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Mechanisms of pulmonary edema clearance: from basic research to clinical implication

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Introduction

Research in the area of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) has focused on several areas including the identification of putative molecular markers and/or targets, optimal strategies of mechanical ventilation and fluid management. More recently, there has been significant interest in the mechanisms whereby pulmonary edema fluid is cleared from the lungs. There already exists data to suggest that clearance of edema fluid, as measured by concentration of bronchoalveolar lavage (BAL) albumin, results in improved outcome in patients with pulmonary edema [1].

Several studies have focused on the mechanisms of edema clearance in animal models. It has been shown that edema is cleared primarily by active Na⁺ transport with water following the Na⁺ movement [2, 3]. Na⁺ enters the cells via apical Na⁺ channels and exits via the basolateral Na,K-ATPases (Fig. 1) [4, 5, 6]. Na,K-ATPases are transmembrane proteins consisting of α and β subunits. The α subunit binds and cleaves the high energy phosphate bond of ATP, whereas the β subunit is apparently responsible for the assembly and normal function of the enzyme complex in the plasma membrane [7, 8]. Na,K-ATPases are ubiquitous in all mammalian cells and can be found in the lung in both alveolar epithelial type I and II cells (ATI, ATII). Na,K-ATPase works in coordination with the apical Na⁺ channel to generate an electrochemical gradient

which results in a vectorial Na⁺ flux from the airspace and subsequent isosmotic movement of water from the airspaces [5, 6].

The mechanisms of transepithelial edema flux are currently under investigation. There have been several studies examining the role of water channels in transcellular versus paracellular water movement. Aquaporins (AQP) are molecular water channels. Aquaporins-1,4, and 5 (AQP1,4,5) are present in the lung: AQP1 in microvascular endothelium [9, 10], AQP4 in basolateral membranes of airway epithelium [11] and AQP5 in apical membrane of ATI cells (Fig. 1) [12]. AQPs presumably have a role in water homeostasis in the lung as evidenced by increased expression in the perinatal period during which rapid fluid absorption is followed by the initiation of alveolar respiration [13, 14].

Fig. 1 Schematic representation of alveolar epithelial cells, types I and II, depicting the apical Na⁺ channels, the basolaterally located Na,K-ATPase, the aquaporins and some of the co-transporters. Sodium enters through the apical membrane via Na⁺ channels and is extruded by the Na,K-ATPase with water following isosmotically. Also shown is an airway epithelial cell with associated basolateral aquaporin

Recent data demonstrate that changes in lung edema clearance correlate with Na,K-ATPase function in both normal and acutely injured animal lungs [15, 16]. There is also experimental evidence in epithelial cell monolayers and animal models that upregulation of Na,K-ATPases increases active Na⁺ transport. For example, several growth factors, dopamine (DA) and catecholamines have been reported to increase the lung's ability to clear edema by upregulating Na,K-ATPase in rat alveolar epithelium [17, 18, 19]. Below are summarized four recent papers that have set the stage for potential clinical trials aimed at enhancing pulmonary edema clearance.

Lecuona D, Saldias F, Comellas A, Ridge K, Guerrero C, Sznajder JI (1999) Ventilator-associated lung injury decreases lung ability to clear edema in rats. *Am J Respir Crit Care Med* 159: 603–609

The authors studied lung edema clearance in an isolated, perfused rat lung model after the rats had been exposed to mechanical ventilation for either 25, 40 or 60 min. Rats were ventilated using either high tidal volume (HVT, 30 ml/kg) resulting in peak airway pressures (Pao) of approximately 35 cmH₂O, low VT (LVT, 10 ml/kg) with Pao of approximately 8 cmH₂O, or moderate VT (MVT, 20 ml/kg) with Pao of about 20 cmH₂O. Control rats were not mechanically ventilated. Gravimetric estimates of pulmonary edema showed that HVT rats had increased extravascular lung water compared to rats ventilated with MVTs or LVTs and controls rats. Lung permeability for small solutes, Na⁺ and mannitol, and albumin were also increased in rats ventilated with HVT for 60 min compared with MVT and LVT rats and control animals. Similarly, epithelial lining fluid (ELF), as measured by the dilution of Evans blue dye (EBD), was also increased in rats ventilated with HVT for 60 min.

Edema clearance in control rats measured approximately 0.5 ± 0.02 ml/h, approximately 10% of liquid instillate. While the rate of clearance was not significantly different in rats ventilated with MVT and LVTs, the rats ventilated with HVT had a significant decrease in clearance at 40 min and 60 min. In parallel, Na,K-ATPase activity in alveolar type II (ATII) cells isolated from rats ventilated with HVT for 40 min decreased by 50%, compared with LVT and control animals.

Saldias F, Lecuona E, Comellas A, Ridge K, Sznajder J (1999) Dopamine restores lung ability to clear edema in rats exposed to hyperoxia. *Am J Respir Crit Care Med* 159: 626–633

This study utilized a hyperoxic model of ALI which results in pulmonary edema. In this model, the lung's ability to clear edema is reduced in association with decreased Na,K-ATPase activity in ATII cells. DA has been shown to increase active sodium transport and

edema clearance by stimulation of Na,K-ATPase activity in the alveolar epithelium of normal rats. The current study demonstrated that DA has the same effect on injured lungs.

There were eight experimental groups. Groups A–D breathed room air (RA). Group A (control) had buffered salt albumin (BSA) instilled into the airspace. Group B had 10⁻⁵ M DA instilled into the airspace. Group C had ouabain perfused into the pulmonary circulation, with or without DA instillation. Group D had colchicine, or β -lumicolchicine, injected intraperitoneally approximately 15 h before the experiment, with or without DA instillation. Ouabain is a specific antagonist of Na,K-ATPase. Colchicine depolymerizes microtubules, impairing intracellular microtubular transport of proteins from intracellular stores to the plasma membrane. β -lumicolchicine is an isomer of colchicine that does not depolymerize the microtubules but, like colchicine, it inhibits protein synthesis. Groups E–H were exposed to 100% oxygen for 64 h. Group E had BSA instilled into the airspace. Group F had 10⁻⁵ M DA instilled into the airspace. Group G had ouabain perfused into the pulmonary circulation, with or without DA instillation. Group H had colchicine, or β -lumicolchicine, with or without DA instillation.

Utilizing the isolated, perfused rat lung model, the ELF volume and permeability to small solutes and to albumin increased in hyperoxic rats compared with RA rats. Lung edema clearance in RA control rats was approximately 0.50 ± 0.02 ml/h. Clearance decreased by about 44% in hyperoxic rats. The addition of DA increased clearance by 50% in the RA rats and by approximately 250% in hyperoxic rats. Ouabain blocked the effect of DA in both hyperoxic and RA control rats, as did colchicine but not β -lumicolchicine. Na, K-ATPase α 1-subunit abundance was determined by Western blot of the basolateral membrane of isolated ATII cells after incubation with DA. DA increased the subunit abundance in the control and β -lumicolchicine treated ATII cells. Colchicine inhibited the increase of the subunit, presumably by disruption of the microtubular transport system.

Factor P, Saldias F, Ridge K, Dumasius V, Zabner J, Jaffe HA, Blanco G, Barnard M, Mercer R, Perrin R, Sznajder J (1998) Augmentation of lung liquid clearance via adenovirus-mediated transfer of a Na,K-ATPase β -1 subunit gene. *J Clin Invest* 102: 1421–1430

The investigators hypothesized that lung edema clearance could be enhanced by increasing the abundance of available Na,K-ATPase via adenoviral transfer of the Na,K-ATPase β -1 subunit gene. A shuttle vector was constructed containing either the α -1 or β -1 subunit of Na,K-ATPase, or neither (null). The adenovirus was constructed using a plasmid containing a human type 5 adenovirus genome cotransfected with the shuttle vec-

tor into human embryonic kidney cells. Rat ATII cells and airway epithelial cells were isolated and infected. Rats were sedated and underwent alveolar instillation of either (1) adenovirus with surfactant, (2) null adenovirus, (3) adenovirus with galactosidase, (4) adenovirus with β -1 subunit or (5) adenovirus with α -1 subunit. The rats were killed after 1 week.

Transfection efficiency in rat ATII cells ranged between 50–90 % depending on the concentration of virus instilled (1,5,10 moi). Northern blot analysis detected a dose-dependent adenoviral-derived β -1 subunit mRNA in ATII cells. Western blot detected dose-dependent adenoviral-derived β -1 subunit immunoreactivity in ATII cells in vitro and rat lung epithelium. Na,K-ATPase function increased twofold in the cells infected with adenovirus containing β -1 subunit, but not α -1 subunit, galactosidase or null. Transepithelial Na⁺ transport also increased twofold in cells infected with adenovirus containing the β -1 subunit.

Using the isolated, perfused rat lung model edema clearance was increased by 100 % in rats infected with the adenovirus containing the β -1 subunit gene as compared to the other experimental groups. When ouabain (an inhibitor of Na,K-ATPase) was added to the perfusate of the adenovirus β -1 animals, clearance decreased by 75 %.

Bai C, Fukuda N, Song Y, Thonghui M, Matthay M, Verkman A (1999) Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. *J Clin Invest* 103: 555–561

AQP knockout mice were used to investigate the role of aquaporins in fluid transport between airspace, interstitium and capillary in the lungs. Control mice were (+/+), AQP1 mice where heterozygous (+/-) or homozygous deficient (-/-), AQP4 mice were homozygous deficient (-/-). (Immunoblot showed decreased AQP1 levels to about 40 % in +/- animals and 0 % in AQP1 or AQP4-/-). Osmotically driven water movement between airspace and capillary was measured by filling the airspace with a solution containing a membrane impermeable fluorescent indicator (FITC-dextran). Pulmonary artery (PA) perfusate osmolality was altered resulting in water movement into or out of the airspace and a change in the FITC concentration. Quantitative measurements of the time course of change in fluorescence intensity were used to calculate osmotic water permeability of the airspace-capillary barrier (Pf). Results were a $t_{1/2}$ of approximately 12 s in AQP +/+ mice, 36 s in AQP1 +/-, and 75 s in AQP1 -/-. Pf was 17 ± 2 cm/sec in +/+ mice, 6.6 ± 0.06 in AQP1 +/- mice, 1.7 ± 0.03 in AQP1 -/- mice, and 12 ± 1 in AQP4 -/-. To confirm reduced microvascular water permeability in AQP1 -/- mice, perfluorocarbon was instilled in the airspace and the PA was perfused with varying osmolalities of FITC-dextran. In +/+ mice, increase in perfusate osmolality resulted

in movement of fluid into the vascular compartment and decreased fluorescence. The amplitude of the change in fluorescence signal relates to microvascular osmotic water permeability and was reduced in AQP1 -/- mice.

Lung weight was measured as PA perfusate pressure was increased from 8 to 18 cmH₂O. A rapid increase in weight represented vascular engorgement and recruitment. A slower increase represented accumulation of lung fluid. Fluid accumulated at a lower rate in the AQP1 -/- mice (45.6 ± 4.2 mg +/+ , versus 14.5 ± 2.4 mg -/-, $p < .001$). Lung wet/dry weights were no different in AQP1 -/- mice at normal hydrostatic pressure, but were reduced when perfusate pressure increased, compared with +/+ controls.

To determine if near-isosmolar, active fluid absorption requires AQP1 or 4, the airspace was infused with isosmolar solution containing ¹²⁵I-albumin. The PA was perfused with isosmolar solution at 8–10 cmH₂O. In some experiments, isoproterenol, terbutaline, amiloride or ouabain were added to perfusate and instillate. The absorption rate was 6.0 ± 0.9 in controls, 15.3 ± 1.3 with isoproterenol, 2.1 ± 0.05 with amiloride and 0.1 ± 1.2 with ouabain. The rate of absorption was the same for AQP1 and 4 -/- mice (16.4 ± 1.5 and 16.3 ± 1.7 , respectively).

Discussion

Effective therapy for ALI and ARDS remains a challenge. Several studies have suggested that recent improvements in outcome are related to improved fluid and ventilator management. As our understanding of the molecular mediators of the pathophysiology improves, new therapeutic avenues will evolve. A growing insight into the mechanism of edema clearance at the molecular level is an example. We now recognize that such clinically relevant injury as ventilator-associated lung injury (VALI) and hyperoxic lung injury are compounded by substantial reduction in lung ability to clear edema at a time when clearance is imperative. The decrease in edema clearance in VALI is paralleled by a decrease in Na,K-ATPase activity, suggesting a possible cause-effect relationship.

Fortunately, it appears that even injured lungs may respond to therapy. Apparently, catecholamines and DA instilled into the airspace, or perfused into the pulmonary circulation, restore the lung's ability to clear edema in hyperoxic injury. In fact, the DA effect was greatest in the injured lung. Inhibition of DA's effect by colchicine, but not β -lumicolchicine, suggests that upregulation is achieved by recruitment of preformed ion-transporting proteins, such as Na,K-ATPase, from intracellular pools to the plasma membrane. Furthermore, the rapid time frame in which upregulation occurs is inconsistent with transcription and translation of new

protein. Available data now confirm that isoproterenol also increases Na,K-ATPase activity by insertion of increased numbers of α subunits, recruited from late endosomes, into the plasma membrane [20].

The optimal means by which to upregulate Na⁺ channels, Na,K-ATPase and edema clearance have not been established. Experimental models have added DA, or other catecholamines, directly into the airspace or the perfusate. Another route may be gene therapy. Efficient gene transfer of β -1 subunits into ATII cells can be achieved with newer, replication-deficient recombinant adenovirus vectors. Increased Na,K-ATPase function after β -1 subunit gene transfer demonstrates that Na,K-ATPase activity in ATII cells can be augmented, resulting in increased vectorial transport of Na⁺ and increased clearance. The time course for viral transfection may prohibit its use in the acute setting, however it may

have clinical implications for therapy of more long-term volume overload states, such as congestive heart failure. Prior to clinical application of gene therapy, the safety of recombinant adenoviruses needs to be established, especially in regard to their potential to induce lung inflammation.

The parallel movement of water following active Na⁺ transport does not appear to be dependent on AQP1 or AQP4. Water movement in this setting may occur through ATI cells, which contain AQP 5, suggesting the barrier for this type of fluid movement is the alveolar epithelium.

Taken together, these studies suggest that, in the near future, therapy aimed at increasing edema clearance may be introduced to the clinical setting and improve the outcome of patients with ALI.

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