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Differential Effects of Buffered Hypercapnia versus *Hypercapnic Acidosis on Shock and Lung Injury Induced by Systemic Sepsis*

Brendan D. Higgins, Ph.D., Joseph Costello, M.B., F.C.A.R.C.S.I.,† Maya Contreras, M.B., F.C.A.R.C.S.I.,† Patrick Hassett, M.B., F.C.A.R.C.S.I.,† Daniel O' Toole, Ph.D.,* John G. Laffey, M.D., F.F.A.R.C.S.I.‡*

Background: **Acute hypercapnic acidosis protects against lung injury caused by nonseptic insults and after both pulmonary and systemic sepsis. The authors wished to dissect the contribution of the acidosis *versus* hypercapnia *per se* to the effects of hypercapnic acidosis on the hemodynamic profile and severity of lung injury induced by systemic sepsis.**

Methods: **In the hypercapnic acidosis series, adult male Sprague-Dawley rats were randomized to normocapnia or hypercapnic acidosis—produced by adding 5% carbon dioxide to the inspired gas—and cecal ligation and puncture performed. In the buffered hypercapnia series, animals were first randomized to housing under conditions of environmental normocapnia or hypercapnia—produced by exposure to 8% carbon dioxide—to allow renal buffering. After 96 h, cecal ligation and puncture was performed. In both series, the animals were ventilated for 6 h, and the severity of the lung injury and hemodynamic deterioration were assessed.**

Results: **Both hypercapnic acidosis and buffered hypercapnia attenuated the development and severity of hypotension and reduced lactate accumulation compared to normocapnia. Hypercapnic acidosis reduced lung injury and inflammation, decreased mean (± SD) bronchoalveolar lavage protein concentration (232 ± 50 *versus* 279 ± 27 $\mu g \cdot ml^{-1}$) and median neutrophil counts (3,370 *versus* 9,120 cells $\cdot ml^{-1}$), and reduced histologic lung injury. In contrast, buffered hypercapnia did not reduce the severity of systemic sepsis induced lung injury.**

Conclusions: **Both hypercapnic acidosis and buffered hypercapnia attenuate the hemodynamic consequences of systemic sepsis. In contrast, hypercapnic acidosis, but not buffered hypercapnia, reduced the severity of sepsis-induced lung injury.**

ADVANCES in our understanding of the potential for high-stretch mechanical ventilation to damage lungs[1,2] has led to the widespread use of "protective" ventilation strategies. These ventilation strategies generally result in elevated arterial carbon dioxide levels termed "permissive hypercapnia," which is tolerated to minimize pulmonary overdistension.[3–5] In addition, the demonstration that acute hypercapnic acidosis (HCA) attenuates acute lung injury in multiple inflammatory [6–9] and septic[10–12] models and in the setting of excessive lung stretch[13–15] has raised the potential that HCA may have therapeutic efficacy in the setting of Acute Lung Injury and Acute Respiratory Distress Syndrome.[16–18]

An important clinical issue is whether or not the acidosis produced by acute hypercapnia should be buffered. Buffering of the hypercapnic acidosis with bicarbonate infusions was permitted in the Acute Respiratory Distress Syndrome network tidal volume study.[3] The effects of buffered hypercapnia in the setting of inflammatory injury may differ considerably from that seen with HCA. Concerns regarding the effects of buffered hypercapnia are underlined by the finding that buffering of a hypercapnic acidosis ablates its protective effects in the setting of pulmonary ischemia–reperfusion *ex vivo*.[19]

The most common cause of severe acute lung injury is sepsis, whether primary pulmonary or secondary to systemic sepsis,[20–25] and it is associated with the poorest outcome.[24,26] We have recently demonstrated that HCA exerts beneficial effects in the setting of severe evolving[11] and established[12] pneumonia-induced lung injury. In contrast, buffering of the hypercapnic acidosis worsens *E. coli*-induced lung injury produced.[27] In the setting of systemic sepsis, our group[10] and others[28] have demonstrated that HCA reduces the severity of lung injury induced after cecal ligation and puncture. The beneficial hemodynamic effects of hypercapnic acidosis in systemic sepsis appear similar to those seen with dobutamine.[28] However, the contribution of acidosis *versus* hypercapnia to the protective effects of HCA in systemic sepsis, and the safety of buffered hypercapnia in this setting, are not known.

Based on the foregoing issues, we wished to dissect the contribution of the acidosis *versus* hypercapnia *per se* to the effects of HCA on the hemodynamic profile and lung injury induced by systemic sepsis. To establish buffered hypercapnia, we exposed rats to a hypercapnic environment for 4 days before the induction of lung injury to induce a renal compensatory response.

Materials and Methods

Specific pathogen-free adult male Sprague-Dawley rats (Harlan, Bicester, United Kingdom) weighing between 400 and 500 g were used in all experiments. All work

* Postdoctoral Research Fellow, † Clinical Research Fellow, ‡ Professor of Anaesthesia and Critical Care Medicine, Department of Anaesthesia, Clinical Science Institute, and Lung Biology Group, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland.

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Address correspondence to Dr. Laffey: Department of Anaesthesia, Clinical Sciences Institute, National University of Ireland, Galway, Ireland. john.laffey@nuigalway.ie.

was approved by the Animal Ethics Committee of the National University of Ireland, Galway, and was conducted under license from the Department of Health, Ireland.

Anesthesia and Dissection

Anesthesia was induced with intraperitoneal ketamine 80 mg · kg^{-1} (Ketalar; Pfizer, Cork, Ireland) and xylazine 8 mg · kg^{-1} (Xylapan; 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 further anesthesia was maintained with an intravenous Saffan infusion (Alfaxadone 0.9% and alfadolone acetate 0.3%; Schering-Plough, Welwyn Garden City, United Kingdom) at 5–20 mg · kg^{-1} · h^{-1}. A tracheostomy tube (2-mm internal diameter) was inserted and secured and intraarterial access (22-gauge cannulae; Becton Dickinson, Cowley, United Kingdom) was sited in the right external carotid artery. After confirmation of depth of anesthesia by using paw clamp, Cisatracurium besilate (0.5 mg; Nimbex, GlaxoSmithKline, Dublin, Ireland) was administered intravenously to produce muscle relaxation. Intravenous access was also sited at the internal jugular vein for fluid infusion and central venous pressure and oxygen saturation measurement. The animals were ventilated by using a small animal ventilator (Model 683, Harvard Apparatus, Kent, United Kingdom) with an inspired gas mixture of 30% oxygen, respiratory rate of 90 breaths/min, tidal volume of 6 ml · kg^{-1}, and positive end-expiratory pressure of 2 cm H_2O. To minimize lung derecruitment, a recruitment maneuver consisting of a positive end-expiratory pressure of 10 cm H_2O for 25 breaths was applied every 15 min throughout the protocol.

Depth of anesthesia was assessed every 15 min by monitoring the cardiovascular response to paw clamp. Body temperature was maintained at 36–37.5°C by using a thermostatically controlled blanket system (Harvard Apparatus, Holliston, MA) and confirmed with an indwelling rectal temperature probe. Systemic arterial pressure, peak airway pressures, and temperature were continuously measured throughout the experimental protocol. After 20 min, an arterial blood gas sample was drawn for blood gas measurement (ABL 705; Radiometer, Copenhagen, Denmark), and lung compliance was measured as described below (Measurement of Physiologic Variables) to confirm baseline stability. These measurements were repeated at hourly intervals over the course of the experimental protocol.

Cecal Ligation and Puncture (CLP) Protocol

The lower half of the abdomen was shaved and disinfected with 100% alcohol, and the cecum was mobilized through an approximately 2-cm-long, median abdominal incision. The cecum was filled by gently "milking back" colon contents and then ligated at 50% of its length with a 3–0 silk ligature distal to the ileo-cecal valve without causing bowel obstruction. The cecum was then subjected to a single through and through perforation with a sterile 18-gauge needle and gently compressed until its contents began to exude, to ensure patency of the perforation sites. The bowel was then repositioned, and the abdominal incision was closed in layers with 4–0 silk sutures. All rats were given 10 ml · kg^{-1} Gelofusine (B. Braun, Dublin, Ireland) intravenously for fluid resuscitation over a 15-min period and then a continuous infusion of 10 ml · kg^{-1} · h^{-1} over the course of the experimental protocol.

Exclusion and Termination Criteria

Before entry into the experimental protocol, the following baseline values were required for continuation with the protocol: arterial oxygen tension greater than 120 mmHg, HCO_3^- greater than 20 mmol · l^{-1}, and temperature of 36.0–37.5°C. Where the criteria were not fulfilled, variables were reassessed after an additional 15 min, during which no specific interventions were performed. Failure to meet the criteria at this point mandated exclusion from the protocol. Thereafter, the experiment was terminated if at any stage during the protocol the mean arterial pressure (MAP) dropped below 30 mmHg for greater than 15 min.

Experimental Protocols

Hypercapnic Acidosis Series. The purpose of this series was to investigate the effect of HCA in the setting of severe systemic sepsis. After anesthesia and dissection, confirmation of the absence of baseline exclusion criteria, the cecum was ligated and punctured, and animals were randomized by using a computerized random number generator to receive normocapnia or HCA. Normocapnia animals were ventilated with an inspired gas mixture of 30% oxygen and 70% nitrogen. HCA animals were ventilated with an inspired gas mixture of 5% carbon dioxide, 30% oxygen, and 65% nitrogen. The animals were then ventilated for 6 h, and the severity of lung and systemic organ injury assessed.

Buffered Hypercapnia Series. The purpose of this series was to investigate the effect of buffered hypercapnia in the setting of severe systemic sepsis. Animals randomized to receive buffered hypercapnia were housed in an environmental chamber in which ambient oxygen was maintained at 21% and carbon dioxide at 8% using automated controllers (ProOx 110 and $ProCO_2$ 120; Biospherix, Lacona, NY). Rats randomized to normocapnia were maintained in 21% oxygen without added carbon dioxide during this time. After 96 h, at which stage tissue buffering of the hypercapnic acidosis was demonstrated to be complete in pilot studies, the animals were anesthetized, and ventilated with an inspired gas mixture of 5% carbon dioxide, 30% oxygen, and 65% nitrogen. Normocapnia animals were ventilated with an inspired by gas mixture of 30% oxygen and 70%

nitrogen. In all animals, the cecum was ligated and punctured, the animals were ventilated for 6 h, and the severity of lung and systemic organ injury was assessed.

Measurement of Physiologic Variables

Intraarterial blood pressure, peak airway pressures, and rectal temperature were recorded continuously for the 6-h duration of the protocol. Hourly assessment of oxygenation, ventilation, and acid-base status was carried out through blood gas analysis. Static inflation lung compliance was measured at baseline and hourly throughout the protocol. Compliance was measured immediately before a recruitment maneuver, ensuring a standardized lung volume history. Incremental 1-ml volumes of room air were injected *via* the tracheostomy tube, and the pressure attained 3 s after each injection was measured, until a total volume of 5 ml was injected.

At the end of the treatment protocol, heparin (400 $IU \cdot kg^{-1}$; CP Pharmaceuticals, Wrexham, United Kingdom) was then administered intravenously, and the animals were then killed by exsanguination.

Tissue Sampling and Assays

Immediately postmortem, the heart-lung block was dissected from the thorax, and bronchoalveolar lavage (BAL) collection was performed as previously described.[29,30] Total cell numbers per milliliter in the BAL fluid were counted, and differential cell counts were performed. The concentrations of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in BAL fluid were determined by using commercially available rat quantitative sandwich enzyme-linked immunosorbent assays (R&D Systems Europe Ltd., Abingdon, United Kingdom). The concentration of total protein in BAL fluid was determined by using a Micro BCA Protein assay kit (Pierce, Rockford, IL) as previously described.[31]

The concentration of bacteria in BAL, blood, and abdominal fluid was determined by plating serial dilutions on blood agar plates and carrying out a colony count 24 h later.

Histologic and Stereologic Analysis

The left lung was isolated and fixed for morphometric examination as previously described.[9,29,32] Briefly, the pulmonary circulation was first perfused with normal saline at a constant hydrostatic pressure of 25 cm H_2O until the left atrial effluent was clear of blood. The left lung was then inflated through the tracheal catheter by using paraformaldehyde (4% $wt \cdot vol^{-1}$) 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. After 30 min, the pulmonary artery and trachea were ligated, and the lung was stored in paraformaldehyde.[32] The extent of histologic lung damage was determined by using quantitative stereological techniques by blinded assessors as previously described.[33,34]

Data Presentation and Analysis

The distribution of all data was tested for normality using the Kolmogorov-Smirnov test. Results are expressed as mean ± (SD) for normally distributed data and as median (interquartile range, IQR) if nonnormally distributed. Data that were obtained at multiple timepoints throughout the experiment, such as arterial oxygen and carbon dioxide tension and pH and airway pressures, were analyzed by using a two-way repeated measures analysis of variance, with group allocation (HCA *vs.* Normocapnia) as the group factor and time as the repeated measure. Lung histology was analyzed by two-way ANOVA, with group as the first factor and histologic classification (airspace, intraalveolar tissue, extraacinar tissue) as the second factor. *Post hoc* testing was carried out by using Student-Newman-Keuls testing, with the Bonferroni correction as appropriate. Underlying model assumptions were deemed appropriate on the basis of suitable residual plots.

Data obtained at baseline and again at the end of the experiment were analyzed by comparing the differences between baseline and final values by using a two-tailed unpaired *t* test. Data obtained at a single timepoint were analyzed by using a two-tailed unpaired *t* test or Mann–Whitney U test, with the Bonferroni correction as appropriate. Mortality data were analyzed by using a Fisher exact test. A two-tailed $P < 0.05$ was considered significant.

Results

Effect of Hypercapnic Acidosis

Twenty-four animals were entered into this study. No animals were excluded before randomization, and all 24 animals were randomized to receive normocapnia (n = 12) or HCA (n = 12). There were no differences between the groups at baseline with regard to animal weight, MAP, central venous pressure, central venous oxygen saturation, arterial oxygen tension, arterial carbon dioxide tension, arterial pH, serum lactate and bicarbonate, peak airway pressure, or static compliance (table 1; figs. 1 and 2). In four animals in the HCA group and five in the normocapnia group, the protocol was terminated early because of sustained hypotension. Eight animals in the HCA group and seven in the normocapnia group survived the entire protocol (table 1).

Arterial Carbon Dioxide Tension and Acid–Base Status. Arterial pH and carbon dioxide tension were similar in the normocapnia and HCA groups at baseline (fig. 1, A and B). Arterial pH decreased significantly in both groups over time. There was an initial rapid decrease in pH and an increase in arterial carbon dioxide tension in the HCA group after the induction of hypercapnia. At each hourly timepoint during the experiment, arterial pH was lower and carbon dioxide tension was higher in the HCA than in the normocapnia group (fig. 1,

Table 1. Effect of Hypercapnic Acidosis

Variable	Normocapnia	HCA
Number of animals	12	12
Animal weight, g	450 ± 30	470 ± 26
Animal survival, %	7/12 (58)	8/12 (67)
Arterial pH		
Baseline	7.43 ± 0.03	7.42 ± 0.03
1 h post-CLP	7.37 ± 0.05	7.19 ± 0.03†‡
Final	7.23 ± 0.09‡	7.11 ± 0.06†‡
Arterial CO_2 tension (mmHg)		
Baseline	34 ± 4.2	37 ± 3.1
1 h post-CLP	34 ± 4.2	61 ± 4.2†‡
Final	31 ± 7.7	60 ± 11.1†‡
Serum bicarbonate, mmol/l		
Baseline	24.2 ± 1.0	24.2 ± 1.0
Final	8.9 ± 3.0‡	13.7 ± 3.0‡
Base excess		
Baseline	–0.8 ± 1.5	–0.6 ± 1.1
Final	–14.6 ± 4.7‡	–8.8 ± 6.8‡*
Time to development of shock, min		
Time to 25% MAP decrease	29 [21, 34]	108 [39, 238]*
Time to 50% MAP decrease	115 [62, 201]	261 [202, 360]*
Central venous pressure, mmHg		
Baseline	4.8 ± 0.9	4.7 ± 0.8
Final	5.1 ± 0.6	5.1 ± 0.8
Central venous oxygen saturation, $S_{cv}O_2$		
Baseline	65 ± 10	67 ± 11
Final	59 ± 6	64 ± 9
Arterial O_2 tension, mmHg		
Baseline	146 ± 10	141 ± 6
1 h post-CLP	133 ± 10‡	150 ± 10‡*
Final	146 ± 10	149 ± 15
Peak airway pressure, mmHg		
Baseline	5.3 ± 0.5	5.4 ± 0.5
Final	6.2 ± 0.5‡	5.8 ± 0.9
Static lung compliance, $ml \cdot mmHg^{-1}$		
Baseline	0.76 ± 0.015	0.74 ± 0.07
Final	0.49 ± 0.10‡	0.54 ± 0.08‡
BAL neutrophil count, ml^{-1}	9120 [4410, 16745]	3370 [2080, 6000]*
BAL TNF-α concentration, $pg \cdot ml^{-1}$	178 ± 114	69 ± 57*
BAL IL-6 concentration, $pg \cdot ml^{-1}$	4103 ± 1657	2936 ± 1712
Bacterial Counts, CFU		
Blood – T180, $\times 10^{10} \cdot ml^{-1}$	1.4 ± 0.4	0.4 ± 0.2*
T360, $\times 10^{10} \cdot ml^{-1}$	1.8 ± 0.9	0.9 ± 0.7
BAL, $\times 10^{7} \cdot ml^{-1}$	7.1 ± 6.3	7.0 ± 5.0
Peritoneal fluid, $\times 10^{12} \cdot ml^{-1}$	12.6 ± 8.4	13.6 ± 9.9

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

* Significantly different from normocapnia ($P < 0.05$). † significantly different from normocapnia ($P < 0.01$). ‡ significantly different from baseline ($P < 0.05$).

BAL = bronchoalveolar lavage; CFU = colony forming units; CLP = cecal ligation and puncture; CO_2 = carbon dioxide; HCA = hypercapnic acidosis; IL-6 = interleukin 6; MAP = mean arterial pressure; TNF-α = tumor necrosis factor alpha; T180 = 180 min post cecal ligation and puncture; T360 = 360 min postcecal ligation and puncture.

A and B). Serum bicarbonate decreased significantly over time in both groups, but there were no differences between the groups at the end of the protocol (table 1). The base excess decreased significantly over time in both groups but decreased to a significantly greater extent with normocapnia *versus* HCA (table 1).

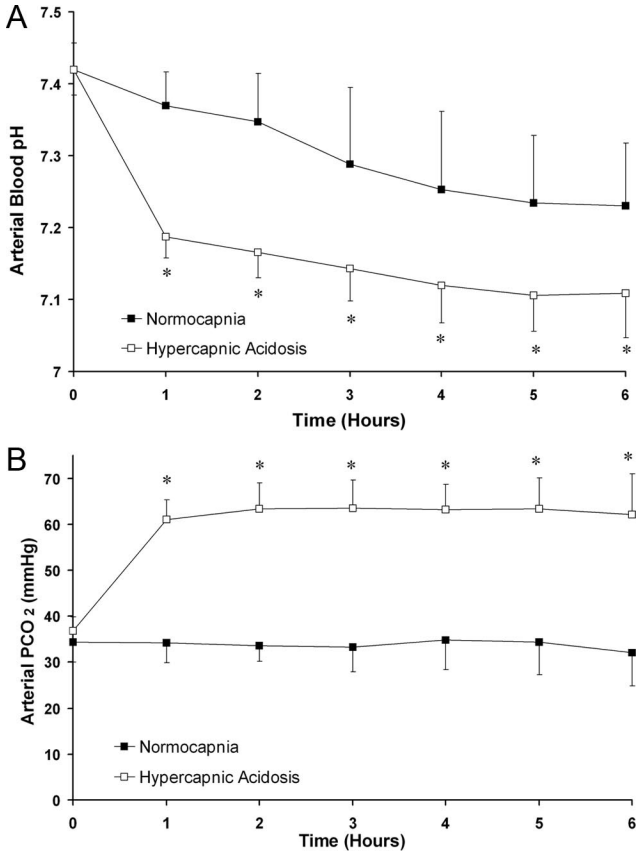

Fig. 1. (*A*) Graph representing mean (SD) arterial pH at baseline and over the course the protocol with hypercapnic acidosis compared to normocapnia. (*B*) Graph representing mean (SD) arterial carbon dioxide tension at baseline and over the course the protocol with hypercapnic acidosis compared to normocapnia. * Significantly different from normocapnia ($P < 0.05$, ANOVA). P_{CO_2} = carbon dioxide tension.

Hemodynamic Data. HCA reduced the development of hypotension and indices of global hypoperfusion compared to normocapnia. MAP decreased significantly in both groups over the course of the protocol. The MAP was significantly lower with normocapnia compared to HCA at 60, 120, and 180 min after CLP, but not at the later time points (fig. 2A). The time required for the MAP to drop by 25% and 50% from baseline values was significantly shorter in the normocapnia group compared to HCA (table 1). There were no changes in central venous pressure in either group over the course of the protocol (table 1). Central venous hemoglobin oxygen saturation did not change significantly in either group over the course of the protocol (table 1). The serum lactate increased progressively over the course of the protocol in both groups, but this increase was significantly greater with normocapnia compared to HCA (fig. 2B).

Lung Injury. HCA decreased the development of lung injury after CLP compared to normocapnia. Arterial oxygen tension decreased significantly with normocapnia and increased with HCA at 60 min after CLP, but not at

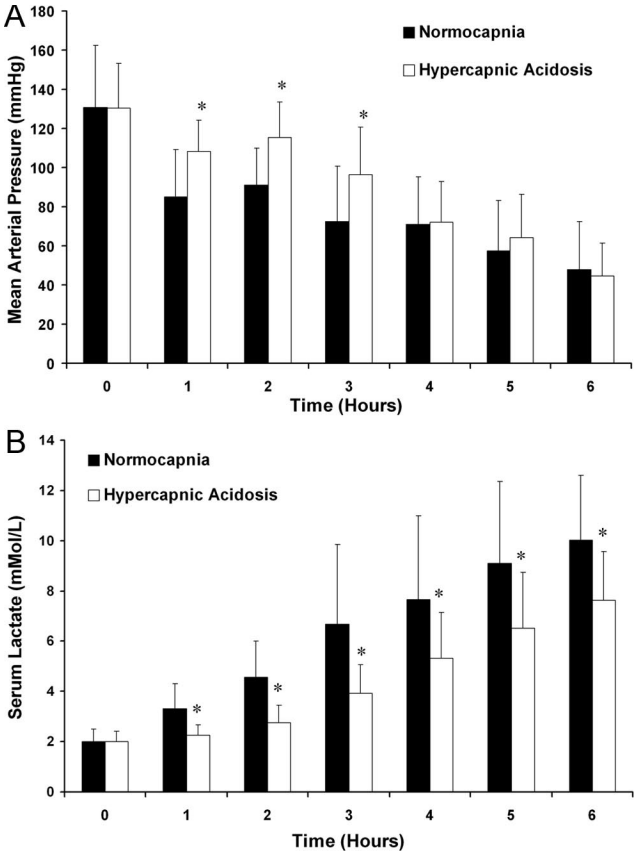

Fig. 2. (*A*) Graph representing mean (SD) arterial blood pressure at baseline and over the course the protocol with hypercapnic acidosis compared to normocapnia. (*B*) Graph representing mean (SD) arterial lactate concentrations at baseline and over the course the protocol with hypercapnic acidosis compared to normocapnia.* Significantly different from normocapnia ($P < 0.05$, ANOVA).

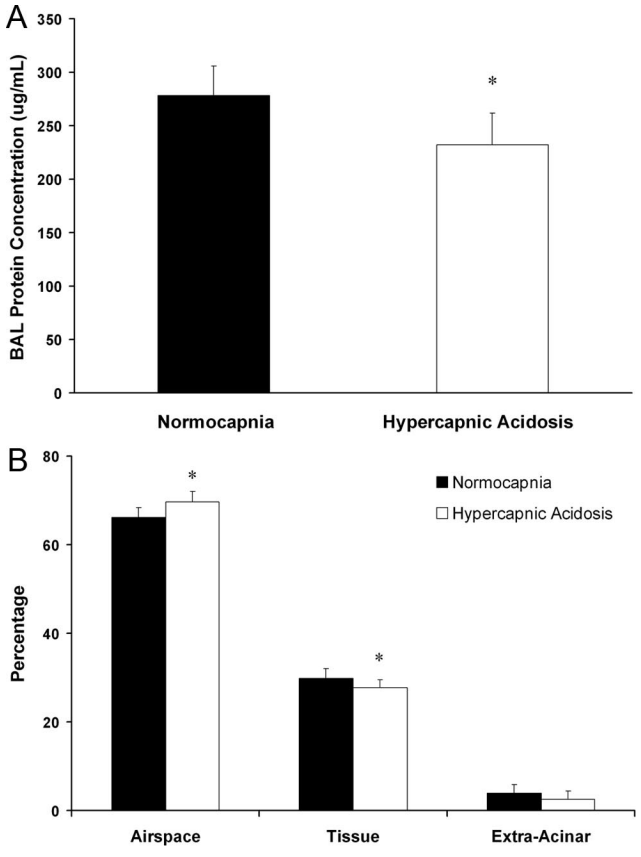

Fig. 3. (*A*) Histogram representing the bronchoalveolar lavage protein concentrations at the end of the protocol with hypercapnic acidosis compared to normocapnia. (*B*) Histogram representing stereologic assessment of the extent of histologic injury with hypercapnic acidosis compared to normocapnia. * Significantly different from normocapnia ($P < 0.05$, *t* test). BAL = bronchoalveolar lavage.

the other time points (table 1). Peak airway pressure increased significantly in the normocapnia group, but was unchanged with HCA over the course of the experiment (table 1). Static inspiratory lung compliance decreased significantly from baseline in both groups over the course of the protocol (table 1). Final static lung compliance was higher with HCA, but this was not statistically significant ($P = 0.09$). HCA significantly reduced BAL protein concentrations compared to normocapnia (fig. 3A). Quantitative stereological analysis demonstrated that HCA reduced the histologic injury produced by CLP. HCA significantly reduced acinar tissue volume fraction and increased acinar air-space volume fraction compared to normocapnia (fig. 3B).

Lung Inflammation. HCA reduced BAL neutrophil counts compared to normocapnia (table 1). BAL TNF-α was significantly lower with HCA, but there was no difference between the groups in BAL IL-6 levels (table 1).

Pulmonary and Systemic Bacterial Load. There were no significant differences between the groups in the bacterial loads of the lungs, as assessed by BAL colony counts (table 1). Of interest, HCA appeared to delay the appearance of bacteria in the blood, as evidenced by reduced blood bacterial load at 180 min following CLP (table 1). However, there were no significant differences between the groups in the bacterial loads in the blood at the end of the protocol (table 1). Peritoneal fluid bacterial loads were similar in both groups.

Effect of Buffered Hypercapnia

Sixteen animals were entered into this study. No animals were excluded before randomization, and all 16 animals were randomized to receive normocapnia (n = 8) or buffered hypercapnia (n = 8). All eight animals in the buffered hypercapnia group and six in the normocapnia group survived the entire protocol (table 2). The protocol was terminated early in two animals in the normocapnia group due to sustained hypotension. There were no differences between the groups at baseline with regard to animal weight, MAP, central venous pressure, central venous oxygen saturation, arterial oxygen tension, peak airway pressure, or static compliance (table 2; fig. 4).

Table 2. Effect of Buffered Hypercapnia

Variable	Normocapnia	Buffered Hypercapnia
Number of animals	8	8
Animal weight, g	470 ± 16	461 ± 24
Animal survival, %	6/8 (75)	8/8 (100)
Arterial pH		
Baseline	7.41 ± 0.01	7.34 ± 0.01†
1 h post-CLP	7.37 ± 0.02‡	7.31 ± 0.01†
Final	7.25 ± 0.10‡	7.22 ± 0.2‡
Arterial CO_2 tension, mmHg		
Baseline	35 ± 3.8	59 ± 4.7†
1 h post-CLP	32 ± 4.0	63 ± 5.5†
Final	24 ± 6.8‡	64 ± 7.9†
Serum bicarbonate, mmol/L		
Baseline	22.5 ± 1.0	27.9 ± 1.7†
Final	12.7 ± 5.0‡	20.3 ± 2.7‡†
Base excess		
Baseline	–2.6 ± 1.3	5.1 ± 2.0†
Final	–15.4 ± 5.2‡	–2.9 ± 3.8‡†
Time to development of shock, min		
Time to 25% MAP decrease	100 ± 82	228 ± 86*
Time to 50% MAP decrease	179 ± 121	343 ± 31†
Central venous pressure, mmHg		
Baseline	4.8 ± 0.8	5.4 ± 1.2
Final	5.3 ± 0.8	5.7 ± 0.9
Central venous oxygen saturation, $S_{cv}O_2$		
Baseline	67 ± 6	68 ± 4
Final	53 ± 8‡	56 ± 12‡
Arterial O_2 tension, mmHg		
Baseline	142 ± 3	146 ± 8
1 h post-CLP	142 ± 8	150 ± 7
Final	146 ± 27	156 ± 10
Peak airway pressure, mmHg		
Baseline	4.3 ± 0.3	4.3 ± 0.5
Final	4.6 ± 0.3	4.4 ± 0.5
Static lung compliance, $ml \cdot mmHg^{-1}$		
Baseline	0.83 ± 0.06	0.81 ± 0.12
Final	0.68 ± 0.04‡	0.68 ± 0.09‡
BAL neutrophil count, $\cdot ml^{-1}$	3867 ± 2097	2852 ± 1379
BAL TNF-α concentration, $pg \cdot ml^{-1}$	158 ± 93	117 ± 91
BAL IL-6 concentration, $pg \cdot ml^{-1}$	3990 ± 1406	2690 ± 1501*
Bacterial counts, CFU		
Blood – T180, $\times 10^{10} \cdot ml^{-1}$	0.4 ± 0.2	0.3 ± 0.1
T360, $\times 10^{10} \cdot ml^{-1}$	0.8 ± 0.4	0.8 ± 0.2
BAL, $\times 10^{7} \cdot ml^{-1}$	4.9 ± 2.3	5.8 ± 2.7
Peritoneal fluid, $\times 10^{12} \cdot ml^{-1}$	15.7 ± 3.1	15.0 ± 1.7

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

* Significantly different from normocapnia ($P < 0.05$). † significantly different from normocapnia ($P < 0.01$). ‡ significantly different from baseline ($P < 0.05$).

BAL = bronchoalveolar lavage; CFU = colony forming units; CLP = cecal ligation and puncture; CO_2 = carbon dioxide; HCA = hypercapnic acidosis; IL-6 = interleukin 6; MAP = mean arterial pressure; TNF-α = tumor necrosis factor alpha; T180 = 180 min post cecal ligation and puncture; T360 = 360 min post cecal ligation and puncture.

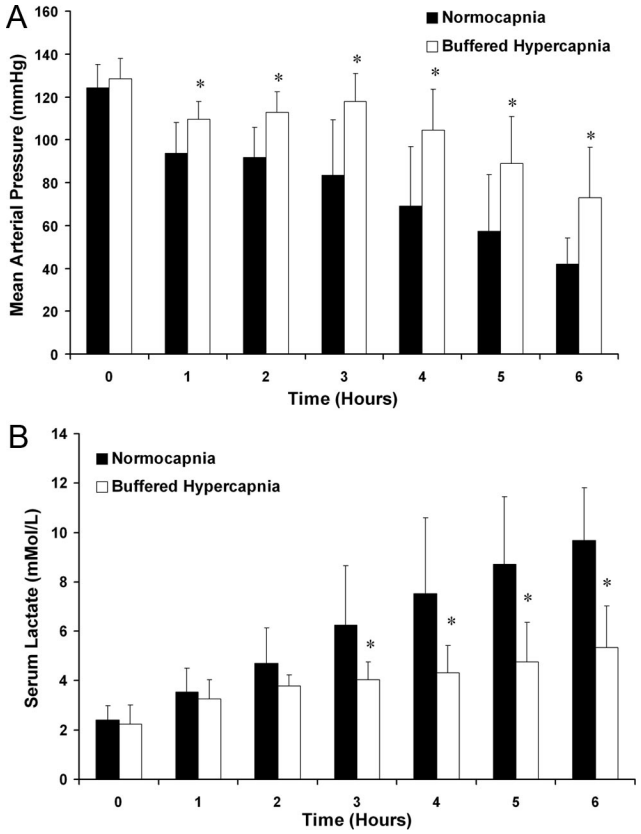

Fig. 4. (*A*) Graph representing mean (SD) arterial blood pressure at baseline and over the course the protocol with buffered hypercapnia compared to normocapnia. (*B*) Graph representing mean (SD) arterial lactate concentrations at baseline and over the course the protocol with buffered hypercapnia compared to normocapnia. * Significantly different from normocapnia ($P < 0.05$, ANOVA).

Arterial Carbon Dioxide Tension and Acid-base Status. At baseline, the serum bicarbonate (HCO_3^-) and the base excess were significantly elevated in the buffered hypercapnia group compared to the normocapnia group, a finding consistent with renal compensation of the hypercapnia-induced acidosis (table 2). Nevertheless, there remained a modest but significant difference in pH between the buffered hypercapnia and normocapnia groups at baseline and up to 120 min post CLP, but not at the later timepoints (table 2). Arterial pH decreased significantly in both groups over time. Arterial carbon dioxide tension was significantly different between the groups at baseline and at all time points throughout the protocol (table 2). Serum bicarbonate decreased significantly over time in both groups, but it remained significantly higher with buffered hypercapnia (table 2). The base excess decreased significantly over time in both groups, but it decreased to a significantly greater extent with normocapnia *versus* buffered hypercapnia (table 2).

Hemodynamic Data. Buffered hypercapnia reduced the development of hypotension and indices of global hypoperfusion compared to normocapnia. MAP decreased significantly in both groups over the course of

the protocol. The MAP was significantly lower with normocapnia compared to buffered hypercapnia at each time point after CLP, and at the end of the experimental protocol (fig. 4A). The time required for the MAP to drop by 25% and 50% from baseline values was significantly shorter in the normocapnia group compared to buffered hypercapnia (table 2). There were no changes in central venous pressure in either group over the course of the protocol (table 2). Central venous hemoglobin oxygen saturation decreased significantly in both groups over the course of the protocol, but there were no differences between groups at the end of the protocol (table 2). The serum lactate increased progressively over the course of the protocol in both groups, but this increase was significantly greater with normocapnia compared to buffered hypercapnia (fig. 4B).

Lung Injury. Buffered hypercapnia did not reduce the development of lung injury after CLP compared to normocapnia. Arterial oxygen tension and peak airway pressure did not change significantly in either group over the course of the experimental protocol (table 2). Static inspiratory lung compliance decreased significantly from baseline in both groups over the course of the protocol, and it was not different between the groups at any time point (table 2). BAL protein concentrations were not significantly different with buffered hypercapnia compared to normocapnia (fig. 5A). Quantitative stereological analysis demonstrated that there was no significant difference between buffered hypercapnia and normocapnia in regard to acinar tissue volume fraction or acinar air-space volume fraction (fig. 5B). These data are consistent with a similar degree of structural lung damage in buffered hypercapnia and normocapnia.

Lung Inflammation. There was no between-group difference in BAL neutrophil counts compared to normocapnia (table 2). BAL IL-6 was significantly lower with buffered hypercapnia, but there was no difference between the groups in BAL TNF-α levels (table 2).

Pulmonary and Systemic Bacterial Load. There were no significant differences between the groups in the bacterial loads of the lungs, as assessed by BAL colony counts (table 2). There were no significant differences between the groups in the bacterial loads in the blood at 180 min or at the end of the protocol (table 2). Peritoneal fluid bacterial loads were similar in both groups (table 2).

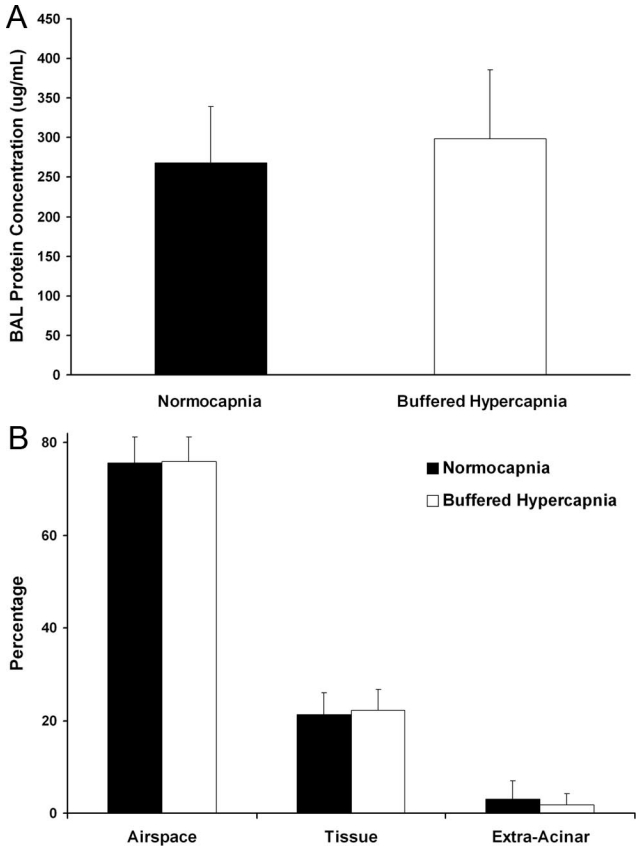

Fig. 5. (*A*) Histogram representing the bronchoalveolar lavage protein concentrations at the end of the protocol with hypercapnic acidosis compared to normocapnia. (*B*) Histogram representing stereologic assessment of the extent of histologic injury with buffered hypercapnia compared to normocapnia. BAL, bronchoalveolar lavage.

Discussion

Buffered hypercapnia is frequently encountered in patients with Acute Lung Injury and Acute Respiratory Distress Syndrome for two reasons. First, the acute buffering of a hypercapnic acidosis with exogenous bicarbonate remains a common, albeit controversial clinical practice. Although buffering of the hypercapnic acidosis with bicarbonate infusions was permitted in the Acute Respiratory Distress Syndrome network tidal volume study,[3] a subsequent analysis demonstrated that hypercapnic acidosis was protective in patients who received high tidal volume ventilation.[35] Second, when hypercapnia persists for more prolonged periods, such as during extended periods of protective ventilation, renal compensation of the hypercapnic acidosis gradually corrects the pH close to the normal range.

Sepsis, whether due to pneumonia or systemic infection, remains a major cause of acute lung injury,[20–25] and it is associated with the poorest outcome.[24,26] In addition, sepsis may complicate critical illness as a result of other causes, as evidenced by the frequency of ventilator-associated pneumonia in the critically ill.[36] We have recently demonstrated that HCA exerts beneficial effects in the setting of severe evolving[11] and established[12] pneumonia and endotoxin[9]-induced lung injury. In contrast, buffering of the hypercapnic acidosis actually worsened the lung injury induced by intrapulmonary endotoxin and *E. coli* instillation,[27] further underlining concerns regarding the effects of buffered hypercapnia in the setting of sepsis.

Acute hypercapnic acidosis exerts beneficial effects on the hemodynamic profile and the extent of lung injury

produced by systemic sepsis.[10,28] However, the contribution of acidosis *versus* hypercapnia to the effects of HCA on the lung and hemodynamic profile in systemic sepsis, and the safety of buffered hypercapnia in this setting, are not known. Hypercapnia activates the sympathetic nervous system, which may account for its beneficial hemodynamic profile in systemic sepsis. In fact, the beneficial hemodynamic effects of hypercapnic acidosis in systemic sepsis appear similar to those seen with dobutamine.[28] In contrast, the beneficial effects of hypercapnic acidosis in experimental lung injury appear to be the result of its antiinflammatory effects, which include: attenuation of cellular immune function[37–39] reduction of free radical generation[6] and oxidant-induced tissue damage[9] and reduction in the levels of key cytokines such as TNF-α, IL-1,[40] and IL-8.[39] Buffering of a hypercapnic acidosis ablated its antiinflammatory effects in the setting of pulmonary ischemia–reperfusion *ex vivo*.[19]

We established buffered hypercapnia in these experiments by exposing rats to hypercapnia over a 4-day period, during which renal mechanisms acted to buffer the initial pH changes. An alternative approach that might have been used to achieve buffered hypercapnia would have been to ventilate previously normocapnic rats with elevated inspired carbon dioxide and to correct the pH to normal by infusing sodium bicarbonate. However, the results of such experiments would be very difficult to interpret because bicarbonate infusions can exert adverse effects on tissues and organs.[41–45] Moreover, bicarbonate infusions can produce complex effects that are not due to changes in pH. For example, bicarbonate infusions have actions caused by their significant sodium content, including immunomodulatory[46] and hemodynamic actions.[47] Bicarbonate infusions can also cause paradoxical increases in carbon dioxide or can worsen intracellular acidosis without further elevating arterial carbon dioxide tension.[48] Tromethamine, an organic amine proton acceptor, which crosses the cell membrane and does not generate carbon dioxide, is an alternative pharmacologic approach to buffer the hypercapnic acidosis. However, tromethamine is not widely available clinically, and it would have required a continuous infusion throughout the experimental protocol to buffer the acidosis. By using sustained exposure to hypercapnia to produce buffered hypercapnia, we avoided these confounding aspects of acute pH correction.

Both hypercapnic acidosis and buffered hypercapnia reduced the severity of early septic shock produced by CLP in these studies. Although not directly compared in these studies, buffered hypercapnia appeared to result in better maintenance of arterial blood pressure than HCA. Both HCA and buffered hypercapnia attenuated the increase in serum lactate, compared to normocapnia. It is possible that the improved hemodynamic picture seen with buffered hypercapina may be due in part to sodium retention and volume expansion caused by hypercapnia-induced sympathetic stimulation. However, there was no difference in the body weight of the animals exposed to buffered hypercapnin compared to normocapnia. Central venous pressures did not change throughout the protocol, further reducing the likelihood that differences in fluid volume status contributed to the hemodynamic picture in either group. These findings regarding the hemodynamic effects of HCA and buffered hypercapnia confirm and extend the previously reported findings of our group[10] and those of Wang *et al.*,[28] who demonstrated that acute HCA improved indices of tissue oxygenation in septic shock produced by CLP.[28]

Hypercapnic acidosis reduced the severity of lung injury induced by systemic sepsis, reducing the decrement in dynamic lung compliance and oxygenation, attenuating the increase in lung permeability, and reducing histologic evidence of injury, compared to normocapnia. Of importance, HCA reduced indices of lung inflammation, namely reduced BAL neutrophil counts and reduced BAL TNF-α but not IL-6 concentrations compared to normocapnia. In contrast, buffered hypercapnia did not demonstrate protective effects in the lung after CLP. There was no effect of buffered hypercapnia on physiologic or histologic indices of lung injury. Of interest, buffered hypercapnia did reduce BAL IL-6 concentrations, but did not alter BAL neutrophil counts or BAL TNF-α levels.

It is possible that HCA simply delays the effects of systemic sepsis on the hemodynamic profile and lung injury, rather than reducing it. HCA appeared to reduce the speed of entry of bacteria into the blood, as evidenced by reduced blood bacterial load in the blood at 180 min. While blood bacterial load was reduced with HCA at 360 min, these differences were not statistically significant. Therefore, the effect of HCA might ultimately be abrogated, as evidenced by the fact that the hemodynamic benefits of HCA were not sustained beyond the first 3 h. In a separate study, we did examine the effects of HCA in prolonged (96 h) CLP-induced systemic sepsis.[10] These studies demonstrate that HCA reduced the severity of lung injury induced by prolonged systemic sepsis and that this effect occurred despite the fact that HCA did not cause any changes in pulmonary, blood, or peritoneal bacterial load.[10]

There was no evidence that either HCA or buffered hypercapnia increased the bacterial load in the bronchoalveolar lavage, blood, or peritoneal fluid after CLP. These findings provide reassurance regarding the safety of HCA and buffered hypercapnia in the setting of bacterial sepsis. However, our group has previously reported that hypercapnia exerted deleterious effects in the setting of prolonged bacterial pneumonia.[30] In this study, sustained hypercapnia worsened pneumonia-induced lung injury and increased bacterial load in the lungs.[30] The increased bacterial numbers in this study appear to have been the result of reduced bacterial

killing, as evidenced by reduced neutrophil phagoctyic activity rather than from hypercapnia-enhanced growth of *E. coli*.[30] In fact, the growth rate of *E. coli* is unaltered by carbon dioxide values greater than 20%, concentrations of carbon dioxide that markedly exceed those used in either study.[49] The finding that prolonged hypercapnia did not worsen the severity of lung injury caused by CLP[10] and studies demonstrating that carbon dioxide pneumoperitoneum increases survival in mice and rabbits after CLP[50,51] provide reassurance regarding the efficacy and safety of hypercapnia in systemic sepsis. The mechanisms underlying the differing findings regarding the effects of prolonged hypercapnia in pulmonary *versus* systemic sepsis are unclear. Taken together, these findings suggest that the effects of prolonged hypercapnia may depend on the location of primary infection.

HCA may be more effective in the setting of more severe lung injury. In the analysis by Kregenow *et al.* of the data from the Acute Respiratory Distress Syndrome Network tidal volume study,[3,35] HCA was associated with benefit in patients who were ventilated with the higher tidal volume strategy, but the effect was not seen with the lower tidal volume strategy. These findings are consistent with the demonstration that HCA directly attenuates high stretch-induced lung injury in experimental models.[13,14] However, where lower levels of lung stretch are used, HCA is less effective[15] or may exert no benefit.[52] In the setting of pulmonary sepsis, although HCA demonstrated therapeutic benefit in the setting of severe acute[11] and established pneumonia,[12] it did not exert beneficial effects in the setting of less severe pneumonia.[29] In these studies, HCA exerted beneficial effects in the context of a relatively mild systemic sepsis-induced lung injury. The effects of HCA in a more severe systemic sepsis-induced lung injury remain to be determined.

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% carbon dioxide, which was based on our previous demonstration that this concentration range was both safe and effective.[8] This produced a degree of hypercapnic acidosis similar to that commonly observed when using protective ventilatory strategies. The effects of higher concentrations of carbon dioxide in systemic polymicrobial sepsis are not known, but they are potentially important given that higher doses are deleterious in other lung injury models.[8] However, the beneficial effects of a carbon dioxide pneumoperitoneum, which likely results in high intraperitoneal carbon dioxide tensions, may allay these concerns in regard to abdominal sepsis. Furthermore, in our experiments hypercapnia, was introduced either before or at the time of commencement of the CLP injury. In the buffered hypercapnia animals, buffering occurred before CLP. In the clinical setting, buffering is generally a gradual process that occurs after the injury. It is not clear what effect HCA or buffered hypercapnia might have if introduced well after the establishment of infection. Finally, the finding that HCA reduced the decrement in arterial oxygen tension in early systemic sepsis may be explained in part by its potential to improve V/Q matching.[53] However, HCA did reduce the severity of other physiologic and histologic indices of lung injury in these studies.

Conclusion

We report that both HCA and buffered hypercapnia attenuate the hemodynamic consequences of systemic sepsis induced by cecal ligation and puncture. In contrast HCA, but not buffered hypercapnia, reduced the severity of sepsis-induced lung injury.

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