Hypercapnic Acidosis Impairs Plasma Membrane Wound Resealing in Ventilator-injured Lungs

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The objective of this study was to assess the effects of hypercapnic acidosis on lung cell injury and repair by confocal microscopy in a model of ventilator-induced lung injury. Three groups of normocapnic, hypocapnic, and hypercapnic rat lungs were perfused *ex vivo*, either during or after injurious ventilation, with a solution containing the membrane-impermeant label propidium iodide. In lungs labeled during injurious ventilation, propidium iodide fluorescence identifies all cells with plasma membrane wounds, both permanent and transient, whereas in lungs labeled after injurious ventilation propidium iodide fluorescence identifies only cells with permanent plasma membrane wounds. Hypercapnia minimized the adverse effects of high-volume ventilation on vascular barrier function, whereas hypocapnia had the opposite effect. Despite CO_2-dependent differences in lung mechanics and edema the number of injured subpleural cells per alveolus was similar in the three groups (0.48 ± 0.34 versus 0.51 ± 0.19 versus 0.43 ± 0.20 for hypocapnia, normocapnia, and hypercapnia, respectively). However, compared with normocapnia the probability of wound repair was significantly reduced in hypercapnic lungs (63 versus 38%; $p < 0.02$). This finding was subsequently confirmed in alveolar epithelial cell scratch models. The potential relevance of these observations for lung inflammation and remodeling after mechanical injury is discussed.

Keywords: permissive hypercapnia; plasma membrane wounding and repair; ventilator-induced lung injury

So-called lung protective mechanical ventilation strategies emphasize lung recruitment and the avoidance of large tidal volumes. Such strategies are often associated with hypercapnic acidosis. Many experts view "permissive hypercapnia" as a necessary evil of a low tidal ventilation strategy (1). Concern about detrimental effects of acidemia on renal and cardiovascular function have motivated attempts to enhance CO_2 removal by tracheal gas insufflation (2) and have led to unsubstantiated recommendations about the use of bicarbonate buffers in hypercapnic patients (3). More recent data suggest that hypercapnia may actually protect the lung from certain manifestations of ischemia–reperfusion, endotoxin, and mechanical ventilation–related injury (4–9). Hypocapnia, in contrast, and correction of acidemia may be harmful (10–12). The specific mechanisms through which hypercapnia influences lung injury and vascular barrier function remain uncertain (13). CO_2 generates H^+ ions, which react with titratable groups in certain amino acids and/or interacts directly with free amine groups in proteins to form carbamate residues (14–16). CO_2-dependent effects on the generation of reactive oxygen species and reactive nitrogen species as well as effects on ion channel conductivity have all been considered (17, 18).

Ventilator-induced lung injury (VILI) is characterized by a mechanical failure of the blood–gas barrier. Widespread endothelial and epithelial cell injury is one of the hallmarks of the entity and accounts at least in part for the increased microvascular permeability of ventilator-injured lungs (19–22). Gajic and coworkers have provided direct evidence that the VILI lesion is associated with a transient loss of endothelial and/or epithelial plasma membrane integrity (23). This is in keeping with data suggesting that removal of mechanical stress causes a rapid restoration of vascular barrier function (24–26). In cell culture failure to reseal a plasma membrane break causes cell death by necrosis, whereas a wounded and resealed cell remains viable for some time (27, 28).

The objective of the experiments described in this article was to assess the effects of CO_2 on lung cell injury and repair in an experimental model of VILI as well as in cell culture. The VILI model is the isolated perfused rat lung preparation as described by Gajic and coworkers (23), in which effect centers on the assessment of cell injury in subpleural airspaces, using confocal microscopy. For this purpose lungs are perfused either during or after injurious mechanical ventilation with a solution containing the membrane-impermeant label propidium iodide (PI). When PI enters a cell through a membrane defect it intercalates with DNA and emits red fluorescence on excitation with blue light. In experiments in which lungs are labeled during injurious ventilation, PI fluorescence identifies all cells with plasma membrane wounds, both permanent and transient. In contrast, in experiments in which lungs are labeled after injurious ventilation PI fluorescence identifies only those cells that have failed to heal, that is, have failed to reseal plasma membrane defects.

Our results confirm earlier reports from other injury models, that CO_2 influences vascular barrier function and thereby the mechanics of the injured lung (4–9). However, we also show that CO_2 tension influences the probability that injured cells will repair plasma membrane defects. The implications of this finding for alveolar wound healing and molecular cell repair mechanisms are discussed. Portions of this study were presented in abstract form at the 2003 International Meeting of the American Thoracic Society (29, 30).

METHODS

Animal Preparation, Equipment, and Monitoring

The isolated perfused rat lung VILI model is detailed in the online supplement and in a previous publication (23). In brief, isolated perfused rat lungs were mechanically ventilated *ex vivo* as dictated by the experimental protocol (*see below*). The membrane-impermeant label PI was added to the perfusate at certain points during the experiment and the number of labeled subpleural cells was measured by confocal intravital microscopy.

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Experimental Protocol

Seventy-two rat lungs were mechanically ventilated for 20 minutes at a rate of 40 cycles per minute with a tidal volume of 40 ml/kg. The lungs were randomized in blocks of six to one of six groups (Figure 1). The groups differed with respect to $F_{I_{CO_2}}$ and the timing of label perfusion. Preparations randomized to CO_2 treatment Groups I, II, and III were supplemented to an $F_{I_{CO_2}}$ of 0.05 (normocapnia), 0.01 (hypocapnia), and 0.12 (hypercapnia), respectively. Preparations randomized to Subgroups A were labeled during the initial 20 minutes of injurious mechanical ventilation. Preparations assigned to Subgroups B were perfused with solutions containing PI for 20 minutes after cessation of injurious ventilation while the lungs were held inflated at a pressure of 20 cm H_2O. Independent confirmation of our findings was sought in 30 additional experiments on lungs that had been injured under either normocapnic or hypercapnic conditions.

In Vitro Cell Injury Model

Effects of CO_2 on plasma membrane wound repair were also tested in a cell culture model as previously described (23). Primary rat Type II alveolar epithelial cells and A549 cells were grown to confluence in Lab-Tek II eight-chambered slides (Nalge Nunc International, Naperville, IL) and injured in the presence of fluorescent dextran (70 kD; Sigma, St. Louis, MO) with a surgical blade at CO_2 tensions of 10, 40, and 80 mm Hg, respectively. Two minutes later the slides were washed with 4°C phosphate-buffered saline, incubated for 2 minutes with PI-containing medium, and washed again. Epifluorescence images were obtained at emission peaks of 510 and 620 nm, using an inverted microscope (Carl Zeiss, Thornwood, NY). The number of fluorescent dextran– and PI-positive cells per ×20 view field was counted. Cells with green cytoplasmic dextran fluorescence were considered wounded but healed, whereas cells with red PI-fluorescent nuclei were considered wounded but permanently injured (27).

Data Analysis

The amount of cell injury in whole lungs was evaluated in a blinded fashion by two independent observers. A cell injury index was defined as the number of PI-positive subpleural cells per alveolus. CO_2 effects on vascular barrier function were inferred from lung weight gain relative to normal predicted values (31), from the changes in lung inflation compliance, and from changes in pressure at the lower inflection point of the dynamic lung inflation pressure–volume curve (32).

Data were analyzed with S-PLUS Professional 6.0 for Windows (Insightful, Seattle, WA) and JMP 5.1 (JMP/SAS, Cary, NC). All data are presented as means (± standard deviation). For each baseline, physiologic response, and change variable, a linear regression model (analysis of variance) was fit with CO_2 treatment group, time group, and the interaction between CO_2 treatment group and time group. The test for interaction provides a test for whether the time group effect is different across CO_2 treatment groups. A reduced model was also fit that included only CO_2 treatment group. Subgroup analysis was done on number of PI-positive cells per alveolus (PI/Alv) to compare each CO_2 treatment group pairwise. The average resealing percentage was estimated for each CO_2 treatment group: $100 \times (1 - b/a)$, where b is the mean PI/Alv for Group B (labeled after injury) and a is the mean PI/Alv for Group A (labeled during injury). A bootstrap resampling procedure was performed to obtain confidence intervals and pairwise comparisons for resealing percent. Statistical significance was accepted at $p < 0.05$.

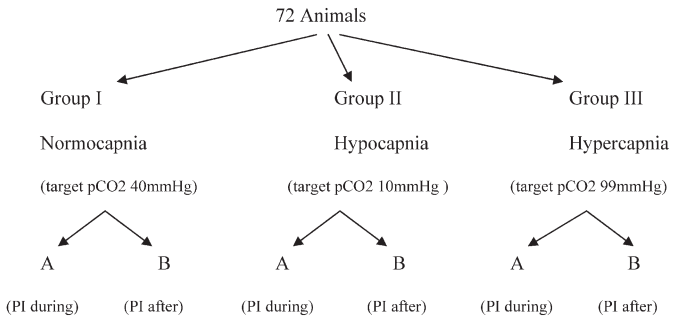

Figure 1. Experimental design. For explanation, *see* text.

RESULTS

CO_2 Effects on Vascular Barrier Function of Mechanically Injured Lungs

Table 1 shows baseline variables by CO_2 treatment group. For all variables, timing group (A versus B) is not statistically significant. Weight, predicted lung weight, peak airway pressure (Paw), pulmonary arterial pressure (Ppa), and dynamic lung compliance (Cdyn) are not significantly different by capnia group. pH decreases and P_{CO_2} in venous effluent increases with increasing CO_2 supplementation ($p < 0.001$ for each).

Table 2 shows physiologic response variables by CO_2 treatment group. For all variables except the wounding index PI/Alv, timing group (A versus B) is not statistically significant. Pulmonary arterial pressure (Ppa), pulmonary arterial pressure change (ΔPpa), and Cdyn change (ΔCdyn) are not significantly different by CO_2 treatment group. As CO_2 tension increases from hypocapnia to hypercapnia, lung weight decreases ($p = 0.003$), Paw decreases ($p = 0.003$), Cdyn increases ($p = 0.002$), airway pressure at the lower inflection point decreases ($p < 0.001$), lung weight change decreases ($p = 0.002$), and Paw change decreases. The lack of CO_2 effect on pulmonary artery pressure is probably explained by relatively low perfusate flow.

CO_2 Effects on Cell Wounding and Repair from Ventilator-induced Injury

Figure 2 shows box plots of PI/Alv by CO_2 treatment group with data points added. Despite CO_2-dependent differences in lung mechanics and edema the number of injured subpleural cells per alveolus (Group A data) was similar in the three CO_2 treatment groups (0.48 ± 0.34 versus 0.51 ± 0.19 versus 0.43 ± 0.20 during hypocapnia, normocapnia, and hypercapnia, respectively). When analyzed across all CO_2 treatment groups, the number of cells with permanent wounds (Group B data) was less than the total number of injured cells (Group A) ($p < 0.001$). The estimated difference between Group A and Group B is 0.07 lower for normocapnia than for hypocapnia ($p = 0.58$), 0.09 lower for hypocapnia than hypercapnia ($p = 0.49$), and 0.15 lower for normocapnia than hypercapnia ($p = 0.082$). The latter suggests that plasma membrane wound repair may be inhibited in the presence of hypercapnic acidosis, a conclusion supported by the analysis shown in Figure 3.

Figure 3 shows estimates of the probability of cell repair (resealing percentage) per CO_2 treatment group and the corresponding 95% confidence intervals obtained via a bootstrap resampling procedure. The estimates (and 95% confidence intervals) are 51% (23–71%) for hypocapnia, 63% (50–72%) for normocapnia, and 38% (16–53%) for hypercapnia. The estimated resealing percent is 11% higher for normocapnia than for hypocapnia ($p = 0.37$), 13% higher for hypocapnia than hypercapnia ($p = 0.41$), and 25% higher for normocapnia than hypercapnia ($p = 0.022$).

In the experiments of Figures 2 and 3, the CO_2 tension of perfusate and inspired gas was rigorously controlled, while that at the lung surface was not. We, therefore, validated our results in 30 additional preparations in which ambient CO_2 tensions were clamped as defined in Protocols IB and IIIB (*see* Figure 1). Indeed, lungs that were label perfused after injurious mechanical ventilation under normocapnic as opposed to hypercapnic conditions had significantly fewer injured and presumably necrotic

TABLE 1. BASELINE CHARACTERISTICS

	Hypocapnia		Normocapnia		Hypercapnia		
	A	B	A	B	A	B	p Value
Weight, kg	0.29	0.29	0.29	t0.28	0.31	0.31	0.16
	(0.06)	(0.06)	(0.06)	(0.05)	(0.05)	(0.04)	
Lung weight, g	1.08	1.08	1.03	1.02	1.15	1.19	0.16
	(0.30)	(0.30)	(0.29)	(0.28)	(0.24)	(0.20)	
Ischemic time, min	2.02	2.43	1.93	2.14	2.19	1.93	0.15
	(0.30)	(0.55)	(0.36)	(0.66)	(0.32)	(0.67)	
Paw, cm H_2O	35.61	37.59	36.34	35.25	34.02	34.40	0.25
	(4.42)	(4.86)	(5.18)	(4.79)	(3.78)	(6.42)	
Ppa, cm H_2O	11.87	12.75	13.41	12.93	13.14	12.35	0.65
	(3.19)	(2.50)	(4.08)	(3.19)	(3.38)	(2.69)	
Cdyn, ml/cm H_2O per kg	1.29	1.29	1.27	1.32	1.35	1.30	0.90
	(0.32)	(0.26)	(0.32)	(0.22)	(0.19)	(0.18)	
pH	7.70	7.68	7.39	7.38	7.11	7.10	< 0.001
	(0.07)	(0.11)	(0.07)	(0.06)	(0.12)	(0.07)	
P_{CO_2}	11.89	12.29	30.33	31.52	64.48	65.14	< 0.001
	(1.30)	(2.19)	(3.40)	(3.97)	(11.09)	(8.70)	

Definition of abbreviations: Cdyn = dynamic lung compliance; Paw = airway pressure; Ppa = pulmonary artery pressure.

Baseline variables are presented as means (standard deviation). For all variables, a comparison of timing group (label applied during injurious ventilation [A] versus label applied after injurious ventilation [B]) produced no statistically significant differences. The p values shown correspond to overall comparisons between the CO_2 treatment groups: hypocapnia, normocapnia, and hypercapnia. Note that mean Paw and Ppa values for hypocapnia Group B and Normocapnia Group A are based on group sizes of 11, not 12; mean Cdyn values for hypocapnia Group B and Normocapnia Group A are based on group sizes of 10 and 11, respectively, not 12; and that mean pH and P_{CO_2} values for normocapnia Group A and hypercapnia Group A are based on group sizes of 11, not 12.

cells (0.22 ± 0.09 versus 0.33 ± 0.1, $p < 0.02$). Raw data are shown in the online supplement.

Effects of CO_2 on Cell Membrane Repair after Scratch Injury in Primary Type II Rat Alveolar Epithelial Cells

To test the hypothesis that hypercapnic acidosis has adverse effects on plasma membrane repair in a perfusion-independent system, we studied cell-resealing responses in a primary Type II rat alveolar (ATII) epithelial cell wound model. Using a dual-labeling method, injured but healed cells were distinguished from permanently damaged cells (27). Our observations confirmed that hypercapnic acidosis impairs plasma membrane resealing. Figure 4 shows plasma membrane-resealing rates after scratch injury under normocapnic (P_{CO_2} = 36.4 mm Hg; pH 7.47), hypocapnic (P_{CO_2} = 20 mm Hg; pH 7.66), and hypercapnic (P_{CO_2} = 119 mm Hg; pH 7.01) conditions.

As was true for the intact lung, there were significantly more cells with permanent plasma membrane defects in hypercapnic, acidotic samples than in normocapnic samples. Accordingly, the rate of plasma membrane repair in cells injured under normocapnic conditions was $73 \pm 13\%$ compared with only 60 ± 25 and $49 \pm 20\%$ in hypocapnic and hypercapnic preparations, respectively ($p < 0.05$). In keeping with results from intact lungs, the resealing responses of cells wounded in a hypocapnic environment were intermediate and were not significantly different from those of normocapnic controls. Subsequent experiments performed in A549 cells, using an identical approach, are shown in the online supplement and also confirm this result.

DISCUSSION

With this series of experiments we have established that hypercapnic acidosis impairs the ability of lung cells to repair plasma membrane wounds. Such wounds are the result of plasma membrane stress failure, which invariably occurs in ventilator-injured lungs (19, 23). In the following sections we briefly consider the strengths and limitations of our experimental approaches and analysis methods. We then review the current understanding of the molecular mechanisms involved in plasma membrane repair and examine how hypercapnic acidosis may impact these mechanisms.

Strengths and Limitations of Experimental Approach and Analysis Methods

We developed the rat lung VILI model so as to study the effects of specific interventions such as hypercapnia on plasma membrane repair in the intact lung. The repair readout, that is, the fraction of injured cells with healed plasma membrane wounds, rests on a comparison of PI uptake between preparations that are labeled during as opposed to after injurious mechanical ventilation. The ventilator settings of 40 ml/kg and zero positive end-expiratory pressure were chosen to maximize the specific injury mechanism of interest, namely deformation-related cell wounding.

Perfusate and tissue CO_2 tension were manipulated by adjusting the inspired gas mixture to a target P_{CO_2} in the venous effluent. Because lungs eliminate CO_2 through the pleural space in the initial series of experiments (Table 1) the P_{CO_2} of the venous effluent was consistently lower than that of the inspired gas. Consequently, the lungs in the group labeled normocapnic in Figures 1 and 2 were actually hypocapnic, but had a normal pH. To the extent to which pleural gas exchange could have biased PI label distributions and confounded our interpretation of results, in confirmatory experiments shown in Figure E3 (*see* the online supplement), all normocapnic preparations were maintained at an inspired and ambient P_{CO_2} of 40 mm Hg.

We observed a significantly greater number of PI-positive cells in hypercapnic preparations that were labeled after removal of the injurious stress. This suggests that hypercapnic acidosis causes a defect in plasma membrane repair and raises concern that permissive hypercapnia promotes lung cell necrosis. Although we did not demonstrate necrosis by independent means it is known from cell culture systems that plasma membrane repair must occur within less than 1 minute (more likely seconds) for cells to survive a plasma membrane tear (28).

TABLE 2. PHYSIOLOGIC RESPONSES TO INJURY

	Hypocapnia		Normocapnia		Hypercapnia		
	A	B	A	B	A	B	p Value
Lung weight, g	8.16	8.70	6.44	7.28	5.62	6.60	0.003
	(2.41)	(3.48)	(1.88)	(2.42)	(1.00)	(2.05)	
Paw, cm H_2O	50.39	48.48	48.67	45.62	41.63	44.85	0.003
	(8.15)	(4.31)	(7.16)	(5.04)	(5.37)	(5.18)	
Ppa, cm H_2O	17.68	15.24	16.59	15.31	15.25	14.15	0.32
	(6.94)	(1.35)	(4.10)	(4.13)	(3.50)	(3.14)	
Cdyn, ml/cm H_2O per kg	0.24	0.27	0.28	0.31	0.42	0.48	0.002
	(0.07)	(0.12)	(0.11)	(0.17)	(0.23)	(0.32)	
LIP, cm H_2O	28.51	26.52	25.52	25.21	22.68	22.50	< 0.001
	(4.43)	(2.49)	(1.92)	(1.73)	(3.01)	(2.97)	
ΔLung weight, g	5.08	5.62	3.41	4.26	2.46	3.41	0.002
	(2.42)	(3.46)	(1.65)	(2.30)	(0.92)	(1.96)	
ΔPaw, cm H_2O	14.78	11.54	12.49	10.37	7.61	10.45	0.029
	(6.69)	(4.20)	(4.18)	(3.87)	(2.73)	(7.79)	
ΔPpa, cm H_2O	5.81	2.73	3.50	2.38	2.12	1.80	0.082
	(5.88)	(2.64)	(4.21)	(1.80)	(3.74)	(1.24)	
ΔCdyn, ml/cm H_2O per kg	−1.05	−1.03	−0.99	−1.01	−0.93	−0.82	0.062
	(0.29)	(0.21)	(0.24)	(0.28)	(0.20)	(0.25)	

Definition of abbreviations: Cdyn = dynamic lung compliance; LIP = airway pressure at lower inflection point; Paw = peak airway pressure; Ppa = pulmonary artery pressure.

Physiologic responses are presented as means (standard deviation). For all variables except PI/Alv (*see* Figure 2), a comparison of timing group (label applied during injurious ventilation [A] versus label applied after injurious ventilation [B]) produced no statistically significant differences. The p values shown correspond to overall comparisons between the CO_2 treatment groups: hypocapnia, normocapnia, and hypercapnia. Note that mean Paw, Ppa, Cdyn, and LIP values for hypocapnia Group B are based on a group size of 10, not 12; mean LIP values for normocapnia Group B, hypercapnia Group A, and hypercapnia Group B are based group sizes of 11, not 12; and mean ΔPaw, ΔPpa, and ΔCdyn values for hypocapnia Group B and normocapnia Group A are based on group sizes of 10 and 11, respectively, not 12.

We have interpreted the differences in cell injury estimates with respect to label timing (Conditions A and B in Figure 1) as a measure of plasma membrane wound repair. This interpretation rests on the assumption that the PI concentration in extracellular (and alveolar) fluid is sufficiently high to label all cells with plasma membrane tears under all experimental conditions. This assumption would be violated if injury, ventilator settings, P_{CO_2}, and pH alone or in combination caused significant changes in pulmonary vascular barrier properties and hence label delivery

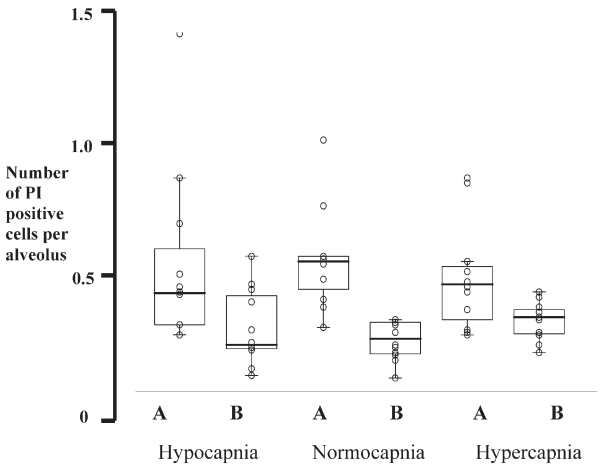

Figure 2. *Box plots* of number of propidium iodide–positive cells per alveolus (PI/Alv) by treatment group with data points added. Fewer than 12 per group may appear because of duplicate values. Groups A and B refer to label timing with respect to injury (labeled during injurious ventilation, *A*; labeled after injurious ventilation, *B*). Mean PI/Alv is 0.25 lower for Group B than Group A for hypocapnia, 0.32 lower for normocapnia, and 0.16 lower for hypercapnia (overall, p = 0.39). The estimated difference between Group A and Group B is 0.07 lower for normocapnia than for hypocapnia (p = 0.58), 0.09 lower for hypocapnia than for hypercapnia (p = 0.49), and 0.15 lower for normocapnia than for hypercapnia (p = 0.082).

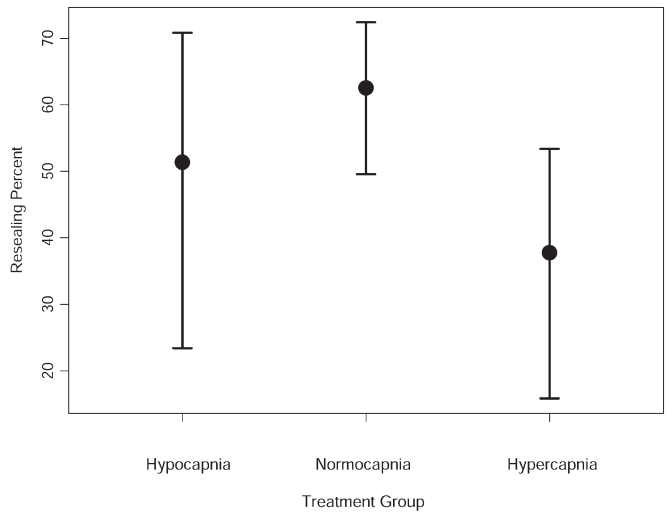

Figure 3. Estimated resealing percentage by capnia group and 95% confidence intervals via a bootstrap resampling procedure. The estimates (and 95% confidence intervals) are 51% (23–71%) for hypocapnia, 63% (50–72%) for normocapnia, and 38% (16–53%) for hypercapnia. The estimated resealing percentage is 11% higher for normocapnia than for hypocapnia (p = 0.37), 13% higher for hypocapnia than for hypercapnia (p = 0.41), and 25% higher for normocapnia than for hypercapnia (p = 0.022).

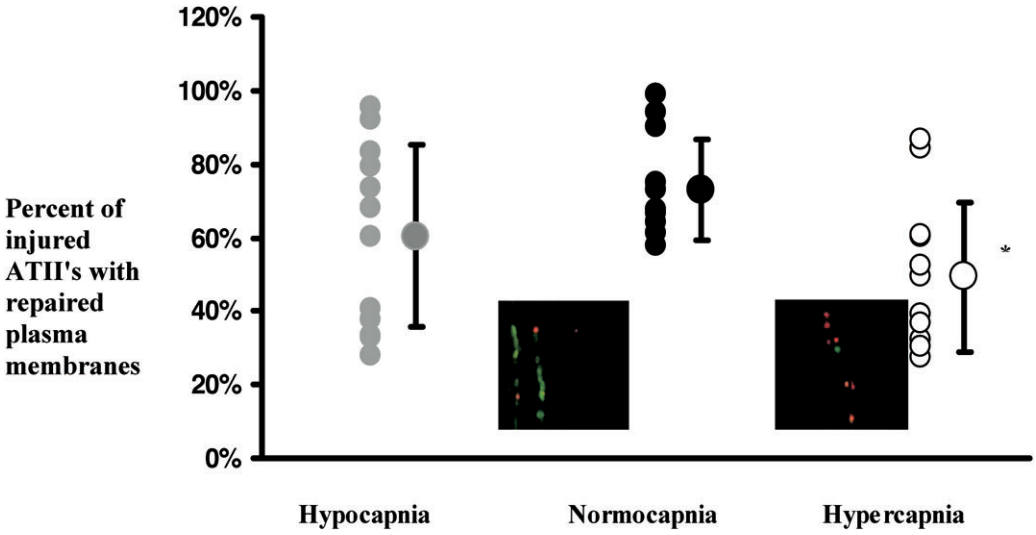

Figure 4. Plasma membrane–resealing rates after scratch injury under normocapnic (P_{CO_2} = 36.4; pH 7.47) hypocapnic (P_{CO_2} = 20; pH 7.66), and hypercapnic (P_{CO_2} = 119; pH 7.01) conditions. The fluorescence image insets demonstrate CO_2-dependent differences in the number of injured cells with transient (*green*) and permanent (*red*) plasma membrane wounds. The rate of plasma membrane repair in cells injured under normocapnic conditions was 73 ± 13% compared with only 60 ± 25 and 49 ± 20% in hypocapnic and hypercapnic preparations, respectively (*$p < 0.05$).

to injured target cells. For example, if after removal of injurious stress (Figure 1, Condition B) a rapid restoration of vascular barrier function excluded PI from entering the alveolar spaces, necrotic alveolar epithelial cells could remain unstained. To guard against this error, we labeled the perfusate after return to noninjurious ventilator settings with fluorescein isothiocyanate–dextran and demonstrated that barrier function is not fully restored in the immediate postinjury period. Confocal images demonstrating green fluorescence in alveolar edema, a surrogate of increased capillary leakiness, are shown in the online supplement. Finally, we reaffirmed earlier observations that alveolar edema fluid of lungs that had been perfused with PI after injury is able to label injured cells in epithelial monolayers (data not shown).

Another potential source of bias is an injury-, ventilator setting-, P_{CO_2}-, and/or pH-dependent redistribution of perfusion between subpleural airspaces that are accessible to intravital microscopy and more central lung regions that are not. It is well known that injury, ventilator settings, P_{CO_2}, and pH modulate pulmonary vascular resistance and that these effects vary with species and experimental setting. We used relatively low perfusate flows, which is why the pulmonary artery pressure, our readout of overall changes in pulmonary vascular resistance, differed little between experimental conditions. To our knowledge, the effects of injury and acid–base balance on the topographic distribution of blood flow and vascular barrier properties have not been studied in *ex vivo* preparations. On the other hand, we know of no compelling physiologic reason why in an experimental setting like ours systematic, CO_2-sensitive differences in flow and vascular properties between lung center and lung periphery should exist. We recognize that ultimate proof of our assumptions would require a mapping of PI concentrations throughout the preparation and a demonstration that PI concentrations everywhere are sufficiently high that all cells with plasma membrane defects are labeled. Although such an approach is not feasible we had observed, in validation studies that preceded our initial publication of the technique (23), that results are not sensitive to the overall concentration of PI in the perfusion solution.

At first glance, therapeutic hypercapnia appears to have had no effect on the susceptibility of lung cells to high-volume ventilation-induced plasma membrane stress failure. However, this interpretation of the "negative" data shown in Figure 2 must be viewed with caution. As has been shown by others in several *in vivo* and *ex vivo* injury models (4–9, 11, 12) we also demonstrate highly significant CO_2 effects on lung barrier function in our preparation. Hypercapnia ameliorates injury-related edema, whereas hypocapnia exaggerates it (Table 2). Because both amount and distribution of edema are important determinants of alveolar microstrain (33, 34) the stress imposed by mechanical ventilation on lung cells has undoubtedly differed between the experimental groups. Accordingly, one might have expected an inverse correlation between the number of injured cells and P_{CO_2}. The fact that this was not observed (Figure 2) means either that we were underpowered to detect small group mean differences in the number of wounded cells or that hypercapnia, in addition to inhibiting membrane repair, also increases the susceptibility of cells to stress failure. One potential mechanism that would link the susceptibility to stress failure with the probability of membrane repair would be a general pH and/or P_{CO_2} dependence of cellular lipid trafficking. We had shown in previous work that deformation-induced lipid trafficking is an important cytoprotective mechanism that in the face of an externally imposed cellular shape change keeps plasma membrane tension below lytic levels (27). In secretory cells membrane trafficking, specifically endocytosis, is inhibited at low pH (35, 36). However, at present we have no direct evidence that would either support or refute a pH and/or P_{CO_2} dependence of deformation-induced lipid trafficking in lung cells, nor do we know whether the PI-positive cells in subpleural airspaces are of endothelial or epithelial origin, and represent Type I or Type II cells.

Electron micrographs of ventilator-injured rat lungs suggested that, compared with endothelial cells and Type I alveolar (ATI) epithelial cells, the ultrastructure of ATII cells appears relatively preserved (37). Consequently it was argued that *in situ* ATII cells are protected from deforming stress by virtue of their location in alveolar corners. This hypothesis is consistent with *in vitro* observations suggesting that ATII cells are actually more susceptible to stretch injury than are transdifferentiated ATI-like cells (38). However, both *in vitro* and *in situ* observations await independent confirmation. For example, Imai and colleagues emphasize ATII necrosis in a rabbit model of ventilator-injured lungs (39).

Molecular Mechanisms of Plasma Membrane Repair

The molecular mechanisms that drive plasma membrane resealing vary with cell type and lesion size (28). Small plasma mem-

brane wounds (< 1 μm) tend to seal spontaneously by lateral plasma membrane lipid flow. This is best demonstrated in red blood cells (40). However, most plasma membrane lesions, particularly if they are large, repair only if intracellular lipids are shuttled to the plasma membrane by an active, energy-dependent, and Ca^{2+}-regulated process (41–43). The insertion of lipids into the plasma membrane causes a fall in plasma membrane tension, which in turn promotes "self-sealing" by lateral lipid flow (44, 45). If the lesion is large, extracellular Ca^{2+} enters the cell and induces lipid vesicles to fuse and form a membrane patch (46–48). Patch formation and the subsequent sealing of the surface break by site-directed exocytosis require the coordinated actions of vesicle fusion and docking proteins such as synaptotagmin, synaptobrevin, synaptosome-associated protein-25, and involves molecular motors of the kinesin family. In most cells the organelles that coalesce to form patches include lysosomes (49, 50). In addition, a widely expressed class of vesicles, called enlargosomes, has been identified in secretory and nonsecretory mammalian cells and has been implicated in membrane repair (51).

Even this extremely sketchy account of plasma membrane resealing identifies a host of potentially pH-sensitive target molecules: vesicle docking and fusion proteins, molecular motors, second messengers of membrane-adhesive interactions such as phosphatidylinositol 4,5-bisphosphate and the proteins that control its activity, protein kinase C–dependent Golgi export, and cellular Ca^{2+} homeostasis itself (52, 53). Although our statistical confidence in the effects of hypercapnia on cell repair is stronger than that pertaining to hypocapnia, all our observations, be they in whole lungs or cell culture models, suggest that positive as well as negative deviations from the eucapnic state alter cellular repair responses. The long yet undoubtedly incomplete list of potentially pH- and Pco_2-sensitive candidate molecules underscores the difficulty of trying to attribute CO_2-mediated effects to a limited number of substrates or signaling pathways. Moreover, the many degrees of freedom in CO_2-mediated free radical reactions underscore the critical dependence of metabolic pathways on the local environment. For example, in aqueous solutions CO_2 scavenges peroxynitrite to form nitrosoperoxycarbonate. This compound is hydrolyzed to nitrate and carbonate and the net effect of this reaction is prevention of nitration and oxidative damage. However, in the nonpolar environment of membranes nitrocarbonate undergoes other reactions promoting nitration of proteins and oxidative damage (15).

Clinical Relevance

Although our observations have generated provocative hypotheses about CO_2- and pH-dependent mechanisms in cell injury and repair it would be inappropriate to derive recommendations for clinical practice. Even without the full appreciation of how the molecular components of the cellular lipid-trafficking machinery are integrated and what makes the machinery sensitive to CO_2 a clinician may ask whether correction of extracellular pH can restore cell repair function. Most clinicians administer bicarbonate buffers to hypercapnic patients when blood pH falls below some critical value. However, bicarbonate buffers are CO_2 donors and transiently lower intracellular pH. For example, the apical membranes of polarized alveolar pneumocytes are impermeable to transepithelial acid–base fluxes, but they are permeable to CO_2 (54). To the extent to which membrane repair processes could be dependent on intracellular pH and not extracellular pH, the infusion of bicarbonate buffers may well be counterproductive.

What is a clinician to think of an intervention, which on the one hand has potentially beneficial immune-modulating effects on cytokine networks (52, 55–57), protects lung barrier function (reviewed in Kregenow and Swenson [13]), has complex effects on reactive intermediates (14–16, 58), but also increases the number of necrotic lung cells? In contrast to apoptosis, which may also be enhanced in a hypercapnic environment (18, 59), cell death by necrosis triggers a proinflammatory response. Whether this response is more intense than one that is initiated by wounded and repaired cells is not known. Cells, which repair and thereby survive plasma membrane tears in culture, express a number of early stress response genes as well as CXC chemokines and adhesion molecules consequent to the activation and translocation of the nuclear transcription factor NF-κB (60). The latter is thought to play a mechanistic role in mediating inflammatory manifestations of adult respiratory distress syndrome and ventilator-induced lung injury (61). To the extent to which injured but living cells could generate a more long-lasting coordinated proinflammatory response by uninjured neighbors, it should not be assumed *a priori* that promoting cell repair and avoiding permissive hypercapnia-induced cell necrosis are beneficial for patient outcomes.

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