Carbonic Anhydrase IX: Scaring Away the Grim Reaper in Acute Lung Injury?

Endothelial injury is a major contributor to the pathophysiology of acute respiratory distress syndrome (ARDS) (1). Promoting survival of injured endothelial cells may confer a clinical benefit to critically ill patients with acute lung injury. Understanding molecular dynamics that promote survival of lung endothelial cells during infection may eventually translate to improved patient survival.

Patients with ARDS experience impaired gas exchange due to damage of alveolar units with protein-rich edema. Clinically, this is in part reflected by hypoxemia with low levels of circulating oxygen (PaO₂) from shunt and low ventilation–perfusion (V/Q) units as well as by hypercapnia with elevated levels of carbon dioxide (PaCO₂) from V/Q mismatch, especially from high V/Q units that contribute to an increase in pulmonary dead space (2). It is uncertain whether these abnormalities confer beneficial (3, 4) or harmful (5, 6) outcomes in patients with ARDS, although hypercapnia and hypoxemia impair alveolar epithelial fluid clearance and the resolution of alveolar edema (7, 8). Furthermore, the mechanism of either protective or detrimental effects of abnormal CO₂ levels on pulmonary microvascular endothelial cells (PMVECs) during infection is uncertain.

In vivo, CO₂ is in part regulated by enzymes termed carbonic anhydrases (CAs). In addition to catalyzing conversion of CO₂ to carbonic acid and thereby regulating pH, CA isoforms, which are abundantly expressed in PMVECs (9), may act as immunomodulators and contribute to improved PMVEC survival during infection. In this issue of the Journal, Lee and colleagues (pp. 630–645) report on the role of CA IX during in vivo human and rat pneumonia as well as CA IX isoforms on in vitro PMVEC survival during Pseudomonas aeruginosa infection under hypoxic and hypercapnic conditions (10).

The authors found that although plasma CA IX levels are similar in critically ill patients and rats with P. aeruginosa pneumonia relative to respective controls, levels of this enzyme in BAL fluid from patients with pneumonia and rat P. aeruginosa pneumonia lung tissue were higher, for the first time providing in vivo data suggesting pneumonia-specific CA IX differential expression in the lung. These observations together with this group’s previous finding that PMVECs express CA IX (9) prompted detailed in vitro characterization of CA IX expression and function under stress conditions relevant to ARDS.

CA IX is composed of an N-terminal proteoglycan-like (PG) domain, a catalytic (CA) domain, a transmembrane domain, and an intracellular (IC) domain (11). To study the mechanism of domain-specific secretion in rat PMVECs, CRISPR-Cas9 was used to generate rat PMVECs with ΔPG, ΔCA, and ΔIC conditionally expressing CA IX functional mutants. The authors identified that ΔIC domain mediated the CA IX membrane localization in PMVECs.

Next, to understand whether increased lung tissue CA IX concentrations originate from pulmonary capillaries, the authors studied CA IX secretory mechanisms by measuring baseline CA IX concentration in PMVEC lysates and supernatants. After determining that CA IX release and cleavage at the ectodomain–transmembrane domain junction was present at baseline, metalloproteinase (MMP) inhibitors were used to demonstrate that MMPs mediate CA IX ectodomain cleavage in the extracellular space after unprocessed CA IX is released from cells. Interestingly, MMP inhibition prevented extracellular CA IX cleavage without affecting CA IX release from PMVECs. Thus, CA IX processing by MMPs warrants further investigation to test the potential protective role of CA IX during infections identified in this study.

Because in vivo data (patient BAL fluid and P. aeruginosa pneumonia rat lung tissue) identified increased tissue-specific CA IX levels, the authors next used an in vitro rat PMVEC P. aeruginosa infection model to study infection-induced changes in CA IX expression, demonstrating that in vitro infection increases both CA IX release and ectodomain cleavage. More specifically, P. aeruginosa infection of CA IX ΔIC PMVECs compared with CA IX wild-type PMVEC was associated with impaired cell survival, pointing to the critical role of the CA IX IC domain during P. aeruginosa infection.

Lastly, the authors tested host environmental conditions associated with pneumonia and ARDS (low O₂ and high CO₂) as well as the enzymatic properties associated with CA IX (CO₂ regulation) using the in vitro rat PMVEC model. These studies provided evidence that during P. aeruginosa infection, severe hypoxia protects PMVECs (less necrosis) while hypercapnia attenuates this protective effect. The negative impact of hypercapnia was more pronounced in CA IX wild-type relative to CA IX ΔIC PMVECs, suggesting that the IC domain increases the cytotoxic effect of hypercapnia under in vitro infection-related hypoxic conditions, providing further evidence that the IC domain of CA IX influences in vitro pulmonary endothelial cell survival. These pathways are summarized in Figure 1.

Translating the authors’ findings into clinical practice is challenging at this time. First, the authors postulate that the lack of a relationship between circulating plasma CA IX levels in patients and in the in vivo rat pneumonia model may be due to rapid degradation of CA IX released from the lung tissue before it reaches the systemic circulation. This could make it challenging to follow levels of this enzyme in response to potential future therapies. Second, the in vitro studies were performed in rat PMVECs, and how they translate to human microvascular responses to infection, hypoxia, and hypercapnia deserves further investigation. Third, although modest levels of hypoxemia (recommended oxygen saturation as low as 88%) and hypercapnia occur in the era of lung protective ventilation (12),
dioxide tension/pressure during infection. Furthermore, how CA IX expression in other nonpulmonary organs is affected in vivo by the conditions tested in this study should be studied before testing therapies targeting CA IX.

This study provides compelling and new evidence that CA IX, in addition to being an enzyme that sustains CO2 metabolism, may be important for mediating endothelial cell survival during infection. Moreover, focusing on the intracellular domain may be particularly relevant. These interesting findings provide a strong rationale for future studies of this enzyme and its expression, processing, and metabolism as potential therapeutic targets for mechanisms of acute lung injury and translational issues in ARDS.

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