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Chapter 38

Angiotensin-Converting Enzyme 2/ Angiotensin-(1-7)/Mas Receptor Axis: Emerging Pharmacological Target for Pulmonary Diseases

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INTRODUCTION

The renin–angiotensin system (RAS) has long been recognized as an important player in the pathogenesis of several lung diseases, including pulmonary arterial hypertension (PAH), pulmonary fibrosis (PF), chronic obstructive pulmonary disease (COPD), and acute respiratory distress syndrome (ARDS). Evidence for this stems from the following observations: (a) PAH and PF are associated with higher circulating levels of angiotensin II (Ang II)[1,2]; (b) increased concentrations of angiotensinogen (the precursor for Ang II peptide) and angiotensin-converting enzyme (ACE), the major generator of Ang II, have been observed in the lungs of fibrotic and pulmonary hypertensive subjects[3,4]; (c) patients carrying the ACE ID/DD genotype, which confers increased ACE levels, are susceptible to COPD and ARDS[5,6]; (d) human lung fibroblasts obtained from patients with PF, but not from normal lungs, generate Ang II,[2] suggesting a causative role for this peptide in disease pathogenesis; (e) Ang II induces apoptosis of the alveolar epithelial cells, a key initiating factor for lung fibrogenesis[7]; (f) Ang II is a potent pulmonary vasoconstrictor with mitogenic properties, that produces migratory, hypertrophic, and proliferative effects on the lung smooth muscles to cause PAH[8]; (g) Ang II mediates oxidative stress and cytokine signaling,[9] factors that contribute to the pathophysiology of lung diseases; and (h) pharmacological blockade of the RAS using ACE inhibitors (ACEi) or angiotensin receptor blockers (ARB) offers protection against animal models of COPD, PAH, and lung fibrosis.[5,10,11] Collectively, these findings indicate an active involvement of the RAS in lung diseases. However, clinical studies with ACEi or ARBs have yielded mixed results, failing to reach a consensus opinion on the use of these agents for treating pulmonary disorders.

Discovery of several new members has led to the emergence of a new arm of the RAS, which has forced a reevaluation of the role of this hormonal system in pulmonary physiology and pathobiology. These new components include the monocarboxypeptidase angiotensin-converting enzyme 2 (ACE2), the vasoactive peptide angiotensin-(1-7) (Ang-(1-7)), and its G protein-coupled receptor Mas, which together form a protective arm, the ACE2/Ang-(1-7)/Mas axis of the RAS.[12] This axis counteracts the vasoconstrictive, proliferative, inflammatory, and fibrotic actions of Ang II in several target organs, including the lungs. Our objective in this chapter will be to (i) summarize the importance of the ACE2/Ang-(1-7)/Mas axis in lung diseases and (ii) explore novel strategies to upregulate this axis that hold great potential for clinical development in the treatment of pulmonary disorders.

EVIDENCE FOR THE EXISTENCE OF VASODELETERIOUS AND VASOPROTECTIVE AXES OF THE RAS IN THE LUNGS

The classical RAS comprises a cascade of proteolytic enzymes and peptides located primarily within the circulatory system. However, in addition to the circulating RAS, a tissue-specific RAS has been identified in several organs, including the heart, kidneys, and lungs. Biochemical and molecular studies have revealed that all the necessary components required for the active biosynthesis of Ang II, namely, renin, angiotensinogen, and ACE, are present within the lung tissue.[13] Furthermore,

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expression of AT_1R and AT_2R at both mRNA and protein levels has been detected in the pulmonary system. Since AT_1R gene expression is more abundant than AT_2R in the adult lungs, this receptor mediates most of the biological effects of Ang II. The intrapulmonary ACE-Ang II-AT_1R axis has been shown to promote vasoconstriction, smooth muscle cell proliferation, and fibrosis, along with induction of oxidative stress and inflammation. Thus, this axis forms the vasodeleterious arm of the RAS. On the other hand, identification of ACE2 and Mas receptor within the lung parenchymal cells has lent credence to the existence of a vasoprotective arm of the RAS.[14] We believe that an imbalance between vasodeleterious and vasoprotective axes of the RAS plays a crucial role in the pathophysiology of lung diseases.

THE ACE2/ANG-(1-7)/MAS AXIS IN PULMONARY DISEASES

ACE2 is a zinc-dependent metalloproteinase that is widely expressed on the pulmonary endothelium, type II alveolar epithelial and smooth muscle cells of the lungs.[15] Its primary physiological function is to metabolize Ang II to Ang-(1-7), a biologically active heptapeptide that binds to the Mas receptor to exert vasodilatory, antiproliferative, and antifibrotic actions in the pulmonary system. It is becoming increasingly evident that downregulation of the ACE2/Ang-(1-7)/Mas axis contributes to the development and progression of lung diseases. The importance of ACE2 in pulmonary physiology was appreciated when this enzyme was identified as a functional receptor for the SARS coronavirus (SARS-CoV), the causative agent of severe acquired respiratory syndrome (SARS).[16] *In vivo* experimental studies revealed that lung ACE2 expression is markedly decreased upon SARS-CoV infection, which consequently leads to respiratory failure and death.[17] The protective role of ACE2 against lung injury was further underscored by studies carried out on ACE2-deficient animals. Knockout mice for ACE2 displayed enhanced vascular permeability, increased lung edema and neutrophil accumulation, which worsened pulmonary function in experimental models of ARDS.[18]

With respect to fibrotic lung diseases, Li et al. were the first to demonstrate severe downregulation of ACE2 and its catalytic activity in the lungs of animals, as well as patients with PF.[19] Also, inhibition/silencing of pulmonary ACE2 in rodents was associated with excessive lung collagen accumulation, suggesting that decreased ACE2 levels play a causal role in lung fibrogenesis. Consistent with this interpretation, mice deficient in ACE2 gene were more susceptible to bleomycin-induced injury, exhibiting excessive deposition of extracellular matrix proteins in the lungs as compared with wild-type animals. [20]

A growing body of studies has focused on the relevance of the ACE2/Ang-(1-7)/Mas axis in PAH. PAH is a fatal lung disease that is associated with increased blood pressure in the pulmonary arteries. Decreased expression of ACE2 and enzymatic activity has been observed in animal models of PAH and pulmonary hypertensive patients, along with a substantial increase in circulating Ang II levels.[1,12] Thus, it is evident that restoring the balance between the vasodeleterious and the vasoprotective axes of the RAS could be therapeutically beneficial in attenuating disease pathogenesis.

THE ACE2/ANG-(1-7)/MAS AXIS PROTECTS AGAINST ARDS AND PAH

ARDS is the most severe form of acute lung injury that is triggered by sepsis, gastric juice aspiration, or pathogenic infections such as SARS-CoV and H5N1 avian influenza virus. In experimental models of ARDS, treatment of wild-type mice and ACE2 knockout mutants with catalytically active recombinant ACE2 protein rescued them from lung failure.[18] Likewise, administration of Ang-(1-7) conferred beneficial effects against acute lung injury, an effect that was abolished by blocking the Mas receptor.[21]

With regard to PAH, the enzymatic activity of ACE2 offers a dual beneficial effect. On one hand, it decreases the levels of Ang II, which are significantly elevated during PAH, and on the other, it increases the concentration of the vasoprotective heptapeptide, Ang-(1-7). We were the first to demonstrate that pulmonary overexpression of ACE2 attenuates monocrotaline (MCT)-induced PAH and associated pathophysiology.[22] Similarly, genetic overexpression of Ang-(1-7) rendered beneficial effects against this disease.[23] Interestingly, coadministration of A-779, a Mas antagonist, abolished the cardiopulmonary protection offered by Ang-(1-7), suggesting the involvement of Mas-mediated signaling pathway. In a separate set of experiments, Kleinsasser et al. demonstrated that administration of recombinant human ACE2 protein (rhACE2) decreased hypoxia-induced pulmonary vasoconstriction.[24] On the clinical front, it was observed that the serum ACE2 activity was significantly reduced in patients with PAH.[25] Apparently, elevating circulating levels of ACE2 in these pulmonary hypertensive patients could yield beneficial results.

THE ACE2/ANG-(1-7)/MAS AXIS IN PF

PF is a debilitating disease marked by scarring in the lungs. Treatment of ACE2 knockout animals with rhACE2 attenuated bleomycin-induced PF and improved lung function.[20] Furthermore, studies from our group have shown that pulmonary

overexpression of either ACE2 or Ang-(1-7) by lentiviral-mediated gene transfer prevents lung fibrosis in the bleomycin model.[23] One of the hallmarks of PF is the loss of alveolar epithelial cells (AECs). Mechanistic studies carried out in cultured AECs revealed that ACE2 mediates cell survival by balancing the proapoptotic Ang II and its antiapoptotic metabolic product Ang-(1-7). This is evidenced by the fact that silencing of the ACE2 gene in AECs or its pharmacological inhibition resulted in increased Ang II and decreased Ang-(1-7) levels, which was associated with apoptotic cell death.[14] On the contrary, treatment of AECs with Ang-(1-7) attenuated Ang II and bleomycin-induced apoptosis. This cell survival effect of Ang-(1-7) was mediated through inhibition of caspases (caspases 3 and 9) and downregulation of the Jun N-terminal kinase (JNK) pathways. Also, these Ang-(1-7)-mediated effects were reversed by blocking the Mas receptor or with antisense nucleotides against the Mas mRNA. Taken together, these findings implicate a potent antifibrotic role for the ACE2/Ang-(1-7)/Mas axis against PF.

THERAPEUTIC STRATEGIES TO ACTIVATE THE ACE2/ANG-(1-7)/MAS AXIS

Recombinant Human ACE2 (rhACE2)

Administration of rhACE2 has been shown to exert promising effects in a variety of disease models with pathologically elevated Ang II levels or a dysregulated RAS. These experimental findings have provided compelling evidence for initiating clinical trials with rhACE2. In this regard, GlaxoSmithKline is currently evaluating the effects of GSK2586881 (formerly APN01), a soluble form of rhACE2, in a multicenter phase II clinical trial for the treatment of acute lung injury. Previous phase I trials have established that administration of single and multiple doses of rhACE2 is well tolerated by healthy humans, with no serious adverse events or dose-limiting toxicities.[26] Furthermore, in a separate clinical study, the efficacy of rhACE2 is being assessed for the treatment of PAH (ClinicalTrials.gov; NCT01884051). Although clinical trials are currently under way, the use of protein therapy faces several challenges. Among these, the cost of manufacturing the recombinant protein, its stability, repetitive intravenous dosing, and patient compliance pose major obstacles in realizing the full therapeutic potential of ACE2 therapy.

ACE2 ACTIVATORS

An ideal approach to overcome the aforementioned limitations of recombinant protein therapy would be to identify small synthetic molecules that can favorably activate endogenous ACE2. Using a structure-based drug design, we have identified synthetic enhancers of ACE2 such as resorcinolnaphthalein, 1-[[2-(dimethylamino)ethyl]amino]-4-(hydroxymethyl)-7-[[(4-methylphenyl)sulfonyl]oxy]-9H-xanthone9 (XNT), and diminazene aceturate (DIZE).[27–29] *In silico* docking studies revealed that binding of these small molecules to a novel pocket in the hinge-bending region of the ACE2 protein stabilizes the enzyme in an open conformation. This is important because only the open conformation favors/accelerates enzyme activity. Among the three compounds, DIZE was found to be the most potent, followed by XNT, and finally resorcinolnaphthalein. Pharmacologically, all these ACE2 activators were found to be effective in attenuating lung injury. In addition, XNT exhibited strong antithrombotic actions along with decreased platelet attachment,[30] whereas DIZE therapy was associated with enhanced endothelial progenitor cell (EPC) function in pulmonary hypertensive animals, factors that could potentiate the cardiopulmonary beneficial effects of these agents. However, there are some critical issues that impede the successful translation of these ACE2 activators into the clinic. XNT has an undesirable pharmacokinetic profile. It is poorly soluble in water and requires acidic pH for solubilization, making it an unsuitable drug candidate. With regard to DIZE, it is associated with toxic side effects. Chronic treatment with DIZE has been shown to harm the liver, kidneys, and brain, which could potentially result in life-threatening situations. In fact, a preslaughter withdrawal period of 21–35 days has been recommended for DIZE when treated animals are intended for human consumption. Besides, DIZE was found to be mutagenic, but not teratogenic. Nonetheless, XNT and DIZE could serve as lead compounds that require structural modification to make them safe and druggable.

STRATEGIES TARGETING ANG-(1-7)

It is evident that Ang-(1-7) holds promise for the treatment of lung diseases. However, the use of Ang-(1-7) as a therapeutic agent is limited because of its short half-life *in vivo.* Also, being of peptide nature, Ang-(1-7) will be rapidly degraded in the gastrointestinal tract when given orally. To overcome these issues, formulations of Ang-(1-7) with extended half-life and improved oral bioavailability are currently being developed. Ang-(1-7) complexed with beta-cyclodextrin (HPβCD/Ang-(1-7)) enabled effective oral administration, with significant increases in plasma Ang-(1-7) levels.[31] In addition,

Kluskens and coworkers synthesized a cyclic analog of Ang-(1-7) (thioether-bridged Ang-(1-7)) that exhibited better *in vivo* pharmacokinetic profile.[32] Alternatively, liposomal delivery system represents an effective method to administer Ang-(1-7). Administration of liposomes containing Ang-(1-7) was also found to increase the half-life of this heptapeptide.[33] Importantly, all these modified forms of Ang-(1-7) were pharmacologically active when tested in experimental models. It is worth noting that TXA127, a synthetic analog of Ang-(1-7) from Tarix Pharmaceuticals, is currently in clinical trials for the treatment of PAH. TXA127 could also be a promising candidate for PF therapy.

MAS RECEPTOR AGONISTS

Given that stimulation of the Mas receptor is protective, it would be reasonable to evaluate the effects of Mas agonists for pulmonary therapeutics. AVE 0991 is the first nonpeptide agonist of the Mas receptor that was shown to promote beneficial effects against several cardiovascular diseases.[34] An important aspect of this compound is that it is orally active and mimics the effects of Ang-(1-7) in several organs by binding to the Mas receptor. Recently, two novel peptides (CGEN 856 and CGEN 857) with high specificity for the Mas receptor have been discovered.[35] All these Mas agonists have the potential to treat pulmonary diseases.

FUTURE PERSPECTIVES

Oral Delivery of ACE2 and Ang-(1-7)

The oral route is the most widely accepted means of drug administration. But oral delivery of therapeutic proteins like ACE2 and Ang-(1-7) is not feasible since they will be easily degraded in the gastrointestinal tract. However, we have developed a low-cost oral delivery system for administering ACE2 and Ang-(1-7) using transplastomic technology. The use of transplastomic technology allows chloroplasts to generate high levels of therapeutic proteins within plant leaves.[36] Since chloroplast expression of ACE2 or Ang-(1-7) enables the bioencapsulation of these therapeutic proteins within plant cells, it protects them against gastric enzymatic degradation upon oral delivery. We observed that oral feeding of rats with bioencapsulated ACE2 or Ang-(1-7) prevented the development of MCT-induced PH and was associated with improved right heart function.[37] It is possible to produce bulk quantities of therapeutically active ACE2 and Ang-(1-7) using transplastomic technology, which holds great potential for clinical development as an oral delivery system.

Genetic Modification of Stem Cells

Reduced number and impaired function of circulating/resident stem cells have been associated with poor cardiopulmonary outcomes in patients and experimental models of lung diseases. In this regard, exogenous administration of stem cells such as endothelial progenitor cells, bone marrow- or adipose-derived mesenchymal stromal cells (MSCs), and umbilical cord blood cells could offer protection. However, such cell-based therapy presents a number of unique challenges before they can be effectively employed in the clinical setting. The hostile environment characterized by inflammation and high oxidative stress within the diseased lungs could threaten survival and impair homing of the injected stem/progenitor cells. Genetic modification of these cells to overexpress ACE2 or Ang-(1-7) could improve survival and enhance their potential to effectively repair lung injury. In fact, we have shown that ACE2 priming of endothelial progenitor cells not only enhances their function but also increases their therapeutic efficacy to render protection against stroke.[38]

CONCLUSIONS

A dysregulated pulmonary angiotensin system contributes to the pathogenesis and progression of lung diseases. Discovery of the protective ACE2/Ang-(1-7)/Mas axis has provided a novel and more promising target for disease treatment than the classical approach of using ACEi or ARBs. Innovative pharmaceutical strategies that can effectively activate this beneficial ACE2/Ang-(1-7)/Mas axis hold great promise for treating pulmonary pathologies.

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