Hypercapnia: A Nonpermissive Environment for the Lung

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Patients with severe acute and chronic lung diseases develop derangements in gas exchange that may result in increased levels of CO₂ (hypercapnia), the effects of which on human health are incompletely understood. It has been proposed that hypercapnia may have beneficial effects in patients with acute lung injury, and the concepts of “permissive” and even “therapeutic” hypercapnia have emerged. However, recent work suggests that CO₂ can act as a signaling molecule via pH-independent mechanisms, resulting in deleterious effects in the lung. Here we review recent research on how elevated CO₂ is sensed by cells in the lung and the potential harmful effects of hypercapnia on epithelial and endothelial barrier, lung edema clearance, innate immunity, and host defense. In view of these findings, we raise concerns about the potentially deleterious effects hypercapnia may have in patients with acute and chronic lung diseases.

Keywords: CO₂; COPD; Na,K-ATPase; alveolar epithelial barrier function; innate immunity

Patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS), chronic obstructive pulmonary disease, and cystic fibrosis develop abnormalities in gas exchange that may result in hypercapnia, the accumulation of increased levels of CO₂ in the blood and tissues (1–3). During acute respiratory failure, mechanical ventilation (MV) is often necessary to maintain gas exchange, which may lead to ventilator-induced lung injury (VILI). VILI can be reduced by ventilating patients with low tidal volume (V_T) (4–6). Because low-V_T ventilation sometimes results in hypercapnia, it has become customary to allow PCO₂ to rise in patients with ARDS, a concept termed “permissive” hypercapnia. Some investigators have hypothesized that hypercapnia *per se* might account for better outcomes in ARDS and have proposed the concept of “therapeutic” hypercapnia (2). Because inflammation is a hallmark of ALI/ARDS and because respiratory acidosis has been shown to inhibit several inflammatory mediators, it seemed logical that hypercapnia might be protective in ARDS (2). Mechanisms by which hypercapnia has been proposed to attenuate lung injury include impairment of neutrophil function, inhibition of free radical generation or action, and reduction in levels of proinflammatory

cytokines (7). However, some of these effects were probably primarily attributable to acidosis rather than to hypercapnia *per se* because buffering hypercapnic acidosis worsened experimental lung injury (8).

Here we review recent findings on how CO₂ is sensed in lung cells; potential detrimental effects of hypercapnia on epithelial and endothelial wound repair, alveolar fluid balance, innate immunity and host defense; and recent clinical data indicating that hypercapnia may not be associated with improved outcome in ALI/ARDS.

CO₂ SENSING IN THE ALVEOLAR EPITHELIUM

Central and peripheral chemoreception of CO₂ in mammalian neurons, such as specialized brainstem neurons and the carotid body glomus cells, was described several decades ago (9). It is well established that an increase in blood PCO₂ leads to depolarization of glomus cells, triggering an increase in alveolar ventilation to maintain normal PCO₂ (10). However, the physiological and pathophysiological effects of CO₂—in particular, the effects of hypercapnia on cells in the lung, the primary site of CO₂ elimination—are incompletely understood. Recent studies in alveolar epithelial cells demonstrated that a short-term elevation in CO₂, independent of changes in intra- or extracellular pH or reactive oxygen species, rapidly activated the AMP-activated protein kinase (AMPK), a metabolic sensor (11), in a Ca²⁺– and Ca²⁺/calmodulin-dependent kinase kinase-β–dependent manner, thereby leading to activation of protein kinase (PK)C-ζ, down-regulation of the Na,K-ATPase, and alveolar epithelial dysfunction (Figure 1) (12, 13). Activation of AMPK by hypercapnia has also been shown to be regulated by the mitogen-activated protein kinase family member extracellular signal-regulated protein kinase, further demonstrating the complexity of the CO₂–induced signaling pathway (14). Elevated CO₂ levels also down-regulated the Na,K-ATPase in *Drosophila melanogaster* (15), suggesting that some molecular effects of CO₂ are evolutionarily conserved. Furthermore, elevated CO₂ levels (independent of pH and hypoxia) induced miR-183, a microRNA, which in turn down-regulated isocitrate dehydrogenase 2, a key enzyme of the tricarboxylic acid cycle that catalyzes the conversion of isocitrate to α-ketoglutarate, leading to mitochondrial dysfunction and impaired proliferation of alveolar epithelial cells (16). Thus, it appears that increased CO₂ levels are sensed by the alveolar epithelium, the site of CO₂ elimination in mammals, leading to deleterious effects on cell function.

EFFECTS OF HYPERCAPNIA ON CELL MEMBRANES

Plasma membrane (PM) wounding of resident alveolar cells contributes to the pathogenesis of ALI and VILI (17, 18). Studies in experimental VILI models indicate that the majority of wounded alveolar cells repair and survive deformation-induced insults (19). This is important insofar as wounded and repaired

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Figure 1. Schematic representation of elevated CO₂-induced signaling pathway leading to rapid down-regulation of Na,K-ATPase in the alveolar epithelial plasma membrane and impaired alveolar fluid clearance.

cells activate stress response genes (20), release proinflammatory mediators, and may thereby contribute to injurious deformation responses, commonly referred to as biotrauma (21). Some studies have shown that increasing the concentration of CO₂ to the inspired gas of animals with injured lungs has protective effects (22). To explore the basis of these observations, we determined if hypercapnia protected resident alveolar cells against wounding by mechanical stress. We found that the rates of PM stress failure in subpleural alveolar epithelial and endothelial cells in ventilation-injured rat lungs were similar across a wide range of alveolar CO₂ tensions (23). However, although it did not protect lung cells from mechanical injury, hypercapnia had a profound effect on the fate of wounded cells. Depending on phenotype and experimental context, most cells in a normocapnic environment repaired even micron-scale PM lesions within 20 to 30 seconds (24). In contrast, under hypercapnic conditions many cells failed to repair such lesions within a time frame compatible with recovery (<2 min), resulting in increased cell death (25). Despite an increased burden of necrotic cells, hypercapnic ventilation-injured lungs gained less weight and exhibited blunted proinflammatory deformation responses, suggesting that CO₂-dependent effects on the integrity and hydraulic permeability of cell-cell junctions may outweigh the effects of epithelial and/or endothelial necrosis on barrier properties in this model of VILI. Moreover, in contrast to necrotic cells, wounded and repaired cells may amplify the proinflammatory response by uninjured neighbors (26).

Successful repair is facilitated by remodeling of the subcortical cytoskeleton in the vicinity of the PM lesion, by the reorientation of polar lipid heads at the wound margin, by the increase in PM fluidity consequent to a decrease in the adhesive interactions between inner PM leaflet lipids and cytoskeleton associated proteins, and by the trafficking of calcium-sensitive endomembranes in coordination with caveolar endocytosis (27, 28). The effect of hypercapnia on cell repair appears to be pH dependent (29), in contrast to its effects on alveolar fluid clearance (12, 13, 30) and innate immunity (15, 31).

HYPERCAPNIA IN ALVEOLAR FLUID BALANCE

Disruption of the alveolo-capillary barrier, a characteristic of ALI/ARDS, results in accumulation of alveolar edema and impaired

gas exchange. Pulmonary edema is cleared via active sodium transport across the alveolar epithelium through apically located sodium channels and basolateral Na,K-ATPases, with water following isosmotically the Na⁺ gradients generated by Na,K-ATPase activity (32, 33). Because clearing the excess alveolar liquid upon lung injury is essential for survival, it is important to investigate whether hypercapnia alters reabsorption of alveolar edema. Briva and colleagues reported that elevated CO₂ levels impaired Na,K-ATPase function and alveolar edema clearance independently of extra- and intracellular acidosis (12). Elevated CO₂ led to activation of PKC-ζ, which directly phosphorylated the Na,K-ATPase α₁-subunit at Ser 18 residue, leading to endocytosis and decreased alveolar fluid clearance (Figure 1).

The CO₂-induced, PKC-ζ-mediated down-regulation of Na,K-ATPase function was independent of cytosolic and mitochondrial ROS (13), contrasting with effects of other stimuli that inhibit the Na,K-ATPase by the action of PKC-ζ in the alveolar epithelium (34–36) and independent of the activity of alveolar epithelial carbonic anhydrases, important regulators of CO₂ metabolism and intracellular pH (30). Rather, elevated CO₂ concentrations resulted in a rapid activation of the metabolic sensor, AMPK, which is known to activate energy-generating pathways while down-regulating energy-consuming events, thereby promoting cellular adaptation (11). Because the Na,K-ATPase accounts for up to 40% of the total resting energy expenditure of alveolar epithelial cells, it is perhaps not surprising that hypercapnia activated AMPK, leading to PKC-ζ phosphorylation and down-regulation of Na,K-ATPase and reduced alveolar fluid clearance (13). This effect was preserved when animals were exposed to sustained hypercapnia up to 7 days, which more accurately mimics the clinical setting. Thus, hypercapnia may have deleterious effects on alveolar epithelial function impairing the resolution of pulmonary edema and therefore on the recovery of patients with ALI/ARDS.

HYPERCAPNIA, INNATE IMMUNITY, AND HOST DEFENSE

Hypercapnia is a marker of poor prognosis in COPD (37–40) and has been associated with increased mortality in hospitalized patients with community-acquired pneumonia (41) and in patients with cystic fibrosis awaiting lung transplantation (1). These observations raised the possibility of a causal link between hypercapnia and poor clinical outcomes and if there is such a link, whether hypercapnia might impair the ability of patients with advanced lung disease to resist infections. The report that hypercapnia worsened acute lung injury in rats with *Escherichia coli* pneumonia (42), in contrast to ventilator- or LPS-induced lung injury (43, 44), was consistent with our hypothesis that hypercapnia might impair lung host defense. To investigate this question, we determined the effect of elevated CO₂ on cytokine expression in LPS-stimulated human and mouse macrophages. We found that normoxic hypercapnia inhibited the expression of tumor necrosis factor and IL-6 at Pco₂ levels encountered in patients with chronic lung disease (31). Inhibition of these cytokines, which play critical roles in antimicrobial host defense (45–51), was not due to cytotoxicity, extracellular or intracellular acidosis, nitric oxide signaling, or general cell stress (31). Furthermore, hypercapnia inhibited cytokine expression at the level of gene transcription. Although tumor necrosis factor, IL-6, and other nuclear factor (NF)-κB-regulated cytokines were suppressed, hypercapnia did not inhibit expression of LPS-induced cytokines that are not directly regulated by NF-κB, such as IL-10 (31).

More recently, in a study assessing changes in global gene expression, we found that hypercapnia modulated a large number

of LPS-regulated genes in human and mouse macrophages. The largest category of CO₂-regulated genes included those related to immune function, but genes encoding transcription factors and proteins involved in signaling, ion/solute transport, cytoskeletal interactions, adhesion, metabolism, and other processes were also affected (52). In addition to altering innate immune gene expression, we found that hypercapnia inhibits phagocytosis (31) and NADPH oxidase-mediated generation of reactive oxygen species (53), both of which are critical for phagocyte antimicrobial activity (Figure 2).

To test the *in vivo* relevance of our findings in phagocytes, we studied the effect of hypercapnia on the outcomes of *Pseudomonas aeruginosa* infection in mice. In these experiments, exposure to 10% CO₂ (with 21% O₂) markedly increased the mortality of *P. aeruginosa* pneumonia (54). Hypercapnia decreased the clearance of *Pseudomonas* from the lungs and increased bacterial dissemination to other organs. The hypercapnia-induced increase in pneumonia mortality was not due to acidosis because mice preexposed to 10% CO₂ for 3 days to allow renal compensation of respiratory acidosis were no less affected than hypercapnic animals without preexposure (and had much lower blood pH due to acute respiratory acidosis).

The immunosuppressive effects of hypercapnia are not limited to mammals. We have found that hypercapnia also alters innate immune gene expression in *Drosophila* (15) and *Caenorhabditis elegans* (55). In *Drosophila*, hypercapnia decreased the expression of multiple antimicrobial peptides, which, like many of the human and mouse immune genes suppressed by hypercapnia, are regulated by NF- κ B pathways. Moreover, hypercapnia increased the mortality of bacterial infection in *Drosophila*, as in our mouse pneumonia model. These and other data suggest that hypercapnia suppresses innate immunity genes by an evolutionarily conserved mechanism. We have therefore used *Drosophila* as a gene discovery platform, leading to the identification of a number of *Drosophila* genes that are required for hypercapnic immune suppression in fruit flies. We are currently investigating the mammalian orthologs of these genes to determine their roles in hypercapnic immune suppression in mice and humans. These studies should help to elucidate the pathway(s) by which hypercapnia inhibits immune gene expression and lung host defense. Understanding these pathways will provide the opportunity to develop new therapeutics that block CO₂-mediated immunosuppression.

EFFECTS OF HYPERCAPNIA IN MECHANICALLY VENTILATED PATIENTS

Mechanical ventilation started to a large extent in the context of anesthesia and the operating room (56). Subsequently, during the 1970s and 1980s, new ventilatory modes were introduced (57), with the objective of normalizing arterial Po₂ and Pco₂ values. However, a retrospective study by Hickling and colleagues (58) suggested that limiting airway pressures to less than 30 cm H₂O and allowing Pco₂ to rise was associated with lower mortality in patients with ARDS. Subsequent clinical trials from the late 1990s reported that the use of low V_T and a low level of PEEP were associated with variable effects on mortality (4–6, 59). However, analysis of the pooled results suggested that low-V_T ventilation reduced mortality overall (60). This pooled analysis and the ARDSNet study showing decreased mortality in patients with ARDS ventilated with lower V_T (6) led to the current recommendation regarding the way patients with ARDS should be mechanically ventilated (60, 61). As expected, patients ventilated with lower V_T in these studies had slightly higher Pco₂ levels.

Recently, Esteban and colleagues conducted two large multicenter, multinational surveys on the use of MV (62). Using

Figure 2. Schematic representation of mechanisms leading to impaired host defense during hypercapnia.

these databases, the investigators analyzed whether V_T has changed over time in light of the recommendation to use a low V_T in ARDS. In the entire population of patients receiving MV, V_T did not change from 1998 to 2004. However, in patients with ARDS, V_T decreased significantly over time, from a mean of 8.9 ml/kg ideal body weight in 1998 to 7.7 ml/kg in 2004. Hypercapnic acidosis was found in 13% of patients with ALI in 1998 and in almost twice as many (22%) in 2004. Whether mortality has changed as a result of these changes in the strategy of MV over time is uncertain. In the aforementioned study (62), hospital mortality of patients with ALI was 40% and did not change from 1998 to 2004.

Based on clinical studies to date, there is no evidence that hypercapnia has a protective effect in ALI/ARDS. Moreover, preliminary results of a prospective international cohort of 3,400 patients receiving MV for at least 48 hours conducted by the VENTILA Group indicate that patients who developed hypercapnia in the first 48 hours had a higher incidence of new ARDS and required MV for longer periods (63).

SUMMARY

Recent studies suggest that CO₂ can act as a signaling molecule via pH-independent mechanisms, resulting in deleterious effects in the lung. These include inhibition of cell membrane repair, impairment of alveolar fluid clearance, and suppression of innate immunity and host defense. Additional studies are needed to further elucidate the molecular mechanisms by which hypercapnia alters the function of cells in the lung. Understanding these mechanisms should help lay the basis for the development of novel approaches for the treatment of lung diseases associated with impaired alveolar ventilation and elevated CO₂.

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