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Molecular Biology Ying Yang in Alveolar Fluid Clearance

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Even as a trained biochemist/molecular biologist, reading the article by Hsueh-Ju and colleagues (1) required considerable focus. However, amidst the abbreviation alphabet soup, double negatives, and signaling pathway complexity lays novel information that ultimately may inform our clinical approach to understanding and treating acute respiratory distress syndrome (ARDS).

As the authors point out in their Introduction, ARDS-associated acute pulmonary edema is a function not only of diffuse pulmonary capillary leak, but also impaired alveolar fluid clearance. Treatment of ARDS has largely been supportive in terms of providing mechanical ventilation and avoidance of fluid overload while the lungs undergo repair. It remains unclear if intervention with corticosteroids is beneficial in this setting (2, 3). Although completely basic science in its conduct, the study by Lin and colleagues encourages us to speculate that modulation of alveolar fluid clearance may represent another therapeutic intervention for ARDS.

In their investigation the authors utilized a murine hyperoxia-exposure lung injury model that reproducibly produces acute hypoxemic respiratory failure associated with pulmonary edema. At the onset it is important to understand the molecular components being investigated. WNK is the focus of investigation. Although its own activation remains somewhat unclear, this investigation examined the WNK downstream pathway that involves phosphorylation of another kinase termed SPAK and in turn phosphorylation activation of the sodium-potassium-chloride co-transporter (NKCC.) The authors point out that alveolar fluid clearance likely involves a number of mechanisms including an epithelial sodium channel, a cystic fibrosis trans-membrane conductance regulator, a sodium/potassium ATPase, as well as the sodium-potassium-chloride co-transporter. The authors utilized well-established methodology in the murine hyperoxia model to derive a number of findings that support the role of the WNK—SPAK—NKCC pathway in alveolar fluid regulation.

The hyperoxia model results in acute pulmonary edema in mice following 60 hours of exposure to 100% oxygen and activates WNK as well as SPAK expression and the sodium-potassium-chloride co-transporter as well as the epithelial sodium channel. It is crucial to

understand that increased activity of the epithelial sodium channel leads to enhanced alveolar fluid clearance, while activation of the sodium-potassium-chloride co-transporter results in impaired alveolar fluid clearance (4, 5). This biologic couplet provides an example of a servo mechanism that is likely involved in tight regulation of fluid balance within the alveolus. Ultimately, the investigators demonstrate that in their model the abundance of the WNK kinase is increased concomitant with increased phosphorylation (activation) of the SPAK kinase, all of which is associated with increased phosphorylation (activation) of the sodium-potassium-chloride co-transporter. This gain of function of the co-transporter is associated with fluid and ion retention within the alveolus and hence reduced alveolar fluid clearance. Concomitantly the investigators show that the same hyperoxia stimulus enhances the activity of the epithelial sodium channel that would have the opposite effect, namely increasing alveolar fluid clearance. If mice with a double knockout of the SPAK gene are utilized in the experiment, then the phosphorylation of the sodium-potassium-chloride co-transporter is decreased, the co-transporter is less activated and hence there is less fluid and ion retention in the alveolus. A similar effect was demonstrated by treating the animals with nebulized furosemide, which inhibits sodium-potassium-chloride co-transporter function with a resultant effect similar to that seen in the SPAK double knockout mice.

These intricate but elegant series of experiments illustrate the complexity of intracellular signaling involved in biologic processes, in this case alveolar fluid clearance. For the clinician, this basic science report provides a possible new therapeutic intervention for acute pulmonary edema, specifically nebulized furosemide. The authors point out that the effect of nebulized furosemide was likely isolated to the lungs, as this intervention had no effect on animal weight or urine output. However, as a cautionary note, although β_2 -adrenergic therapy to enhance the activity of the alveolar sodium potassium ATPase was shown to be beneficial in a variety of pre-clinical settings, a definitive clinical trial among patients with ARDS did not demonstrate benefit (6). However, that approach really addressed only one side of the equation. It is possible that inhibiting the sodium-potassium-chloride co-transporter, for example with nebulized furosemide, as well as augmenting the sodium potassium ATPase with β_2 -adrenergic agonists could simultaneously reduce alveolar fluid and ion retention as well as enhance ion and water transport out of the alveolus. The approach seems logical with both of the pharmacologic interventions relatively nontoxic, particularly when provided with local nebulized drug delivery.

In summary, Lin and colleagues provide us with new information that further untangles water and ion regulation in the alveolus. Deciphering the complex, redundant, inter-connected signaling pathways involved in biologic processes can only really be undertaken with these types of investigations. Hopefully these positive findings will eventually make their way to translational investigations. It would be nice to have a pharmacological intervention to augment to mechanical ventilation support for our patients with ARDS.

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