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# Novel Pharmacologic Approaches for the Treatment of ARDS

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## Introduction

Acute respiratory distress syndrome (ARDS) is a heterogeneous disease, which is defined by the acute onset of hypoxemic respiratory failure with bilateral infiltrates on chest radiography due primarily to non-cardiogenic pulmonary edema [1]. Only two supportive strategies have shown to improve survival in patients with acute lung injury (ALI)/ARDS, namely lung protective ventilation that reduces the stretch of the lungs and a conservative fluid strategy [2, 3]. The mortality of ARDS, however, remains high and no pharmacological therapies have effectively improved the outcome of these patients so far.

Studies of the pharmacologic management of ARDS, such as trials of anti-inflammatory agents, anticoagulants, surfactant, vasodilators, and  $\beta_2$  agonists, have shown conflicting results [4]. Potential reasons for such conflicting results may be:

- 1) the heterogeneity of the pathophysiologic processes leading to lung injury;
- 2) failure to apply therapies to subgroups of patients with an homogeneous disease process; and
- 3) inappropriate dosing and route of administration of the drugs.

In the last decade, there has been extensive research into potential therapeutic targets and methods of drug administration that could help in the development of specific pharmacological drugs to prevent or mitigate the development of ARDS.

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## Pathophysiology

Early in ARDS, there is widespread neutrophilic alveolitis with disruption of the alveolar epithelial and endothelial barriers, which leads to the formation of protein-rich edema in the interstitium and alveolar spaces [5]. There is activation of alveolar macrophages and neutrophil recruitment, cell death in the alveolar epithelium and vascular endothelium, deposition of hyaline membranes on the denuded basement membrane, deficient surfactant production that contributes to alveolar collapse, and formation of microthrombi. This scenario evolves to a fibro-proliferative process that fills the airspaces with granulation tissue containing proliferating alveolar type II cells, as well as new blood vessels and extracellular matrix rich in collagen and fibrin. In some patients, this process progresses to irreversible lung fibrosis [6].

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## Modulation of Neutrophil Migration and Activation

A considerable quantity of neutrophils is present in the bronchoalveolar lavage (BAL) fluid from patients with ARDS. Persistence of elevated numbers of neutrophils correlates with worsening gas exchange and with poor prognosis [7]. The regulation of neutrophil lifespan is known to be critical for maintaining an effective host response and preventing excessive inflammation. One of the mechanisms that controls the number of neutrophils in the lung is the induction of apoptosis. Interestingly, neutrophils are less susceptible to undergo apoptosis under hypoxic conditions, as occurs in the alveoli in ARDS. In this line, the hypoxia-inducible factor (HIF) oxygen-sensing pathway has been shown to play a role in prolonging neutrophil survival during hypoxia. The expression and transcriptional activity of HIF are regulated by the oxygen-sensitive prolyl hydroxylases (PHD1-3) [8]. This suggests that modulation of HIF and PHD1-3 expression aimed at enhancing neutrophil apoptosis may be a potential approach to downregulate inflammation in the early phase of ARDS.

Active neutrophils release a series of enzymes that contribute to lung damage. Neutrophil elastase is a serine protease stored in the azurophilic granules of leukocytes that has been implicated in the pathology of ARDS. Neutrophil elastase seems to promote inflammation, endothelial and epithelial cell injury, extracellular matrix damage and collagen deposition that leads pulmonary fibrosis [9–11]. Sivelestat was the first neutrophil elastase inhibitor tested in humans and it suppressed the production of neutrophil elastase and interleukin (IL)-8, resulting in improved respiratory function in patients with ARDS secondary to cardiopulmonary bypass [12]. In animal models of lung fibrosis, sivelestat not only decreased inflammatory cell recruitment in the acute phase, but also reduced collagen deposition in lung parenchyma in the fibroproliferative phase of ARDS resulting in less fibrosis and better static compliance of the lung. This beneficial effect was also associated with a decrease in transforming growth factor (TGF)- $\beta$ 1 activation and expression of phospho-SMAD2/3, which are mechanisms involved in lung fibrosis [9, 11]. AZD9668 (N-{[5-(methanesulfonyl)pyridin-2-yl]methyl}

-6-methyl-5-(1-methyl-1H-pyrazol-5-yl)-2-oxo-1-[3-(trifluoromethyl)phenyl]-1,2-dihydropyridine-3-carboxamide) is another novel neutrophil elastase inhibitor that can be administered orally. It exerts selective and rapid inhibition of neutrophil elastase that is also reversible. Oral administration of AZD9668 to mice or rats prevented human neutrophil elastase-induced lung tissue injury as assessed by decreased matrix protein degradation products in BAL fluid and reduced inflammatory responses in the lung in acute and chronic lung injury models. Therefore, neutrophil elastase inhibitors may be drugs that could potentially reduce lung inflammation and diminish structural and functional pulmonary changes in human ARDS [13].

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## Endothelial Protection

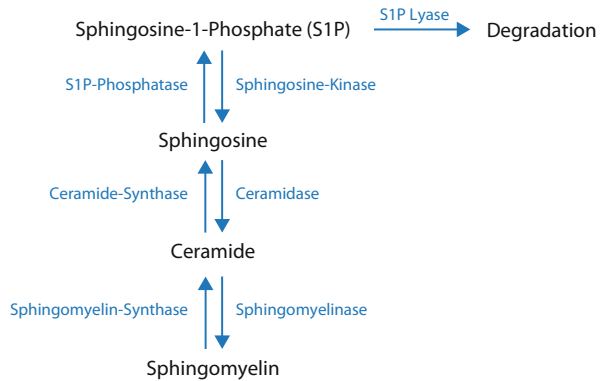
Because the cardinal feature of ALI/ARDS is an increase in lung vascular permeability, often precipitated by an exuberant inflammatory response with subsequent endothelial barrier disruption, strategies aimed at promoting endothelial barrier function could serve as novel therapies in this setting. In this regard, several promising agonists have been identified, including lipid mediators (sphingosine 1-phosphate, resolvins), statins, ang-1/2-Tie2 system and activated protein C (APC). All these agonists have in common the ability to directly mediate endothelial cell signaling and induce characteristic actin cytoskeletal rearrangement leading to endothelial cell barrier protection [14].

## Sphingosine 1-phosphate

There is increasing evidence that sphingolipids contribute to different pulmonary disorders, including ALI. Sphingolipids are essential constituents of plasma membranes and regulate many pathophysiological cellular responses inducing apoptosis and cell survival, vascular permeability, mast cell activation, and airway smooth muscle functions [15].

Sphingosine-1-phosphate (S1P) exerts a protective effect against ALI. S1P is a potent angiogenic factor that preserves human lung endothelial cell integrity and barrier function [16] preventing the alveolar flooding demonstrated in animal models of ARDS. S1P is a naturally occurring bioactive sphingolipid that acts extracellularly (via its G protein-coupled receptors, S1P1-5) and intracellularly on various targets. The synthesis of S1P is catalyzed by sphingosine kinases 1 and 2, and the degradation of S1P is mediated by lipid S1P phosphatases and S1P lyase, contributing to the control of the S1P cellular responses [15, 16] (Fig. 1). S1P enhances vascular barrier function through a series of cellular events initiated by activation of its G-protein coupled receptor, S1P1, and the subsequent downstream activation of Rho and Rac1. Activation of these intracellular signals results in endothelial cytoskeletal reorganization to form strong cortical actin rings in the cell periphery, and reinforcement of focal adhesions and paracellular junctional complexes

**Fig. 1** Overview of sphingolipid metabolism. *Arrows* indicate regulatory enzymes



(cadherin, paxillin, catenins, zona occludens), that prevent excessive vascular permeability [16, 17].

Recent studies show that stimulating the S1P-S1P1 axis reduces vascular permeability, which can be of great value to treat ARDS. S1Ps and S1P analogs, such as FTY720 and ftysiponate, serve as protective agents limiting the disruption of the pulmonary microvascular endothelial barrier and lung edema formation, as well as attenuating parenchymal accumulation of inflammatory cells, which limit the development of ARDS [16, 18]. Another strategy is the prevention of degradation of S1P via inhibition of S1P lyase (S1PL). All these results can be particularly relevant as lung injury was associated with an enhanced S1PL expression and decreased S1P levels in lung tissue in some models of lipopolysaccharide (LPS)-induced ARDS. 2-acetyl-4(5)-[1(R),2(S),3(R),4-tetrahydroxybutyl]-imidazole inhibited S1PL in lung tissue and BAL fluid and reduced lung injury and inflammation in animal models [19].

## Resolvins

Resolvins are docosahexaenoic acid (DHA)-derived lipid mediators that constitute novel endogenous pathways of local-acting mediators that possess both anti-inflammatory and pro-resolvin properties [19]. A novel resolvin D1 (RvD1) suppressed production of pro-inflammatory mediators by primary human cells, such as small airway epithelial cells and monocytes, in a dose-dependent manner *in vitro* [20] and attenuated inflammation in LPS-induced ALI *in vivo* [21]. This attenuated lung inflammation was mediated by the suppression of nuclear factor-kappa B (NF- $\kappa$ B) activation through a mechanism partly dependent on peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) activation. Also, RvD1 significantly reduced macrophages and neutrophil exocytosis and upregulated the expression of the anti-inflammatory cytokine, IL-10, which accelerated the resolution of lung inflammation [20]. In addition, these endogenous mediators seem to have anti-fibrotic and host-directed antimicrobial actions [19]. Thus, resolvins consti-

tute a novel genus of chemical mediators that could be useful for the design of new therapies for ARDS.

## Statins

Statins are a class of 3-hydroxy-3-methylglutaryl-coenzyme A-reductase inhibitors that have pleiotropic properties. Statins not only lower cholesterol, but also have other non-lipid-lowering effects that can be beneficial for the management of sepsis and sepsis-induced ARDS, particularly associated with pneumonia [22]. In this context, statins confer lung protection by preserving endothelial function and integrity, reducing vascular leak, and exerting anti-inflammatory and anti-thrombotic effects. In experimental studies of endotoxin-induced lung injury, simvastatin preserved protein permeability preventing lung edema formation, attenuated neutrophil migration to the lung, and reduced endothelial cell-derived cytokine expression (IL-6, IL-8, monocyte chemoattractant protein [MCP]-1, regulated upon activation normal T cell expressed and presumably secreted [RANTES]). These protective effects of statins are mediated through the upregulation of the activity of integrin- $\beta$ 4 in endothelial cells. These findings support statins as useful drugs and integrin- $\beta$ 4 as a novel therapeutic target in patients with ALI [23].

## Angiopoietin-1/2 System

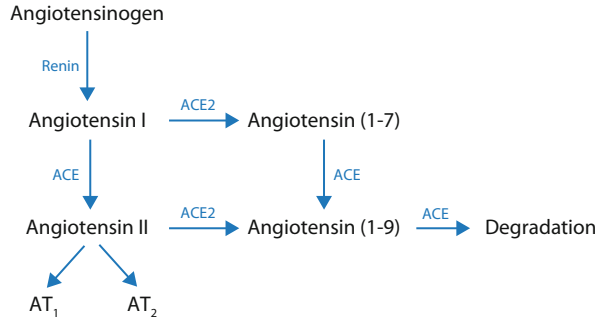
The angiopoietin (Ang)-1/2 system has been recognized to play a major role in various features of human sepsis and ALI. Ang-1 and Ang-2 bind to the endothelial and the soluble form of the Tie2 receptor (sTie2). Ang-1 induces activation of the endothelial Tie2 receptor, and reduces pulmonary inflammation and endothelial cell permeability. Ang-2 prevents Ang-1 from binding to the endothelial Tie2 receptor, and consequently leads to pulmonary inflammation and increased endothelial cell permeability [24, 25]. This suggests that the Ang-1/2-Tie2 system should be further investigated as it constitutes a potential target for treating patients with ARDS.

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## Activated Protein C

APC, an endogenous protein that promotes fibrinolysis and inhibits thrombosis, can modulate the coagulation and inflammation associated with ALI. Restoring normal alveolar levels of APC was expected to prevent the progression of pulmonary coagulopathy and to mitigate the intensity of lung damage. However, a recent clinical trial demonstrated that APC did not improve outcomes in patients with ALI, suggesting that the method of APC administration may be important. It has been speculated that APC inhalation, instead of intravenous administration, might provide the expected benefit to patients with ARDS [26, 27]. Furthermore, combination of APC with other anticoagulant agents (including anti-thrombin and

**Fig. 2** Schematic of the renin-angiotensin system. ACE: angiotensin-converting enzyme; ACE2: angiotensin-converting enzyme 2; AT<sub>1</sub>: angiotensin II type 1 receptor; AT<sub>2</sub>: angiotensin II type 2 receptor



heparin) and pro-fibrinolytic agents (such as plasminogen activators) could be more effective in attenuating pulmonary coagulopathy and inflammation, but this has not been tested yet.

## Regulation of the Renin-angiotensin System

Recent studies indicate that the renin-angiotensin system plays a critical role in acute lung diseases. Angiotensin II, a key effector peptide of the system, causes vasoconstriction and exerts multiple biological functions. Angiotensin-converting enzyme (ACE) and ACE2 are homologs with different key functions in the renin-angiotensin system. ACE cleaves angiotensin I to generate angiotensin II, whereas ACE2 reduces angiotensin II levels and thereby functions as a negative regulator of the angiotensin system (Fig. 2). Capillary blood vessels in the lung are a major site of ACE expression and angiotensin II production in the human body [28]. ACE, angiotensin II and the angiotensin II type 1 receptor (AT<sub>1</sub>) induce apoptosis in the alveolar wall, alter alveolar capillary barrier integrity leading to lung edema, and promote collagen deposition, which can result in lung fibrosis with the consequent impairment of lung function. In contrast, ACE2 and the angiotensin II type 2 receptor (AT<sub>2</sub>) protect against all these effects (Fig. 2). Indeed, inhibiting ACE and AT<sub>1</sub> as well as increasing ACE2 and AT<sub>2</sub> activity attenuated lung injury in several animal models of ARDS, and may represent novel approaches for the treatment of ARDS [29–32].

## Endotoxin Signaling

Many drugs targeting endotoxin or the endotoxin-induced cytokine storm have been developed with therapeutic purposes in sepsis and sepsis-induced ARDS. Toll-like receptor 4 (TLR4) is activated by LPS or endotoxin, the major component of the outer membrane of Gram-negative bacteria, and constitutes a potent inflammatory stimulus implicated in septic shock and septic-induced ARDS. Activation of TLR4 by LPS depends on LPS binding protein (LBP)-catalyzed extraction and transfer

of individual LPS molecules from aggregated LPS to CD14 and then from CD14 to MD2, followed by engagement and dimerization of TLR4. TLR4 signaling may also be activated by stimuli other than LPS. Chemical (acid aspiration) or virus (influenza) insults may cause ALI via the generation of host-derived, oxidized phospholipids that potently stimulate TLR-4 [33]. Eritoran is a potent, well-tolerated, synthetic TLR4 antagonist that protects against influenza-induced ARDS in mice, improving the survival of these animals even when administered as late as 6 days post-infection. Eritoran binds to CD14, which inhibits ligand binding to MD2:TLR4 complexes preventing TLR4 dimerization and activation [34]. In viral infection, eritoran blocks oxidized phospholipid-induced TLR4 signaling, mitigates the subsequent cytokine storm, and also helps to control viral titer. Despite the lack of benefit in severe septic patients (ACCESS trial) [38], eritoran could still be effective in influenza infection and, perhaps, in ALI mediated by non-infectious agents, but its most effective timing and dosing remain unknown. Based on endotoxin challenge studies in animals, the prophylactic or very early administration of eritoran in patients at-risk or with ARDS could be more effective in controlling the 'sepsis cascade'. Antagonism of TLR4 signaling could therefore become a useful therapy for ARDS [35, 36].

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## Cell Adhesion Molecules

The cell adhesion molecules, integrins and selectins, are transmembrane cell surface receptors that regulate leukocyte migration and activation, endothelial/epithelial cytokine expression and alveolar-capillary protein permeability in the lung [37]. The  $\alpha v\beta 5$  and  $\alpha v\beta 6$  integrins have an important role on pulmonary endothelial and epithelial cells, respectively. Dysfunction of these molecules alters alveolar endothelial- and epithelial-cell functions and barrier integrity, leading to alveolar flooding. In addition,  $\alpha v\beta 6$  integrin, which is upregulated in injured epithelial cells, plays a robust role in the development of lung fibrosis via activation of the TGF- $\beta$  (a pro-fibrotic cytokine). Prevention of leukocyte-epithelial adhesion by using antagonists of integrins, such as XVA143 (antagonist of  $\alpha-L/\beta-2$  integrin) or BIO1211 (antagonist of  $\alpha-4/\beta-1$  integrin), protects alveolar epithelial cells from injury in inflammatory lung diseases [38].

These results identify integrins and selectins as promising therapeutic targets for ALI, in particular in sepsis-induced ARDS [39]. Inhibition of these integrins by specific antibodies has shown therapeutic effects in models of septic shock [40, 41]. In fact, these families of adhesion molecules have been under intense investigation by the pharmaceutical industry. In this regard, antagonists of integrin  $\alpha v\beta 3$  (e. g., abciximab), integrin  $\alpha L\beta 2$  (efalizumab), and integrin  $\alpha 4\beta 1$  (natalizumab) are currently US FDA-approved for acute coronary syndromes, psoriasis and multiple sclerosis, respectively. However, none has been approved for indications related to ARDS. Recently, positive results in phase II clinical trials with a small-molecule selectin antagonist (bimosiamose) and a small-molecule integrin  $\alpha 4\beta 1$  antagonist (valategrast, R411) for the management of asthma and chronic obstructive pul-

monary disease (COPD) have generated great enthusiasm [37]. The efficacy of these molecules against the development of ARDS may therefore warrant further investigation.

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## MicroRNA

ARDS is a disease affected by various genetic and non-genetic factors. MicroRNAs (miRNAs) have emerged as a novel class of gene regulators which play critical roles in complex diseases including ARDS. From animal models of ALI, including LPS- and ventilator-induced lung injury (VILI), several miRNAs (e. g., miR-146a, miR-155, miR-16) have been identified as important regulators of inflammatory responses in the lung. For example, upregulation of miR-16 significantly downregulates IL-6 and TNF- $\alpha$  expression in LPS-induced lung injury [42]. In addition, some miRNAs (hsa-miR-374a, hsa-miR-374b, hsa-miR-520c-3p, and hsa-miR-1290) have been involved in endothelial cell barrier dysfunction and in the increased vascular permeability and formation of lung edema in injured lungs. These miRNAs seem to control endothelial cell barrier via regulation of non-muscle myosin light chain kinase (nmMLCK) expression in human lung endothelium *in vitro*. Thereby, modulation of miRNAs could have important beneficial effects on controlling the development and progression of ARDS [43].

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## Antihistamines

Endogenous histamine is widely distributed in the lungs and is an important mediator of early and late inflammatory responses. Histamine promotes neutrophil migration, increases cytokine expression, and enhances capillary permeability leading to protein leak. In animal models of ALI, inhaled endotoxin causes an increase in histamine concentration in BAL fluid. Administration of mepyramine (H<sub>1</sub>-receptor antagonist) or ranitidine (H<sub>2</sub>-receptor antagonist) attenuates the increased histamine concentration, but only the administration of ranitidine was associated with a significant reduction in neutrophil numbers and in protein permeability. This study suggests that endogenous histamine may be involved in the recruitment of neutrophils and protein leaks in endotoxin-induced ALI via the H<sub>2</sub> receptors [44]. Also, scavenging of endogenous histamine by the arthropod-derived histamine binding protein, EV131, mitigates endotoxin-induced lung injury, supporting the notion that histamine-mediated signaling could be a potential target in ARDS [45].



## Stimulators of Lung Repair

### Growth Factors

A series of growth factors, such as keratinocyte-, epithelial-, basic fibroblast-, hepatocyte- and vascular endothelial-growth factor, are emerging as important candidates to repair the damaged pulmonary alveolar capillary membrane. To this end, the synergistic interaction of these growth factors, which directly or indirectly affect pulmonary endothelial and epithelial cells, appears to promote the repair process of lung damage. As an example, the combined effect of trefoil factor family peptides and growth factors, such as epithelial growth factor (EGF), stimulated epithelial proliferation and repair [46].

### Erythropoietin

A number of preclinical studies have revealed beneficial effects of exogenous erythropoietin (EPO) administration in various experimental models of ARDS. EPO provides protection by modulating multiple early signaling pathways involved in apoptosis, inflammation and peroxidation. Furthermore, EPO appears to confer vascular protection by promoting angiogenesis. However, only preliminary studies exist and more experimental and clinical data are necessary to clarify the efficacy and cytoprotective mechanisms of EPO [47].

### Hyaluronan

Hyaluronan constitutes the major glycosaminoglycan in lung tissue and has diverse functions in normal lung homeostasis and pulmonary disease. Hyaluronan and its degradation products, produced by hyaluronidase enzymes and reactive oxygen species (ROS), are implicated in different aspects of the ALI process. The different activities of hyaluronan and its degradation products are regulated by multiple hyaluronan-binding proteins, including CD44, TLR4, hyaluronan-binding protein 2 (HABP2), and receptor for hyaluronan-mediated motility (RHAMM). Recent research indicates that exogenous administration of high molecular weight hyaluronan can serve as a novel therapeutic intervention for lung diseases, including LPS-induced ALI, sepsis/VILI, and airway hyperactivity [48].

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## Nanomedicine

Novel long-acting biocompatible and biodegradable phospholipid micelles have been developed to inhibit different targets implicated in ALI. For example, nano-sized micelles have been developed to trigger receptor expressed on myeloid cells 1 (TREM-1), ROS and heat shock protein (Hsp)90, all effectors thought to be in-

involved in ALI. The main limitations of some peptide drugs for clinical use are the short half-life (minutes) and their water-insolubility. To overcome these limitations, some groups have developed micellar-targeted peptides in which the peptide drug remains in its active form for longer (hours) and increases its stability and bioavailability [49]. These long-acting micellar nanomedicines have provided significant advancement in the treatment of ARDS in experimental models, which could be extended to the clinical setting. Another advance is the novel nanovesicle aerosols composed of non-lamellar phospholipids that were developed as pulmonary surfactant aerosols and have been successfully used as therapy in mice with acid-induced lung injury. Currently existing intratracheal surfactants are ineffective because they are inactivated by albumin leakage and other mechanisms. The nanovesicle aerosols of non-lamellar lipids improve the resistance of pulmonary surfactants to inhibition, and may therefore be promising as a non-invasive aerosol therapy in ALI [50].

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## Conclusions

Despite decades of clinical trials and experimental research, there is no effective pharmacologic therapy for the treatment of all patients with ARDS. Multiple biochemical and biological targets, however, have been identified that could give rise to future drug discovery and development. Several aspects should be taken into account in order to guarantee the efficacy of those potential new drugs. Better stratification may help to identify subgroups of patients with ARDS that benefit from targeted interventions, given the heterogeneity of patients with ARDS and the multiple etiologies of this disease (direct versus indirect ARDS). Combinations of different therapeutic strategies could also improve outcomes for these patients compared with therapies based on single agents. Finally, we also need to optimize the dose, timing and route of administration as well as to determine the potential toxicity of the novel pharmacologic agents for their future use in the management of patients with ARDS.

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