed using a two-way repeated-measures analysis of variance, with group allocation (HCA *vs.* control) as the group factor and time as the repeated measure. Lung histology was analyzed by two-way analysis of variance, with group as the first factor and histologic classification (airspace, intraalveolar tissue, extraacinar tissue) as the second factor. Other data obtained at a single time point were analyzed using a *t* test or Mann–Whitney U test, with the Bonferroni correction as appropriate. Mortality data were analyzed using a Fisher exact

test. A two-tailed *P* value less than 0.05 was considered significant.

Results

HCA without Antibiotic Therapy

Twenty animals underwent bacterial inoculation. All animals survived bacterial inoculation, developed lung injury, and were entered into the treatment protocol. No animals were excluded after randomization into the study. There were no significant between-group differences in the number of bacteria instilled into each animal (table 2). Five animals in the control group and four animals in the HCA group survived the complete protocol, indicating a 55% overall mortality in the study. There was no significant between-group difference in mortality rate or in the duration of survival.

Both groups sustained a comparable degree of pneumonia-induced lung injury at entry into the treatment component of the protocol. Specifically, there were no significant differences between the groups at entry to the treatment protocol in regard to lung function as indicated by arterial oxygen and carbon dioxide tension, arterial pH, peak airway pressure, static compliance, and alveolar–arterial oxygen gradient (table 2 and figs. 1–3).

Table 2. Effects of Hypercapnic Acidosis in Established *Escherichia coli* Pneumonia in the Absence of Antibiotic Therapy

Variable	Normocapnia, n = 10	HCA, n = 10
Animal weight, g	332 ± 26	351 ± 19
Instilled *E. coli* bacterial load, CFU × 10^9/ml	5.6 ± 1.9	5.9 ± 1.6
Mean arterial blood pressure, mmHg		
Baseline	150 ± 19	155 ± 17
Final	84 ± 28†	89 ± 28†
Serum bicarbonate, mM		
Baseline	24.8 ± 1.3	25.3 ± 1.3
Final	12.7 ± 5.1†	13.1 ± 3.8†
Base excess		
Baseline	−0.4 ± 1.6	−0.5 ± 1.9
Final	−15.1 ± 6.9†	−9.1 ± 11.0†
Alveolar–arterial oxygen gradient, mmHg		
Final (F_{IO_2} = 1.0)	306 ± 170	325 ± 149
BAL neutrophil count, × 10^6/ml	1.2 ± 0.4	1.3 ± 0.8
BAL TNF-α concentration, pg/ml	301 ± 186	363 ± 190
BAL IL-6 concentration, pg/ml	3,612 ± 717	4,926 ± 1,422*
End protocol bacterial counts		
BAL, CFU × 10^4/ml	16 [10–18]	17 [13–27]
Blood, CFU × 10^2/ml	64 [16–108]	51 [24–100]

Data are expressed as mean ± SD or as median [interquartile range]. Final data were collected upon completion of the experimental protocol.

* Significantly different from control ($P < 0.05$). † Significantly different from baseline ($P < 0.05$).

BAL = bronchoalveolar lavage; CFU = colony-forming unit; F_{IO_2} = fraction of inspired oxygen; HCA = hypercapnic acidosis; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α.

Arterial pH and carbon dioxide tension were similar in the normocapnia and HCA groups at baseline (fig. 1A and B). There was an initial rapid increase in arterial carbon dioxide tension and decrease in pH in the HCA group after alteration of the F_{ICO_2}. There were further significant decreases in pH over the course of the protocol in both groups. At the end of the experiment, arterial carbon dioxide tension was significantly higher and pH was significantly lower in the HCA group than in the normocapnia group (fig. 1A and B). There was no significant between-group difference in serum bicarbonate or in base excess, at entry into or at the end of the protocol (table 2).

Arterial oxygen tension decreased to a similar extent in the two groups over the course of the experiment (fig. 2A). At the end of the protocol, there was no significant difference in alveolar–arterial oxygen gradient, measured on 100% O_2, between the control and HCA groups (table 2). There was no significant between-group difference in the final alveolar–arterial oxygen gradient, measured after ventilation with F_{IO_2} of 1.0 for 15 min at the end of the experimental protocol (table 2). Peak airway pressure increased significantly in the normocapnia group over time but was unchanged in the HCA group (fig. 2B). Static inspiratory lung compliance decreased significantly from baseline in the normocapnia group over time but was unchanged in the HCA group (fig. 3A). Mean arterial blood pressure decreased significantly in both groups over the course of the treatment protocol (table 2). There were no significant between-group differences in mean arterial blood pressure over the course of the protocol.

There was no significant difference in BAL neutrophil counts or BAL TNF-α levels in the control group *versus* the HCA group (table 2). Of interest, BAL IL-6 levels were significantly higher in the HCA group (table 2). Quantitative stereologic analysis demonstrated that there were no significant between-group differences in airspace or tissue fractions (fig. 3B). There were no significant between-group differences in the bacterial loads in the lungs, as determined by analysis of BAL, or in the blood, at the end of the protocol (table 2).

HCA with Antibiotic Therapy

Twenty-four animals underwent bacterial inoculation. All animals survived bacterial inoculation, but four animals died as a result of the development of a severe lung injury before entry into the treatment protocol. Twenty animals were entered into this study. No animals were excluded after randomization into the study. There were no significant between-group differences in the number of bacteria instilled into the animals (table 3). All animals survived for the entire duration of the treatment protocol in both groups.

The normocapnia and HCA groups sustained a comparable degree of pneumonia-induced lung injury at entry into the treatment protocol. Specifically, there were no significant differences between the groups at entry to the treatment protocol in regard to lung function as indicated by

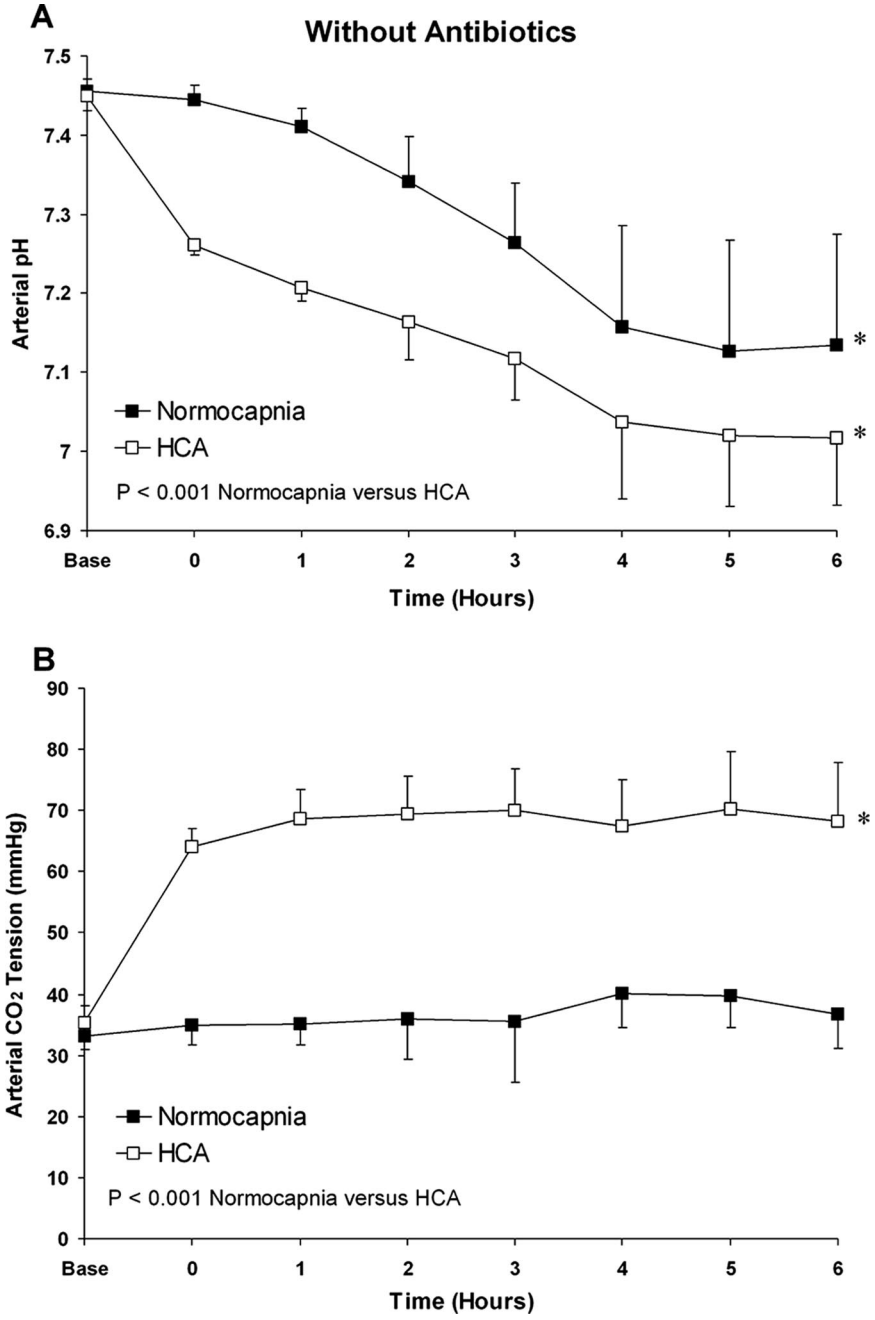

Fig. 1. Evolution of pH (*A*) and arterial carbon dioxide (CO_2) tension (*B*) in the absence of antibiotic therapy in normocapnia (n = 10) and hypercapnic acidosis (HCA; n = 10). Data are mean ± SD. * *P* < 0.05 indicates significant within group change from baseline. The *P* value refers to the overall comparison between HCA and normocapnia.

arterial oxygen and carbon dioxide tension, arterial pH, peak airway pressure, static compliance, and alveolar–arterial oxygen gradient (table 3 and figs. 4 and 5). Although not directly compared, the degree of lung injury at entry into the treatment protocol was greater than that sustained in series 1. This may be because of batch-to-batch animal variation in susceptibility to bacterial infection.

Arterial pH and carbon dioxide tension were similar in the normocapnia and HCA groups at baseline (table 3). There was an initial rapid increase in arterial carbon dioxide tension and decrease in pH in the HCA group after commencement of inspired carbon dioxide. There were further significant decreases in pH over the course of the protocol in the HCA group but not in the normocapnia group. At the end of the experiment, arterial carbon dioxide tension was significantly higher and pH was significantly lower in the HCA group than in the normocapnia group (table 3). The serum bicarbonate levels were significantly lower in the HCA group at the end of the protocol (table 3).

Arterial oxygen tension increased significantly in both groups over the course of the experiment (fig. 4A). There were no significant between-group differences in arterial oxygen tension at any point during the protocol. At the end of the protocol, there was no significant difference in alveolar–arterial oxygen gradient, measured at an F_{IO_2} of 1.0, between the control and HCA groups (table 3). Peak airway pressures did not change significantly in either group over time (fig. 4B).

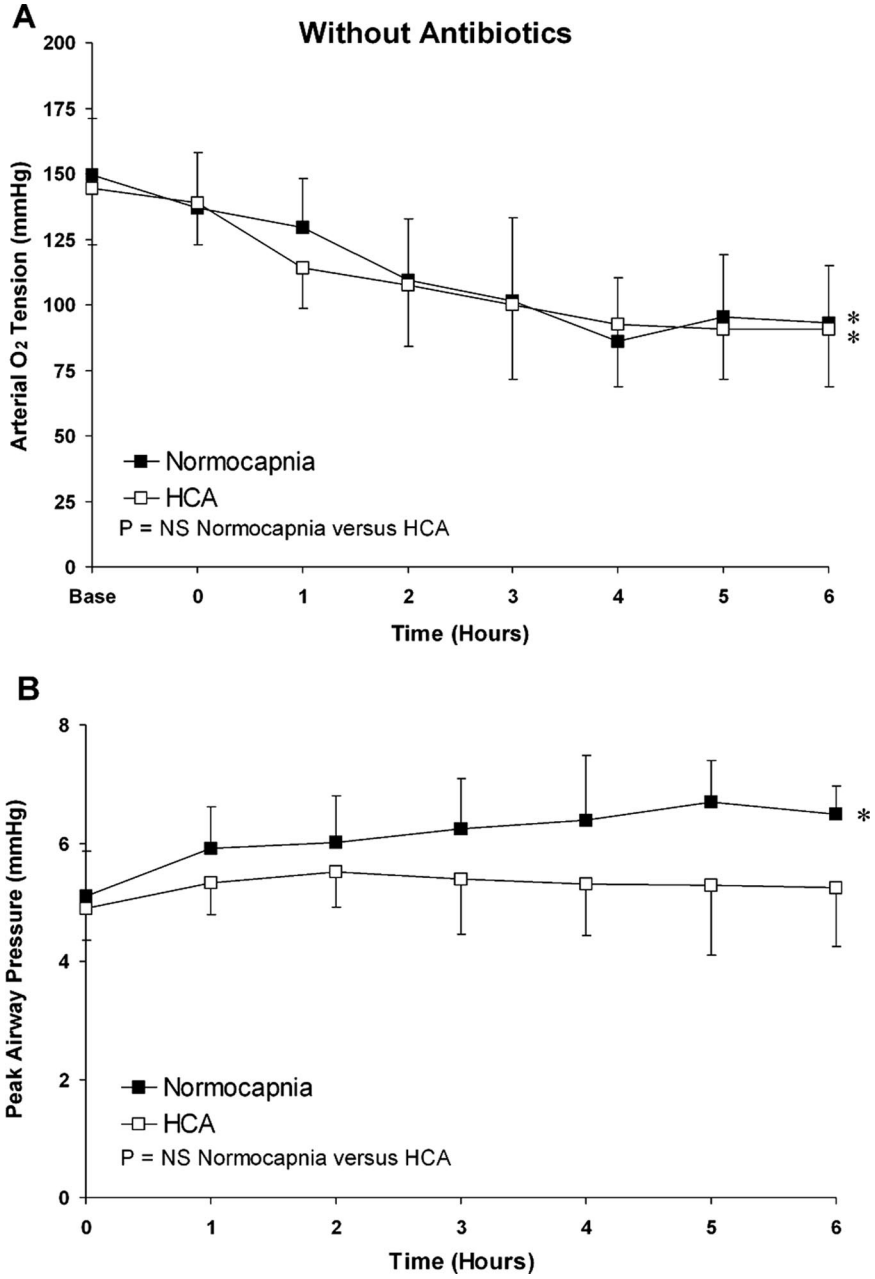

Fig. 2. Graphs representing mean (SD) arterial oxygen (O_2) pressures (*A*) and peak airway pressures (*B*) at baseline and over the course of the protocol in the absence of antibiotic therapy in normocapnia (n = 10) and hypercapnic acidosis (HCA; n = 10). Data are mean ± SD. * $P < 0.05$ indicates significant within group change from baseline. P = NS indicates that the overall comparison between HCA and normocapnia was not significantly different.

At the end of the experiment, there was no significant between-group difference in peak airway pressure (fig. 4B). Static inspiratory lung compliance did not change significantly in either group over time. At the end of the experiment, there was no significant difference in static inspiratory compliance between the normocapnia and HCA groups (fig. 5A). Mean arterial blood pressure decreased significantly in both groups over the course of the treatment protocol (table 3). There were no significant between-group differences in mean arterial blood pressure over the course of the protocol.

There was no significant difference in BAL neutrophil counts, BAL TNF-α levels, or BAL IL-6 levels in the in normocapnia group *versus* the HCA group (table 3). Quantitative stereologic analysis demonstrated that there was significantly less histologic injury in the HCA group. The HCA group had a significantly greater alveolar airspace fraction and a significantly reduced alveolar tissue fraction compared with the normocapnia group (fig. 5B). These data are consistent with a reduced degree of structural lung damage in the HCA group. The numbers of bacteria recovered from the BAL was greatly reduced compared with the animals that received antibiotic therapy. There were no significant between-group differences in the bacterial loads of the lungs, as determined by analysis of BAL. No viable bacteria were present in the bloodstream in any animal from either group (table 3).

Discussion

Deliberately induced HCA has been demonstrated to attenuate ALI in several *ex vivo* and *in vivo* laboratory

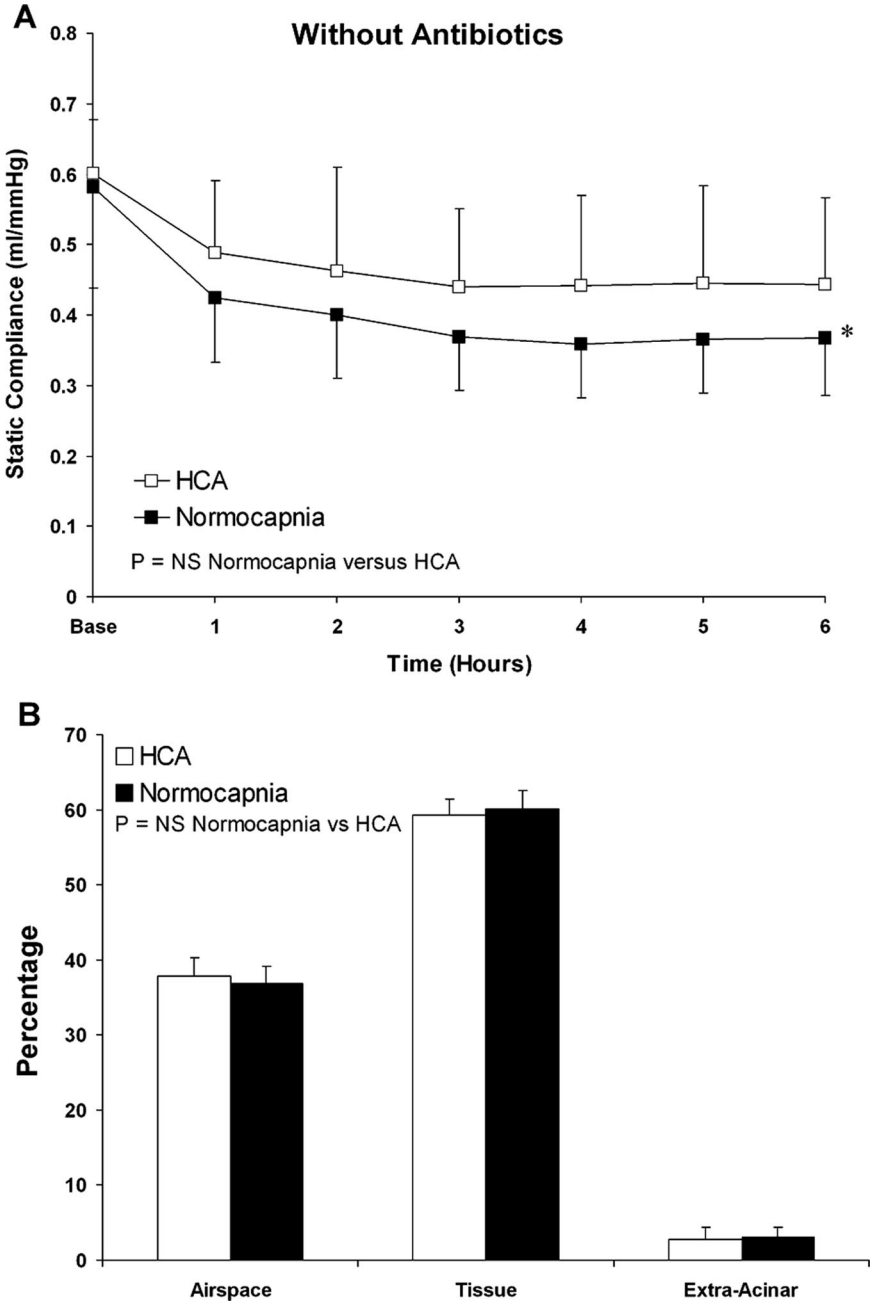

Fig. 3. ***A*** **represents mean (SD) static lung compliance at baseline and over the course of the protocol in the absence of antibiotic therapy in normocapnia (n = 10) and hypercapnic acidosis (HCA; n = 10). * $P < 0.05$ indicates significant within group change from baseline.** ***B*** **represents a stereologic assessment of the extent of histologic injury in established *Escherichia coli* pneumonia in the absence of antibiotic therapy. P = NS indicates that the overall comparison between HCA and normocapnia was not significantly different.**

models of ALI. In the isolated perfused rabbit lung, HCA attenuates the increase in lung permeability seen after free radical–,[5] ischemia-reperfusion–,[5,6] and ventilator–induced[10] ALI. HCA has been demonstrated to attenuate the impairment in oxygenation, the deterioration in lung mechanics, and the increase in lung permeability seen after *in vivo* pulmonary[6] and mesenteric[7] ischemia–reperfusion. HCA also directly protects against endotoxin-induced lung injury—a sterile model of sepsis-induced ARDS.[8] These and other findings have led to the suggestion that HCA may have potential as a therapy in clinical ARDS.[3,12,30]

The safety and potential for adverse effects of HCA in the context of live bacterial sepsis is a key issue given prevalence of hypercapnia in patients treated with contemporary lung ventilation strategies. The concern is that HCA may worsen lung damage and injury caused by live bacterial infection.[12,13] This issue is of central importance given the prevalence of sepsis as a cause of admission to critical care units,[31] the frequency of nosocomial infection in the critically ill,[32] and the fact that severe sepsis associated with multiorgan failure remains a leading cause of death in these patients. The basis for this concern is the known antiinflammatory properties of HCA. While these may be advantageous in reducing the extent of host-induced tissue injury in nonsepsis models, its effects during established bacterial replication and proliferation are as yet unknown.

Table 3. Effects of Hypercapnic Acidosis in Established *Escherichia coli* Pneumonia in the Presence of Antibiotic Therapy

Variable	Normocapnia, n = 10	HCA, n = 10
Animal weight, g	331 ± 31	345 ± 38
Instilled *E. coli* bacterial load, CFU × 10^9/ml	5.6 ± 1.9	5.8 ± 2.8
Mean arterial blood pressure, mmHg		
Baseline	135 ± 22	125 ± 26
Final	101 ± 26†	89 ± 34†
Arterial carbon dioxide tension, mmHg		
Baseline	44 ± 5	43 ± 6
1 h after bacterial instillation	46 ± 9	74 ± 12*†
Final	43 ± 5	71 ± 5*†
Arterial pH		
Baseline	7.35 ± 0.03	7.37 ± 0.03
1 h after bacterial instillation	7.30 ± 0.07	7.17 ± 0.03*†
Final	7.32 ± 0.06	7.13 ± 0.06*†
Serum bicarbonate, mM		
Baseline	23.5 ± 1.6	23.5 ± 1.2
Final	20.7 ± 2.2†	17.8 ± 2.9*†
Alveolar–arterial oxygen gradient, mmHg		
Final (F_{IO_2} = 1.0)	293 ± 104	223 ± 64
BAL neutrophil count, × 10^6/ml	1.6 ± 0.6	1.7 ± 0.3
BAL TNF-α concentration, pg/ml	938 ± 294	869 ± 316
BAL IL-6 concentration, pg/ml	5,748 ± 730	5,446 ± 366
End protocol bacterial counts		
BAL, CFU × 10^3/ml	32 [8–40]	28 [20–72]
Blood, CFU/ml	0 [0–0]	0 [0–0]

Data are expressed as mean ± SD or as median [interquartile range]. Final data are collected upon completion of the experimental protocol.

* Significantly different from normocapnia ($P < 0.05$). † Significantly different from baseline ($P < 0.05$).

BAL = bronchoalveolar lavage; CFU = colony-forming unit; F_{IO_2} = fraction of inspired oxygen; HCA = hypercapnic acidosis; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α.

Hypercapnic acidosis may have a differential impact on outcome depending on the phase of the infection process when it is introduced. Bacteria such as *E. coli* cause tissue damage through several mechanisms, including toxins, direct bacterial damage, and induction of the host immune response. The significance of each depends on the time course of the infection. Early in the infection process, the contribution of bacterial toxins and the host response to the infection may predominate over direct bacterial injury and damage which occur later. The antiinflammatory effects of HCA[3] and its potential to reduce endotoxin-induced ALI[8] may thus limit early tissue injury. Our previous finding that HCA does not increase the severity of evolving bacterial pneumonia[24] provides reassurance regarding the effects of HCA in early lung infection.

In contrast, HCA may impair the host response to established bacterial sepsis. The phagocytic activity and bactericidal capacity of neutrophils and macrophages is central to an effective host response to invading bacteria. HCA also attenuates free radical activity, which is central to the bactericidal activity of neutrophils and macrophages.[6] Therefore, as infection becomes more established, HCA may reduce neutrophil recruitment and microbicidal[33,34] and chemotactic activity,[35] impairing bacterial killing, leading to increased bacterial load and overwhelming bacterial invasion, ultimately exacerbating tissue damage.[12,13]

We used a well-characterized animal model of infection-induced lung injury that mimics the clinical development of ARDS very closely.[36–40] Intratracheal *E. coli* instillation resulted in physiologic and pathologic changes consistent with a significant lung injury at 6 h after bacterial instillation, as evidenced by an overall mortality of 20% before entry into the treatment protocol and the higher airway pressures and lower oxygenation seen at baseline in our animals. These changes were similar to those previously described, and included decrements in lung compliance,[40] decreased oxygen exchange,[40] and alveolar infiltration of neutrophils.[38] This *in vivo* model of established pneumonia has direct clinical relevance, particularly in the context of the critically ill patient, where lung infection and injury is generally well established by the time therapy is instituted.

Based on these issues, we hypothesized that HCA would worsen lung injury in the setting of established pulmonary bacterial sepsis in the absence of effective antibiotic therapy. In contrast to our hypothesis, we found that HCA exerted modest protective effects, reducing the decrement in lung static and dynamic compliance compared with normocapnia. Of importance, there was no evidence to suggest that HCA exerted deleterious effects in established sepsis. In particular, there was no evidence that HCA increased BAL or bloodstream *E. coli* counts, which might have been expected had HCA inhibited bacterial killing.

In this study, we used ceftriaxone, a third-generation cephalosporin with broad spectrum antibacterial effects against both gram-negative and gram-positive organisms and a prolonged elimination half-life. Preliminary laboratory work confirmed that our *E. coli* isolate was sensitive, which is in keeping with reports in the literature.[41] The administration of ceftriaxone at the time of randomization of the animals to normocapnia or HCA reduced the progression of the lung injury and damage. All animals survived the treatment component of the protocol, which contrasts with a mortality of more than 50% without antibiotics. Furthermore, arterial oxygenation improved and airway pressures were reduced in both groups during the treatment protocol. Of importance, HCA reduced the severity of histologic injury, increasing the alveolar airspace fraction, while reducing alveolar tissue fraction, compared with the group that received normocapnia. There were no between-group differences in the bacterial loads of the lungs, which were greatly reduced compared with those seen in animals that did not receive antibiotic therapy.

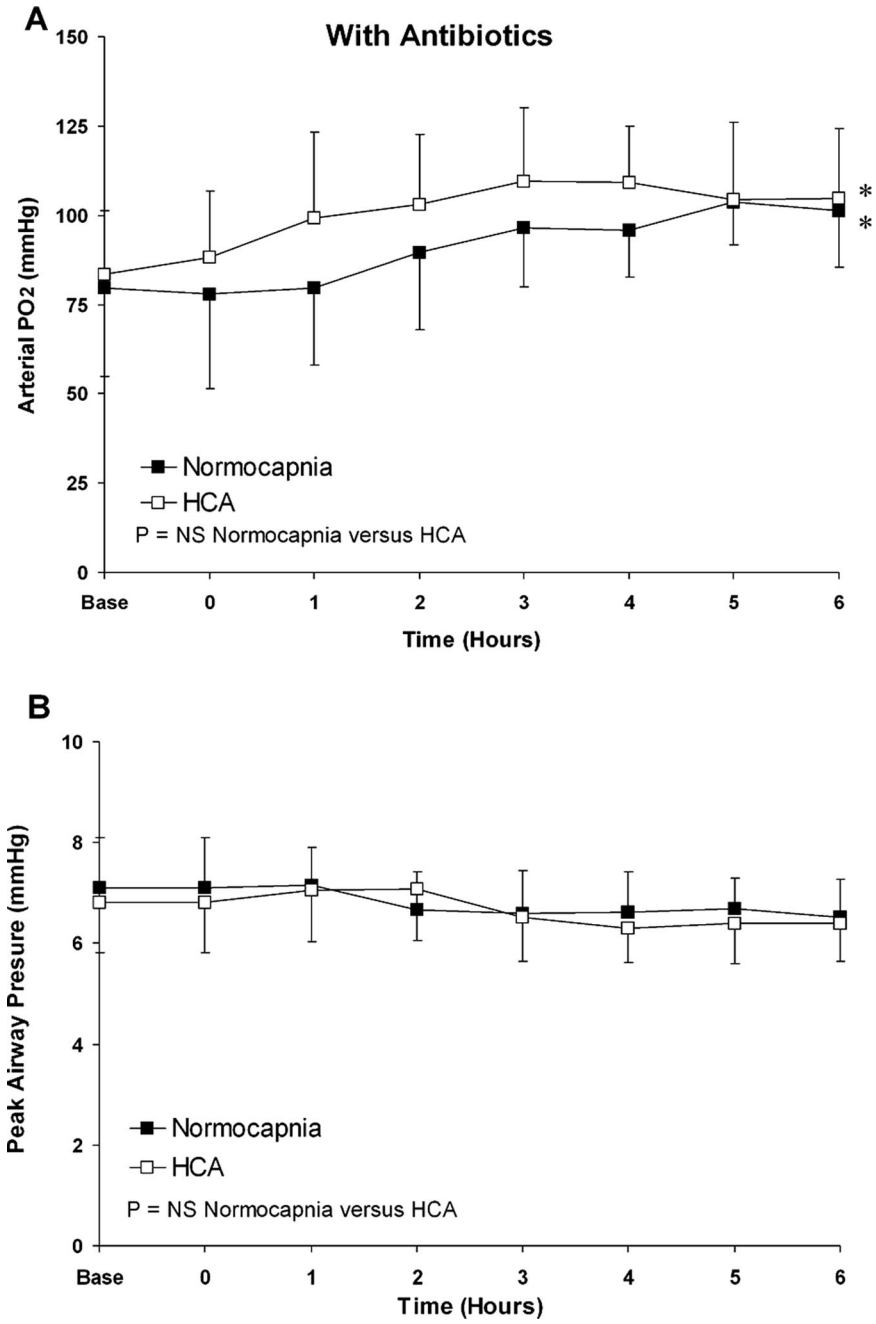

Fig. 4. Graphs representing mean (SD) arterial oxygen pressures (Po_2; *A*) and peak airway pressures (*B*) at baseline and over the course of the protocol in the presence of antibiotic therapy in normocapnia (n = 10) and hypercapnic acidosis (HCA; n = 10). Data are mean ± SD. **P* < 0.05 indicates significant within group change from baseline. *P* = NS indicates that the overall comparison between HCA and normocapnia was not significantly different.

Hypercapnic acidosis seems to exert net beneficial effects in the setting of established bacterial pneumonia, both in the presence and in the absence of effective antibiotic therapy. Potential mechanisms underlying these protective effects include reduction of free radical generation[5]; decreased oxidant-induced tissue damage[8]; attenuation of the concentration of key cytokines, such as TNF-α, IL-1β,[42] and IL-8[43]; and attenuation of cellular immune function.[43–45] The beneficial effects of HCA on lung compliance seem to be due to an attenuation of the increase in alveolar tissue and reduction in alveolar airspace seen in control animals in response to established *E. coli* infection. Other potential mechanisms, such as alterations in alveolar–capillary permeability[46,47] and in surfactant synthesis or function,[48] may also play a role in this protective effect.

Hypercapnic acidosis could lead to an increase in bacterial load *via* two mechanisms, namely enhancement of bacterial growth or reduced bacterial killing. In regard to the potential for HCA to enhance bacterial growth, the concentrations of carbon dioxide used in this study, and indeed seen clinically, have minimal potential to alter the growth of *E. coli*. In fact, the growth rate of *E. coli* has been demonstrated to be unaltered by carbon dioxide values greater than 20%, concentrations of carbon dioxide that markedly exceed those used in this study.[49] Concerns regarding the potential for HCA to reduce bacterial killing are allayed by the finding that HCA did

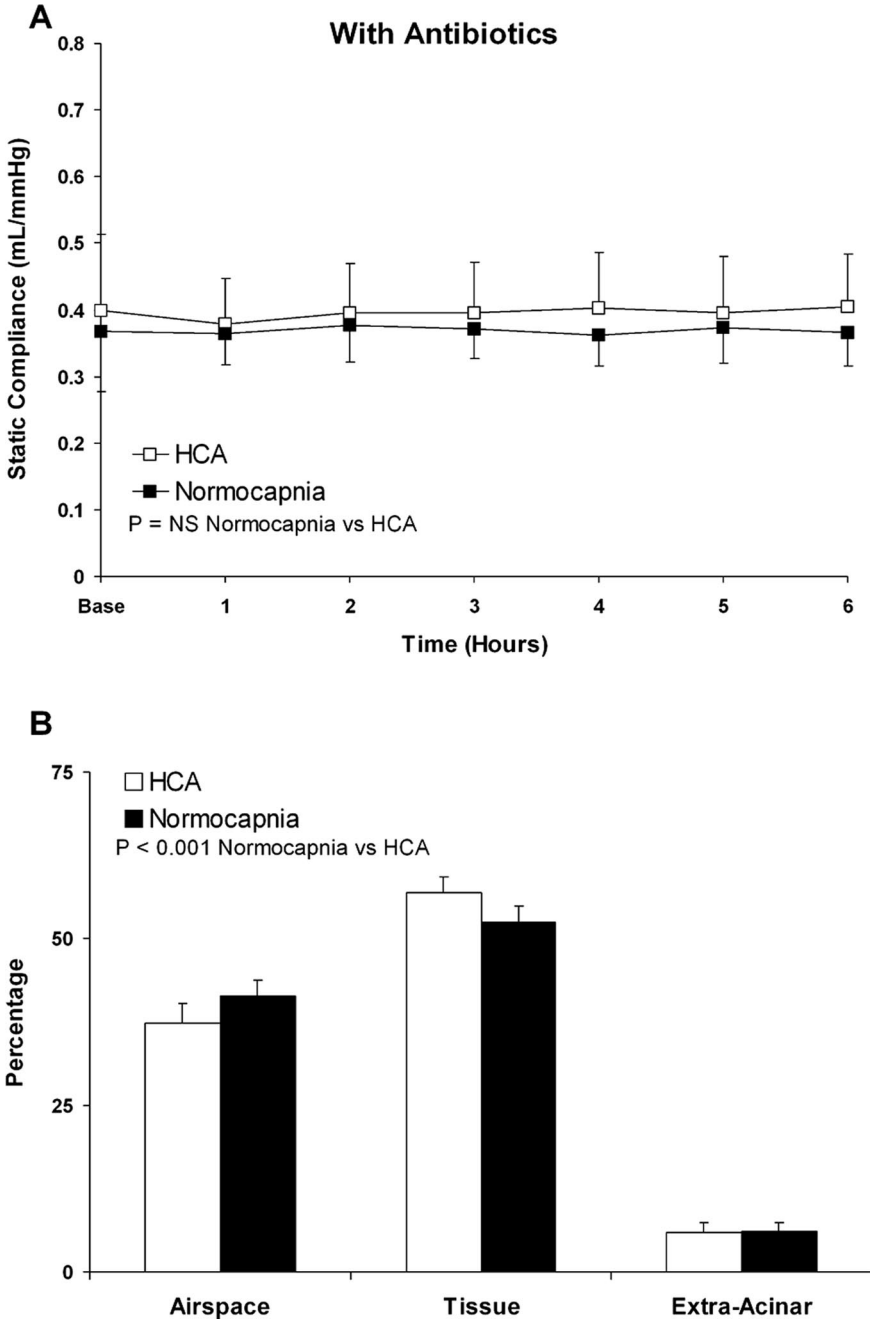

Fig. 5. ***A*** **represents mean (SD) static lung compliance at baseline and over the course of the protocol in the presence of antibiotic therapy in normocapnia (n = 10) and hypercapnic acidosis (HCA; n = 10).** ***B*** **represents a stereologic assessment of the extent of histologic injury in established** ***Escherichia coli*** **pneumonia in the absence of antibiotic therapy. The** ***P*** **value refers to the overall comparison between HCA and normocapnia.**

not increase lung or bloodstream *E. coli* counts in this model. Therefore, it seems that the antiinflammatory effects of HCA limited tissue injury in the setting of established bacterial infection but did not lead to an increase in bacterial load, a major potential concern in the clinical setting.

There are a number of aspects of this study that indicate the need for caution before extrapolation to the clinical scenario. First, this study used a concentration of 5% CO_2, which was based on our previous experience that this was both safe and effective.[7] This produced a degree of hypercapnia and acidosis observed commonly when using protective ventilatory strategies in intensive care settings. Second, ALI and ARDS generally have time courses significantly longer than the 12 h assessed by this model. Differences between groups that were not apparent at 6 h after institution of HCA might be clear after a number of days. Further experiments with a longer observation period might be useful in giving insights into the likely clinical impact of HCA in infection-induced ARDS. A third limitation is the fact that the degree of lung injury 6 h after bacterial instillation at entry into the treatment protocol was greater in the second compared with the first experimental series. The reasons for this degree of variability, although not uncommon in *in vivo* studies, are unclear but may be due

to batch-to-batch animal variation in susceptibility to bacterial infection. The use of anesthesia may have altered the degree of lung injury in this model.[50] However, the anesthetic regimen was identical in all groups in each series. Finally, the effects of HCA in the setting of lung injury produced by other pathogens that are important in the clinical setting, such as *Haemophilus influenzae*,[51] remain to be determined.

We have demonstrated potential beneficial effects of HCA in the setting of established pneumonia-induced ALI. The effects of HCA in this setting are seen in the presence and absence of effective antibiotic therapy. Additional experimental work is needed over longer time periods to further clarify the interactions between HCA and pulmonary infection.

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