FLUID TRANSPORT IN THE DEVELOPING AND PERINATAL LUNG

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Running Title: Lung Fluid Clearance

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**Introduction:**

How epithelia actively transport salt and water into the lung’s airspaces or from the lung’s airspaces is critical to our understanding of lung development, physiology and pathophysiology. *In utero,* the placenta carries out fetal gas exchange whilst the fetal lung secretes $\text{Cl}^-$, with $\text{Na}^+$ and water following, into the lumen of the lung. This process of epithelial liquid secretion is essential for normal fetal lung development. However, at birth the lung’s lumen must be free of liquid and when there is failure to efficiently clear airspace fluid then Transient Tachypnea of the Newborn (TTN) occurs (1) and when combined with surfactant deficiency the more severe respiratory disorder, Hyaline Membrane Disease (HMD) results (2). After birth if there is an increase in the transvascular pressure, abnormal permeability of the alveolar capillary membrane to solutes, or a combination of these two processes, fluid pathologically fills the airspaces resulting in pulmonary edema. Regardless if the patient has congestive heart failure or the acute respiratory distress syndrome (ARDS), both morbidity and mortality are related to the lungs’ ability to actively transport salt and water from the airspaces (3;4).

**Cellular Mechanisms that Result in Epithelial $\text{Na}^+$ Transport**

Epithelia that transport $\text{Na}^+$ have tight junctions and are polarized with $\text{Na}^+$/K$^+$ ATPase located at their basolateral membrane and $\text{Na}^+$ permeant ion channels on their apical membranes. Vectorial transport of $\text{Na}^+$ results in $\text{Cl}^-$ and water passively following through paracellular and/or intracellular pathways (5). In respiratory epithelium the activity of the $\text{Na}^+$ permeant ion channels in the apical membrane represent the rate-limiting step in lung epithelial $\text{Na}^+$ transport. Early studies, combined with much work done on frog skin or mammalian kidneys, resulted in a commonly held belief that all epithelial $\text{Na}^+$ transport was sensitive to amiloride and that the responsible $\text{Na}^+$ permeant ion channel was the amiloride sensitive epithelial $\text{Na}$ channel (ENaC). ENaC does play an important role in lung epithelial $\text{Na}^+$ transport and is composed of $\alpha$, $\beta$ and $\gamma$ sub-units (6). However, many studies from different laboratories have now determined that approximately 1/3 to 1/2 of the lung’s epithelial active transport of $\text{Na}^+$, with $\text{Cl}^-$ and water following, occurs via amiloride-insensitive conductances in the apical membrane in mammalian whole lung and distal lung epithelia in culture (7).

**Clinical Implications of Deficient Epithelial $\text{Na}^+$ Transport**

The biologic importance of this active $\text{Na}^+$ transport in the transition from fetal to postnatal life was suggested by fetal experiments (8;9), however, studies using amiloride, an inhibitor of $\text{Na}^+$ transport, provided the first direct evidence that defective $\text{Na}^+$ transport was clinically relevant (10). Otherwise normal newborn animals who received airspace instillation of amiloride prior to the first breath had markedly impaired postnatal lung liquid clearance and associated respiratory distress and hypoxemia (10). After the amiloride-sensitive epithelial $\text{Na}^+$ channel (ENaC) was cloned (6), it was demonstrated that $\alpha$-ENaC knockout mice, although having apparently normal fetal lung development, died shortly after birth of defective ability to clear their lung fluid (11). Interestingly pseudohypoaldosteronism patients, the “human $\alpha$-ENaC subunit knockout” (12), do not have a comparable marked impairment in clearing lung liquid at birth as they don’t have nRDS at birth (13). There are several potential explanations for this variation between
species including the possibility that low levels of α-ENaC subunits rescue the pseudohypoaldosteronism lung (14), that the β (15), or β and γ-ENaC subunits (16), or other amiloride insensitive Na\(^+\) channels (7) may be able to rescue the human lung.

TTN is a result of too much fetal lung liquid remaining in the lungs’ distal units after birth. Infants with TTN are most frequently full term, have mature surfactant pathways and more frequently are products of Caesarian section. Clinically, they have hyperinflated chests and their chest radiographs illustrate marked overinflation with evidence of peribronchial interstitial and some air space edema. Usually these infants do well with minimal or modest respiratory support and occurs within 48 to 72 hours after birth (17). Studies showing that infants with TTN have low nasal epithelium amiloride-sensitive potential difference (PD) compared to control infants without TTN and that this amiloride-sensitive PD increased as the infants recovered from their disease (18) suggested that TTN results from immature amiloride sensitive epithelial Na\(^+\) transport capacity. Fortunately, these infants have the “usual normal full term newborn” 10 fold greater amount of surfactant as does the adult (19). This helps prevent acute lung injury by keeping the alveolar capillary membrane fully intact, which eventually enables slow absorption of the excess fluid and relatively benign clinical course.

Prematurely born infants frequently suffer from the more severe form of respiratory distress syndrome, HMD. It is characterized by diffuse microatelectasis with accompanying acute lung injury, air space fluid, hyaline membranes composed of fibrin and desquamated epithelium, and proteinaceous exudate that arise from the increase in alveolar capillary membrane permeability. Advances in ventilation modalities, along with exogenous surfactant replacement has led to improved morbidity and mortality of infants with nRDS. It is important to note that premature infants suffering from nRDS also have less amiloride-sensitive transepithelial PD in their nasal respiratory epithelium (20) and have low levels of ENaC in their respiratory epithelium (21). This strongly suggests that inadequate lung epithelial Na transport plays a mechanistic role, in addition to the well documented relative surfactant deficiency, in the pathogenesis of HMD.

There is leakage of protein rich pulmonary edema fluid in nRDS as lung damage occurs as a result of the respiratory efforts required to overcome the combination of surfactant deficiency and airspace fluid filling from the inability to clear fetal lung liquid (2). However, pulmonary edema may occur in an infant with mature lungs or in an older child or adult either from an increase in the transvascular pressure gradients, as clinically occurs in congestive heart failure or when there is abnormally high permeability of lung’s blood vessels, such as occurs in ARDS, which allows water and solutes to move more easily out of intravascular space toward interstitium and airspaces. This raises the question of how does the mature lung, whether it is the infant or adult lung, clear the edema from its airspaces?

Matthay and his colleagues first showed that passive classic Starling forces could not explain how fluid was reabsorbed from the alveolar space of the adult mammalian lung (22). The rate of alveolar liquid fluid clearance in humans is amongst the fastest of all species, being approximately ~25% per hour (5). Is this active transport dependent clearance of fluid from airspaces is clinically important? First, it is biologically reasonable. Second, as described above when there is inadequate Na\(^+\) transport in the newborn then respiratory distress occurs. Finally, the reabsorption of fluid from airspaces has been related to clinical outcome (amount of mechanical ventilation and
death rate) in adults suffering from CHF or ARDS. It has been shown that approximately 60% and 90% of CHF and ARDS patients respectively, have impaired airspace fluid clearance and that this correlates with clinical outcomes (3;4). A recent single center randomized controlled trial has also demonstrated that when a β₂ agonist, which admittedly has several effects including the ability to increase lung epithelia Na⁺ transport, is infused into adults with ARDS there is a decrease in lung water content and physicians can use lower ventilator pressures to ventilate their patients (23). Many labs are currently investigating a variety of approaches to increase lung epithelial Na⁺ transport at the transcriptional, translational and post-translational steps.


