Lung

Giselle S. Magalhães, Maria Jose Campagnole-Santos,
and Maria da Glória Rodrigues-Machado

Renin-Angiotensin System Components in the Lungs

There is a considerable body of evidence for the existence of local, tissue-based, renin-angiotensin system (RAS) in which angiotensin (Ang) peptides production is independent of circulating precursors [13, 56]. Expression of angiotensinogen, the type 1 (AT1) and type 2 (AT2) Ang II receptors in rat and human lung tissue support local generation of Ang II [56]. Membrane angiotensin-converting enzyme (ACE), primarily responsible for conversion of Ang I to Ang II in the circulation, is abundantly expressed in vascular endothelium of pulmonary circulation.

Ang II can modulate inflammatory response promoting cytokine production, expression of endothelial adhesion molecules, inflammatory cell migration, epithelial cell apoptosis, oxidative stress, lung fibroblast growth and fibrosis [56]. The majority of these actions are mediated through the AT1 receptor involving complex intracellular signaling pathways [38]. AT1 receptor, coupled to Gq/11 protein, can stimulate multiple signaling pathways including MAPK/ERK, Rho/ROCK kinase,

---

G. S. Magalhães

Department of Physiology and Biophysics, Federal University of Minas Gerais,
Belo Horizonte, MG, Brazil

Post-Graduation Program in Health Sciences, Medical Sciences Faculty of Minas Gerais,
Belo Horizonte, MG, Brazil

M. J. Campagnole-Santos

Departamento de Fisiologia e Biofísica, INCT-Nanobiofar, Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

e-mail: mjcs@icb.ufmg.br

M. da Glória Rodrigues-Machado (✉)

Post-Graduation Program in Health Sciences, Medical Sciences Faculty of Minas Gerais,
Belo Horizonte, MG, Brazil

e-mail: maria.machado@cienciasmedicasmg.edu.br

PLC β /IP3/diacylglycerol, tyrosine kinases, and NF- κ B [3]. ACE/Ang II/AT1 receptor axis is involved in many lung diseases.

ACE2, an ACE homologous enzyme, has emerged as a potent negative regulator of the RAS. ACE2 regulates RAS signaling, reducing Ang II/AT1 receptor signaling and activating the counterregulatory angiotensin-(1-7) [Ang-(1-7)]/Mas receptor pathway. ACE2 protein is expressed in the lungs, mainly in the vascular endothelium, Clara cells, type I and type II alveolar epithelial cells [26, 27, 48], as well as in smooth muscle of small and medium vessels in the mouse lung [92]. Mas receptor, a functional receptor for Ang-(1-7) [76], is present in thin areas of the bronchial epithelium and smooth muscle [52]. ACE2/Ang-(1-7)/Mas receptor pathway often serves to counterregulate the pro-inflammatory, pro-proliferative, and pro-fibrotic effects of the ACE/Ang II/AT1 receptor pathway [77].

Ang-(1-7) and Pulmonary Arterial Hypertension

Pulmonary hypertension (PH) is a disorder characterized by an increase in mean pulmonary arterial pressure (PAP) ≥ 25 mmHg at rest as assessed by right heart catheterization (RHC) [32]. The term pulmonary arterial hypertension (PAH) describes a group of PH patients characterized hemodynamically by the presence of pre-capillary PH, defined by a pulmonary artery wedge pressure (PAWP) ≤ 15 mmHg and a pulmonary vascular resistance (PVR) > 3 Wood units (WU) in the absence of other causes of pre-capillary PH, such as PH due to lung diseases, chronic thromboembolic pulmonary hypertension (CTEPH), or other rare diseases [32]. A hallmark of PAH is a vascular remodeling process that increases PVR and subsequent right ventricular hypertrophy and premature death [78]. Regardless of the underlying disease, chronic cor pulmonale is associated with progressive clinical deterioration and a poor prognosis in most cases. Incidence and prevalence of PAH is very similar in USA (2.0 and 10.6 cases of PAH per million inhabitants, respectively) and in UK (1.1 and 6.6 cases of PAH per million inhabitants, respectively) [49, 62].

Clinical classification of PH categorizes multiple clinical conditions into five groups, according to their similar clinical presentation, pathological findings, hemodynamic characteristics, and treatment strategy [20].

Diagnosis of PH is based on clinical suspicion established by symptoms, typically induced by exertion (shortness of breath, fatigue, weakness, angina, and syncope). Symptoms at rest occur only in advanced circumstances. Abdominal distension and ankle edema will develop with progressing right ventricle (RV) failure. Diseases that cause or are associated with PH as well as other concurrent diseases can modify the presentation of PH [20].

Advances in basic and clinical research into PAH have led to improved understanding of disease pathogenesis and identification of novel therapeutic targets [42]. The aim of specific therapies for PH is to reduce PVR and thereby improve RV function. Currently, five classes of drugs have been applied for PAH: endothelin receptor antagonists (ERAs), prostanoids, phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, and selective prostacyclin receptor agonists

[89]. Despite improvement in patient symptoms and well-being with these agents, mortality rates remain high (~65% survival at 5 years). New therapies are needed targeting alternative pathways that can reverse pulmonary vascular remodeling, inhibit disease progression, and improve survival [23]. The RAS is being intensively studied as an alternative therapeutic target [90].

A large number of studies have shown that the RAS is importantly involved in PAH pathophysiology [12, 38, 86]. Lungs of patients with PAH express high levels of ACE in the intra-acinar arteries, suggesting that locally increased production of Ang II, a potent pulmonary vasoconstrictor with mitogenic actions, may contribute to the process of pulmonary vascular remodeling [69]. Ang II is also capable of inducing an inflammatory response in the vascular wall. Ang II, via the type 1 (AT1) receptors, enhances the production of reactive oxygen species (ROS) through stimulation of NAD(P)H oxidase in the vascular wall, leading to endothelial dysfunction and vascular inflammation by stimulating the redox-sensitive transcription factors (NF- κ B) and by upregulating adhesion molecules, cytokines, and chemokines [9]. De Man et al. [12] demonstrated increased serum levels of renin, Ang I, and Ang II and correlations with disease progression and mortality in patients with idiopathic PAH. Taken together, these findings indicate an active role for RAS in the pulmonary hypertensive process.

There is a body of evidence suggesting that ACE2, either by itself or through its catalytic product Ang-(1-7), opposes the proliferative, hypertrophic, and fibrotic effects of Ang II in many organs, including the lungs, pointing for a plausible protective role against PAH. Ang II appears to be the main substrate for ACE2, and is effectively hydrolyzed to Ang-(1-7). ACE2 protein is expressed in various human organs and in the lungs, it is expressed mainly on the vascular endothelium, and type I and type II alveolar epithelial cells [26, 27].

Studies demonstrate that serum ACE2 was decreased in patients with PAH due to congenital heart disease, and mean PAP was negatively correlated with serum levels of ACE2 [11]. Similar results were found for Ang-(1-7), suggesting the decrease in Ang-(1-7) shifts the balance of the RAS toward the ACE/Ang II/AT1 receptor axis, resulting in increases in vascular remodeling, fibrosis and PAH in congenital heart disease patients [10]. Consistent with these findings, several ACE2 activators such as diminazene aceturate (DIZE) [84], xanthenone (XNT, e 1-[(2-dimethylamino)ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyloxy]-9H-xanthene-9-one) [31], resorcinolnaphthalein [44, 45], and NCP-2454 [24] have been reported in various preclinical models of PAH.

In a recent trial, Hemnes et al. [30] assessed the mechanism, safety, and efficacy of ACE2 (single IV infusion of GSK2586881) in the treatment of patients with idiopathic and heritable PAH (18 years) with functional class I-III. PAH patients had a significant decrease in ACE2 activity as reflected by the increased Ang II/Ang-(1-7) ratio in PAH patients compared with controls. After treatment, PAH patients had a decrease in Ang II/Ang-(1-7) ratio, suggesting increased activity of ACE2. In addition, levels of superoxide dismutase (SOD2) protein were approximately 25% lower in PAH plasma compared with controls. After treatment, there was significant induction of plasma SOD2 protein levels by 2 weeks suggesting induction in the enzymatic activity by GSK2586881. Compared with control, patients with PAH had increased

levels of cytokines (IL-10, IL-1 β , TNF- α , IL-13, IL-8, and IL-4). After GSK2586881 administration, there was suppression of IL-10, IL-1 β , IL-2, and TNF- α that could be detected as early as 2 hours after drug administration and was associated with sustained anti-inflammatory effects with reduced levels of IL-1 β , IL-6, IL-8, and TNF- α at 2 weeks [30]. Taken together, these data showed that treatment with ACE2 reduced the markers of oxidant and inflammatory mediators and improved the balance between ACE/Ang II/AT1 receptor and ACE2/Ang-(1-7)/Mas receptor axis.

Ang-(1-7) promotes the release of prostanoids from endothelial cells (EC) and smooth muscle cells (SMC) and the release of nitric oxide (NO). In addition, Ang-(1-7) inhibits proliferation of vascular SMC and EC *in vitro* and *in vivo* and opposes the mitogenic effects of Ang II [77]. Drugs that inhibit the synthesis of Ang II (ACE inhibitors) or that antagonize AT1 receptors (Ang II receptor blockers – ARBs) have been shown to decrease right ventricular hypertrophy, decrease medial thickening and peripheral muscularization of small pulmonary arteries in hypoxic animals [65]. In addition, ACE2 [17, 94] or Ang-(1-7) itself, by targeted gene transfer, protects the lungs in a model of pulmonary hypertension [82]. The effects of Ang-(1-7) appear to be associated with upregulation of endothelial nitric oxide synthase (eNOS) activation via AKT pathway [7]. Recently, Zhang et al. [96] showed that phosphorylation of ACE2 by AMPK enhanced the stability of ACE2, which increased Ang-(1-7) and nitric oxide synthase (eNOS)-derived NO bioavailability in endothelial cells.

Shenoy et al. [85] developed a plant-based oral delivery of ACE2 or Ang-(1-7) to protect against gastric enzymatic degradation and facilitates long-term storage at room temperature. Further, fusion to a transmucosal carrier helped effective systemic absorption from the intestine on oral delivery. Rats fed with bioencapsulated ACE2 or Ang-(1-7) presented attenuation in the development of monocrotaline-induced PH and improvement of cardiopulmonary pathophysiology. Furthermore, in the reversal protocol, oral ACE2 or Ang-(1-7) treatment significantly arrested disease progression, along with improvement in right heart function, and decrease in pulmonary vessel wall thickness. In addition, a combination therapy with ACE2 and Ang-(1-7) augmented the beneficial effects against monocrotaline-induced lung injury. According to the authors, these results provided proof-of-concept for a novel low-cost oral ACE2 or Ang-(1-7) delivery system using transplastomic technology for pulmonary disease therapeutics.

Microvesicles derived from mesenchymal stem cells (MSCs) improve the outcome of PAH [43]. Recently, Liu et al. [50] investigated whether the effect of MSC-derived microvesicles on PAH induced by monocrotaline was correlated with RAS. Animals treated with microvesicles from MSCs notably attenuated the pulmonary artery pressure, reversed the RV hypertrophy and pulmonary vessel remodeling, the inflammation score and the collagen fiber volume fraction. In addition, ACE2 mRNA in the lung tissues and plasma levels of Ang-(1-7) were both upregulated in animals treated with MSC microvesicles. These protective effects were diminished by the use of A-779, a selective inhibitor of the Mas receptor (Fig. 1).

Fig. 1 Effects triggered by treatment with angiotensin-converting enzyme 2 (ACE2), ACE inhibitors and angiotensin II receptor blockers in pulmonary hypertension

Table 1 Origin of the inflammatory insult in ARDS

Pulmonary ARDS	Extrapulmonary ARDS
Pneumonia (bacterial, viral, fungal)	Sepsis syndrome
Aspiration of gastric contents	Non-thoracic trauma
Lung contusion	Transfusion
Inhalation injury	Cardiopulmonary bypass
Near-drowning	Pancreatitis
Fat emboli	Drug overdose
Reperfusion injury	Burn injury
Mechanical ventilation (barotrauma, volutrauma)	

Ang-(1-7) in Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is a life-threatening form of respiratory failure, that globally accounts for 10% of intensive care unit admissions, representing more than three million patients with ARDS annually [16]. Its first description dates 50 years ago [2]. Since then, ARDS has been redefined several times to ameliorate the accuracy of clinical diagnosis [4, 66, 73]. The last one was the Berlin definition [73] that proposed three categories of ARDS based on the severity of hypoxemia, timing of acute onset, origin of edema, and the chest radiograph or computed tomographic (CT) findings.

ARDS results from a wide spectrum of different risk factors, which can be either local or systemic (Table 1). According to the origin of the inflammatory insult, ARDS can be classified in pulmonary ARDS (ARDS_p), as local or direct lung insult and extrapulmonary ARDS (ARDS_{exp}), as systemic or indirect lung

injury [21]. There are important clinical differences between ARDS_p and ARDS_{exp} in pathology, radiography, respiratory mechanics, response to treatment, and outcomes [21, 80].

ARDS remains a serious clinical problem with the main treatment being supportive in the form of mechanical ventilation. However, if the mechanical ventilation is used improperly, it can exacerbate the tissue damage caused by ARDS, known as ventilator-induced lung injury (VILI). To date, the only intervention demonstrated to improve clinical outcomes in ARDS is the use of a protective ventilatory strategy that uses low tidal volumes (VT) of 6 mL/kg predicted body weight compared with traditionally applied VT of 12 mL/kg [5].

Different animal models of experimental lung injury have been used to investigate mechanisms of lung injury [1]. In 2011, a committee assembled by the American Thoracic Society (ATS) published a workshop report determining the main features that characterize ARDS in animals and then identifying the most relevant measurements to assess these features. Important traits include (1) histological evidence of tissue injury, (2) alteration of the alveolar capillary barrier, (3) the presence of an inflammatory response, and (4) evidence of physiological dysfunction [59].

A body of evidence demonstrates that the RAS is involved in the pathogenesis of ARDS. In addition to its cardiovascular functions, Ang II is involved in inflammatory and fibrogenic processes in the lung [18, 25, 55]. Association between ACE polymorphism and susceptibility, progression, and outcome in ARDS has been demonstrated [36, 57]. Moreover, several studies have shown that inhibition of ARDS by AT1 receptor blockade or inhibition of Ang II formation by ACE has a protective effect on ARDS [34, 72, 81].

Protective effect of losartan has been tested on different models of ARDS. Losartan delayed the onset of ARDS in Wistar rats challenged by *i.t.* instillation of Bordetella bronchiseptica, prevented progressive deterioration of gas exchange and delayed the mortality of infected rats [72]. The signs of inflammation, thickened alveolar septae, and a marked increase in cellularity dominated by polymorphonuclear leukocytes were much less evident in losartan-treated rats. Although this effect was associated with a significant inhibition of lung-neutrophil recruitment, lung bacterial clearance was not impaired but rather, it was significantly improved. Similar results were found with irbesartan. Differently, neither the ACE inhibitor captopril, nor the nonselective peptide inhibitor of Ang II receptors, saralasin, reproduced these effects. The protective effects of losartan on ARDS were attributed, at least in part, to NF- κ B and MAPK mechanisms. In a sepsis-induced ARDS using cecal ligation and puncture (CLP), Shen et al. [81] demonstrated that losartan treatment significantly led to inhibition of lung tissue NF- κ B activation, attenuated degradation of I κ B- α , and inhibited phosphorylation of p38MAPK, extracellular signal-regulated kinase 1/2, and c-Jun N-terminal kinase, critical pathways for cytokine release. Similarly, results of Raiden et al. [72] showed that losartan delays the onset of ARDS triggered by a bacterial infection, prevents blood gas deterioration and histopathologic appearance of ARDS, and significantly improved survival after sepsis.

The effects of captopril and losartan have also been tested in fat embolism (FE) and the consequent fat embolism syndrome (FES) that occurs after trauma or surgery and can lead to serious pulmonary injury, including ARDS and death [63]. There was a reduction in pulmonary inflammation, along with a significant decrease in interseptal edema and hemorrhage. Pathologic changes induced by FE in the lumen patency were also diminished with RAS inhibitors. Extending the evidence for the involvement of the RAS in this syndrome, Fletcher et al. [18] demonstrated that aliskiren, a renin inhibitor, protects rat lungs from the histopathological effects of fat embolism.

ACE inhibition or blockade of AT1 receptor favors an increase in Ang-(1-7) levels [77]. In ARDS, an ACE/ACE2 imbalance occurs in favor of increased ACE activity and correlates with lung injury. Previous studies have found that ACE2 mRNA, protein, and enzymatic activity were severely downregulated in human and experimental lung tissue injuries [34, 41]. The decrease in ACE2 expression was importantly involved in severe acute respiratory syndrome (SARS), in which the pathogen, coronavirus (SARS-CoV), triggers severe pneumonia and acute, often lethal, lung failure. Kuba et al. [41] demonstrated that ACE2 is a crucial SARS-CoV receptor *in vivo*, and both SARS-CoV infections and the Spike protein of the SARS-CoV reduced ACE2 expression, contributing to the severity of lung pathology. In addition, the injection of SARS-CoV Spike into mice worsens acute lung failure *in vivo*. This effect was associated with an increase in Ang II in the lung and it was attenuated by blocking AT1 [41].

In 2005, Imai et al. reported that lack of ACE2 expression (ACE2KO animals) precipitated ARDS, suggesting that ACE2 could present an important role in the prevention of ARDS. ARDS resulted in reduced ACE2 expression and increased Ang II production in ACE2^{+/+} animals as a result of insults. Elastance of the respiratory system, as well as pulmonary edema, was significantly higher in sepsis groups, mainly in ACE2^{-/-} mice. In addition, it was observed thickening of the alveolar wall, edema and pulmonary congestion, infiltration of inflammatory cells and hyaline membrane in sepsis-induced ACE2^{-/-} mice. After 6 hours of observation, all animals in the ACE2^{+/+} group were alive and only 2 of the 10 animals in the ACE2^{-/-} group survived. Moreover, intraperitoneal injection of recombinant human ACE2 protein (rhuACE2) in ARDS induced in ACE2^{-/-} mice prevented the increase in elastance of the respiratory system and formation of pulmonary edema. In contrast to ACE2^{-/-} mice, mice with genetic deletion of ACE (ACE^{-/-}) are protected against acid aspiration-induced ARDS and inactivation of ACE in ACE2^{-/-} animals attenuates ARDS. Likewise, pharmacological inhibition or genetic deletion of AT1a (AgTr1a^{-/-}) receptors significantly attenuated lung function and edema formation. On the other hand, inactivation of AT2 receptors aggravated acute lung injury (ALI) [34].

Recently, bone marrow-derived mesenchymal stem cells (MSCs) overexpressing ACE2 served as a vehicle for gene therapy in lipopolysaccharide (LPS)-induced ARDS mice [28]. MSCs were transduced with ACE2 gene (MSC-ACE2) by a lentiviral vector and then infused into wild-type (WT) and ACE2 knockout

(ACE2^{-/-}) mice following an LPS-induced intratracheal lung injury. MSC-ACE2 improved the lung histopathology, inflammation (decreased the neutrophil counts in the BALF, downregulated the expression of IL-1 β and IL-6, and upregulated IL-10 in the lung). Additionally, MSC-ACE2 significantly reduced lung edema, in part by improving lung endothelial permeability, and normalized lung eNOS expression. Increased activity of ACE2 decreased the Ang II and increased the Ang-(1-7) in the lung, thereby inhibiting the detrimental effects of accumulating Ang II.

Protective mechanisms of ACE2 on experimental ARDS are not fully understood. ACE2 regulates RAS signaling, reducing Ang II/AT1 receptor signaling and activating the counterregulatory Ang-(1-7)/Mas receptor pathway. Treatment with lentiviral packaged ACE2 cDNA reduced and ACE2 shRNA increased Ang II/Ang-(1-7) ratio in the bronchoalveolar lavage, LPS-induced lung injury and inflammatory response. These responses were associated with alteration in the phosphorylation of MAPK and were all abolished by A779, a Mas receptor antagonist, suggesting these effects were mediated by Ang-(1-7) [47]. These data indicate that ACE2 protects lung injury via an increase in Ang-(1-7), which in turn stimulates Mas-mediated signaling to inhibit ERK1/2 and NF- κ B activation [46, 47]. A recent study indicates that early initiation of therapy after experimental ALI induced by oleic acid and continuous drug delivery are most beneficial for optimal therapeutic efficiency of Ang-(1-7) treatment [88].

The cornerstone of ARDS management remains mechanical ventilation. However, mechanical ventilation with high tidal volumes causes lung hemorrhage and edema and activates inflammatory pathways, process referred as ventilator-induced lung injury (VILI). Jiang et al. [37] demonstrated an increase in lung Ang II levels induced by VILI. Deleterious effects were attenuated by captopril, an ACE inhibitor. These results suggested that local tissue angiotensin mediates these harmful events in VILI. Using the same VILI model of high tidal volumes, Jerng et al. [35] demonstrated that the lung injury score, bronchoalveolar lavage fluid protein concentration, pro-inflammatory cytokines, and NF- κ B activities were significantly increased in the high-volume group compared with controls. In addition, the lung Ang II and mRNA levels of angiotensinogen and AT1 and AT2 receptors were also significantly increased in the high-volume group. Pretreatment with captopril or concomitant infusion with losartan or PD123319 in the high-volume group attenuated the lung injury and inflammation. Losartan and a protease-resistant, cyclic form of Ang-(1-7), showed similar lung protective effects, but losartan caused a significant decrease in blood pressure in the LPS-exposed ventilated animals [93].

Intravenous effect of Ang-(1-7) or its non-peptide agonist, AVE0991, was evaluated in ARDS induced by intravenous injection of oleic acid [40]. Ang-(1-7) or AVE0991 infusion 30 minutes after oleic acid administration reversed lung edema, and attenuated increased myeloperoxidase activity, which reflects neutrophil invasion. In addition, administration of Ang-(1-7) or AVE0991 restored arterial pressure and kept throughout experimental protocol (4 h), which falls rapidly by approximately 40% in untreated animals. Ang-(1-7) or its analog AVE0991 also

prevented a decrease in pulmonary vascular resistance, characteristic for the acute phase of ARDS. Further, Ang-(1-7) or AVE0991 blocked the increase in TNF- α concentration in bronchoalveolar (BALF). These effects were antagonized by A779 and D-Pro⁷-Ang-(1-7) [40]. Corroborating with the results of Imai et al. [34], treatment with ibesartan, an AT1 blocker, normalized systemic blood arterial pressure, pulmonary arterial resistance, wet-to-dry lung weight ratio, BALF protein concentration, and myeloperoxidase activity in lung tissue. The beneficial effect of ibesartan was prevented by co-treatment with either A779 or d-Pro⁷-Ang-(1-7) on systemic and pulmonary hemodynamics. Thus, the protective effect of recombinant ACE2 or AT1 antagonization in ALI may be related at least in part to increased formation of Ang-(1-7) and stimulation of its specific receptor signaling pathways [40]. In this same study, the effect of Ang-(1-7) was tested in two murine ARDS models, ventilator-induced lung and acid aspiration injury. Ang-(1-7) reversed the effects in both models [40].

Ang-(1-7) has a antiremodeling role in pulmonary fibrosis that occurs after ARDS [8]. Recently, Zambelli et al. [95] evaluated the potential for Ang-(1-7) to attenuate ARDS severity and lung fibrosis in a preclinical ARDS model. These authors evaluated if Ang-(1-7) would reduce the severity of early ARDS induced by the combined ‘insults’ induced by unilateral acid aspiration model followed by high stretch mechanical ventilation. Ang-(1-7) acute infusion showed a significant improvement of arterial oxygenation and inflammatory response (in terms of polymorphonuclear recruitment into alveoli) in acute ARDS. In other protocol, two weeks of Ang-(1-7) infusion increased blood oxygen saturation and the right lung from treated rats showed a significant reduction in collagen deposition. Thus, the inhibitory effect of Ang-(1-7) on inflammatory cells recruitment seen in the acute phase may be related to the reduction of fibrosis in the later phase. The beneficial effects observed by Jiang et al. [37] and Jerng et al. [35] may be related to the formation of Ang-(1-7) from the use of captopril and/or losartan.

More recently, Khan et al. [39] reported results of a phase II trial examining the safety and efficacy of using GSK2586881, a recombinant human ACE2 (rhACE2), in 18 and 80 years old patients with ARDS, which had been mechanically ventilated for less than 72 h. The use of twice-daily doses of GSK2586881 infusion (0.4 mg/kg) for 3 days resulted in a decrease in plasma Ang II associated with an increase in Ang-(1-7) and Ang-(1-5) that remained elevated for 48 h. There was also a trend to decrease in IL-6. Although no episodes of hypotension were associated with infusion of GSK2586881, no significant improvement in oxygenation was observed in patients (ratio of partial pressure of arterial oxygen to fraction of inspired oxygen-PaO/FIO₂, oxygenation index), or Sequential Organ Failure Assessment-SOFA score between treated and placebo groups was observed, which the authors attributed to numerous factors that were not adequately controlled for in this trial [39]. However, this study reinforces the need for further evaluation of the impact of RAS modulation on pulmonary hemodynamics and markers of pulmonary injury (Fig. 2).

Fig. 2 Alterations in renin-angiotensin system components in acute respiratory distress syndrome (ARDS). (ACE angiotensin-converting enzyme, Ang angiotensin)

Ang-(1-7) in Asthma

Epidemiological studies show that asthma is currently the most common chronic disease in children, being the major cause of missed days at school and, in adults, loss of working days. In addition, asthma is associated with a significant rate of mortality [74]. The large increase in incidence of asthma is becoming a major global health problem and has encouraged studies aimed at increasing the knowledge of the pathophysiology of asthma, as well as development of new treatments to improve clinical management of the disease, mainly to meet asthma patients who do not respond well to current therapies [51].

Asthma is defined as a reversible airway obstructive disease, caused by airway mucosal edema, inflammation, increased mucus secretion, smooth muscle contraction, and airway hyperreactivity and remodeling [83]. Multiple cells and multiple mediators play a crucial pathophysiological role. The inflammatory response in allergic asthma is characterized by excess production of IgE, mast cell degranulation, and the infiltration of eosinophils and lymphocytes [22, 83]. However, the recruitment and activation of these cells depend on the expression and release of several classes of proteins, such as cytokines, particularly Th2-derived. Inflammatory mediators that increase influx of leukocytes, activity, and survival of eosinophils are positively correlated with asthma severity [14, 19]. Failure to resolve the inflammatory process causes a persistent inflammation with consequent tissue destruction and loss of pulmonary function [14].

There is experimental and clinical evidence indicating that activation of the pulmonary RAS is involved in the pathophysiology of allergic pulmonary disease,

especially through an inappropriate increase in angiotensin II (Ang II) [67, 68]. However, the Ang-(1-7)/Mas receptor axis, recognized as a counterregulatory peptide system within the RAS, exhibits anti-inflammatory effects and prevents inappropriate remodeling in different pathophysiological states, such as asthma. Here, we show the effects of treatment with Ang-(1-7) on the three main changes observed in chronic asthma: inflammation, pulmonary remodeling, and bronchial hyperresponsiveness.

Experimental studies try to clear up aspects of the pathophysiology of asthma mimicking human disease. They classically include two phases: sensitization and challenge. Sensitization is traditionally performed by intraperitoneal and subcutaneous routes, and the challenges with allergens are performed through aerosol, intranasal, or intratracheal instillation. Sensibilization increases IgE levels in the circulation, but does not induce signs of inflammation or pulmonary remodeling. IgE binds to receptors in eosinophils, mast cells, and basophils. When the challenge occurs with the same allergen, the allergen provokes an antigenic-antibody reaction that induces the degranulation of these cells. Degranulation releases inflammatory mediators that initiate and propagate the process. Ovalbumin (OVA) is a widely used allergen, because promote to an intense allergic lung inflammation. In addition, the most common species studied in the last two decades is mice, particularly BALB/c [15, 52].

In an experimental model of acute asthma (BALB/c mice), Ang-(1-7) treatment resulted in inhibition of the OVA-induced increase in total cell counts, eosinophils, lymphocytes, and neutrophils. Ang-(1-7) also significantly reduced the OVA-induced perivascular and peribronchial inflammation (Fig. 3). Moreover, Ang-(1-7) attenuated OVA-induced increase in the phosphorylation of I κ B- α and ERK 1/2, suggesting that Ang-(1-7) could mediate an anti-inflammatory pathway in allergic asthma [15]. In chronic allergic lung inflammation that administration of Ang-(1-7) or a synthetic analog, AVE 0991 (Mas receptor agonist), decreased inflammatory cell infiltrate in the peribronchial, perivascular, and alveolar regions of the lung [52, 75]. Furthermore, Ang-(1-7) treatment decreased chemokines (CCL2 and CCL5), cytokines (IL-4, IL-5 and GM-CSF), IgE, and two signaling pathways associated with asthma, the ERK1/2, and possibly the JNK pathways. Altogether, these results suggest that Ang-(1-7) treatment decreases chemokines and cytokines essential for the initiation and maintenance of the inflammatory process, as well as those important for the migration of eosinophils to the site of injury and reduction of their apoptosis. These effects were associated with the inhibition of ERK1/2 pathway [52].

It has been demonstrated that genetic Mas deficiency increased chronic allergic pulmonary inflammation. FVB/N mice with genetic deletion of the Mas receptor subjected to a model of chronic allergic lung inflammation presented a significant increase in the number of eosinophils in BALF and inflammatory cell infiltrate in the lung [53]. Furthermore, there was an increase in ERK1/2 phosphorylation and proinflammatory cytokine (IL-13) and chemokines (CCL2/MCP-1 and CCL5/RANTES) in the lungs of mice asthmatic with genetic deletion of the Mas receptor [53]. Thus, Mas receptor-induced effects are important

Fig. 3 Representative histological images of lung sections stained with H&E from OVA-sensitized and challenged mice and treated with Ang-(1-7). The OVA produced a pronounced increase in the density of inflammatory cell infiltrate around the airways and blood vessels and alveolar parenchyma (a). Treatment with Ang-(1-7) attenuated the inflammatory infiltrate in the peribronchial, perivascular and alveolar regions of the lung (b). In addition, OVA mice exhibited significantly greater thickening and inflammation of the alveolar wall and bronchial wall thickness. Ang-(1-7) presented reduced inflammation in the interalveolar space with normal appearance of the alveolar lumen [52]

counterbalancing mechanisms of the RAS for attenuating the inflammatory process in asthma. Moreover, impairment of the Ang-(1-7)/Mas receptor pathway may lead to the deterioration of the pathophysiology of asthma.

Defective apoptosis of eosinophils, the main leukocyte in the pathogenesis of asthma, and delay in its removal lead to lung damage and loss of pulmonary function due to failure in the resolution of inflammation [14, 19]. Recently, we demonstrated a novel action of Ang-(1-7), resolution of allergic lung inflammation [54]. Balb/c mice were sensitized and challenged with OVA and treated with Ang-(1-7) at the peak of the inflammatory process. Treatment with Ang-(1-7) reduced the accumulation of eosinophils in the lung by inducing apoptosis. In addition, Ang-(1-7) treatment reduced the phosphorylation of intracellular signaling pathway, associated with cytokine production and leukocyte survival, the NF- κ B. Increase in apoptosis of leukocytes and their clearance by macrophages are essential events to promote resolution of inflammation [70]. Ang-(1-7) treatment increased the clearance of the apoptotic cells by macrophages [54]. This result added important criteria to establish Ang-(1-7) as an endogenous pro-resolutive mediator.

Unregulated or prolonged inflammatory responses in the lungs can lead to tissue damage, pulmonary remodeling, and consequently compromised lung function [33]. There is evidence that lung inflammation and remodelling in both asthmatic patients and in experimental models of asthma are not restricted to the airway and extend into the parenchyma and pulmonary vessels [33]. In addition to leukocytes migrating to the lung, structural cells, airway epithelium and smooth muscle cells secreting a variety of inflammatory mediators and extracellular matrix proteins, can participate in immunomodulation and airway remodelling in asthma [91]. In a model of chronically OVA-sensitized and challenged mice, there was an increase in

the deposition of collagen fibres in the airway wall, an increase in the expression of collagen I and III in the lung, along with thickening of the alveolar wall and smooth muscle of the arterioles. In addition, the OVA-mice showed right ventricular hypertrophy, probably due to a functional and structural adaptation in response to chronic pulmonary artery pressure overload [52]

Lung sections from mice that were challenged intranasally with OVA (four consecutive days, with 20 μ g OVA) showed severe perivascular and peribronchial fibrosis and marked goblet cell hyper/metaplasia suggesting airway remodeling. In contrast, lung sections from OVA-challenged mice treated with Ang-(1-7) decreased in the perivascular and peribronchial fibrosis and goblet cell hyper/metaplasia [15].

In other studies, mice were sensitized and challenged with OVA three times per week (for four weeks). OVA mice exhibited significantly greater thickening and inflammation of the alveolar wall. The epithelial thickness and collagen deposition in airways and lung parenchyma were increased. In addition, OVA induced an increase in the mRNA expression of collagen I and collagen III. However, OVA-sensitized and challenged animals treated with Ang-(1-7) or AVE0991 presented reduced inflammation in the interalveolar space with normal appearance of alveolar lumen and reduce epithelial thickness [52, 75]. Furthermore, OVA-sensitized and challenged mice treated with Ang-(1-7) presented a marked reduction in collagen deposition in airway walls, lung parenchyma, and mRNA expression of collagen I and III (Fig. 4. [52]).

The model of chronic asthma in mice with lack of the Mas receptor induces an intense degree of lung inflammation and remodeling in a mice strain (FVB/N) less sensitive to an experimental model of asthma. Indeed, FVB/N-WT (wild-type) mice presented an attenuated response to OVA challenge compared with the response observed in Balb/C mice subjected to the same protocol. However, deletion of Mas receptor induces worsening of the development of chronic allergic lung inflammation in mice. These data show that impairment of the Ang-(1-7)/Mas receptor pathway may lead to the deterioration of the pathophysiology of asthma [53].

A recent study, showed that treatment with Ang-(1-7) at the peak of the inflammatory process induced resolution of eosinophilic inflammation in an experimental model of asthma. Balb/c mice were sensitized and challenged with ovalbumin and treated with Ang-(1-7), 24 h after the last OVA challenge. The inclusion of Ang-(1-7) into an oligosaccharide HP β CD cavity protects the peptide during its passage through the gastrointestinal tract. Resolution of inflammation is an active process that allows cessation of inflammation and re-establishment of tissue homeostasis. Therefore, oral treatment with Ang-(1-7) promoted prevention of excessive trafficking of eosinophil to the lung, shutdown intracellular signaling molecules associated with cytokine production and eosinophil survival, apoptosis of recruited eosinophil, and promotion of clearance of apoptotic leukocytes, i.e., efferocytosis. These effects induced the return of pulmonary homeostasis through a decrease in extracellular matrix accumulation and a great reduction in collagen I and III genes expression in the lung [54].

Fig. 4 Representative histological images of lung sections stained with Gomori's trichrome from OVA-sensitized and challenged mice (**a**) and treated with Ang-(1-7) (**b**). OVA-challenged mice presented marked peribronchial and perivascular fibrosis (**a**-asterisks), which was prevented by Ang-(1-7) treatment (**b**). (**c** and **d**) Representative histological images of lung sections stained with periodic acid schiff (PAS) from OVA-sensitized and challenged mice and treated with Ang-(1-7). The OVA-challenged mice presented increased mucus deposition in airways (**c**-arrows). In addition, the treatment with Ang-(1-7) decreased mucus deposition in airways in mice with allergic pulmonary inflammation (**d**) [52]

These data will accelerate the research efforts for the development of new Ang-(1-7)-based pharmacological strategies to control, prevent, and treat chronic inflammation-related diseases, such as asthma. Thus, the observation that Ang-(1-7) is effective through oral route can provide clinical benefits for treatment of allergic asthma, as it can be better tolerated than nebulization or than standard drugs, and it can act sistemically reducing overall inflammation and optimizing health of patients

Ang-(1-7) in Pulmonary Fibrosis

Pulmonary fibrosis (PF) is a fatal lung disease of unknown cause. The disease is characterized by progressive scarring of the lung tissue accompanied by fibroblast proliferation, the sudden onset of lung parenchyma, with thickening of the alveolar septa, hyperplasia of type II pneumocytes (PII), and myofibroblasts, causing narrowing of airways, all leading to a loss of lung function and decreased quality of life

[79]. The estimated prevalence of PF is around 30 cases per 100,000 people, reaching more than 100 individuals per 100,000 people aged 75 years or more [71]. Treatment for PF with anti-inflammatory, immunosuppressive, and antifibrotic agents has not shown promising results to abate the progression of the disease or to improve the quality of life [71]. Therefore, it becomes essential to better understand the disease pathophysiology and to identify novel therapeutic targets/agents for the treatment of PF.

Bleomycin (BLM), used and described method to cause PF in rodents, is a chemotherapeutic used in the treatment of several neoplasias. Challenged with BLM in intratracheal administration causes some lung lesions such as parenchyma inflammation, lesion of the alveolar epithelial cells with reactive hyperplasia, activation and fibroblast to myofibroblast differentiation and pulmonary fibrosis [64]. In addition, the presence of PH secondary to fibrotic lung diseases, called cor pulmonale, indicates poor prognosis with a compromised cardiac function.

Studies demonstrate that Ang II/AT1 receptor is required for the pathogenesis of experimental lung fibrosis. Ang II has a number of profibrotic effects on lung parenchymal, such as induction of growth factors for mesenchymal cells, extracellular matrix deposition, production of cytokines, and increased motility of lung fibroblasts [55, 58]. Recent evidence shows that the counterregulatory molecule Ang-(1-7), the product of the ACE2 acts as an antifibrotic pulmonary survival factor [87].

Shenoy et al. [82] showed that endotracheal instillation of bleomycin evoked a severe fibrotic response, characterized by the accumulation of interstitial lung collagen. In addition, increased lung mRNA levels of an important cytokine that plays a key role in fibrogenesis, the transforming growth factor- β (TGF- β), were also observed. Collagen deposition and TGF- β were significantly decreased by overexpression of ACE2 or Ang-(1-7). Furthermore, this study did detect pulmonary hypertension (PH) and right ventricular hypertrophy (RVH) after bleomycin administration. However, treatment with Ang-(1-7) prevented the development of both PH and RVH. The treatment with Ang-(1-7) or overexpression of ACE2 presented similar beneficial effects, possibly mediated via generation of Ang-(1-7). It is conceivable that the protective effects of ACE2 and Ang-(1-7) on the heart may be secondary to the reduction in the lung fibrosis.

Meng et al. [60] investigated whether the upregulation of the ACE2/Ang-(1-7)/Mas axis protects against BLM-induced pulmonary fibrosis by inhibiting the mitogen-activated protein kinase (MAPK)/NF- κ B pathway. In this experimental protocol, male Wistar rats were submitted the PF by BLM and/or AngII. The results showed that Ang-(1-7) regulates the balance of the RAS from the ACE/AngII/AT1R axis toward the ACE2/Ang-(1-7)/Mas axis. The BLM-treated animals presented characteristic histological changes in lung tissue, including areas of inflammatory infiltration, thickening of the alveolar walls, increased interstitial collagen deposition, and a fibroblastic appearance. Chronic infusion with Ang-(1-7) resulted in a protective effect against lung fibrosis. Furthermore, treatment with Ang-(1-7) and lenti-ACE2 protect against BLM- or AngII-induced inflammation and extracellular matrix (ECM) accumulation by inhibiting the MAPK/NF- κ B and NF- κ B signaling pathways. These results suggest that treatment with Ang-(1-7) decreased activation

of MAPKs pathways (ERK1/2, p38, JNK) and NF- κ B, which are crucial for lung fibrogenesis [60].

Study in vitro shows that human fetal lung-I cells were pretreated with compounds that block the activities of AT1 receptor, Mas (A-779), and MAPKs before exposure to Ang II or Ang-(1-7). The human fetal lung-I cells were infected with lentivirus-mediated ACE2 before exposure to Ang II. Ang-(1-7) and lentivirus-mediated ACE2 inhibited the Ang II-induced MAPK/NF- κ B pathway, thereby attenuating inflammation and α -collagen I production, which could be reversed by A-779, Mas receptor antagonist. Ang-(1-7) inhibited Ang II-induced lung fibroblast apoptotic resistance via inhibition of the MAPK/NF- κ B pathway and activation of the mitochondrial apoptotic pathway [60].

It is well known that in addition to MAPK and NF- κ B activation, the reactive oxygen species (ROS) generated by NADPH oxidase-4 (NOX4) initiates lung fibrosis. ROS generation plays a relevant role in lung fibrosis, and recent studies suggest that NADPH oxidases (NOXs) are key sources of ROS in the fibrotic lung [6]. The NOX4 in mediating fibroblast functions during the lung fibrosis process has been stressed. In addition, mice with genetic deletion of NOX4 are protected against BLM-induced pulmonary fibrosis [29]. Meng et al. [61] showed that NOX4-dependent ROS caused by the activation of the ACE/Ang II/AT1 receptor axis contributes to the development of AngII- or BLM-induced lung fibrosis by fibroblast migration and α -collagen I synthesis. Ang(1-7) and lentiACE2 treatment protect against BLM-induced pulmonary fibrosis by shifting the balance of the RAS toward the ACE2/Ang(1-7)/Mas axis and by inhibiting the generation of ROS. In addition, Ang-(1-7) and lentiACE2 protected against BLM- or Ang II-induced lung fibroblast migration and ECM accumulation by inhibiting the NOX4-derived. These results suggest that the ACE2/Ang(1-7)/Mas axis could be a novel pharmacological antioxidant target for lung fibrosis induced by Ang II-mediated ROS [61].

References

1. Aeffner F, Bolon B, Davis IC. Mouse models of acute respiratory distress syndrome: a review of analytical approaches, pathologic features, and common measurements. *Toxicol Pathol.* 2015;43(8):1074–92.
2. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet.* 1967;2:319–23.
3. Balakumar P, Jagadeesh G. A century old renin-angiotensin system still grows with endless possibilities: AT1 receptor signaling cascades in cardiovascular physiopathology. *Cell Signal.* 2014;26:2147–60.
4. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American-European consensus conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;149:818–24.
5. Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. The acute respiratory distress syndrome network: ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med.* 2000;342:1301–8.

6. Bocchino M, Agnese S, Fagone E, Svegliati S, Grieco D, Vancheri C, Gabrielli A, Sanduzzi A, Avvedimento EV. Reactive oxygen species are required for maintenance and differentiation of primary lung fibroblasts in idiopathic pulmonary fibrosis. *PLoS One*. 2010;5:e14003.
7. Chen L, Xiao J, Li Y, Ma H. Ang-(1-7) might prevent the development of monocrotaline induced pulmonary arterial hypertension in rats. *Eur Rev Med Pharmacol Sci*. 2011;15(1):1–7.
8. Chen Q, Yang Y, Huang Y, Pan C, Liu L, Qiu H. Angiotensin-(1-7) attenuates lung fibrosis by way of Mas receptor in acute lung injury. *J Surg Res*. 2013;185:740–7.
9. Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. *Med Sci Monit*. 2005;11(6):RA194–205.
10. Dai HL, Dai H, Gong Y, Xiao Z, Guang X, Yin X. Decreased levels of serum angiotensin-(1-7) in patients with pulmonary arterial hypertension due to congenital heart disease. *Int J Cardiol*. 2014;176:1399–401.
11. Dai HL, Guo Y, Guang XF, Xiao ZC, Zhang M, Yin XL. The changes of serum angiotensin-converting enzyme 2 in patients with pulmonary arterial hypertension due to congenital heart disease. *Cardiology*. 2013;124:208–12.
12. de Man FS, Tu L, Handoko ML, Rain S, Ruiter G, François C, Schali J, Dorfmueller P, Simonneau G, Fadel E, Perros F, Boonstra A, Postmus PE, van der Velden J, Vonk-Noordegraaf A, Humbert M, Eddahibi S, Guignabert C. Dysregulated renin-angiotensin-aldosterone system contributes to pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2012;186(8):780–9.
13. Duncan JC. Clinical relevance of local renin angiotensin systems. *Front Endocrinol (Lausanne)*. 2014;5:113.
14. Duncan CJA, Lawrie A, Blaylock MG, Douglas JG, Walsh GM. Reduced eosinophil apoptosis in induced sputum correlates with asthma severity. *Eur Respir J*. 2003;22:484–90.
15. El-Hasmin, et al. Angiotensin-(1-7) inhibits allergic inflammation, via MAS1 receptor, through suppression of ERK1/2 and NF-Kb-dependent pathways. *Br J Pharmacol*. 2012;166(6):1964–76.
16. Fan E, Brodie D, Slutsky AS. Acute respiratory distress syndrome: advances in diagnosis and treatment. *JAMA*. 2018;319(7):698–710.
17. Ferreira AJ, Shenoy V, Yamazato Y, Sriramula S, Francis J, Yuan L, Castellano RK, Ostrov DA, Oh SP, Katovich MJ, Raizada MK. Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. *Am J Respir Crit Care Med*. 2009;179(11):1048–54.
18. Fletcher AN, Molteni A, Ponnareddy R, Patel C, Pluym M, Poisner AM. The renin inhibitor aliskiren protects rat lungs from the histopathologic effects of fat embolism. *J Trauma Acute Care Surg*. 2017;82(2):338–44.
19. Fulkerson PC, Rothember ME. Targeting eosinophils in allergy, inflammation and beyond. *Nat Rev Drug Discov*. 2013;12:117–29.
20. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M, ESC Scientific Document Group. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension: the joint task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2016;37(1):67–119.
21. Gattinoni L, Pelosi P, Suter PM, Pedoto A, Vercesi P, Lissoni A. Acute respiratory distress syndrome caused by pulmonary and extrapulmonary disease. Different syndromes? *Am J Respir Crit Care Med*. 1998;158(1):3–11.
22. Georas SN, Guo J, De Fanis U, Casolaro V. T-helper cell type-2 regulation in allergic disease. *Eur Respir J*. 2005;26:1119–37.
23. Ghataorhe P, Rhodes CJ, Harbaum L, Attard M, Wharton J, Wilkins MR. Pulmonary arterial hypertension progress in understanding the disease and prioritizing strategies for drug development. *J Intern Med*. 2017;282(2):129–41.

24. Haga S, Tsuchiya H, Hirai T, Hamano T, Mimori A, Ishizaka Y. A novel ACE2 activator reduces monocrotaline-induced pulmonary hypertension by suppressing the JAK/STAT and TGF- β cascades with restored caveolin-1 expression. *Exp Lung Res*. 2015;41(1):21–31.
25. Hagiwara S, Iwasaka H, Matumoto S, Hidaka S, Noguchi T. Effects of an angiotensin-converting enzyme inhibitor on the inflammatory response in in vivo and in vitro models. *Crit Care Med*. 2009;37:626–33.
26. Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD, Timens W, Turner AJ, Navis G, van Goor H. The emerging role of ACE2 in physiology and disease. *J Pathol*. 2007;212(1):1–11.
27. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*. 2004;203(2):631–7.
28. He H, Liu L, Chen Q, Liu A, Cai S, Yang Y, Lu X, Qiu H. Mesenchymal stem cells overexpressing angiotensin-converting enzyme 2 rescue lipopolysaccharide-induced lung injury. *Cell Transplant*. 2015;24(9):1699–715.
29. Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, Pennathur S, Martinez FJ, Thannickal VJ. NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. *Nat Med*. 2009;15(9):1077–81.
30. Hemnes AR, Rathinasabapathy A, Austin EA, Brittain EL, Carrier EJ, Chen X, Fessel JP, Fike CD, Fong P, Fortune N, Gerszten RE, Johnson JA, Kaplowitz M, Newman JH, Piana R, Pugh ME, Rice TW, Robbins IM, Wheeler L, Yu C, Loyd JE, West J. A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur Respir J*. 2018;51(6).
31. Hernández Prada JA, Ferreira AJ, Katovich MJ, Shenoy V, Qi Y, Santos RA, Castellano RK, Lampkins AJ, Gubala V, Ostrov DA, Raizada MK. Structure-based identification of small-molecule angiotensin-converting enzyme 2 activators as novel antihypertensive agents. *Hypertension*. 2008;51(5):1312–7.
32. Hoeper MM, Bogaard HJ, Condliffe R, Frantz R, Khanna D, Kurzyna M, Langleben D, Manes A, Satoh T, Torres F, Wilkins MR, Badesch DB. Definitions and diagnosis of pulmonary hypertension. *J Am Coll Cardiol*. 2013;62(25 Suppl):D42–50.
33. Holgate ST. Asthma: a simple concept but in reality a complex disease. *Eur J Clin Investig*. 2011;41:1339–52.
34. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005;436(7047):112–6.
35. Jerng JS, Hsu YC, Wu HD, Pan HZ, Wang HC, Shun CT, Yu CJ, Yang PC. Role of the renin-angiotensin system in ventilator-induced lung injury: an in vivo study in a rat model. *Thorax*. 2007;62(6):527–35.
36. Jerng JS, Yu CJ, Wang HC, Chen KY, Cheng SL, Yang PC. Polymorphism of the angiotensin converting enzyme gene affects the outcome of acute respiratory distress syndrome. *Crit Care Med*. 2006;34:1001–6.
37. Jiang JS, Wang LF, Chou HC, Chen CM. Angiotensin-converting enzyme inhibitor captopril attenuates ventilator-induced lung injury in rats. *J Appl Physiol*. 2007;102(6):2098–103.
38. Kaparianos A, Argyropoulou E. Local renin-angiotensin II systems, angiotensin-converting enzyme and its homologue ACE2: their potential role in the pathogenesis of chronic obstructive pulmonary diseases, pulmonary hypertension and acute respiratory distress syndrome. *Curr Med Chem*. 2011;18(23):3506–15.
39. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, Hall R, Poirier G, Ronco JJ, Tidswell M, HARDS K, Powley WM, Wright TJ, Siederer SK, Fairman DA, Lipson DA, Bayliffe AI, Lazaar AL. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. 2017;21(1):234.
40. Klein N, Gembardt F, Supé S, Kaestle SM, Nickles H, Erfinanda L, Lei X, Yin J, Wang L, Mertens M, Szaszi K, Walther T, Kuebler WM. Angiotensin-(1-7) protects from experimental acute lung injury. *Crit Care Med*. 2013;41(11):e334–43.

41. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. 2005;11:875–9.
42. Latus H, Delhaas T, Schranz D, Apitz C. Treatment of pulmonary arterial hypertension in children. *Nat Rev Cardiol*. 2015;12(4):244–54.
43. Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, Sdrimas K, Fernandez-Gonzalez A, Kourembanas S. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation*. 2012;126(22):2601–011.
44. Li G, Liu Y, Zhu Y, Liu A, Xu Y, Li X, Li Z, Su J, Sun L. ACE2 activation confers endothelial protection and attenuates neointimal lesions in prevention of severe pulmonary arterial hypertension in rats. *Lung*. 2013;191(4):327–36.
45. Li G, Xu YL, Ling F, Liu AJ, Wang D, Wang Q, Liu YL. Angiotensin-converting enzyme 2 activation protects against pulmonary arterial hypertension through improving early endothelial function and mediating cytokines levels. *Chin Med J*. 2012;125(8):1381–8.
46. Li Y, Cao Y, Zeng Z, Liang M, Xue Y, Xi C, Zhou M, Jiang W. Angiotensin-converting enzyme 2/ angiotensin-(1-7)/ Mas axis prevents lipopolysaccharide-induced apoptosis of pulmonary microvascular endothelial cells by inhibiting JNK/NF- κ B pathways. *Sci Rep*. 2015;5:8209.
47. Li Y, Zeng Z, Cao Y, Liu Y, Ping F, Liang M, Xue Y, Xi C, Zhou M, Jiang W. Angiotensin-converting enzyme 2 prevents lipopolysaccharide-induced rat acute lung injury via suppressing the ERK1/2 and NF- κ B signaling pathways. *Sci Rep*. 2016;6:27911.
48. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003;426:450–4.
49. Ling Y, Johnson MK, Kiely DG, et al. Changing demographics, epidemiology, and survival of incident pulmonary arterial hypertension: results from the pulmonary hypertension registry of the United Kingdom and Ireland. *Am J Respir Crit Care Med*. 2012;186:790–6.
50. Liu Z, Liu J, Xiao M, Wang J, Yao F, Zeng W, Yu L, Guan Y, Wei W, Peng Z, Zhu K, Wang J, Yang Z, Zhong J, Chen J. Mesenchymal stem cell-derived microvesicles alleviate pulmonary arterial hypertension by regulating renin-angiotensin system. *J Am Soc Hypertens*. 2018;12(6):470–8.
51. Loftus PA, Wise SK. Epidemiology and economic burden of asthma. *Int Forum Allergy Rhinol*. 2015;5(Suppl 1):S7–10.
52. Magalhães GS, Rodrigues-Machado MG, Motta-Santos D, Silva AR, Caliari MV, Prata LO, Abreu SC, Rocco PR, Barcelos LS, Santos RA, Campagnole-Santos MJ. Angiotensin-(1-7) attenuates airway remodelling and hyperresponsiveness in a model of chronic allergic lung inflammation. *Br J Pharmacol*. 2015;172(9):2330–23342.
53. Magalhaes GS, Rodrigues-Machado MG, Motta-Santos D, Alenina N, Bader M, Santos RA, Campagnole-Santos MJ. Chronic allergic pulmonary inflammation is aggravated in angiotensin-(1-7) Mas receptor knockout mice. *Am J Physiol Lung Cell Mol Physiol*. 2016;311(6):L1141–8.
54. Magalhaes GS, Barroso LC, Reis AC, Rodrigues-Machado MG, Gregório JF, Motta-Santos D, Oliveira AC, Perez DA, Barcelos LS, Teixeira MM, Santos RAS, Pinho V, Campagnole-Santos MJ. Angiotensin-(1-7) promotes resolution of eosinophilic inflammation in an experimental model of asthma. *Front Immunol*. 2018;9:58.
55. Marshall RP, Gohlke P, Chambers RC, Howell DC, Bottoms SE, Unger T, McAnulty RJ, Laurent GJ. Angiotensin II and the fibroproliferative response to acute lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2004;286:L156–64.
56. Marshall RP. The pulmonary renin-angiotensin system. *Curr Pharm Des*. 2003;9(9):715–22.
57. Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, RJ MA, Humphries SE, Hill MR, Laurent GJ. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2002;166(5):646–50.

58. Marshall RP, McAnulty RJ, Laurent GJ. Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor. *Am J Respir Crit Care Med*. 2000;161:1999–2004.
59. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM. An official American thoracic society workshop report: features and measurements of experimental acute lung injury in animals. *Am J Respir Cell Mol Biol*. 2011;44:725–38.
60. Meng Y, Yu C-H, Li W, Li T, Luo W, Huang S, Wu P-S, Cai S-X, Li X. Angiotensin-Converting Enzyme 2/Angiotensin-(1-7)/Mas Axis Protects against Lung Fibrosis by Inhibiting the MAPK/NF- κ B Pathway. *Am J Respir Cell Mol Biol*. 2014;50(4):723–36.
61. Meng YTL, Zhou G-s, Chen Y, Yu C-H, Pang M-X, Li W, Li Y, Zhang W-Y, Li X. The Angiotensin-Converting Enzyme 2/Angiotensin (1-7)/Mas Axis Protects Against Lung Fibroblast Migration and Lung Fibrosis by Inhibiting the NOX4-Derived ROS-Mediated RhoA/Rho Kinase Pathway. *Antioxid Redox Signal*. 2015;20(22(3)):241–58.
62. McGoon MD, Benza RL, Escribano-Subias P, et al. Pulmonary arterial hypertension: epidemiology and registries. *J Am Coll Cardiol*. 2013;62:D51–9.
63. McIlff TE, Poisner AM, Herndon B, Lankachandra K, Molteni A, Adler F. Mitigating effects of captopril and losartan on lung histopathology in a rat model of fat embolism. *J Trauma*. 2011;70(5):1186–91.
64. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? *Int J Biochem Cell Biol*. 2008;40:362–82.
65. Morrell NW, Morris KG, Stenmark KR. Role of angiotensin-converting enzyme and angiotensin II in development of hypoxic pulmonary hypertension. *Am J Phys*. 1995;269(4 Pt 2):H1186–94.
66. Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis*. 1988;138:720–3.
67. Myou S, Fujimura M, Kamio Y, Ishiura Y, Kurashima K, Tachibana H, et al. Effect of losartan, a type 1 angiotensin II receptor antagonist, on bronchial hyperresponsiveness to methacholine in patients with bronchial asthma. *Am J Respir Crit Care Med*. 2000;162:40–4.
68. Myou S, Fujimura M, Kamio Y, Kita T, Watanabe K, Ishiura Y, et al. Effect of candesartan, a type 1 angiotensin II receptor antagonist, on bronchial hyper-responsiveness to methacholine in patients with bronchial asthma. *Br J Clin Pharmacol*. 2002;54:622–6.
69. Orte C, Polak JM, Haworth SG, Yacoub MH, Morrell NW. Expression of pulmonary vascular angiotensin-converting enzyme in primary and secondary plexiform pulmonary hypertension. *J Pathol*. 2000;192(3):379–84.
70. Perez DA, Vago JP, Athayde RM, Reis AC, Teixeira MM, Sousa LP, et al. Switching off key signaling survival molecules to switch on the resolution of inflammation. *Mediat Inflamm*. 2014;2014:829851.
71. Prata LO, et al. ACE2 activator associated with physical exercise potentiates the reduction of pulmonary fibrosis. *Exp Biol med*. 2017;242(1):8–21.
72. Raiden S, Nahmod K, Nahmod V, Semeniuk G, Pereira Y, Alvarez C, Giordano M, Geffner JR. Nonpeptide antagonists of AT1 receptor for angiotensin II delay the onset of acute respiratory distress syndrome. *J Pharmacol Exp Ther*. 2002;303:45–51.
73. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. 2012;307:2526–33.
74. Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci*. 2012;8:1281–90.
75. Rodrigues-Machado MG, et al. AVE 0991, a non-peptide mimic of angiotensin-(1-) effects, attenuates pulmonary remodeling in a model of chronic asthma. *Br J Pharmacol*. 2013;170(4):835–46.
76. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos

- MJ, Schultheiss HP, Speth R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A*. 2003;100(14):8258–63.
77. Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, Campagnole-Santos MJ. The ACE2/Angiotensin-(1-7)/MAS axis of renin-angiotensin system: focus on angiotensin-(1-7). *Physiol Rev*. 2018;98(1):505–3.
78. Schannwell CM, Steiner S, Strauer BE. Diagnostics in pulmonary hypertension. *J Physiol Pharmacol*. 2007;58 Suppl 5(Pt 2):591–602.
79. Selman M, King TE, Pardo A, American Thoracic Society; European Respiratory Society; America College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypothesis about its pathogenesis and implications for therapy. *Ann Intern Med*. 2001;134(2):136–51.
80. Shaver CM, Bastarache JA. Clinical and biological heterogeneity in acute respiratory distress syndrome: direct versus indirect lung injury. *Clin Chest Med*. 2014;35(4):639–53.
81. Shen L, Mo H, Cai L, Kong T, Zheng W, Ye J, Qi J, Xiao Z. Losartan prevents sepsis-induced acute lung injury and decreases activation of nuclear factor kappa B and mitogen-activated protein kinases. *Shock*. 2009;31(5):500–6.
82. Shenoy V, Ferreira AJ, Qi Y, Fraga-Silva RA, Díez-Freire C, Dooies A, Jun JY, Sriramula S, Mariappan N, Pourang D, Venugopal CS, Francis J, Reudelhuber T, Santos RA, Patel JM, Raizada MK, Katovich MJ. The angiotensin-converting enzyme 2/angiogenesis-(1-7)/Mas axis confers cardiopulmonary protection against lung fibrosis and pulmonary hypertension. *Am J Respir Crit Care Med*. 2010;182(8):1065–72.
83. Shelhamer JH, Levine SJ, Wu T, Jacoby DB, Kaliner MA, Rennard SL. Airway inflammation. *Ann Intern Med*. 1995;123:288–304.
84. Shenoy V, Gjymishka A, Jarajapu YP, Qi Y, Afzal A, Rigatto K, Ferreira AJ, Fraga-Silva RA, Kearns P, Douglas JY, Agarwal D, Mubarak KK, Bradford C, Kennedy WR, Jun JY, Rathinasabapathy A, Bruce E, Gupta D, Cardounel AJ, Mocco J, Patel JM, Francis J, Grant MB, Katovich MJ, Raizada MK. Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models. *Am J Respir Crit Care Med*. 2013;187(6):648–57.
85. Shenoy V, Kwon KC, Rathinasabapathy A, Lin S, Jin G, Song C, Shil P, Nair A, Qi Y, Li Q, Francis J, Katovich MJ, Daniell H, Raizada MK. Oral delivery of Angiotensin-converting enzyme 2 and Angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension. *Hypertension*. 2014;64(6):1248–59. Erratum in: *Hypertension*. 2015;65(3):e8.
86. Shenoy V, Qi Y, Katovich MJ, Raizada MK. ACE2 a promising therapeutic target for pulmonary hypertension. *Curr Opin Pharmacol*. 2011;11(2):150–5.
87. Simoes e Silva AC, Silveira KD, Ferreira AJ, Teixeira MM. ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis. *Br J Pharmacol*. 2013;169:477–92.
88. Supé S, Kohse F, Gemhardt F, Kuebler WM, Walther T, et al. Therapeutic time window for angiotensin-(1-7) in acute lung injury. *Br J Pharmacol*. 2016;173(10):1618–28.
89. Taichman DB, Ornelas J, Chung L, Klinger JR, Lewis S, Mandel J, Palevsky HI, Rich S, Sood N, Rosenzweig EB, Trow TK, Yung R, Elliott CG, Badesch DB. Pharmacologic therapy for pulmonary arterial hypertension in adults: CHEST guideline and expert panel report. *Chest*. 2014;146(2):449–75.
90. Tan WSD, Liao W, Zhou S, Mei D, Wong WF. Targeting the renin-angiotensin system as novel therapeutic strategy for pulmonary diseases. *Curr Opin Pharmacol*. 2018;40:9–17.
91. Van Wetering S, Zuyderduyn S, Ninaber DK, van Sterkenburg MA, Rabe KF, Hiemstra PS. Epithelial differentiation is a determinant in the production of eotaxin-2 and -3 by bronchial epithelial cells in response to IL-4 and IL-13. *Mol Immunol*. 2007;44:803–11.
92. Wiener RS, Cao YX, Hinds A, Ramirez MI, Williams MC. Angiotensin converting enzyme 2 is primarily epithelial and is developmentally regulated in the mouse lung. *J Cell Biochem*. 2007;101(5):1278–91.
93. Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, van Goor H, Kamilic J, Florquin S, Bos AP. Acute respiratory distress syndrome leads to

- reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. *J Pathol.* 2011;225:618–27.
94. Yamazato Y, Ferreira AJ, Hong KH, Sriramula S, Francis J, Yamazato M, Yuan L, Bradford CN, Shenoy V, Oh SP, Katovich MJ, Raizada MK. Prevention of pulmonary hypertension by angiotensin-converting enzyme 2 gene transfer. *Hypertension.* 2009;54(2):365–71.
95. Zambelli V, Bellani G, Borsa R, Pozzi F, Grassi A, Scanziani M, Castiglioni V, Masson S, Decio A, Laffey JG, Latini R, Pesenti A. Angiotensin-(1-7) improves oxygenation, while reducing cellular infiltrate and fibrosis in experimental acute respiratory distress syndrome. *Inten Care Med Exp.* 2015;3(1):44.
96. Zhang J, Dong J, Martin M, He M, Gongol B, Marin TL, Chen L, Shi X, Yin Y, Shang F, Wu Y, Huang HY, Zhang J, Zhang Y, Kang J, Moya EA, Huang HD, Powell FL, Chen Z, Thistlethwaite PA, Yuan ZY, Shyy JY. AMPK phosphorylation of ACE2 in endothelium mitigates pulmonary hypertension. *Am J Respir Crit Care Med.* 2018;198(4):509–20.