Hypercapnic Acidosis May Attenuate Acute Lung Injury by Inhibition of Endogenous Xanthine Oxidase

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Relative hypoventilation, involving passively—or "permissively"—generated hypercapnic acidosis (HCA), may improve outcome by reducing ventilator-induced lung injury. However, the effects of HCA per se on pulmonary microvascular permeability ($K_{f,c}$) in noninjured or injured lungs are unknown. We investigated the effects of HCA in the isolated buffer-perfused rabbit lung, under conditions of: (*1*) no injury; (*2*) injury induced by warm ischemia–reperfusion; and (*3*) injury induced by addition of purine and xanthine oxidase. HCA (fraction of inspired carbon dioxide [$F_{I_{CO_2}}$] 12%, 25% versus 5%) had no adverse microvascular effects in uninjured lungs, and prevented ($F_{I_{CO_2}}$ 25% versus 5%) the increase in $K_{f,c}$ following warm ischemia–reperfusion. HCA ($F_{I_{CO_2}}$ 25% versus 5%) reduced the elevation in $K_{f,c}$, capillary (Pcap), and pulmonary artery (Ppa) pressures in lung injury induced by exogenous purine/xanthine oxidase; inhibition of endogenous NO synthase in the presence of 25% $F_{I_{CO_2}}$ had no effect on $K_{f,c}$, but attenuated the reduction of Pcap and Ppa. HCA inhibited the *in vitro* generation of uric acid from addition of xanthine oxidase to purine. We conclude that in the current models, HCA is not harmful in uninjured lungs, and attenuates injury in free-radical–mediated lung injury, possibly via inhibition of endogenous xanthine oxidase. Shibata K, Cregg N, Engelberts D, Takeuchi A, Fedorko L, Kavanagh BP. Hypercapnic acidosis may attenuate acute lung injury by inhibition of endogenous xanthine oxidase. AM J RESPIR CRIT CARE MED 1998;158:1578–1584.

Limiting pressure and/or tidal volumes in patients with respiratory failure often involves the development of permissive hypercapnia. This approach constitutes an accepted clinical strategy in the management of patients with respiratory failure (1). It involves limiting the airway pressure or tidal volumes thereby allowing the Pa_{CO_2} to increase during mechanical ventilation, and has been reported to improve outcome in patients with acute respiratory distress syndrome (ARDS) (2). Because of the evidence linking lung distension with acute lung injury (ALI) (3) and the expert recommendations advising against exposure to elevated airway pressures in patients with ARDS (4), conventional wisdom assumes that the hypercapnic acidosis (HCA) reflects the underlying protective hypoventilatory strategy. However, the possibility that HCA per se may exert specific effects on the pathogenesis of ALI has not been explored.

Although the effects of severely elevated Pa_{CO_2} on systemic organ function and pulmonary vascular tone have been described in some detail (5), the interaction of CO_2 and pulmonary capillary permeability has received little attention. Several lines of evidence are important in this regard. First, although hypocapnia is associated with increased airway microvascular permeability (6), the effects of hypercapnia on permeability at the airway or alveolar level have not been described. Second, hypercapnia or acidosis may have protective effects in tissue ischemia in the central nervous system (7) and the myocardium (8), and metabolic acidosis is known to be protective against ischemia–reperfusion lung injury in the isolated buffer-perfused rat lung (9). However, the effects of HCA have not been explored in ALI. Third, hypercapnia increases endogenous production of nitric oxide (NO) in the lung (10) and cyclic nucleotides in neural tissue (11). Given the protective roles of these molecules in ALI (12, 13), HCA might be expected to exert protective effects. Fourth, oxygen-derived free radicals are central to the pathogenesis of many types of ALI (14), and in tissue homogenates, hypercapnia has been shown to attenuate production of free radicals and reduce lipid peroxidation (15). Taken together, these lines of evidence point to the need to evaluate the effects of HCA on important microvascular indices of ALI.

We therefore investigated the effects of HCA on microvascular permeability and hydrostatic pressures in the isolated buffer-perfused rabbit lung, under conditions of: (*1*) no lung injury; (*2*) injury induced by warm ischemia–reperfusion, when purine, but not xanthine, was added to the perfusate; and (*3*) injury induced by oxygen-derived free radicals, generated by addition of purine and xanthine oxidase, with/without inhibition of endogenous NO synthase. We investigated, in addition, the effects of HCA on the *in vitro* activity of exogenous xanthine oxidase.

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METHODS

Male New Zealand White rabbits approximately 3.0 to 4.0 kg were used in all experiments. All experimental work conformed to the guidelines of the Canadian Committee for Animal Care, and was approved by the Animal Care Committee of The Toronto Hospital.

Experimental Outline

In this study, the experiments were organized into three series of *ex vivo* experiments, and one series of uric acid assays. Series I examined the effects of graded HCA (fraction of inspired carbon dioxide [$F_{I_{CO_2}}$] 5% versus 12% versus 25%) on pulmonary vascular pressures and pulmonary microvascular permeability on uninjured lungs. Series II examined $F_{I_{CO_2}}$ 25% versus 5% on lung injury produced by warm ischemia–reperfusion. Series III assessed the effects of HCA ($F_{I_{CO_2}}$ 25%) versus normocapnia ($F_{I_{CO_2}}$ 5%), with and without inhibition of endogenous nitric oxide, on lung injury induced by addition of exogenous purine and xanthine oxide. Series IV examined the *in vitro* effects of HCA ($P_{CO_2} \approx 110$ mm Hg, pH $\approx$ 6.9 versus $P_{CO_2} \approx 40$ mm Hg, pH $\approx$ 7.4) on the enzymatic activity of xanthine oxidase. For this series, we measured the generation of uric acid from purine by addition of xanthine oxidase, in prepared perfusate.

Surgical Dissection

After premedication with intramuscular ketamine (85 mg $\cdot$ kg^{-1}), anesthesia was induced with intravenous pentobarbital sodium (range 15 to 25 mg $\cdot$ kg^{-1}), and heparin (1,000 IU) was administered. The surgical preparation used in this study was similar to that previously reported (13), with several modifications. Incremental boluses of pentobarbital (5 mg $\cdot$ kg^{-1}) were administered as required. Briefly, a tracheotomy was performed and pancuronium bromide (1 mg intravenously) was administered after depth of anesthesia was confirmed by absence of response to paw clamp. The lungs were ventilated using a small animal ventilator (Model 683; Harvard Apparatus, MA) with fraction of inspired oxygen ($F_{I_{O_2}}$) 1.0, rate 20 min^{-1}, tidal volume 4 ml $\cdot$ kg^{-1}, and 2 cm H_2O positive end-expiratory pressure (PEEP). The carotid artery was cannulated for arterial pressure measurement, with additional pentobarbital administered for any elevation in baseline mean arterial pressure $\geq$ 10%. A sternotomy was performed, and the pulmonary artery and left atrium were cannulated. The lungs were then ventilated with 5% CO_2–95% O_2 and flushed with blood-free Krebs-Henseleit solution containing 3% bovine serum albumin (pH 7.4), using a peristaltic pump (Model M312; Gilson, Villier, France). The heart and lungs were excised from the chest and suspended by the tracheotomy tube from counterbalance force-displacement transducer (Model 60-2995; Harvard Apparatus, MA), connected to a chart recorder (Recordall Series 5000; Fisher Scientific, Nepean, ON, Canada) for continuous measurement of lung weight. The lungs were then perfused in a recirculating manner at a flow rate of 150 ml $\cdot$ min^{-1}. The perfusate temperature was maintained at 37° C, and the total volume in the circuit was 500 ml. Pulmonary artery (Ppa) and left atrial pressures (Pla) were referenced to the height of the left atrium and measured continuously via side holes in the cannulae. The venous reservoir height was adjusted to maintain Pla at +2 mm Hg. The left atrium was wrapped loosely with string and glued to maintain constant left atrial volume. All gas mixtures were supplied premixed in cylinders (VitalAire, Mississauga, ON, Canada).

Measurements

Perfusate gasses. Samples of perfusate were taken via the side hole in the left atrial cannula and were measured for pH, P_{CO_2}, and P_{O_2}, using an ABL-300 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Vascular and airway pressures. Ppa, Pla, and airway pressures were recorded from a standard monitor (Spacelabs Monitor Model 90303B; Spacelabs Medical Products Ltd., Mississauga, ON, Canada), with hard copy tracings for determination of microvascular pressures recorded using a Gould 5 channel recorder (Gould 8 channel Recorder 2800; Gould Inc., Instruments Division, Cleveland, OH).

Pulmonary capillary hydrostatic pressure (Pcap). To determine Pcap, mechanical ventilation was stopped, and the lungs inflated with continuous positive airway pressure (CPAP) of 3 mm Hg. The pulmonary arterial and left atrial cannulae were simultaneously occluded using the double-occlusion technique (13, 16), and Pcap was calculated as the mean of the arterial and venous pressures at 3 s after occlusion.

Pulmonary vascular resistance. After determination of Pcap, pulmonary vascular resistance (R_p) was divided into precapillary (arterial, R_a) and postcapillary (venous, R_v) components such that $R_p = R_a + R_v$, where R_a = (Ppa − Pcap)/Q, and R_v = (Pcap − Pla)/Q, where Q is pulmonary flow (13, 16).

Isogravimetric pressure. Isogravimetric pressure (Piso) is the Pcap at which the lung neither gains nor loses weight, indicating that the Starling forces are balanced (13, 17). Piso was determined by discontinuing flow through the lung and opening a shunt between arterial and venous tubing so that Ppa and Pla were identical and thus equal to Pcap. Pla was then altered in 1 mm Hg increments, by alteration of the height of the venous reservoir, and the effect on lung weight was examined. Piso was defined as the highest Pcap at which the lung did not gain weight over a 3-min period.

Pulmonary capillary filtration coefficient ($K_{f,c}$). $K_{f,c}$ was then calculated by a modification of the methods of Drake and coworkers (13, 17). When Pcap equals Piso, the Starling forces are balanced so that there is no net transfer of fluid across the pulmonary capillary membrane. When the Pcap is suddenly increased from Piso to Piso +7 mm Hg, the other Starling forces initially remain unchanged so that pulmonary edema formation occurs at rate of $7 \times K_{f,c}$. However, lung weight gain after the increase in Pcap is due to both intravascular volume expansion and the edema formation. The expansion is rapid and essentially complete within 3 min. Therefore, the rate of weight gain was recorded on semi log plot every minute from 3 to 10 min and extrapolated back to time 0 by linear regression.

Compliance. Static lung compliance was determined after reversal of visible residual atelectasis, with applied positive end-expiratory pressure. A fixed tidal volume (12 mg $\cdot$ kg^{-1}) was then injected, and the plateau airway pressure was used to calculate static compliance.

Wet-to-dry weight. The left lung was excised after the end of the experiment, and the lung wet:dry weights ratio was determined after sequential weighs demonstrated maximal dehydration in a drying oven.

Exclusion criteria. Prior to randomization in all experiments (Series I–III), baseline perfusion was commenced for 10 min with 95% O_2–5% CO_2. For all preparations, the following exclusion criteria were applied: weight gain $>$ 2 g during baseline perfusion; weight gain $>$ 3 g during estimation of $K_{f,c}$; perfusate leakage $>$ 0.3 ml/min from preparation during baseline perfusion; Ppa $>$ 20 mm Hg. Preparations were discarded where any of these parameters were exceeded during the baseline stage.

Series I: noninjured controls. After baseline values were determined during the ventilation with 5% CO_2–95% O_2 and exclusion criteria had not been met, preparations were randomly allocated to one of three groups (n = 6 in each) as follows: normocapnia ventilated with 5% CO_2–95% O_2; moderate HCA ventilated with 12% CO_2–88% O_2; or severe HCA ventilated with 25% CO_2–75% O_2. Final values were determined after 3 h of perfusion, followed by wet:dry ratios.

Series II: injury induced by warm ischemia and reperfusion. A model of warm ischemia–reperfusion was developed, modified from a previous report (18). After baseline perfusion as before, purine (0.006 g) was added to replace physiologic sources, and reperfusion continued for an additional 5 min. An additional exclusion criterion in this series was weight gain during this time of 1 g. The preparations were then randomized to: ventilation with 5% CO_2, 50% O_2, 45% N_2; or ventilation with 25% CO_2, 50% O_2, 25% N_2. Ventilation and perfusion was continued for 25 min, and then perfusion and ventilation stopped. The lung inflation was maintained using CPAP 3 mm Hg with the same gas mixture, and the temperature maintained at 37° C in a humidified chamber. After 45 min, mechanical ventilation was recommenced. Perfusion was then commenced, and increased in a stepwise fashion, as follows: approximately 30, 55, 90, and 120 ml $\cdot$ min^{-1} for intervals of 1 min each, and resumed thereafter at the baseline rate of 150 ml $\cdot$ min^{-1}. The perfusion continued for an additional 30 min, at which stage final values for Ppa, Pcap, Piso, and $K_{f,c}$ were measured. Wet:dry weight was then measured.

Series III: injury induced by exogenous purine and xanthine oxidase. After baseline values were determined during the ventilation with 5% CO_2–95% O_2 and exclusion criteria had not been met, preparations were randomly allocated to one of three groups (n = 6 each)

TABLE 1

EFFECTS OF HCA IN UNINJURED LUNGS*

	Inspired Concentration of CO_2		
	5%	12%	25%
Ppa baseline, mm Hg	7.0 ± 0.7	7.7 ± 0.8	5.8 ± 0.4
Ppa final, mm Hg	9.0 ± 0.7†	8.7 ± 0.9	6.7 ± 0.6
Pcap baseline, mm Hg	4.2 ± 0.5	5.2 ± 0.4	4.2 ± 0.2
Pcap final, mm Hg	4.6 ± 0.7	4.9 ± 0.7	4.2 ± 0.3
Piso baseline, mm Hg	5.5 ± 0.2	5.7 ± 0.4	4.7 ± 0.3
Piso final, mm Hg	4.2 ± 0.2†	4.5 ± 0.5	3.7 ± 0.3
$K_{f,c}$ baseline, ml · min^{-1} · mm Hg^{-1} · 100 g^{-1}	0.35 ± 0.05	0.40 ± 0.04	0.42 ± 0.05
$K_{f,c}$ final, ml · min^{-1} · mm Hg^{-1} · 100 g^{-1}	0.44 ± 0.04	0.45 ± 0.04	0.46 ± 0.07
Wet:dry ratio	5.2 ± 0.8	4.4 ± 0.5	4.4 ± 0.4
Lung compliance, mm Hg · ml^{-1}	5.2 ± 1.9	3.8 ± 0.9	3.8 ± 1.1

Definition of abbreviations: $K_{f,c}$ = capillary filtration coefficient; Pcap = pulmonary capillary hydrostatic pressure; Piso = isogravimetric pressure; Ppa = pulmonary artery pressure.

* Results are means ± SEM; n = 6 lungs in each group.

† $p < 0.05$ versus baseline values (within-group).

as follows: normocapnia ventilated 5% CO_2, 50% O_2, 45% N_2; severe HCA without N^G-nitro-L-arginine methyl ester (L-NAME) ventilated with 25% CO_2, 50% O_2, 25% N_2; or severe HCA with L-NAME ventilated with 25% CO_2, 50% O_2, 25% N_2, where L-NAME 0.1 mM (Sigma Chemical, St. Louis, MO) was added to the perfusate 5 min before alteration of inspired gas mixture. Twenty minutes after alteration of inspired gas mixtures, oxidant-induced lung injury was generated, based on previous models (13, 19), by addition of purine (0.5 mM) (Sigma Chemical) and xanthine oxidase (0.002 U · ml^{-1}) (type IV, milk source; Sigma Chemical) to the perfusate in all groups. Final values were determined when 3 h elapsed, or when lung weight gain reached 20 g. Wet:dry ratios were subsequently measured.

Series IV: in vitro *purine–xanthine oxidase (PXO) activity.* In order to assess the effects of perfusate HCA on the actions of exogenous xanthine oxidase, the generation of uric acid was measured in the same perfusate solution (Krebs-Henseleit solution containing 3% bovine serum albumin; temperature 37° C; 500 ml per beaker) that was used in the perfused lung experiments. The perfusate preparations were randomly allocated to either normocapnia (bubbled with a gas mixture of 5% CO_2, 50% O_2, 45% N_2); or to HCA (bubbled with a gas mixture of 25% CO_2, 50% O_2, 25% N_2). The perfusate pH and P_{CO_2} were targeted to 7.4 and 40 mm Hg respectively in Group N, and were targeted to 6.9 and 110 mm Hg respectively in Group H. Upon stability of baseline parameters, purine (1.5 mM) was followed 2 min later by xanthine oxidase (0.006 U · ml^{-1}). Oxypurinol (2.4 mM) was used to terminate the reaction (20) in samples taken at timed intervals, and the concentration of uric acid was assayed using an Olympus AU 800 (Olympus, New York, NY) clinical chemistry system, using a coupled uricase enzyme assay (21).

TABLE 2

EFFECTS OF HCA IN ISCHEMIA–REPERFUSION LUNG INJURY*

	Inspired Concentration of CO_2	
	5%	25%
pH	7.39 ± 0.03	6.84 ± 0.02
P_{CO_2}	32.1 ± 2.2	120.2 ± 5.3
P_{O_2}	383.4 ± 14.9	393.8 ± 11.2
Ppa baseline, mm Hg	7.7 ± 0.5	6.0 ± 0.5
Ppa final, mm Hg	9.2 ± 1.0	6.8 ± 0.5
Pcap baseline, mm Hg	5.5 ± 0.3	4.9 ± 0.3
Pcap final, mm Hg	5.7 ± 0.3	5.3 ± 0.5
Piso baseline, mm Hg	5.0 ± 0.3	4.2 ± 0.2
Piso final, mm Hg	4.0 ± 0.3	4.0 ± 0.0
Weight gain, g	6.9 ± 2.1	2.4 ± 0.3
Wet:dry ratio	8.9 ± 0.7	6.9 ± 0.6

For definition of abbreviations, *see* Table 1.

* Results are means ± SEM; n = 6 lungs in each group.

Data Analysis and Statistics

All data were entered into a standard spreadsheet (Excel 7.0; Microsoft Corp.), and exported for analysis using Sigmastat (Jandel no. 2). The data are summarized as means ± standard error of the mean (SE). Statistical analysis used Kruskal-Wallis and Student-Newman-Keuls tests for nonparametric data, and analysis of variance (ANOVA) with Student-Newman-Keuls or paired *t* tests for parametric data. We considered differences significant where $p < 0.05$.

RESULTS

Series I (Noninjured Controls)

Graded HCA (FI_{CO_2} 5% versus 12% versus 25%) had no effect on pulmonary vascular pressures and pulmonary microvascular permeability. There were no significant between-group differences with respect to lung weight ratio, Ppa, Pcap, Piso, or $K_{f,c}$ at any points (Table 1). After 3 h of perfusion, the Ppa and Piso showed small changes in the normocapnic group. In contrast, no significant changes were found in either of the HCA (12% or 25% CO_2) groups.

Series II (Injury Induced by Warm Ischemia and Reperfusion)

All baseline parameters for FI_{CO_2} 5% and 25% are reported (Table 2). Perfusate gas tensions and pH are presented (Table

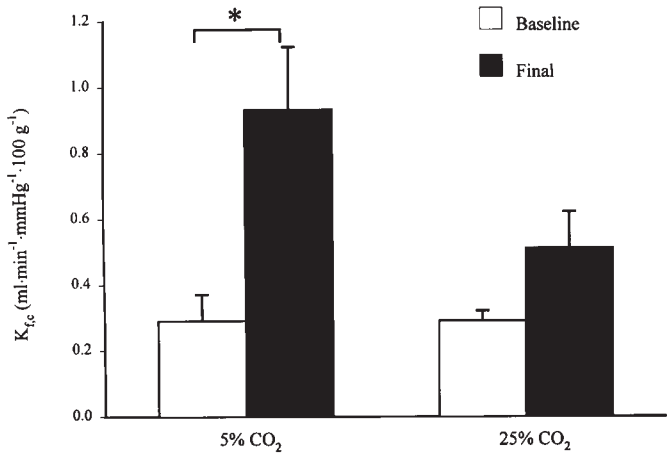

Figure 1. $K_{f,c}$ measured at baseline and after warm ischemia–reperfusion injury (Series II). Results are mean ± SEM of the data. n = 6 per group.

2). Final versus baseline $K_{f,c}$ was significantly greater in the normocapnic but not in HCA group (Figure 1). All other measured parameters were not significantly different between the two groups (Table 2).

Series III (Injury Induced by Exogenous Purine and Xanthine Oxidase)

All baseline variables were similar for the three groups (Table 3). The perfusate values of pH, P_{CO_2} and P_{O_2} are presented (Table 3). The final values of $K_{f,c}$ were significantly higher than baseline in the normocapnic group, but not in either of the HCA (with or without L-NAME) groups (Figure 2). The final Piso was lower than baseline in the normocapnic group, but not in either of the HCA groups (Table 3). Ppa and Pcap were significantly increased in the normocapnic group and the HCA with L-NAME group, but not in the HCA without L-NAME group (Table 3). Analysis of the longitudinal distribution of the pulmonary vascular resistance revealed that the final versus baseline values of R_a and R_v were not increased in the HCA without L-NAME group. Addition of L-NAME to 25% CO_2 resulted in significantly greater elevation of R_p, which was accounted for by elevation in R_a (Table 3). Lung compliance significantly decreased in the 5% CO_2 group, but not in either of the 25% CO_2 groups (Table 3). The wet:dry ratio was significantly greater in the normocapnic group versus either of the HCA groups (Table 3).

Series IV (*In Vitro* Inhibition of Xanthine Oxidase)

The rate of generation of uric acid from the addition of xanthine oxidase to purine in the perfusate solution was significantly less in the presence of hypercarbic acidosis versus normocapnic conditions (Figure 3).

DISCUSSION

HCA—Rationale and Risks

Permissive hypercapnia has been advocated as a beneficial strategy in ARDS (1), and its use has been reported to improve clinical outcome (2). Although these findings have yet to be supported by prospective controlled studies (22), the rationale for instituting permissive hypercapnia reflects reduced minute ventilation, minimized pulmonary stretch—with potentially less ventilator-induced lung injury—and tolerance to the resultant elevated Pa_{CO_2}. The reported levels of Pa_{CO_2} and pH (mean maximal Pa_{CO_2} 67 mm Hg, mean pH 7.2) in the study of Hickling and coworkers (2) reflect typical levels observed with institution of this technique. However, the perfusate levels achieved in the current study (mean values of P_{CO_2} 110 mm Hg and pH 6.9 where ventilated with 25% CO_2) fall well within the extremes of Pa_{CO_2} and pH reported in the clinical literature (158 mm Hg and 6.78, respectively) (2). Although not usually associated with adverse effects in the clinical management of patients with ARDS (1), HCA can be associated with dangerous elevations in intracranial pressure

TABLE 3

EFFECTS OF HCA IN PURINE–XANTHINE OXIDASE LUNG INJURY*

	Inspired Concentration of CO_2		
	5%	25%	25% + L-NAME
pH	7.44 ± 0.01	6.89 ± 0.02†	6.92 ± 0.01†
P_{CO_2}, mm Hg	31 ± 1	113 ± 4†	103 ± 4†
P_{O_2}, mm Hg	279 ± 5	278 ± 14	302 ± 11
T20, h	2.2 ± 0.4	3 ± 0†	3 ± 0†
Ppa baseline, mm Hg	7.0 ± 1.0	7.5 ± 0.3	7.7 ± 0.6
Ppa final, mm Hg	20.8 ± 5.1§	9.2 ± 1.2	13.7 ± 1.7‡§
Pcap baseline, mm Hg	4.7 ± 0.4	4.4 ± 0.2	4.7 ± 0.4
Pcap final, mm Hg	7.0 ± 0.6§	5.2 ± 0.6	7.0 ± 0.8§
R_a baseline, mm Hg · L^{-1} · min^{-1}	16 ± 4.0	21 ± 1.8	20 ± 3.0
R_a final, mm Hg · L^{-1} · min^{-1}	92 ± 33§	27 ± 5†‡	44 ± 6‡§
R_v baseline, mm Hg · L^{-1} · min^{-1}	18 ± 2.7	16 ± 1.3	18 ± 2.7
R_v final, mm Hg · L^{-1} · min^{-1}	33 ± 4§	21 ± 4	33 ± 6§
Piso baseline, mm Hg	4.3 ± 0.2	4.8 ± 0.3	5.2 ± 0.2
Piso final, mm Hg	2.5 ± 0.4§	4.2 ± 0.3†	4.3 ± 0.2†
Cl baseline, mm Hg · ml^{-1}	5.9 ± 0.8	4.5 ± 0.5	4.9 ± 0.4
Cl final, mm Hg · ml^{-1}	3.1 ± 0.2§	3.5 ± 0.2	3.3 ± 0.2§

Definition of abbreviations: Cl = lung compliance; Pcap = pulmonary capillary hydrostatic pressure; Piso = isogravimetric pressure; Ppa = pulmonary artery pressure; R_a = precapillary resistance; R_v = postcapillary resistance; T20 = time to lung weight gain of 20 g.

* Values are means ± SEM; n = 6 lungs in each group.

† $p < 0.05$ versus 5% CO_2 group.

‡ $p < 0.05$ 25% CO_2 versus 25% CO_2[+L-NAME] groups.

§ $p < 0.05$ versus baseline (within-group).

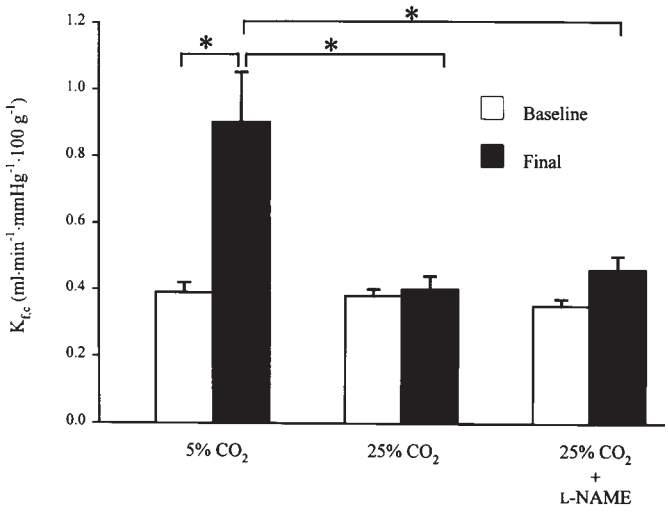

Figure 2. $K_{f,c}$ measured at baseline and after injury induced by addition of purine–xanthine oxidase to perfusate (Series III). Results are mean ± SEM of the data. n = 6 per group.

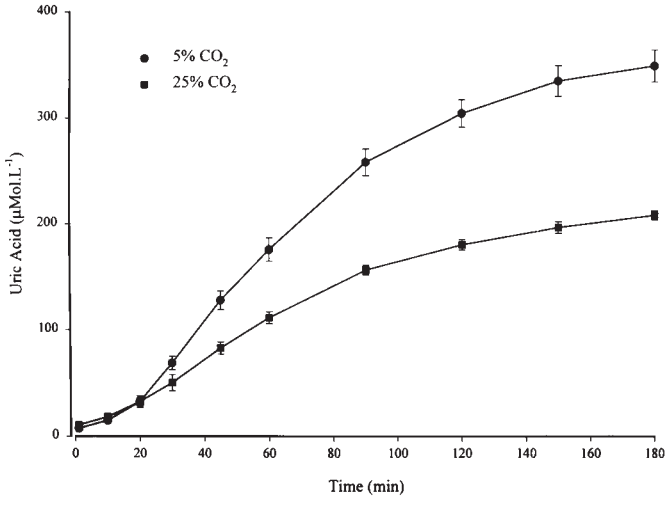

Figure 3. Uric acid concentration at specified time points after the *in vitro* addition of xanthine oxidase to purine under conditions of 5% versus 25% CO_2 (Series IV). Results are mean ± SEM of the data. $p < 0.05$, ANOVA. n = 4 per group.

and potential sympathomimetic effects. The relative risk–benefit assessments have not been clarified in clinical trials.

Cellular Effects and Protective Mechanisms

Metabolic acidosis is known to protect lungs against ischemia–reperfusion injury (9), and also protects the myocardium against hypoxia (23) and reperfusion injury (8). In intact animals, hypercapnia protects against central nervous system (CNS) hypoxic–ischemic injury (7). Acidosis, on either a metabolic or respiratory basis, may therefore be protective in a variety of organ injuries. However, in the myocardium at least, intracellular acidosis is more rapidly augmented when the extracellular acidosis is due to hypercapnia as opposed to a metabolic source (24). Myocardial contractility, and perhaps more importantly, myocardial oxygen consumption, is far more sensitive to elevations in extracellular P_{CO_2} than elevations in extracellular H^+ concentration (25). The reason for this is that elevated levels of P_{CO_2} result in rapid inward diffusion of highly soluble CO_2 molecules, thus lowering the intracellular pH by subsequent dissociation into H^+ and HCO_3^- ions. This occurs more rapidly than is possible by inward equilibration of relatively impermeant H^+ ions associated with metabolic acidosis. Intracellular protection may be of importance in ALI (14), and the rapid entry of CO_2, as opposed to "metabolic" acids, may access endothelial cells and more rapidly exert protective effects.

Despite the available data concerning protective effects of respiratory and/or metabolic acidosis states, the exact mechanisms of protection are undefined. Several possible explanations are possible. First, it is likely that physiologic pH is necessary for Na/H exchanger activation, and that this activation in turn, is necessary for ischemia–reperfusion induced tissue injury (9). Thus, acid pH may protect against ischemia–reperfusion injury via inhibition of Na/H exchanger function. Second, increased xanthine oxidase activity in heart muscle augments membrane permeability to calcium (26) and may potentiate ischemic injury (26). Inhibition of xanthine oxidase by HCA may therefore reduce injury via reduction of intracellular calcium. Third, hypercapnia increases production of cAMP by cerebral microvascular cells (11), and such cyclic nucleotides have been shown to protect against pulmonary ischemia–reperfusion injury (12). Fourth, there are multiple mechanisms whereby NO protects against injury, outlining potential areas of interplay with protective effects of hypercapnic acidosis. Nitric oxide inhibits XO activity (27) and neutrophil production of superoxide radicals (28), and inhaled NO has been shown to reduce both the function of superoxide free radicals (29), and oxygen radical–induced lung injury (13, 30).

Knowledge that NO is produced by normal lungs and is present in exhaled gas (31), together with recent documentation that pulmonary production of NO is increased by hypercapnia in rabbit lungs (10), prompted us to investigate the impact of inhibition of endogenous NO synthase on modulation of ALI by HCA. Inhibition of endogenous NO synthase in the presence of 25% CO_2 resulted in potentiation of postinjury Ppa and Pcap, but did not alter the markers of microvascular permeability. These findings support previous data (13) describing the impact of inhibition of endogenous NO synthase on xanthine oxidase–induced lung injury. In the current study, the relative elevation in Pcap, even in the absence of an effect on $K_{f,c}$ points to a potential protective role of endogenous NO on modulation of microvascular hydrostatic pressure, in the presence of hypercapnia. It is clear from the current study, however, that protection afforded by HCA against increased microvascular permeability is not mediated by endogenous NO.

Effects on Uninjured Lungs

In uninjured lungs, hypercapnia had minimal effects on pulmonary vascular pressures, and capillary permeability. In other uninjured organ systems, the effects of alteration of acid–base status on microvascular permeability are complex, with increasing external H^+ ion concentration increasing cerebral microvascular permeability (32) and decreasing myocardial capillary permeability (33). In uninjured brain, moderate hypercapnia appears to minimally increase cerebral capillary recruitment, does not alter capillary permeability, but may reduce glucose transporter activity in uninjured brain (34). The data from the current study suggest however, that short episodes of profound HCA are not associated with even slight demonstrable adverse effects at the pulmonary microvascular level.

Effects on Ischemia–Reperfusion

Ischemia–reperfusion is an important cause of ALI, the exact mechanisms of which are incompletely understood. Recent studies have shed light on the relative contributions of xanthine oxidase and neutrophils (35), adhesion molecules (36), thromboxane A2 (18), and flavoprotein activation (37). The attenuation of ischemia–reperfusion lung in the current study may reflect inhibition of endogenous xanthine oxidase. We know from the current study that HCA does inhibit exogenous *in vitro* xanthine oxidase, and attenuates lung injury resulting from the action of xanthine oxidase on purine in perfusate. However, it is possible that the HCA inhibited ischemia–reperfusion injury due to mechanisms involving, for example, neutrophil function, adhesion molecule activation, thromboxane or cytokine activity, or flavoprotein modification. The clinical significance of lung ischemia–reperfusion injury lies in its central role in clinical contexts such as lung transplantation, pulmonary embolism, and possible clinical ARDS or multisystem organ dysfunction. Thus, the effects observed with HCA may have considerable clinical implications. In the current study, the exact O_2 tension in the precapillary, capillary, or postcapillary vascular segments was unknown during the ischemic period, and for the initial stages following reperfusion. It is known that alveolar or perfusate O_2 tension (38, 39) can have a significant impact on the development of lung injury after ischemia–reperfusion. These issues may have significance beyond the role of xanthine oxidase, as flavoprotein enzymatic activity appears to function as an important source of O_2-derived free radicals under conditions of anoxia–reoxygenation in the rat lung (37). However, the current study did not focus on these issues.

Role of Xanthine Oxidase Inhibition

Xanthine oxidase is a ubiquitous and complex enzyme system (40). Its rate of conversion from the dehydrogenase to the oxidase form is accelerated by local hypoxia or ischemia (41), thus accelerating its role in the generation of free radicals in the context of many forms of tissue injury. Several studies point to the central importance of xanthine oxidase in ALI/ARDS. Plasma levels of xanthine oxidase are increased in patients with ARDS (42), and the circulating substrate concentrations are higher in nonsurvivors versus survivors of ARDS (43). Severity of ALI following splanchnic ischemia–reperfusion (44) depends on plasma xanthine oxidase activity, and in preterm infants with respiratory failure, plasma xanthine oxidase activity is a predictor for poor outcome (45). Furthermore, xanthine oxidase is a pivotal enzyme system in the pathogenesis of lung ischemia–reperfusion injury, including pulmonary ischemia–reperfusion in the rabbit (35). Hence, the experimental focus on xanthine oxidase modulation is well

founded, based on the underlying mechanisms and clinical relevance.

Limitations of Study

A critical limitation of the current study is the lack of a definite mechanism to explain the observed effects. While the inhibition of xanthine oxidase has been demonstrated, and while it is known that xanthine oxidase plays a pivotal role in tissue injury, we do not have definitive proof that the protection afforded by hypercapnic acidosis was in fact mediated via inhibition of endogenous xanthine oxidase. Extrapolation of our results to the clinical situation must be restricted by the inherent limitations of an isolated buffer perfused model. The perfused lung is denervated, isolated from the systemic circulation, and perfused with a blood-free perfusate. The particular concerns relate to the lack of pulmonary–systemic interactions in the context of organ injury and the potential systemic effects of profound hypercapnia. Because hemoglobin avidly binds and inactivates NO, the use of a blood-free perfusate could result in reduced inhibition or clearance of endogenous NO (46). However the effects of L-NAME in series III of the current study should minimize that limitation.

The current study does not address the relative contributions of the specific components of HCA, i.e., the individual contributions of acidosis versus hypercapnia. Furthermore, the study does not examine the effects of prolonged exposure to hypercapnia with the potential for renal compensatory regeneration of extracellular bicarbonate.

An important issue that is not explained by the results is the apparent discrepancy between the modest inhibition of *in vitro* xanthine oxidase activity versus the complete protection against purine–xanthine oxidase–induced lung injury. We used a dose of purine and xanthine oxidase that was just above the threshold for causation of lung injury, and therefore even modest inhibition of xanthine oxidase resulted in complete abrogation of the lung injury. Use of significantly higher doses of xanthine oxidase can be associated with overwhelming lung injury that is difficult to prevent.

An additional issue with the study is the uncertainty of the oxygenation status of the ischemia–reperfusion preparation. We did not assess effluent gas tensions at or following reperfusion, and it must be recognized that lung inflation with CPAP and $F_{I_{O_2}}$ of 0.5 might have been sufficient to permit ongoing oxidative metabolism, which may alter the mechanism of injury. Nevertheless, injury occurred, and was clearly attenuated in the presence of HCA.

We conclude that in the current models, HCA (*1*) is not harmful in uninjured lungs, and (*2*) attenuates injury in free radical–mediated lung injury, possibly via inhibition of endogenous xanthine oxidase. These findings have significant implications for our understanding of critical tissue injury states including ARDS/ALI, pulmonary embolism, reperfusion during lung transplantation, and multisystem organ dysfunction, where various forms of xanthine oxidase play a central role.

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