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Alveolar epithelial fluid transport in acute lung injury: new insights

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Alveolar epithelial fluid transport in acute lung injury: new insights. C. Sartori, M.A. Matthay. ©ERS Journals Ltd 2002.

ABSTRACT: Pulmonary oedema is a life-threatening condition that frequently leads to acute respiratory failure. From a physiological perspective, pulmonary oedema develops either because of an increase in lung vascular hydrostatic pressure or an increase in lung vascular permeability. Resolution of alveolar oedema depends on the active removal of salt and water from the distal air spaces of the lung across the distal lung epithelial barrier.

Much has been learned about the molecular and cellular basis for oedema fluid reabsorption, including the role of apical ion transporters for sodium (epithelial sodium channel) and chloride (cystic fibrosis transmembrane conductance regulator), as well as the central importance of the sodium pump. The rate of fluid clearance can be upregulated by both catecholamine-dependent and -independent mechanisms.

Injury to the alveolar epithelium can disrupt the integrity of the alveolar barrier or downregulate ion transport pathways, thus, reducing net alveolar fluid reabsorption and enhancing the extent of alveolar oedema. Endogenous catecholamines upregulate alveolar fluid clearance in several experimental models of acute lung injury, but this upregulation may be short term and insufficient to counterbalance alveolar flooding. There is new evidence, however, that pharmacological treatment with β_2-adrenergic agonists and/or epithelial growth factors may influence a more sustained stimulation of alveolar fluid reabsorption and in turn facilitate recovery from experimental pulmonary oedema. Similar results have been achieved experimentally by gene transfer to enhance the abundance of sodium transporters in the alveolar epithelium.

Clinical studies show that impaired alveolar fluid transport mechanisms contribute to the development, severity and outcome of pulmonary oedema in humans. Very recent data suggest that mechanisms that augment transepithelial sodium transport and enhance the clearance of alveolar oedema may lead to more effective prevention or treatment for some types of pulmonary oedema.

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Pulmonary oedema is a life-threatening condition resulting from an imbalance between forces driving fluid into the airspaces and biological mechanisms for its removal. Although, for many years, Starling forces (hydrostatic and protein osmotic pressures) were thought to play a major role in maintaining the alveolar space free of fluid [1], there is now strong evidence that active ion transport across the alveolar epithelium creates an osmotic gradient that leads to water reabsorption both during the perinatal period [2–4] and in the adult lung [5]. The present article reviews 1) new insights into the basic mechanisms regulating vectorial transport of salt and water across the alveolar epithelium of normal adult lung; 2) summarises current understanding of the role of transport mechanisms in the setting of experimental acute lung injury (ALI); 3) describes current pharmacological interventions designed to upregulate alveolar fluid clearance; and 4) considers how this new knowledge regarding salt and water transport in the alveolar epithelium in pathological conditions may be relevant to human clinical medicine.

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Sodium and water transport across the normal respiratory epithelium

The epithelial sodium channel and sodium/potassium adenosine triphosphatase

Results from several studies indicate that sodium can enter the apical membranes of alveolar epithelial cells through amiloride-sensitive cation channels, such as the amiloride-sensitive epithelial sodium channel (ENaC) [6–8], and is then transported across the basolateral membrane into the interstitium by the ouabain-inhibitable sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) [8, 9]. The pathway for chloride transport has not been worked out but recent studies have begun to explore this issue [10]. Water follows passively, probably through water channels (the aquaporins (AQPs) [11, 12]), although the presence of these water channels is not required for maximal alveolar epithelial fluid transport in the lung [13].

In situ hybridisation studies, as well as Northern blot analyses, have identified the presence of messenger ribonucleic acid (mRNA) encoding all three subunits of the ENaC in alveolar epithelial cells both *in vivo* and *in vitro* [14–20]. The physiological importance of the α-subunit of the ENaC in the lung has been demonstrated in a mouse model in which the α-subunit gene was deleted. These α-ENaC-knockout mice were unable to clear liquid from their lungs during the perinatal period, developed respiratory distress and died within 40 h of birth [2]. Recent studies of alveolar epithelial transport indicate that 80–90% of basal alveolar fluid clearance is amiloride-inhibitable in adult mice, suggesting that the ENaC may indeed be the predominant cation channel in that species. However, in other species such as sheep [21], rats [22] and humans [23], amiloride only inhibits 40–50% of basal alveolar fluid clearance (fig. 1). Therefore, either modifications of the ENaC or non-ENaC channels may be involved in active sodium transport in species other than the mouse [24]. Consistent with this hypothesis, patch-clamp studies in rat alveolar epithelial cells have failed to identify the classical amiloride-sensitive sodium channel in alveolar epithelial cells, suggesting that apical sodium entry may also be mediated by other cation channels with different single channel conductance [18, 19].

Several other cation channels have been identified in alveolar type II cells [18]. One of these, the non-selective cation channel, can be inhibited by amiloride, further suggesting that, in parallel with the ENaC, it could contribute to sodium transport across the alveolar epithelium [25, 26]. Molecular characterisation of these other channels is needed.

Na^+/K^+-ATPase is a ubiquitous plasma membrane, ion-transporting ATPase that maintains transmembrane gradients of Na^+ and K^+ by pumping Na^+ out of the cell and K^+ into the cell against their respective concentration gradients. It is widely believed that a heterodimeric form comprising the α_1- and β_1-subunits is the predominant sodium pump isoform expressed in alveolar epithelial type II cells, although expression of the α_2-subunit has also been reported in rat cells [27]. The catalytic α-subunit binds to and cleaves the high-energy phosphate bond of adenosine triphosphate, whereas the β-subunit is apparently responsible for the assembly and normal insertion of the enzyme complex into the plasma membrane [28, 29]. A recent review provides fuller details regarding the possible interaction and cellular localisation of the ENaC and Na^+/K^+-ATPase [30].

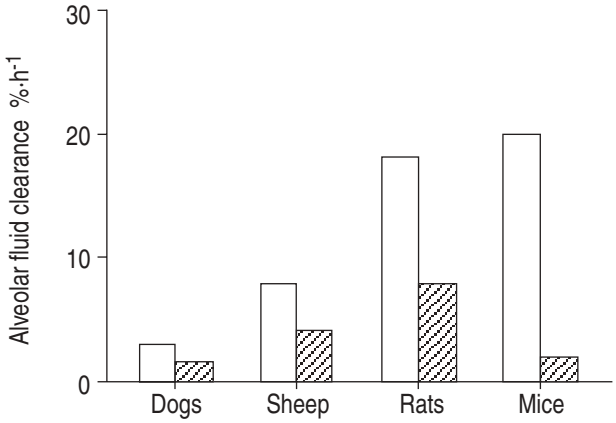

Fig. 1. – Alveolar fluid clearance at baseline (□) and after intratracheal amiloride instillation (▨) in different animal species. Important interspecial differences in basal alveolar fluid clearance rates have been identified. The slowest have been measured in dogs, intermediate in sheep and goats, and highest in rabbits and rats, and most recently in mice. The basal alveolar fluid clearance rate in the human lung has been difficult to estimate, but, based on the isolated nonperfused human lung model, appears to be intermediate to fast. Although, in most other species, humans included, amiloride inhibits at best 50% of alveolar fluid clearance, this amiloride sensitivity reaches almost 90% in mice, suggesting a unique major contribution of the apical sodium channels in this species.

Regulation of alveolar transepithelial sodium transport

Studies *in vitro* have indicated that there is usually parallel independent regulation of apically localised sodium transport processes and basolaterally located Na^+/K^+-ATPase in response to a variety of stimuli, including hormones, such as catecholamines, dopamine, glucocorticoids, thyroid hormone and insulin, and growth factors (fig. 2, table 1). The intracellular sodium concentration appears to be responsible, in certain cases, for the coupling of the sodium pump and cation channel activities [31, 32].

Catecholamine-dependent mechanisms. This is the most extensively studied stimulatory mechanism of transepithelial sodium transport and alveolar fluid clearance. Several *in vivo* studies have demonstrated that β_2-adrenergic agonists can upregulate alveolar fluid clearance in rats [33], sheep [21] and dogs [34], as well as in mice [35, 36]. In most of these studies, a rise in alveolar protein concentration in the distal airspaces of the lungs provided an index of alveolar fluid clearance since alveolar fluid is removed much more rapidly than protein [37]. The β_2-agonist effect is mediated in part by cyclic adenosine monophosphate (cAMP)-dependent mechanisms [38], is partially inhibited by

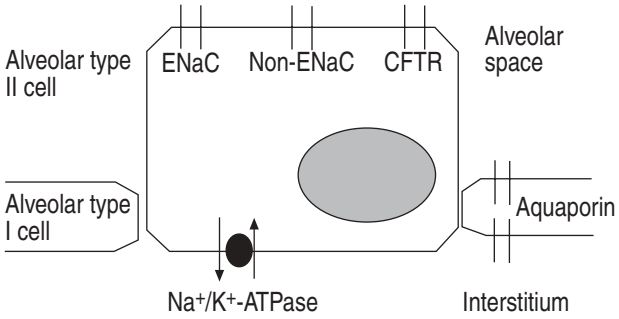

Fig. 2. – Schematic representation of the possible transporters involved in ion and water transport in alveolar cells. ENaC: apical amiloride-sensitive epithelial sodium channel; CFTR: cystic fibrosis transmembrane conductance regulator; Na^+/K^+-ATPase: sodium/potassium adenosine triphosphatase.

amiloride and seems to be unrelated to the increased pulmonary blood flow simultaneously induced by these drugs [21]. It is noteworthy that endogenous catecholamines do not play a major role under normal conditions, as demonstrated by the unaltered alveolar fluid clearance in adrenalectomised animals [39], as well as in studies showing no effect of β-blockers on basal alveolar clearance in sheep [21], rats [22] and the *ex vivo* human lung [23].

β_2-Adrenergic agonists are effective when delivered intravenously or directly into the distal airspaces of the lung [21, 40]. Although early studies indicated that the primary stimulating effect was mediated by β_2-receptors, recent work indicates that β_1 stimulation is also effective in upregulating alveolar fluid clearance [41]. Consistent with these data, β_1- and β_2-receptors are present on both the apical and basolateral surface of alveolar epithelium [42].

Interestingly, some species do not respond to β_2-adrenergic agonists with an increase in alveolar fluid transport, in particular rabbits and hamsters [43, 44]. Therefore, studies in the *ex vivo* human lung have been particularly important in establishing the response of human alveolar epithelium to β_2-adrenergic agonists. Several studies have indicated that β_2-adrenergic agonists markedly enhance the rate of alveolar fluid clearance in *ex vivo* human lung preparations [23, 45]. The magnitude of this effect is similar to that observed in other species, with a β_2-agonist-dependent doubling of alveolar fluid clearance over the baseline level.

Proposed mechanisms for stimulation of active sodium absorption across the alveolar epithelium include increases in sodium pump α-subunit phosphorylation and quantity delivered to the basolateral cell membrane [18, 46], augmented apical sodium channel (both ENaCs and nonselective cation channels [47]) abundance and open probability [8, 48], augmented α-ENaC and α_1-Na^+/K^+-ATPase gene expression [46], and increased transport of the ENaC from the cytoplasm to the cell membrane [49].

Other reports indicate that, in the airways, cystic fibrosis transmembrane conductance regulator (CFTR) can regulate the ENaC [50]. This hypothesis has gained new strength recently. Indeed, indirect stimulation of transcellular sodium movement by stimulation of an apical chloride conductance in the presence of β_2-agonists or cAMP has been reported by different groups [10, 51, 52]. This hypothesis suggests that a critical factor in upregulating fluid clearance might be chloride, rather than simply transepithelial sodium absorption. Consistent with this possibility, preliminary data suggest that β_2-agonist stimulation of alveolar fluid clearance is absent in mutant mice deficient in the CFTR channel and in *in situ* lung of wildtype mice instilled with the chloride channel inhibitor, glibenclamide [53].

Sodium channels and sodium pump activity in the plasma membrane can be regulated by other mechanisms, including acute regulation by covalent or allosteric modification, and acute intracellular trafficking between the endoplasmic reticulum, intracellular endosomal pools and the plasma membrane [49]. Colchicine, which impairs intracellular microtubular transport of proteins from intracellular stores to the plasma membrane, inhibits the increase in Na^+/K^+-ATPase subunit abundance induced by β_2-agonists, suggesting that upregulation is achieved, at least in part, by recruitment of preformed ion-transporting proteins from intracellular pools to the plasma membrane [54]. Recent data also indicate that β_2-agonists can increase Na^+/K^+-ATPase activity by insertion of increased numbers of α-subunits, recruited from late endosomes, into the plasma membrane [55].

Chronic regulation of the abundance of channels and pumps in the membrane is mediated not only by changes in channel synthesis but also by their rates of degradation. Indeed, ENaC activity appears to depend on channel stability at the membrane, a process

Table 1. – Summary of possible mechanisms of regulation of ion and water transport in the lung

Stimulation	Inhibition	Possible implicated mechanisms
Catecholamines and β-agonists	Hypoxia	Transporter gene expression
Growth factors (EGF, KGF, HGF)	Oxidants	Intracellular trafficking
Cytokines (TGF-α)	Nitric oxide	Membrane abundance
Hormones (glucocorticoids, thyroid hormone, insulin)		Degradation/ubiquitination
Oxidants		Transporter activity, open probability and conductance
Serine protease (CAP1)		Cell proliferation

EGF: epidermal growth factor; KGF: keratinocyte growth factor; HGF: hepatocyte growth factor; TGF: transforming growth factor; CAP1: channel-activating protease 1.

regulated by degradation *via* ubiquitination [56]. Finally, there is increasing evidence supporting a new, previously undiscovered, mechanism for autocrine regulation of the ENaC by a serine protease (channel-activating protease 1) expressed in kidney, gut, lung, skin and ovary [57]. The mechanisms underlying these latter processes are not yet known.

Vasoactive agents and hormones. Dobutamine markedly upregulates alveolar epithelial fluid clearance in rats by stimulating β_2-receptors [40], whereas dopamine upregulates alveolar fluid transport by stimulating the dopaminergic D_1 receptor [54, 58].

Glucocorticoids and thyroid hormone increase respiratory transepithelial sodium transport during the foetal and perinatal period in several animal species [59, 60]. Recent observations suggests that these hormones may modulate ENaC and Na^+/K^+-ATPase gene expression in the lung [15, 17] and also play a significant role in upregulating fluid clearance in adult animals [61].

Insulin can cause increased sodium transport across cultured alveolar type II cells, especially when added to the basolateral membrane [62], by increasing the open probability of ENaCs [63]. There is also some data showing that oestrogens may increase ENaC expression [64]. However, it is not clear whether the concentrations needed to increase sodium transport *in vitro* occur in the lung interstitium *in vivo*.

Other catecholamine-independent mechanisms. In addition to the well-studied effects of β_2-adrenergic agonists, several catecholamine-independent pathways can increase the rate of alveolar fluid clearance. Incubation of epidermal growth factor with isolated alveolar type II cells for 24–48 h increases their capacity to transport sodium [65]; it also upregulates alveolar fluid clearance in rats [66]. Keratinocyte growth factor (KGF), an important alveolar epithelial type II cell mitogen, induces a similar effect, primarily by stimulating alveolar type II cell proliferation [67–69]. In a recent study, one dose of KGF (5 mg·kg body weight^{-1}) produced sustained upregulation of alveolar fluid clearance over 5 days in rats [67]. Interestingly, addition of terbutaline, a β_2-adrenergic agonist, further accelerated the rate of alveolar fluid clearance by 50% per hour. Transforming growth factor-α (TGF-α) can increase alveolar fluid clearance acutely in anaesthetised ventilated rats [70]. Interestingly, since cAMP levels were only minimally increased in alveolar type II cells isolated from rats exposed to TGF-α, it is possible that the TGF-α effect may be mediated by an alternative signal transduction pathway that does not require elevation of cAMP levels. Since the effect was inhibited by genistein, the mechanism may involve a tyrosine kinase pathway [71].

Downregulation of alveolar fluid transport can occur with reactive oxygen or nitrogen species or with severe hypoxia. Reactive oxygen/nitrogen at concentrations similar to the one potentially released by activated macrophages downregulates the activity of alveolar type II cell sodium channels [71, 72] as well as amiloride-sensitive currents in oocytes injected with ENaCs [73]. Similar effects have been observed in alveolar type II cells exposed to hypoxia for 12–18 h (see below) [74].

Initial immunocytochemical evidence *in situ* demonstrated apical sodium channels and Na^+/K^+-ATPase were present in type II cells, but not in type I cells [75]. From these observations, it was initially inferred that alveolar transport was only regulated by type II cells [7, 76] and distal airway epithelial cells [77, 78]. Based on more recent data, however, this hypothesis may need to be re-evaluated. New data show that freshly isolated type I cells express subunits of both Na^+/K^+-ATPase and the amiloride-sensitive ENaC, suggesting that this cell type might also play a role in active vectorial ion and water transport [79, 80]. Moreover, freshly isolated type I cells exhibited the highest known water permeability of any mammalian cell type, thereby probably explaining the very high water permeability of the lung [11].

Role of water channels in alveolar water homeostasis

Water transport across the alveolar epithelial barrier occurs during fluid absorption from the alveolar spaces because a miniosmotic gradient is created by the vectorial transport of sodium, and perhaps chloride, that results in water absorption across the alveolar epithelium. Water permeabilities have been measured across several of the major barriers in lung [81]. Osmotically-driven water movement across pulmonary epithelial barriers in the lung is fast [82, 83], weakly temperature-dependent and inhibited by mercurials [84].

Four specialised water-transporting proteins, AQPs, have been localised in lung to date: AQP1 in microvascular endothelia and some pneumocytes; AQP3 in basal cells of the nasopharynx, trachea and large airways; AQP4 at the basolateral membrane of airway epithelium; and AQP5 at the apical membrane of type I alveolar epithelial cells [85]. In order to define the role of AQP water channels in water transport across the various barriers in the intact lung, each of the four lung AQPs has been deleted in mice by targeted gene disruption [13, 85]. Deletion of AQP1 or AQP5 produced an ~10-fold decrease in osmotically driven water transport between the airspace and capillary compartments [86, 87], thus, demonstrating major roles in osmotically driven water movement across the alveolar endothelial and epithelial barriers, respectively. AQP1 deletion also caused a moderate decrease in isosmolar transcapillary water movement in response to a modest increase in lung vascular pressure [86]. AQP4 deletion alone had little effect on airspace-to-capillary water permeability, but studies comparing water permeability in AQP1 null mice *versus* AQP1/AQP4 double-knockout mice showed a small but significant contribution of the distal airways to lung water permeability [88]. Interestingly, and most importantly, isosmolar alveolar fluid clearance was not affected by AQP1 or AQP5 deletion, even under conditions in which the clearance rate was maximised by pretreatment with isoproterenol and KGF [13]. Consistent with these observations, recent studies show no effect of AQP1, AQP4 or AQP5

deletion, alone or in combination, on the formation or clearance of lung oedema following lung injury from hyperoxia, thiourea or acid instillation [13].

Thus, although the precise role of AQPs in lung physiology remains uncertain, the studies to date demonstrate that active isosmolar alveolar fluid clearance in the newborn or adult lung does not require lung water channels. Current studies are focused on the possible role of AQPs in movement of water across the conducting airways of the lung.

Fluid transport across the respiratory epithelium in acute lung injury

Two separate barriers form the alveolar/capillary barrier, the microvascular endothelium and the alveolar epithelium. The acute phase of ALI/acute respiratory distress syndrome (ARDS) is characterised by the influx of protein-rich oedema fluid into the airspaces as a consequence of increased permeability of the alveolar/capillary barrier [89]. The severity and outcome of ALI depends in part on the balance between vascular endothelial and/or alveolar epithelial injuries and their repair mechanisms. The importance of endothelial injury and increased vascular permeability to the formation of pulmonary oedema in this disorder is well established. The critical importance of epithelial injury to both the development of and recovery from alveolar flooding has recently become better recognised [90–92].

Several observations confirm that transepithelial sodium transport plays a major role in the clearance of fluid from the airspace not only under normal conditions but also during experimental lung injury. First, as already explained, α-ENaC-deficient mice die shortly after birth due to lung oedema [2]. Transgenic expression of α-ENaC in α-ENaC (-/-) mice rescues the lethal pulmonary phenotype [93], but results in a subclinical defect of transepithelial sodium transport. This defect is associated with an ~50% lower rate of alveolar fluid clearance and significantly greater hypoxia- and thiourea-induced pulmonary oedema [94]. Secondly, instillation of phenamil (an irreversible blocker of epithelial sodium channels) into the lungs of rats exposed to hyperoxia resulted in a significant increase in extravascular lung fluid volume [95]. Thirdly, systemic administration of amiloride facilitates the development of thiourea-induced pulmonary oedema in mice (unpublished data). Finally, pharmacological stimulation of this transport facilitates recovery from lung injury in several experimental models of ALI (discussed below).

Upregulation of alveolar fluid transport in acute lung injury

Many different experimental animal models of ALI have been established. Several of these have been used to study the mechanisms regulating transepithelial sodium and water transport in such pathological conditions. In many but not all of the existing *in vitro* and *in vivo* models of ALI, sodium and fluid transport is upregulated. This interesting finding is well established in conditions such as some models of hyperoxia [48, 96–100], thiourea-induced injury [101], haemorrhagic shock [102], septic shock [103] and neurogenic pulmonary oedema [104].

Besides a possible, but not yet confirmed, direct stretch-sensitive mechanism in the alveolar wall for detecting volume overload [105], the major underlying mechanism responsible for such stimulation appears to be increased endogenous release of catecholamines (for example, as in the case of short-term studies of septic and hypovolaemic shock in rats [102, 103] or in the early phase of neurogenic pulmonary oedema [104]). Consistent with this hypothesis, adrenalectomy reduces alveolar fluid clearance and facilitates thiourea-induced pulmonary oedema in lung injury models (unpublished data).

Alternatively, new evidence suggests that cytokines can stimulate sodium uptake and net alveolar fluid clearance in injured lung. For example, instillation of exotoxin A from *Pseudomonas aeruginosa* or endotoxin from *Escherichia coli* into the distal airspaces of rats can stimulate alveolar fluid clearance by a catecholamine-independent pathway [106, 107]. The mechanisms of the endotoxin effect may depend on release of tumour necrosis factor-α, since a monoclonal antibody directed against tumour necrosis factor-α inhibited the increase in alveolar fluid clearance that occurred 24 h after development of Gram-negative bacterial pneumonia in the rat lung [108].

Proliferation of alveolar epithelial type II cells provides another catecholamine-independent mechanism for accelerating fluid transport across the alveolar epithelial barrier as shown in the subacute phase of bleomycin-injured rat lungs [109]. This mechanism may be very important as it can result in sustained upregulation of alveolar fluid clearance [67].

Finally, there may also be an oxidant-dependent mechanism that can increase the sodium transport capacity of individual type II cells exposed to hyperoxia for several days [48, 110], although not all studies of hyperoxia have demonstrated this effect [9, 96].

Downregulation of alveolar fluid transport in acute lung injury

In contrast, conditions associated with lung injury may downregulate transepithelial sodium transport. Hypoxia affects respiratory epithelial function [111] and inhibits transepithelial sodium transport in alveolar type II cells *in vitro* [74] and in *ex vivo* lungs [23] by impairing both amiloride-sensitive and -insensitive sodium transport. Moreover, exposure to prolonged hypoxia has been shown to decrease both nasal potential difference [112] and alveolar fluid clearance and Na^+/K^+-ATPase hydrolytic activity in rats [113]. Recent *in vivo* work shows that hypoxia downregulates alveolar fluid clearance in rats by 50% but α-ENaC expression and mRNA levels are modestly upregulated, not downregulated [114]. Interestingly, terbutaline increased alveolar fluid clearance to high levels, suggesting that perhaps cAMP agonists

increase insertion of ENaC into the cell membrane of type II cells, as proposed by SNYDER [49].

Ventilator-associated lung injury decreases the ability of the lung to clear oedema in rats. Moreover, sodium pump activity in alveolar type II cells isolated from rats with ventilator-associated lung injury is significantly downregulated compared with control animals [115]. The mechanisms involved are not clear but may involve an increase in lung endothelial and epithelial paracellular permeability and/or downregulation of alveolar transport proteins.

Release of nitric oxide (NO) by activated alveolar macrophages can contribute to inhibition of sodium transport in lung injury models associated with airways inflammation [18, 71, 116, 117]. Interestingly, administration of NO inhibitors can reverse the effect of pulmonary hypotension on downregulation of alveolar fluid clearance in rats [118]. Finally, oxidants can also downregulate *in vivo* alveolar fluid clearance, as shown by MODELSKA *et al.* [119] and recently by SAKUMA *et al.* [120].

Even when lung endothelial injury occurs, the alveolar epithelial barrier may remain normally impermeable to protein and retain a normal or upregulated fluid transport capacity [92, 121] with confinement of the oedema to the pulmonary interstitium [122]. However, more severe systemic and pulmonary endothelial injury may be associated with a marked increase in epithelial permeability to protein and inability to transport fluid from the airspaces of the lung which, in turn, lead to alveolar flooding. The inability to remove excess fluid from the airspaces in these conditions may be related to both a marked increase in paracellular permeability due to injury to the epithelial tight junctions and loss or inhibition (NO or other oxidants) of the transport capacity of the alveolar epithelium. In some cases, epithelial integrity may recover rapidly (*e.g.* after parenteral oleic acid administration) [123] or gradually (*e.g.* bleomycin-induced lung injury) [109]. In other cases, however, the injury to the epithelial barrier is so severe that its function is compromised and recovery may not occur (*e.g.* severe acid aspiration-induced lung injury) [124].

Effect of pharmacological stimulation of alveolar fluid clearance on experimental acute lung injury

During ALI/ARDS, the ability to remove alveolar fluid rapidly is associated with improved oxygenation, shorter duration of mechanical ventilation and increased likelihood of survival [125–127]. The alveolar epithelium is remarkably resistant to injury, particularly compared to the adjacent lung endothelium [122]. Even when mild-to-moderate alveolar epithelial injury occurs, the capacity of the alveolar epithelium to transport salt and water is often preserved [92]. As discussed earlier, several mechanisms may result in upregulation of the fluid transport capacity of the distal pulmonary epithelium. However, this upregulation may not be sufficient to counterbalance alveolar flooding. Therefore, efforts to attenuate the lung endothelial injury and alveolar epithelial injury [128] are as important as treatments that might enhance the reabsorptive capacity of the alveolar epithelium. This section reviews pharmacological catecholamine-dependent and -independent interventions that can accelerate the resolution of alveolar oedema [8, 10, 12, 98, 100, 129].

Catecholamine-dependent mechanisms. β_2-Agonists are attractive as therapeutic agents because they are already in widespread clinical use and have minimal side-effects, even in critically ill patients [130]. Treatment with β_2-agonists may also increase the secretion of surfactant and perhaps exert an anti-inflammatory effect, thus, helping to restore the vascular permeability of the lung [131, 132].

The previously discussed upregulation of alveolar fluid clearance by endogenous β-adrenergic stimulation has been clearly demonstrated in several clinically relevant animal models. In addition to endogenous upregulation, there is growing evidence that alveolar fluid clearance can be further stimulated by exogenous β-adrenergic therapy in the presence of lung injury [98, 129, 133].

In experimental hydrostatic pulmonary oedema, β_2-agonists can accelerate the resolution of alveolar oedema [134, 135]. In sheep, aerosolised salmeterol resulted in effective treatment, with high concentrations of the drug in the alveolar oedema fluid [135]. β_2-Adrenergic agonists have also been reported to augment the rate of alveolar epithelial fluid transport in rats in the presence of moderate lung injury due to hyperoxia [98, 129,133] and to restore the ability of the rat lung to clear oedema in ventilator-associated lung injury. In the latter studies, disruption of the cell's microtubular transport system by colchicine inhibited this stimulatory effect [133].

In contrast, following prolonged haemorrhagic shock in rats, reactive oxygen species can inhibit the response of alveolar epithelium to β_2-agonist stimulation [119]. This suggests that, under certain circumstances, the epithelium may not respond to β_2-agonist stimulation because of extensive injury and loss of alveolar type II cells or because of a reactive effect of the inflammatory environment on the normal ability of type II cells to increase alveolar fluid clearance.

Growth factors. Since acute injury to alveolar epithelial type I cells frequently causes denudation of the alveolar epithelium [91, 136], an additional approach to hastening the resolution of ALI and ARDS would be to accelerate re-epithelialisation of the alveolar barrier [137].

The provision of a new epithelial barrier with alveolar type II cells may have beneficial effects in addition to restoration of the air-liquid interface. Indeed, the rate of alveolar epithelial fluid clearance in the subacute phase of bleomycin-induced ALI in rats was increased by >100% over baseline levels [109]. The enhanced alveolar fluid clearance depended mainly on the extensive proliferation of alveolar epithelial type II cells.

Recent information suggests that hepatocyte growth factor (HGF) and KGF are major mitogens for alveolar

epithelial type II cells. Intratracheal pretreatment of rats with KGF before induction of lung injury with radiation [138], thiourea [68, 139], bleomycin [138, 140, 141], hyperoxia [142–144] or acid instillation [145] decreased the severity of lung injury and overall mortality. This effect required high doses of the growth factor delivered by the intratracheal route and the maximal effect occurred only after 48–72 h. After 48 h, however, KGF produces sustained upregulation of alveolar fluid clearance for several days [67].

The mechanism of protection is thought to be due to an increase in alveolar fluid transport secondary to increased numbers of alveolar type II cells even though other mechanisms (including cytoprotection [142, 145], antioxidant effect [146], increased release of surface-active material [147] and, perhaps, reduction of lung endothelial injury [148, 149]) may play additive roles.

Interestingly, the combination of KGF and β_2-agonist treatment results in additive upregulation of alveolar fluid clearance [150]. This suggests that there may be mechanisms for providing both short-term (β_2-agonists) and longer-term (growth factor) upregulation of alveolar fluid transport that might hasten the resolution of clinical pulmonary oedema.

Gene therapy. Several experimental studies have demonstrated that alveolar oedema clearance may correlate with Na^+/K^+-ATPase activity in both normal and acutely injured animal lungs [21, 58, 96, 98, 151]. Thus, another potential approach to increasing sodium transport and alveolar fluid reabsorption would be to overexpress the Na^+/K^+-ATPase gene in the alveolar epithelium.

Accordingly, overexpression (*via* adenoviral gene transfer) of the β_1- or α_2-subunit increased sodium pump expression and function in the adult rat lung [152, 153]. Furthermore, pretreatment of the lungs was associated with increased survival in rats exposed to hyperoxia for 64 h [154] or in mice exposed to thiourea [155]. Surprisingly, for unknown reasons, overexpression of the catalytic (α_1) subunit of the pump did not induce a similar effect [152].

Gene transfer technology [156] or transgenic overexpression [39] has also been used to overexpress the β_2-adrenergic receptor. This method has been shown to stimulate liquid clearance from the normal lung by increasing sensitivity to endogenous catecholamines in rats and mice [39, 156]. However, preliminary data suggest that β_2-agonist-stimulated alveolar fluid clearance is not markedly different in these animals compared to their wild-type littermates, and that this upregulated clearance may not be sufficient to prevent pulmonary oedema induced by hyperoxia in mice (N. Fukuda, Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA, USA, personal communication). Further studies are needed to assess whether sufficient downregulation of β-receptors occurs in clinically relevant exposures to justify gene therapy with β_2-receptors to the alveolar epithelium.

Alveolar epithelial transport: clinical medicine

The study of the role of alveolar epithelial sodium and water transport in human clinical medicine is a new area of research. Although only limited data exist, currently available results confirm that active alveolar epithelial transport plays a critical role in maintaining lung fluid balance in humans (table 2).

Several approaches have been used to assess such importance in clinical medicine. First, in the *ex vivo* human lung, standard agonists and antagonists [23, 45, 159, 160] have been employed to demonstrate that active alveolar transepithelial sodium transport in the human lung is inhibited by amiloride and/or ouabain and stimulated by β_2-adrenergic agonists. Furthermore, this transport is preserved in resected human

Table 2. – Potential clinical applications of treatments designed to enhance the resolution of alveolar oedema

Intervention	Substance	Potential clinical condition[#]	Reference
Systemic β-agonists	Dobutamine (intratracheal and intravenous)	Congestive heart failure	[40]
Aerosolised β-agonists	Salmeterol	High-altitude pulmonary oedema	[157]
	Salmeterol	Hydrostatic pulmonary oedema; experimental	[134, 135]
	Isoproterenol/terbutaline	Acute lung injury from hyperoxia	[98, 129, 133]
	Isoproterenol/terbutaline	Ventilator-associated lung injury	[133]
Vasoactive agents	Dopamine	Acute lung injury from hyperoxia	[54, 58]
	Adrenaline		[158]
Growth factors	Keratinocyte growth factor (intratracheal)	Radiation	[138]
		Thiourea	[68]
		Bleomycin	[138]
		Hyperoxia	[142–144]
		Acid instillation	[145]
Glucocorticoids	Solumedrol/dexamethasone	Acute lung injury	[61]
Gene therapy	Na^+/K^+-ATPase β_1-subunit overexpression	Hyperoxia	[152]
	Na^+/K^+-ATPase α_2-subunit overexpression	Hyperoxia	[153]

[#]: based on experimental or clinical studies; Na^+/K^+-ATPase: sodium/potassium-adenosine triphosphatase.

lungs that are exposed to rewarming after severe hypothermia (7°C) [159]. Secondly, the cellular content and protein composition of samples of undiluted oedema fluid collected from mechanically ventilated patients with ALI/ARDS have been compared to those of appropriate control samples collected from ventilated patients with hydrostatic pulmonary oedema. Thus, markers of endothelial and epithelial lung injury have been detected and their appearance correlated with clinical outcome [90, 136]. Thirdly, alveolar fluid clearance has been measured in patients with ALI/ARDS and compared to control patients with hydrostatic pulmonary oedema. Fourthly, *in vivo* respiratory transepithelial sodium transport has been evaluated by measurement of nasal transepithelial potential difference (a marker of this transport in the distal airways) in a small number of patients with defined clinical diseases such as neonatal respiratory distress syndrome and high-altitude pulmonary oedema.

Pulmonary oedema fluid studies

Several studies have compared the concentrations of different biologically active substances in pulmonary oedema fluid collected from patients with ARDS *versus* controls with hydrostatic pulmonary oedema. These studies indicate that, in patients with ALI, oedema fluid concentrations of several substances are increased compared to control subjects.

For example, the concentration of intercellular adhesion molecule-1 (ICAM-1), an adhesion molecule found in high concentrations on the alveolar endothelium and epithelium and released in soluble form into the alveolar space in the setting of lung injury, was two-fold higher in the pulmonary oedema fluid of ALI patients than in that of control patients with hydrostatic oedema [161]. In contrast, plasma concentrations of ICAM-1 were similar in both groups, suggesting that this substance is released directly into the alveolar space when the lungs are injured. Also, preliminary data indicate that high pulmonary oedema fluid levels of ICAM-1 were associated with impaired alveolar fluid clearance and prolonged duration of assisted ventilation [161].

Similarly, the concentration of biologically active HGF (but not KGF) was seven-fold higher in the oedema fluid than in the plasma of patients with ALI. Moreover, the oedema fluid concentration of HGF was higher in patients with ALI than in those with hydrostatic pulmonary oedema [162], and higher oedema fluid levels of HGF were associated with higher mortality in patients with ALI.

Soluble TGF-α is also present, in biologically significant concentrations, in the pulmonary oedema fluid of patients with ALI but not those with hydrostatic pulmonary oedema. The concentrations of TGF-α in pulmonary oedema have potent *in vivo* and *in vitro* effects on alveolar epithelial sodium transport and alveolar epithelial cell mobility [163].

The clinical significance of these and other recently discovered (such as leukotriene D_4, leukotriene B_4, substance P, interleukin-8, interleukin-6, fibroblast-associated (Fas; CD95)/Fas-ligand (CD95L) [164] and HTI-56 (a new integral membrane antigen specific for alveolar epithelial type I cells [136]) increased oedema fluid concentrations are still unknown. However, their role in injuring or repairing the alveolar epithelial barrier in patients with ALI, their utility as possible markers of respiratory epithelial injury and their potential contribution in the development of multiorgan failure secondary to ARDS are currently being studied [90].

Alveolar fluid clearance in patients

Two properties of the epithelial barrier can be assessed clinically. First, since the epithelial barrier is normally impermeable to protein, the ratio of oedema fluid to plasma protein concentration is a good index of epithelial permeability [34, 126]. Secondly, since the concentration of protein in alveolar fluid reflects net clearance of alveolar fluid, the measurement of protein concentration in sequential alveolar oedema fluid samples provides a useful estimate of the ability of the epithelial barrier to remove alveolar oedema fluid. With this method, it has been shown that clearance of alveolar fluid can occur surprisingly early and is often apparent within the first few hours after intubation and initiation of mechanical ventilation in patients with either hydrostatic or increased permeability oedema [125, 126, 165].

In one study, ~40% of patients with ARDS were able to reabsorb some of the alveolar oedema fluid within 12 h after intubation [126]. These patients showed more rapid recovery from respiratory failure and lower mortality. In contrast, patients with no evidence of net reabsorption of alveolar oedema fluid in the first 12 h following ALI experienced protracted respiratory failure and higher mortality [125, 126]. A recent study has extended these initial findings to a larger number of patients [127].

In contrast, 75% of 65 mechanically ventilated patients with severe hydrostatic pulmonary oedema showed intact alveolar fluid clearance. In this study, impaired alveolar fluid clearance was associated with lower arterial pH and higher Simplified Acute Physiology Score II at the time of oedema fluid sampling. Both of these factors may be markers of systemic hypoperfusion, which has been reported to impair alveolar epithelial fluid transport by oxidant-mediated mechanisms in experimental models [119]. Conversely, administration of β_2-agonist (albuterol) had a positive predictive value of 85% for the presence of intact alveolar fluid clearance, although this finding did not reach statistical significance. Finally, intact alveolar fluid clearance was associated with a greater improvement in oxygenation at 24 h, along with a trend towards shorter duration of mechanical ventilation and lower hospital mortality [166].

Intact and rapid alveolar fluid clearance was also found in a group of patients with ischaemia/reperfusion pulmonary oedema after lung transplantation, confirming that a functionally intact alveolar epithelium may be preserved despite clinically severe reperfusion lung injury. Furthermore, alveolar epithelial fluid transport was preserved in most patients despite

evidence of marked lung endothelial injury with a substantial increase in alveolar-capillary barrier permeability. Intact alveolar fluid clearance was correlated with less histological injury, rapid resolution of hypoxaemia, more rapid resolution of radiographic infiltrates, and a trend towards a shorter duration of mechanical ventilation and a shorter intensive care unit stay [165].

Finally, since intact alveolar fluid clearance is a favourable prognostic finding in lung transplant patients with reperfusion pulmonary oedema [165], alveolar fluid clearance and degree of pulmonary oedema were measured in 13 human lungs rejected for transplantation. Interestingly, alveolar fluid transport was intact in the majority of lungs studied, suggesting that many of these lungs might have functioned well had they been used in transplantation [167].

Nasal potential difference

Newborn infants with either transient tachypnoea [168] or neonatal distress syndrome [169] demonstrate a lower nasal potential difference, a marker of transepithelial sodium transport across the epithelium in the distal airways [170, 171]. In these patients, the nasal potential difference is reduced weakly by amiloride. These results suggest that impairment of sodium absorption across the respiratory epithelia of very premature infants may be one factor contributing to the pathogenesis of neonatal respiratory distress syndrome.

Other investigators hypothesised that a similar mechanism may contribute to susceptibility to pulmonary oedema in adults. High-altitude pulmonary oedema (HAPO) was chosen as a paradigm of pulmonary oedema because it occurs in predisposed, but otherwise healthy, subjects, making it possible to study underlying mechanisms in the absence of drugs or cardiac dysfunction [172]. At low altitude, the nasal potential difference was ~30% lower in HAPO-prone than in HAPO-resistant subjects. Amiloride superfusion induced a significantly smaller decrease in nasal potential difference in HAPO-prone than in HAPO-resistant subjects. These findings provide the first evidence for genetic impairment of respiratory transepithelial sodium and water transport, at least in part related to ENaC dysfunction, in a human form of pulmonary oedema [173]. However, the ENaC may not be the only important cation channel facilitating alveolar fluid clearance [26, 160].

Pharmacological stimulation of alveolar fluid clearance

Since impaired alveolar epithelial function and blunted alveolar fluid clearance mechanisms contribute to the development and persistence of pulmonary oedema in humans, another important question is whether pharmacological interventions intended to stimulate alveolar sodium transport might be beneficial for the clearance of pulmonary oedema, not only in animal models but also in humans. The preliminary data on this issue, from a recent placebo-controlled clinical study, suggest that this might be true. In this study, inhalation of the lipid-soluble β_2-agonist, salmeterol, prevented the development of HAPO in predisposed subjects, providing evidence that the impairment in respiratory transepithelial fluid transport in these subjects [173] may be reversed by administration of a β_2-adrenergic agonist [157].

Conclusion

In considering the potential use of β_2-adrenergic agonist therapy for hastening the resolution of alveolar oedema in patients, the method of drug delivery is important. In one experimental study in sheep, a lipid-soluble β_2-agonist, salmeterol, was delivered *via* a simple nebuliser, similar to the method used for aerosolisation in ventilated, critically ill patients. Interestingly, 5 mg salmeterol resulted in a high concentration (10^{-6} M) in alveolar oedema fluid, even 3 h after administration of the aerosolised salmeterol [135]. This concentration was at the plateau of the dose/response curve based on *ex vivo* human lung studies [45]. Recent data show similar or even higher concentrations of albuterol are present in the undiluted oedema fluid of mechanically ventilated patients with ARDS after a single inhalation by standard aerosolisation [174]. Therefore, these results indicate that aerosolisation of a β_2-agonist may be sufficient to provide therapeutic concentrations of β_2-adrenergic agonists in the distal airspaces of the lung. An alternative delivery route could be the intravenous route, although the potential for unwanted haemodynamic side-effects would be greater. Another important issue to consider when using β_2-adrenergic agonists is the occurrence of possible downregulation of β-receptors with a diminishing therapeutic effect over time [175]. However, recently published studies demonstrated no evidence of downregulation when high doses of adrenalin were delivered to rats over a period of 4 h [158].

In addition to β_2-adrenergic agonists, other treatments, such as stimulation of alveolar fluid clearance by gene therapy and/or stimulation of alveolar type II cell proliferation by growth factors, should be further evaluated experimentally. Gene therapy may provide a novel approach for the treatment of pulmonary oedema. Short-term expression may be sufficient for clinical benefit. However, several questions should be addressed before its clinical use can be evaluated. The required levels of expression, the speed of onset, and the thresholds for beneficial effect and duration are still to be defined. The potential of increasing inflammation in response to viral vectors in an already damaged lung and the possibility of development of a secondary systemic injury may limit its beneficial effects. The efficacy of transfection and expression and its benefit need to be established when administered after injury occurs. Further, it is important to determine whether transfection of type I or type II alveolar epithelial cells, or both, is important for upregulation of alveolar fluid clearance. Finally, the optimal delivery method in pulmonary gene therapy

remains to be determined. It is also possible that the time course of viral transfection may prohibit its use in the acute setting. However, it may have clinical implications for the therapy of longer-term, volume-overload states such as congestive hearth failure or the pretreatment of donor lungs to prevent development of reperfusion pulmonary oedema.

Is there potential value in using growth factors to stimulate the proliferation of alveolar type II cells in the clinical setting? There are concerns about their short- and long-term efficacy, potential side-effects, and the possible induction of epithelial dysplasia. Since glucocorticoids can upregulate alveolar fluid clearance experimentally [61, 176, 177], the potential clinical value of this therapy in patients with pulmonary odema due to ALI may deserve further evaluation. However, enthusiasm for this approach has been diminished, in part by clinical studies demonstrating that acute treatment with high doses of glucocorticoids has no beneficial effect on outcome in the early acute phase of clinical ALI [178, 179]. Furthermore, the potential deleterious effects of glucocorticoids on host susceptibility to infection make this form of therapy less attractive, at least as a targeted approach to upregulating alveolar fluid clearance. Nevertheless, a large prospective National Institutes of Health study is evaluating the effects of glucocorticoid therapy in patients with persistent ARDS as a follow-up to the studies of MEDURI and co-workers [180, 181].

In conclusion, the value of therapies to enhance alveolar fluid clearance in patients with acute lung injury and/or other forms of pulmonary oedema, ultimately, will need to be demonstrated by evidence of improved clinical outcomes, such as decreased duration of mechanical ventilation and/or decreased mortality [137].

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