Effects of Hypercapnia in Lung Tissue Repair and Transplant

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Abstract Mammalian cells sense and transduce signals in response to high levels of carbon dioxide. Hypercapnia has a variety of effects on epithelial and immune cells which can be beneficial or detrimental depending on the biologic context. For instance, hypercapnia-mediated suppression of cell proliferation and migration can delay wound repair. Similarly, suppression of macrophages and the inflammatory response during hypercapnia can limit the host's ability to clear pathogens. However, the suppressive effects of hypercapnia on immunity have potential benefits in the setting of transplantation. Here, we discuss the effects of high levels of carbon dioxide on lung healing and the potential applications in lung transplantation.

Keywords Lung repair · Lung transplant · Hypercapnia · Carbon dioxide · Immunity · Cell migration · Pulmonary edema · Inflammation · Injury response · ARDS

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Introduction

Carbon dioxide (CO_2) is a by-product of oxidative metabolism. The idea that CO_2 regulates intracellular events is innovative and is currently being investigated in our laboratories and others [1•, 2•, 3–7]. There is accumulating evidence indicating that mammalian cells sense and transduce signals in response to high levels of CO_2. Although a variety of cells can sense and respond to CO_2, the focus of this review is primarily on lung tissue repair and transplantation. Here, we will discuss our recently published data and hypotheses on the detrimental effects of high CO_2 levels on the lung repair process. We will also present potential therapeutic benefits of elevated CO_2 in lung transplantation.

CO2 sensing

CO_2 is a byproduct of oxidative metabolism and is produced by the citric acid cycle within mitochondria. It is a small non-polar molecule capable of passive diffusion across cell membranes. In some systems, especially those without a gradient, CO_2 transport can be channel-mediated [8, 9]. Once inside the cell, enzymes such as carbonic anhydrases can act as sensors of CO_2 and mediate CO_2 metabolism and signaling. In addition to sensing CO_2, carbonic anhydrase can also facilitate its transport [10].

Another enzyme that mediates CO_2 sensing is adenylyl cyclase (AC), responsible for the production of cyclic adenosine monophosphate (cAMP). Transmembrane adenylyl cyclases (TMACs) that are sensitive to G-proteins get activated secondary to ligand binding. Activation of TMAC leads to cAMP production that acts as a second messenger to induce effector proteins like protein kinase A, cAMP response element binding protein, phosphodiesterase domains, and cyclic nucleotide-gated ion channels. Elevated CO_2 has been shown

to suppress cAMP production and can, thereby, affect a variety of intracellular processes. There is evidence for direct incorporation of CO2 into a CO2-sensitive TMAC, implying that there is a direct pathway for CO2 sensing [11]. Additionally, CO2 can also affect soluble adenylyl cyclases.

In mammalian cells, regulation of CO2 is important since it affects ventilation, alveolar function, cell proliferation, inflammation, and innate immune responses. Neurons located in the brainstem detect changes in arterial pCO2 and in turn regulate breathing [12, 13]. Central and peripheral chemoreceptors modulate each other so that the carotid body can fine tune the sensitivity of central chemoreceptors under hypercapnia [14]. An increase in blood arterial CO2 can lead to depolarization of glomus cells, triggering an increase in alveolar ventilation to maintain normal pCO2 [15]. CO2 also regulates a variety of other cellular responses, including those of the respiratory tract.

CO2 and the respiratory tract

The lung epithelium is an interface with the environment and can be subject to many injury mechanisms. This interface is composed of alveolar epithelium consisting of type I and type II pneumocytes and the endothelium of pulmonary capillaries. Damage to the alveoli has the biggest effect on respiratory physiology. During normal homeostasis, lung cells exhibit a low rate of cell regeneration. After injury, they have the capacity to rapidly regenerate through activation of progenitor cells and proliferation of remaining uninjured cells. Lung repair results in either complete resolution of injury, persistent chronic inflammation, remodeling with fibrosis, or progressive ongoing fibrosis. Without complete resolution, permanent reduction in lung function occurs.

While an increase in blood pCO2 is known to trigger an increase in respiration by central regulation [16], there is growing evidence to support CO2 sensing and regulation of intracellular events in the epithelial and immune cells of the respiratory tract. The effects of CO2 are particularly important in the context of lung injury. Severe injury to the lungs results in the disruption of the alveolar-capillary barrier. Such an impact is a hallmark of acute respiratory distress syndrome (ARDS) and leads to impaired gas exchange. After resolution of the inciting event, the lung undergoes repair. Although lung repair is a highly complex process, there are some key components including the following: A) Clearance of cellular debris by phagocytic cells; and B) Proliferation and migration of alveolar type II cells to cover the injured area. Once the injury response has occurred, removal of edema fluid is necessary for lung function to recover. Impairment of this process leads to pulmonary fibrosis and to a decline in lung function.

Effects of CO2 on lung macrophages

Macrophages are the most well-established scavengers and, therefore, important in the wound healing process. There is now strong evidence to support that hypercapnia can alter macrophage function. This includes both regulation of the phagocyte function as well as cytokine/chemokine production. West and colleagues investigated why laparoscopic surgery that utilizes CO2 was associated with decreased peritoneal inflammation [17]. They found that peritoneal macrophages did not produce tumor necrosis factor (TNF) and interleukin (IL)-1beta upon lipopolysaccharide (LPS) stimulation under hypercapnia. A similar observation was made in rat alveolar macrophages [18]. Investigation performed on LPS-stimulated human and mouse macrophages revealed that elevated CO2 reduced the expression of TNF and IL-6 [5]. In the above studies, hypercapnia affected NF-κB–regulated cytokines but not those that are not regulated by this pathway. The NF-κB pathway plays a key role in the regulation of innate immunity and inflammation [19]. Hence, this pathway may be important in hypercapnia-mediated gene regulation. As such, the suppression of these cytokines by hypercapnia occurs at the gene level and not due to cytotoxicity. Takeshita and colleagues showed that hypercapnia inhibited endotoxin-stimulated RelA nuclear translocation that is an important component of NF-κB signaling [20]. Hypercapnia might also affect the non-canonical NF-κB pathway through the regulation of IKKα and RelB signaling [21•, 22, 23]. Furthermore, elevated CO2 can alter pulmonary inflammation in an NF-κB -dependent manner [24].

Macrophages are known to phagocytose pathogens. In addition, through the process of efferocytosis, they clear cellular debris. Besides altering innate immune gene expression, hypercapnia also inhibits phagocytosis [5] and NADPH oxidase–mediated generation of reactive oxygen species [2•], which is necessary for antimicrobial activity. Hypercapnia was found to worsen lung injury in rats with *Escherichia coli* pneumonia [25]. Further, hypercapnia markedly increases the mortality of *P. aeruginosa* pneumonia in a rodent model by decreasing the clearance of *Pseudomonas* from the lungs and increasing bacterial dissemination to other organs [2•]. The hypercapnia-induced increase in pneumonia mortality was not due to acidosis because mortality in mice pre-exposed to hypercapnia for three days, to allow for metabolic compensation, was similar to those that were not pre-exposed. In our ongoing studies, we have further found that the efferocytosis of apoptotic alveolar epithelial cells by alveolar macrophages is inhibited in hypercapnia. This leads to impairment of wound healing due to persistence of apoptotic cells at the injury site. Although both the engulfment of the apoptotic cells and digestion of phagosomes are affected in hypercapnia, the latter is more pronounced. Transfer of macrophages that have engulfed apoptoti cells in normocapnic conditions to

hypercapnia significantly slows phagosome digestion. It appears that this might be mediated by suppression of Rab5 GTPase that is important for phagosome-lysosome fusion (manuscript under preparation). Such a delay in clearance of cellular debris can results in poor healing following lung injury. In fact, there is now evidence to support that defective macrophage function and reduced ability to clear apoptotic cells can lead to progressive pulmonary fibrosis both in native lungs [26] and transplanted lung allografts [27].

Effects of CO2 on lung epithelium

The disruption of the plasma membrane is an important component of a variety of injury mechanisms including ARDS [28, 29]. Hypercapnia can have a profound effect on the lung injury response. Most cells under a normocapnic environment repair even micron-scale defects in the plasma membranes within 30 seconds [30]. However, under hypercapnia, the recovery is significantly delayed, resulting in cell death. CO_2 also has direct intracellular effects on lung epithelial cells. Alveolar type II cells are key mediators of alveolar injury response. They proliferate in response to the injury and are responsible for differentiating into type I cells as well as closing the injury defect. In a previously published study, hypercapnia was found to induce mitochondrial dysfunction in alveolar type II cells, suppressing cell proliferation and wound closure [31]. This was a result of suppression of isocitrate dehydrogenase-2 (IDH-2), a citric acid cycle enzyme. Further, suppression of IDH-2 was mediated by hypercapnia-induced upregulation of microRNA 183 (miR-183). MicroRNAs belong to the family of short post-transcriptional inhibitor RNAs. Transfection with IDH-2 reversed the delayed wound closure under hypercapnia. Interestingly, treatment with anti-miR-183 (antagomir) reverses the suppression of cell proliferation under hypercapnia but nota the delayed wound closure (manuscript under preparation). This indicates that hypercapnia suppresses cell proliferation and migration through an independent pathway. Studies are underway to elucidate the mechanisms that lead to impairment of cell migration in our laboratories.

Effects of CO2 on pulmonary edema clearance

Clearance of pulmonary edema is dependent on the sodium channel Na,K-ATPases located on the alveolar epithelium [32, 33]. Clearance of the excess alveolar fluid is essential for recovery following lung injury. It is noteworthy that ventilator management for patients with ARDS includes protective lung ventilation using low tidal volume ventilation that invariably leads to hypercapnia. The hypercapnia is considered "permissive" in order to allow the protective lung ventilation. However, high CO_2 is not without consequences. Hypercapnia impairs Na,K-ATPase function and alveolar edema clearance

[34]. Hypercapnia led to activation of PKC-ζ, which directly phosphorylates the Na,K-ATPase α1-subunit at the Ser 18 residue, leading to endocytosis of this pump and decreased alveolar fluid clearance. The activation of PKCζ is dependent on the JNK-required AMP-activated protein kinase (AMPK) [34, 35]. Hence, down-regulation of Na,K-ATPase in hypercapnia is distinct from other stimuli that mediate the same effect by the production of cytosolic and mitochondrial reactive oxygen species [35–38]. Further, these effects are independent of extracellular and intracellular acidosis. Cumulatively, hypercapnia results in epithelial cell dysfunction.

CO2 in ARDS and pneumonia

ARDS is characterized by severe damage to the alveolar-capillary membranes. Protein-rich fluid seeps into the alveoli and lead to interstitial edema. This leads to characteristic decreased lung compliance and diffuse bilateral infiltrates on chest radiographs. The loss of this mechanical barrier predisposes patients to bacterial invasion and sepsis. There is also loss of fluid and ion transport, exposure of the immunogenic basement membrane, and activation of neutrophils and macrophages. The management of ARDS typically includes protective lung ventilation using low tidal volume and airway pressure ventilation. This mechanical ventilation strategy was first described for ARDS in the 1990s and has since been proven to be associated with lower hospital mortality. Low tidal volumes are thought to reduce atelectotrauma and break the cycle of recruitment and collapse of atelectatic lung. However, low tidal volume also leads to hypercapnia and acidosis. Over time, the acidosis gets corrected but hypercapnia usually persists as long as the patient is on low tidal volume ventilation. A current area of controversy is whether or not hypercapnia itself is beneficial in the treatment of ARDS [39–43]. Proponents of hypercapnia argue that it attenuates oxygen free radical formation, NF-κB activation, and TNF-α, IL-1, IL-6, and IL-8 cytokine production [1•, 22]. Indeed, hypercapnia, has been shown to attenuate lung injury in animal models of barotrauma [44, 45] and by endotoxin in rats [46]. However, opponents argue that hypercapnia delays bacterial clearance and worsens lung injury in long standing pneumonia and sepsis [25]. Furthermore, hypercapnia causes mitochondrial dysfunction and impairs alveolar cell proliferation [31]. We favor that hypercapnia is detrimental to lung repair and recovery during pneumonia and ARDS. This is further supported by the fact that hypercapnia is an independent risk factor for mortality in patients with community-acquired pneumonia and multiple adverse clinical outcomes, including pneumonia, sepsis, and mortality [47, 48].

CO2 and lung transplantation

Lung transplants are increasingly being used as a treatment option for a variety of lung disorders, including emphysema, cystic fibrosis, pulmonary fibrosis, and pulmonary vascular diseases, among others. Although there has been significant improvement in the surgical technique and immunosuppression protocols, the long-term outcomes are still limited due to the development of chronic allograft rejection, called bronchiolitis obliterans syndrome (BOS). It is estimated that up to 50 % of lung transplant recipients will develop BOS after 5 years and the median survival rate following BOS is only 2-3 years [49, 50]. Hence, BOS currently remains the bane of lung transplants. Unfortunately, the pathogenesis of BOS is still undefined, although a variety of injury mechanisms have been postulated [51]. These include primary graft dysfunction (PGD), acute cellular rejection, humoral rejection, respiratory viral infections, gastroesophageal reflux, and autoimmunity. Recent studies have demonstrated that PGD is one of the most important risk factors for BOS [52, 53]. PGD occurs within the first 72 hours following lung transplantation and can affect over 25 % of lung transplant recipients. Ischemia reperfusion injury is the main contributor to the development of PGD and there is an emerging role of innate immunity. The suppressive effects of hypercapnia on innate immunity might therefore suggest a potential therapeutic role in ameliorating ischemia reperfusion injury. Li et al demonstrated that suppression of NF-κB activation lead to a reduction in rat hepatic ischemia reperfusion injury under hypercapnia [54]. Similar observations have been made in lung [55] and gut ischemia-reperfusion models [56]. Hence, transient exposure to hypercapnia might be beneficial in reducing ischemia reperfusion injury.

A number of immune pathways have been implicated in the development of PGD. Kohno and colleagues demonstrated that Rho GTPase and Rho-associated coiled-coil forming protein kinase (ROCK) regulate the contraction of endothelial cells by modulating the intracellular cytoskeleton via phosphorylation of the endothelial

Table 1   Hypercapnia and Lung Healing

Inflammatory response inhibition	Repair/regeneration inhibition
NADPH oxidase generation of O2 radicals	
Macrophage production of TNF alpha, IL-1, IL-6, IL-8	Alveolar edema clearance via Na,K-ATPase inhibition
NF-kappaB mediated cytokine release	Cell proliferation via inhibition of Krebs cycle
Macrophage phagocytosis	Cell migration via inhibition of Rac1-GTPase

Table 2   Hypercapnia and Lung Transplant

Therapeutic potential	Negative potential
Decreased ischemia-reperfusion injury	Increased risk of infection
Suppression of donor-derived macrophages	Decreased pathogen-specific immunity
Suppression of endothelial Rho-GTPase	Pulmonary artery vasoconstriction
Suppression of NF-kappaB activation	Pulmonary artery hypertension

myosin light chain. Contraction of the endothelial cells allows for transendothelial cell migration and increases permeability, contributing to reperfusion injury. Inhibition of this pathway by the addition of Y-27632, a specific ROCK inhibitor, to the preservative solution during the cold ischemia significantly ameliorated the ischemia reperfusion injury [57, 58]. Peng and colleagues demonstrated that inhibition of ROCK can be achieved to the same degree by 10 % CO2 [59]. Hence, hypercapnia might be protective against ischemia-reperfusion injury.

Several prior studies have demonstrated that infiltration of recipient-derived macrophages, neutrophils, NK cells, and T cells are important for immunity against pathogens contained in the donor lung [60]. However, they also mediate ischemia reperfusion injury and PGD following transplantation. Inflammatory cytokines produced as a result of the cellular infiltration are important for the development of PGD. Furthermore, the inflammatory milieu triggers development of alloimmunity against donor HLA antigens as well as autoimmunity against lung tissue-restricted antigens. Sharma and colleagues reported that IL-17 and TNF-α from NK T cells and alveolar macrophages contribute significantly to ischemia reperfusion injury and PGD. Both these cytokines synergistically mediate CXCL1 production (neutrophil chemoattractant) by alveolar type II epithelial cells via a NADPH oxidase-dependent mechanism [61]. In fact, NADPH oxidase is important for all bone marrow-derived immune cells that contribute to ischemia reperfusion injury in lung transplant recipients [62] which is also suppressed by hypercapnia [2•]. Hence, there is a potential benefit of using hypercapnia in lung transplantation to reduce ischemia reperfusion injury. The caveat remains that hypercapnia can also lead to suppression of pathogen-specific immunity. Infections remain the predominant cause of morbidity and mortality during the early post-transplant period. Hence, the potential immunomodulatory benefits of hypercapnia should be weighed against increased infection risk. Nevertheless, we postulate that transient hypercapnia in the immediate post-transplant period would have a more favorable impact in PGD while not significantly increasing the infection risk. If used in the context of

lung transplantation, the timing of hypercapnia will be important. Hypercapnia increases pulmonary arterial pressures. Clamping the pulmonary artery (right or left) for the implantation will further raise the pulmonary arterial pressures making it difficult to perform the transplantation without the need for cardiopulmonary bypass. Hence, it might be difficult to use this strategy intraoperatively. However, most lung transplant recipients remain intubated for a period of time after transplantation. Following completion of the lung transplant, the pulmonary pressures are usually low and permissive hypercapnia would be well tolerated. Another possibility is inducing hypercapnia during cold storage. The role of donor-derived macrophages in ischemia reperfusion injury remains unclear. Nevertheless, it has been postulated that elimination or suppression of donor lung macrophages reduces ischemia reperfusion injury. Therefore, hypercapnia-mediated suppression of these macrophages prior to reperfusion is potentially beneficial. Furthermore, suppression of Rho-GTPase in the endothelial cells by hypercapnia during the cold ischemia could also reduce capillary permeability and cellular infiltration in the allograft following reperfusion. The development of the ex vivo lung perfusion system [63] presents an exciting opportunity to test some of these hypotheses.

Conclusions

The effects of hypercapnia on mammalian cells are being defined. Depending on the clinical situation, hypercapnia can be detrimental or beneficial (Table 1). When the suppression of the immune response is important, for example, in ischemia-reperfusion injury in organ transplantations, it can be utilized for therapeutic benefit (Table 2). However, when the immune response is important, for example in the setting of pneumonia, it can be detrimental. In the setting of lung repair, although it has benefits in reducing the inflammatory response, the overall effects are detrimental due to the reasons outlined above. Ongoing investigations are important to delineate the effects of hypercapnia in lung repair, transplantation, and perhaps regeneration.

Compliance with Ethics Guidelines

Conflict of Interest   Stephen Chiu, Jacob Kanter, Haiying Sun, Anchit Bharat, Peter H. S. Sporn, and Ankit Bharat declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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