Impact of buffering hypercapnic acidosis on cell wounding in ventilator-injured rat lungs

Sean M. Caples,1 Deborah L. Rasmussen,1 Won Y. Lee,1 Marla Z. Wolfert,1 and Rolf D. Hubmayr1,2

1Thoracic Diseases Research Unit, Division of Pulmonary and Critical Care Medicine, Department of Medicine, and 2Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota

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Caples SM, Rasmussen DL, Lee WY, Wolfert MZ, Hubmayr RD. Impact of buffering hypercapnic acidosis on cell wounding in ventilator-injured rat lungs. Am J Physiol Lung Cell Mol Physiol 296: L140–L144, 2009. First published November 7, 2008; doi:10.1152/ajplung.90339.2008.—We measured the effects of raising perfusate pH on ventilator-induced cell wounding and repair in ex vivo mechanically ventilated hypercapnic rat lungs. Lungs were randomized to one of three perfusate groups: 1) unbuffered hypercapnic acidosis, 2) bicarbonate-buffered hypercapnia, or 3) tris-hydroxymethyl aminomethane (THAM)-buffered hypercapnia. The membrane-impermeant label propidium iodide was added to the perfusate either during or after injurious ventilation providing a means to subsequently identify transiently wounded and permanently wounded cells in optical sections of subpleural alveoli. Normalizing perfusate pH in hypercapnic preparations attenuated ventilator-induced cell injury, particularly in THAM-buffered preparations. This was observed despite greater amounts of edema and impaired lung mechanics compared with other treatment groups. Protective effects of buffering of hypercapnic acidosis on injury and repair were subsequently confirmed in a cell scratch model. We conclude that buffering of hypercapnic acidosis attenuates plasma cell injury induced by mechanical hyperinflation.

WITH STRONG EVIDENCE THAT mechanical ventilation delivering high tidal volumes results in further damage to injured lungs (25), referred to as ventilator-associated lung injury, many clinicians now routinely prescribe a low tidal volume, so-called lung-protective strategy. The attendant increases in blood carbon dioxide tension and acidosis has been referred to as “permissive” hypercapnia, a consequence of low tidal volume ventilation that for years was largely regarded as a tolerated adverse effect. In the absence of clear supporting data, some have advocated alkali administration to counteract the perceived deleterious effects of this hypercapnic acidosis (6, 25). In fact, the ARDSNet protocol provided for the use of sodium bicarbonate and cited the buffering of acidosis and partial correction of hypercapnia as potential mechanisms of survival benefit associated with the low tidal volume ventilatory strategy (25).

There are mounting experimental evidence, however, that hypercapnia may have protective effects independent of mechanically delivered tidal volumes (11). So-called “therapeutic” hypercapnia has been shown to guard against hypoxic (10), oxidative (22), and inflammatory (24) insults across a variety of cellular and isolated organ models. Furthermore, hypocapnia may have deleterious effects (14, 15), whereas bicarbonate buffering of hypercapnic acidosis has been shown to worsen lung barrier function in animal models of ischemia-reperfusion lung injury (13, 18), augment intracellular oxidative stress (4), and blunt hypercapnia-enhanced organ blood flow (3). Supporting data have also come from clinical studies, where a recent secondary analysis of ARDSNet data showed protective effects of hypercapnia on mortality in those patients ventilated with high tidal volumes, findings potentially at odds with mechanistic hypotheses put forth by the authors of the original ARDS low tidal volume trial (12).

Not all investigators have reported benefit from hypercapnia, which has been implicated in pulmonary (6, 16, 17) and extrapulmonary (21, 28) organ dysfunction, findings that have likely spurred further interest in the use of buffers. Bicarbonate buffers react to liberate carbon dioxide, which, because of high membrane permeability, may raise concern for further intracellular acidosis (9), a potentially operative, although unproven, mechanism in studies demonstrating worsened lung barrier function with buffering of hypercapnic acidosis (13, 18). Tris-hydroxymethyl aminomethane (THAM), acting as a proton acceptor independent of carbon dioxide pathways, has been shown in animal and clinical models to reverse cardio-depressant effects attributed to hypercapnic acidosis (21, 23, 28).

We have previously shown in an experimental model of ventilator-induced lung injury (VILI) of isolated perfused rat lungs that hypercapnic acidosis impairs resealing of plasma membrane wounds. Utilizing a confocal microscopic assessment of subpleural air spaces after timed administration of fluorescent labels, as initially described by Gajic et al. (7), we measured the effects of various carbon dioxide tensions on rates of injury and repair as well as on vascular barrier function. We found that, despite preservation of lung barrier function specific to the hypercapnic group, rates of injury across all carbon dioxide tensions were similar, suggesting pH- and/or PCO2-dependent effects on cellular lipid trafficking.

The current set of experiments was designed to measure the impact of raising extracellular pH with administration of bicarbonate or THAM buffers on lung barrier function and plasma membrane wounding and repair in an ex vivo rat lung model of VILI.

METHODS

Experimental approach, animal preparation, equipment, and monitoring. The protocol was approved by the Mayo Clinic Institutional Review Board (IRB) and the Institutional Animal Care and Use
Buffering hypercapnia in lung injury

Effects of buffering on cell wounding and repair from ventilator-induced injury. Figure 2 shows data plots of PI/Alv by buffering group. Based on reductions in the PI/Alv ratio in the A subgroups, buffering of hypercapnic acidosis attenuates cellular injury in this model. The effect of THAM buffering was statistically significant, despite effecting greater lung

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Bicarbonate</th>
<th></th>
<th>Krebs</th>
<th></th>
<th>THAM</th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Rat weight, g</td>
<td>241.6 (23.9)</td>
<td>247.2 (23.8)</td>
<td>231.5 (17.9)</td>
<td>240.4 (20.1)</td>
<td>232.8 (32.1)</td>
<td>243.4 (20.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>0.80 (0.13)</td>
<td>0.83 (0.13)</td>
<td>0.75 (0.09)</td>
<td>0.77 (0.11)</td>
<td>0.75 (0.12)</td>
<td>0.81 (0.11)</td>
<td>NS</td>
</tr>
<tr>
<td>(predicted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic time (min)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Paw, cmH2O</td>
<td>30.4 (0.6)</td>
<td>30.4 (0.7)</td>
<td>30.1 (0.4)</td>
<td>30.1 (0.5)</td>
<td>30.2 (0.3)</td>
<td>30.5 (0.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Ppa, cmH2O</td>
<td>6.7 (1.1)</td>
<td>6.7 (1.7)</td>
<td>7.0 (1.5)</td>
<td>7.4 (2.1)</td>
<td>6.1 (0.9)</td>
<td>6.6 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 (0.06)</td>
<td>7.39 (0.13)</td>
<td>7.12 (0.18)</td>
<td>7.10 (0.18)</td>
<td>7.44 (0.07)</td>
<td>7.40 (0.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Osmolality</td>
<td>293.4 (6.8)</td>
<td>292.2 (9.5)</td>
<td>292.9 (9.6)</td>
<td>287.3 (11.6)</td>
<td>295.1 (0.0)</td>
<td>297.3 (7.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Paw, peak airway pressure; Ppa, pulmonary arterial pressure, both measured at the moment of initiation of injurious ventilation.
end of injurious ventilation. THAM buffered. Paw and Ppa were both measured at the conclusion of injurious ventilation. Krebs B). The line within the boxes represents the mean values.

Comparison with corresponding Krebs group (i.e., THAM A compared with any of the treatment groups, suggesting, in this experimental model, no measurable effects of buffering on repair of injured cells.

Effects of buffering on cell membrane repair after scratch injury in cell culture models. To test the effects of buffering of hypercapnic acidosis on plasma membrane repair in a perfusion-independent system, we studied cell-resealing responses in a wounding model utilizing human A549 tumor cells. Using a dual-labeling method, injured but healed cells were distinguished from permanently damaged cells (27). These experiments replicated our previous observation (5) that hypercapnic acidosis impairs plasma membrane resealing and confirmed our current whole lung results suggesting protective effects of buffering on cellular injury. Figure 3 shows rates of cells with evidence for permanent plasma membrane wounds following scratch injury under the various buffer conditions and with a normocapnic control, suggesting lack of wound repair and cell death.

DISCUSSION

These experiments show that buffering of hypercapnic acidosis influences pulmonary mechanics and rates of cell membrane wounding and repair when lungs are exposed to injurious ventilation. To the extent that buffering of hypercapnic acidosis was associated with changes in measured variables suggests the importance of pH-dependent mechanisms in protecting against cellular injury as well as preventing irreversible deformation-induced plasma membrane wounds.

The current data are consistent with our previous series of experiments that utilized a similar lung injury model across a range of inspired carbon dioxide concentrations (5, 7). With the differential timing of administration of the fluorescent label PI, we showed that, under normocapnic conditions, most plasma membrane wounds inflicted by injurious ventilation repair themselves (7). Subsequent experiments by Doerr et al. (5) demonstrated that this repair process is impaired under conditions of hypercapnic acidosis. We have replicated those findings with the current set of experiments, showing similar rates of subpleural cellular injury but little repair as determined from the PI/Alv ratios during (group A) and after (group B) fluorescent dye labeling under unbuffered conditions (Fig. 2).

We utilized two compounds with distinct buffering mechanisms to test our hypotheses. Bicarbonate buffer is widely used in a number of clinical conditions, but, because it acts as a CO2 donor, it has the potential to further reduce intracellular pH because of the free diffusion of CO2 across cell membranes, including those of alveolar epithelial cells (9). THAM, on the other hand, acts as a proton acceptor and is subsequently renally excreted (2), avoiding local production of CO2 and therefore having little additive effect on intracellular acid-base balance. However, we should point out that under the conditions of our experiment, in which CO2 tensions in alveolar gas and lung perfusate were clamped, intracellular reductions in pH resulting from CO2 liberation during bicarbonate buffering were likely very short lived.

### Table 2. Physiological responses to injury

<table>
<thead>
<tr>
<th></th>
<th>Bicarbonate</th>
<th>Krebs</th>
<th>THAM</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Lung weight, measured, g</td>
<td>5.3 (1.2)</td>
<td>7.1 (2.1)</td>
<td>5.3 (1.2)</td>
</tr>
<tr>
<td>∆Lung weight, g</td>
<td>4.5 (1.2)</td>
<td>6.2 (2.1)</td>
<td>4.4 (1.4)</td>
</tr>
<tr>
<td>Ppa, cmH2O</td>
<td>8.3 (3.9)</td>
<td>8.9 (2.1)</td>
<td>7.4 (2.5)</td>
</tr>
<tr>
<td>Paw, cmH2O</td>
<td>40.3 (3.8)</td>
<td>44.1 (7.4)</td>
<td>40.5 (4.9)</td>
</tr>
<tr>
<td>∆Paw, cmH2O</td>
<td>10.0 (3.8)</td>
<td>10.3 (5.1)</td>
<td>10.3 (4.7)</td>
</tr>
</tbody>
</table>

Physiological responses are presented as means (SD). The P values correspond to overall comparisons between the groups: bicarbonate buffered, unbuffered, THAM buffered. Paw and Ppa were both measured at the conclusion of injurious ventilation. ∆Paw, mean change in peak airway pressures from beginning to end of injurious ventilation.
In our lung injury model, both THAM and bicarbonate buffers protected against hyperinflation-associated lung cell injury. Statistically, this effect reached significance only for the THAM group, but given the spread in the data and the limited statistical power, we hesitate to interpret the nonsignificant difference between the buffer modalities in a mechanistic sense.

Interestingly, the cytoprotective effect of buffer solutions was observed even though lungs perfused with pH buffers, in particular THAM, developed more edema. While this could reasonably be expected to result in a fall in lung compliance and consequent exposure to higher alveolar pressures during volume preset mechanical ventilation, one would have to consider the possibility that, by acting as a barrier to force transmission, alveolar edema may actually protect flooded regions from overdistension, a conclusion supported by another set of experiments conducted in our laboratory where edema formation was found to be inversely related to endothelial cell injury in a lung hyperinflation model (Berlin ESICM 2007). On the other hand, there are several arguments to refute this explanation. First, an alveolus, in which deformation is large enough to produce a change in barrier properties, is also more likely to experience lytic cell strains. Once the region floods and becomes derecruited, the injurious stress may no longer be transmitted to the cells, but the damage would have already been done. In a short-term physiological experiment like ours, we can ignore repair mechanisms that involve cell proliferation or removal as having obscured such a sequence of events. Second, Bilek et al. (1) have put forth strong evidence in favor of cell injury by interfacial tensions, which should expose the cells of flooded regions to additional stress. Third, in our hands, the interregional variability in cell injury index is not correlated with measures of overall lung weight gain or mechanical impedance. We recognize that our model is to some extent based on the unproven assumption that the injury index correlates with alveolar barrier dysfunction, and, therefore, edema formation. If this were true, as an increasing number of flooded regions become derecruited, one would expect that the vanishing aerated and recruitable lung would be subject to increased injury from hyperinflation. As a result, we would have anticipated an increase in the interregional variability in cell injury index in the most edematous lungs. However, this was not observed.

The whole lung data is augmented by convincing A549 cell scratch injury data suggesting that the impaired wound resealing noted in our previous experiments under conditions of hypercapnic acidosis is pH sensitive. Unfortunately, the data from the ex vivo perfused lungs are less convincing. We have shown previously that a high pH buffer on resealing correlates with alveolar barrier dysfunction, and, therefore, edema formation. If this were true, as an increasing number of flooded regions become derecruited, one would expect that the vanishing aerated and recruitable lung would be subject to increased injury from hyperinflation. As a result, we would have anticipated an increase in the interregional variability in cell injury index in the most edematous lungs. However, this was not observed.

There are myriad potential pH- and CO2-dependent molecular targets that are critical for the maintenance of cell structural integrity in the face of deforming stress (8, 20). In functional terms, they involve complex systems mediating cytoskeletal remodeling as well as lipid and vesicular trafficking, and from the plasma membrane (27). It would be erroneous to think of the cytoprotective effects of pH buffers in hypercapnic preparations in a binary manner, i.e., to simply attribute all mechanisms to pH as opposed to CO2 tension. The interactions between CO2, hydrogen ion concentration, reactive oxygen species (ROS), and reactive nitrogen species (RNS) are complex and very much dependent on the milieu in which unstable intermediates are generated. Vesela and Wilhem (26) state that in an aqueous environment, the net effect of CO2’s interaction with ROS and RNS is the scavenging of peroxynitrite, thereby limiting oxidative damage. However, in the non-polar environment of membranes, the same compounds cause nitration of proteins and oxidative damage.

Translation to clinical outcomes. We do not believe that effects of interventions on cell injury and repair in preclinical injury models should be used to justify management decisions in patients. While there is likely a cause and effect relationship between cell injury, cell necrosis, and the subsequent innate immune response, hypercapnia and acidosis influence such responses in ways that cannot be captured in a short-term physiological experiment with narrow surrogate endpoints. Little is known if and how the regional variability in alveolar CO2 tension that results from V/Q mismatch influences local injury and repair responses. This may be one reason why in some models CO2 supplementation with inspired gas produces different responses than CO2 retention caused by deliberate hypoventilation (11). Low tidal volume ventilation will continue to be widely practiced as one of a very small number of interventions shown to improve the disappointingly high mortality of ARDS. In the absence of further large clinical trials, the debate over management of the attendant hypercapnic acidosis will therefore remain. There is strong animal data suggesting the many potential benefits of hypercapnic acidosis (3, 11, 22, 24), recently augmented by secondary analysis of the ARDSNet data (12). On the other hand, skepticism persists among some, with experimental and clinical data touting drawbacks to hypercapnic acidosis (16) and benefits of various buffering strategies (17, 23, 28), with some compelling clinical arguments for the use of THAM.

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GRANTS

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REFERENCES

