Role of the Renin-Angiotensin System in ARDS

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Introduction

The renin-angiotensin system (RAS) is a powerful biological system that plays an important role in regulation of systemic blood pressure through the maintenance of fluid and salt homeostasis. It is a multifactorial system since it includes different components (**Fig. 1**): The first, renin, was discovered in 1898 [1], whereas the discovery of the last component, angiotensin-converting enzyme 2 (ACE 2), is relatively recent, from 2000 [2, 3]. Three kinds of RAS are known: A) circulating, B) local, and C) intracellular.

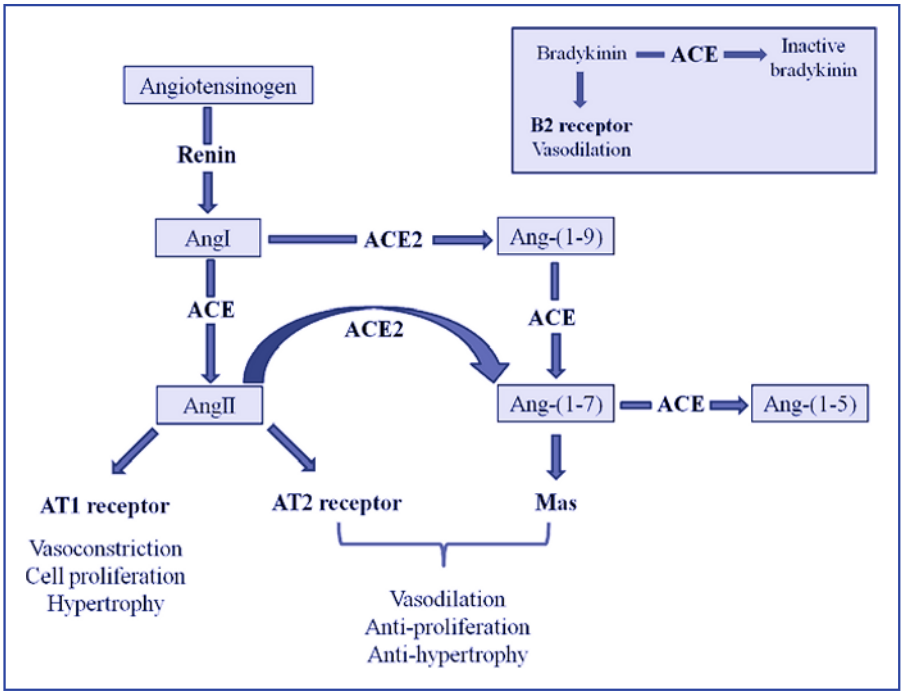

Fig. 1. Renin-angiotensin system cascade. AngI: angiotensin I; ACE: angiotensin-converting enzyme; AT1 receptor: AngII type 1 receptor; AT2 receptor: AngII type 2 receptor; Mas: Ang-(1-7) receptor; B2 receptor: bradykinin receptor.

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a) The circulating RAS acts at a systemic level: Its substrate is angiotensinogen, which is released from the liver and cleaved, to form angiotensin I (AngI), by renin, an enzyme secreted from the juxtaglomerular cells of the kidney. The main effector peptide of the system is angiotensin II (AngII), generated from AngI by ACE and acting as a powerful vasoconstrictor.
b) Local tissue RASs, described in several organs and tissues, can synthesize AngII locally, since they possess the whole RAS enzymatic machinery.
c) More recent studies have revealed an intracellular complete RAS, which can produce AngII.

The RAS can, therefore, be considered an endocrine, paracrine and intracrine system [4].

RAS and Inflammation

The endothelium is a continuous layer of simple squamous epithelial cells (endothelial cells), which contributes to systemic homeostasis through different functions: Barrier, maintenance of coagulation and thrombolytic processes, participation in immune reactions, and synthesis of different vasoactive compounds [5]. Direct injury (mechanical, chemical or infective) of the endothelium exposes the basement membrane components to the blood, leading to a series of events (the so called inflammatory cascade), including alteration of vascular integrity and production and release of cytokines, adhesion molecules, procoagulant agents and reactive oxygen species (ROS). In addition to its well-known regulatory role in hemodynamic homeostasis, RAS, and in particular AngII (**Fig. 2**), is also involved in the modulation of the inflammatory process [6]. Indeed AngII can regulate vascular permeability, the first step of the inflammatory response, by inducing the synthesis of prostaglandins and vascular endothelial cell growth factor (VEGF) [7, 8]. Moreover, RAS participates in leukocyte extravasation, a critical step in the inflammatory response, at different levels. AngII activates leukocytes and increases their adhesion to endothelial cells [9] via the induction of pro-inflammatory mediators like selectins, adhesion molecules, chemokines, cytokines and other factors (e.g., transforming growth factor [TGF]-β). AngII effects are mediated by its receptor angiotensin receptor 1 (AT1R), which leads to the activation of transcription factors, including activating protein-1 (AP-1) and nuclear factor-κB (NF-κB), and the ensuing expression of genes involved in inflammation and tissue injury [10]. In patients with cardiovascular disease, the increased selectin expression and plasma chemokine levels are attenuated by treatment with losartan (AT1R antagonist) [11]. Experimental studies have demonstrated that AngII infusion (at dosages unable to induce vasoconstriction) increases leukocyte transmigration, and that treatment with angiotensin receptor antagonists suppresses this leukocytes response [12]. Finally RAS participates in the last phase of inflammation: Cell growth and tissue repair. Preclinical studies on the kidney [13] have revealed that AngII induces cellular growth and release of several growth factors, resulting in organ hypertrophy, massive matrix synthesis and formation of scarring. Further studies demonstrated that AngII also induces fibrosis in other tissues, such as heart [14] and lung [15].

It is now well known that RAS is involved in the pathophysiology of several diseases, occurring in different organs (e.g., heart and kidney). Overactivation of RAS is linked with the development of atherosclerosis, hypertension and cardio-

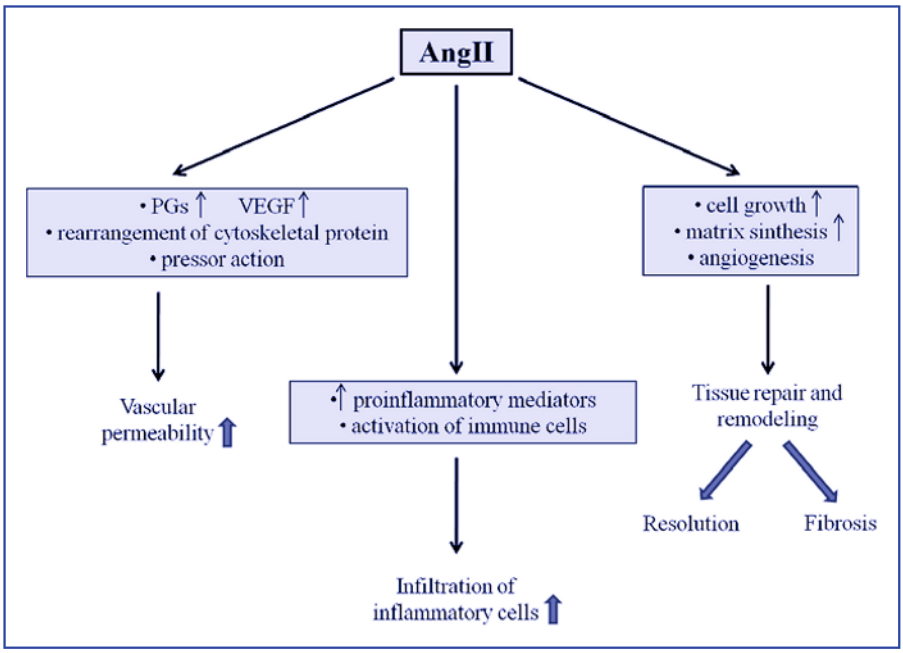

Fig. 2. Pro-inflammatory effects of angiotensin II (AngII). PGs: prostaglandins; VEGF: vascular endothelial cell growth factor.

vascular and renal events, such as myocardial infarction, stroke, congestive heart failure, diabetes, nephrosclerosis [16] and tumorigenesis [17]. Several studies have focused on lung diseases, such as pulmonary hypertension and pulmonary fibrosis, in order to better understand the role of RAS in this field. Capillaries in the lungs are one of the main sites of ACE expression [18], and thereby lungs are considered an important source of AngII. Pulmonary hypertension is characterized by increased vascular resistances and endothelial remodeling, eventually leading to right heart failure and death. In this context, AngII seems to mediate pro-inflammatory signaling (rather than vasoconstriction), resulting in pulmonary vascular remodeling [19]. Indeed AngII can induce smooth muscle cells proliferation [20], with consequent increase in pulmonary artery wall thickness. Evidence has demonstrated the involvement of RAS in the pathophysiology of pulmonary fibrosis. AngII upregulates the expression of TGF-β, thus the differentiation of fibroblasts to myofibroblasts and increase in extracellular matrix deposition [21], as well as alveolar epithelial cells apoptosis [22].

Among the various scenarios in which RAS can have a role, this chapter focuses on current knowledge of the role of RAS in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

Angiotensin-converting Enzyme: The 'Dark Side' of RAS

ACE is a dipeptidylcarboxypeptidase, which converts AngI to AngII by cleavage of C-terminal dipeptides (**Fig. 3**). ACE is also responsible for the breakdown of the

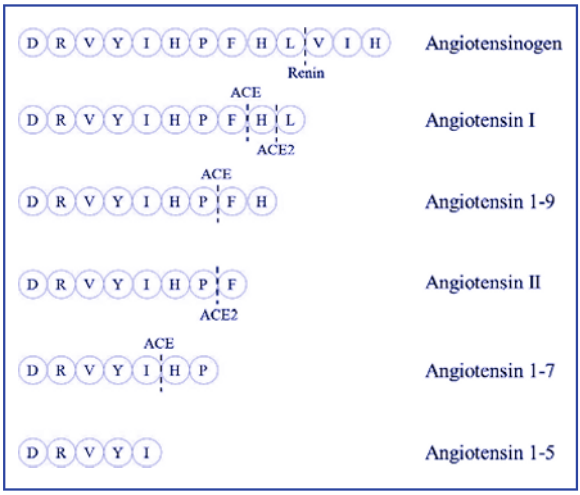

Fig. 3. Amino acid sequences and cleavage sites of components of the renin-angiotensin system (RAS). D: aspartic acid; R: arginine; V: valine; Y: tyrosine; I: isoleucine; H: histidine; P: proline; F: phenylalanine; L: leucine; ACE: angiotensin-converting enzyme

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nonapeptide of bradykinin (**Fig. 1**). ACE is the key enzyme of the RAS and is highly expressed on lung endothelial cells, where it represents an ectoenzyme uniformly distributed along the luminal surface, with its catalytic site exposed to the blood-borne substrates [5].

Some clinical data seem to indicate that ACE activity is related to ALI pathogenesis and outcome. In 1987, Idell et al. [23] found that ACE levels were augmented in bronchoalveolar lavage (BAL) fluid of ARDS patients, compared to normal subjects and to patients affected by sarcoidosis and fibrosis. In particular ACE levels were highest in BAL fluid from patients with ARDS of infectious origin. The authors speculated that ACE in BAL fluid could be a marker of endothelial damage. Two years earlier, in 1985, Fourrier et al. [24] had performed sequential measurements of ACE levels in the serum of patients with ARDS or with sepsis (without ARDS). They found that, in ARDS survivors, ACE levels had a biphasic evolution, decreasing in the early phases of the illness, and increasing at the time of weaning. Conversely, ACE levels in ARDS non-survivors decreased in the early phases without the later increase. Finally, in patients with sepsis without ARDS the levels of ACE remained elevated. The authors postulated that a low level of circulating ACE was an index of pulmonary endothelial injury correlating with the presence and severity of ARDS.

ARDS appears to have a relatively low incidence compared to the number of subjects who are potentially at risk of developing this syndrome. Thus, some genetic background predisposing to the illness has been postulated. The ACE gene is located on chromosome 17q23 and contains a restriction fragment length polymorphism consisting of the insertion or deletion of an Alu repeated sequence in intron 16. Deletion of the Alu sequence is associated with greater ACE activity in plasma and peripheral blood mononuclear cells [25]. Marshall et al. [26] performed a retrospective study in 2002 comparing patients with ARDS to patients with non-ARDS respiratory failure. They found that the deletion of the Alu sequence in the ACE gene was associated with a higher incidence of ARDS and increased mortality in the ARDS group, suggesting that an increase in ACE activity correlates with the risk and outcome of ARDS.

Different results were obtained by Orfanos et al. in 2000 [27]. In order to assess whether pulmonary capillary endothelium bound (PCEB)-ACE activity could be a marker of endothelial injury, thus predicting the development and severity of ALI, patients were grouped according to lung injury score (LIS) and PCEB-ACE activity was measured. The authors observed that PCEB-ACE activity was decreased in patients with ALI/ARDS compared with non-ARDS patients, with an inverse correlation with LIS and APACHE II score. These data suggest that PCEB-ACE dysfunction is an early marker of the presence and severity of endothelial cell damage.

The fact that deletion of the Alu sequence, which leads to a higher activity of ACE, is related to the pathogenesis of ARDS, appears in contrast with the fact that PCEB-ACE activity is reduced as a consequence of pulmonary endothelial dysfunction. Maniatis et al. [28] interpreted this paradox by inferring that increased ACE and AngII activity could generate superoxide anions. The interactions between ROS and nitric oxide (NO) lead to the synthesis of reactive nitrogen species, that damage the pulmonary endothelium. The consequent reduction in ACE activity counterbalances its pro-inflammatory activity. Another hypothesis is that the crude losses of endothelium result in reduced PCEB-ACE activity, whereas it is normally, or even more than normally, active in healthy zones of the lungs.

Angiotensin II

The main biological activity of ACE is the cleavage of AngI to give AngII, which thus appears the main effector through which ACE is responsible for ARDS pathogenesis. AngII is an octapeptide with several biological activities, many of which have been already described in this review. Its action is mediated by two different receptors, AT1R and AT2R, that show opposite biological effects (**Fig. 2**). The role of AngII in the pathogenesis of ALI/ARDS seems to be strongly linked to its pro-inflammatory properties.

Kuba et al. [29] showed that in a murine model of ALI caused by acid aspiration or by the injection of severe acute respiratory syndrome (SARS) coronavirus spike proteins, the levels of AngII in the lungs were increased, and the degree of increase was proportional to the degree of lung injury assessed by histopathology (AngII levels were significantly higher in the lungs of mice subjected to a double injury, acid aspiration and viral infection). However, pulmonary edema, the hallmark of ALI/ARDS, can be due to increased hydrostatic pressure (the typical effect of AngII is augmentation of systemic pressure, which may promote pulmonary edema) or to enhanced permeability: Some studies [6, 30] demonstrated that local AngII was able to act directly on vascular permeability by stimulating VEGF and prostaglandins, thus exerting a primarily pro-inflammatory effect, not mediated by hemodynamic alterations, yielding important evidence in the assessment of the role of AngII in the pathogenesis of ALI/ARDS. The same group [29] demonstrated that the receptor implicated in AngII action during development of ALI is AT1R: Inhibition of AT1R attenuated ALI, as shown by increased compliance and attenuated pulmonary edema.

Ang II may also play a role in the regulation of apoptosis of type II pneumocytes, an important phenomenon in the late phases of ARDS. In fact, while proliferation of type II pneumocytes is characteristic of the early phases of ALI as a reparative phenomenon, their apoptosis indicates evolution towards a fibrotic

phase of the disease [31]. AngII seems to promote apoptosis by modulating the Fas/Fas ligand system [32], which bears major implications in the pathogenesis and evolution of ALI by apoptosis of alveolar epithelial cells. Wang et al. [22] confirmed the role of AngII in modulation of apoptosis: Alveolar epithelial cells were cultured in a medium supplemented with different doses of AngII or angiotensinogen. Both these proteins were able to induce apoptosis in alveolar epithelial cells in a concentration-dependent manner. Moreover, the authors observed that the non-selective angiotensin receptor antagonist, saralasin, could inhibit apoptosis induced by both proteins, while lisinopril, an ACE-inhibitor, blocked apoptosis induced by angiotensinogen but not by AngII. These data showed the relationship between AngII and alveolar epithelial cell apoptosis. Although alveolar epithelial cells express mRNAs for both AngII receptors (AT1R and AT2R), only selective inhibition of AT1 blocked apoptosis caused by AngII [33]. Other findings by Marshall et al. showed that AngII could influence the progression of ALI towards a fibrotic disease: AngII is mitogenic for human adult fibroblasts via the AT1 receptor and stimulates the autocrine release of TGF-β (a pro-fibrotic cytokine) and pro-collagen production from fetal lung fibroblasts [34]. Finally, in a rat model of pulmonary fibrosis, bleomycin augmented lung AngII concentration, whereas treatment with an ACE inhibitor or an AT1 inhibitor was able to attenuate lung collagen accumulation and TGF-β expression [34].

Several animal models [33, 35, 36] helped to disclose the relationship between lipopolysaccharide (LPS)-induced ALI and AngII mainly by using selective inhibitors of AT1 receptors. First, sepsis-induced ALI was characterized by increased levels of AngII in lung tissue. Treatment with an inhibitor of AT1 led to improvements in permeability index *in vivo*, such as wet/dry weight ratio, quantification of Evans blue dye extravasation, histological evidence of edema. *In vitro* studies [35] supported these data, since monolayers of rat pulmonary microvascular endothelial cells had increased permeability following exposure to AngII; this increase in permeability was partially corrected by adding an inhibitor of AT1 to the culture medium. The role of AngII in the inflammatory cascade and endothelial dysfunction was further demonstrated by cytokine levels: Sepsis induced the secretion of several pro-inflammatory cytokines in the lungs and plasma, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6; this secretion was partially inhibited by the infusion of an AT1 inhibitor. Two groups [33, 36] performed additional studies to understand the mechanisms underlying AngII's pro-inflammatory actions: Both groups found that inhibition of AT1 led to a lesser activation of the NF-κB pathway, mediated by increased levels of IκB (inhibitor of NF-κB). Signal transduction through the mitogen-activated protein kinase (MAPK) cascade, c-jun N-terminal kinase (JNK) and AP-1 seems also to be involved in the pro-inflammatory effects of AngII.

Angiotensin-converting Enzyme 2: The 'Bright Side' Of RAS

In 2000, ACE2, the first human homolog of ACE, was discovered by two independent groups [2, 3]. Human ACE2 is expressed in the endothelium, but predominantly in the heart, kidney and testis [2], although its expression is also distributed to lung, liver, small intestine and brain [37]. Human ACE2 consists of 805 amino acids and is a type I integral membrane glycoprotein with an extracellular domain, containing a potential metalloprotease zinc binding site, a single trans-

membrane domain, and a cytoplasmic tail. It has only one catalytic domain, which shows 42 % identity with the amino domain of ACE. ACE2 is a carboxypeptidase, since it cleaves only one C-terminal residue from its substrate, rather than a dipeptide, as ACE does (**Fig. 3**). This particular difference between the two enzymes makes ACE2 resistant to the action of ACE inhibitors, since they mimic the dipeptidyl C-terminal binding sites of ACE substrates. ACE2 converts AngI to Ang 1-9 and AngII to Ang 1-7 [2, 3]. Ang 1-9 can be converted by ACE to Ang 1-7 [2]. Rice and colleagues [38] demonstrated that ACE2 hydrolyzed AngII more efficiently than AngI; therefore, the production of Ang 1-7, a powerful vasodilator, is believed to be the primary goal of ACE2 activity (Ang 1-7 will be discussed in depth later in the chapter). Moreover, other vasoactive peptides are substrates of ACE2 catalytic activity, such as neurotensin, kinetensin, des-Arg bradykinin and apelin-13 [2, 3].

ACE2 is present in the lungs of healthy and diseased humans; it is expressed by type I and type II alveolar epithelial cells and in bronchiolar epithelial cells [30, 39]. ACE2 may play an important role in ALI as shown by experimental studies. Imai et al. [30] examined extensively ACE2-induced protection from severe acute lung failure in two murine models of ALI (acid aspiration and sepsis). Loss of *Ace2* (*Ace2* knockout mice) resulted in decreased compliance, pulmonary edema and leukocyte infiltration in both animal models. The pulmonary edema seemed to be predominantly due to the altered vascular permeability (as demonstrated by Evans blue accumulation), rather than to hemodynamic causes. Indeed no differences in heart contractility or pulmonary vascular tone were found, although the authors did not exclude a potential effect of AngII on blood vessels. To further underline the protective action of ACE2, which counterbalances ACE functions, Imai et al. demonstrated that ACE, AngII and AT1R promoted ALI in different types of knockout mice, whereas ACE2 and AT2R protected against it. ACE2 negatively regulates AngII levels, which, if ACE2 is lacking, increase both in lungs and plasma.

ACE2 appears to play a role also in the pathogenesis of SARS [29]. ACE2 is now considered the SARS virus receptor: The interaction of ACE2 and the virus leads to endocytosis of viral particles and to viral fusion with cells. The absence of ACE2, in *Ace2* knockout mice, results in a dramatic reduction in SARS-coronavirus infection, with subsequent reduced histologic alterations and leukocyte infiltration. Kuba et al. [29] demonstrated *in vivo* the mechanism underlying the elevated severity and mortality of the viral infection: SARS-coronavirus infection downregulated ACE2 expression. During SARS infection, the protective arm of the RAS is impaired by the loss of ACE2 and of its ability to negatively regulate AngII, the levels of which are significantly increased in lung tissue. These authors demonstrated that the inhibition of AT1R, thus inhibition of AngII activity, attenuated the severity of lung injury, in terms of lung mechanical properties and pulmonary edema, in SARS-coronavirus challenged mice.

Angiotensin-(1 – 7) and Mas Receptor

Ang-(1-7) is a biologically active heptapeptide (**Fig. 3**) released from AngI or AngII by different peptidases, of which ACE2 is one of the most important. Ang-(1-7) exerts its action via the receptor, Mas, an 'orphan' G protein-coupled receptor [40] although it can also bind AT2R, but the affinity with AT2R is modest as compared to that of AngII. The ACE2-Ang-(1-7)-Mas arm is now consid-

ered the principal counterregulatory mechanism for the other RAS arm, ACE-AngII-AT1R. Indeed the two arms of the system act in opposite ways, the first leading to formation of Ang-(1-7) and simultaneously decreasing AngII, and the second forming the powerful vasoconstrictor AngII and catabolizing Ang-(1-7) and bradykinin [41]. Ang-(1-7) is both substrate and inhibitor of ACE, since it is cleaved by the N-domain of ACE to form Ang-(1-5) but it can also inhibit the function of ACE C-domain [42]. Ang-(1-7) acts as a vasodilator through the inhibition of ACE and, in addition, through the release of NO achieved by the indirect potentiation of bradykinin [43].

In addition to its vasodilatory properties, Ang-(1-7) interaction with the receptor Mas exerts an antiproliferative effect on vascular smooth muscle cells, fibromuscular tissue, and lung cancer cells. Moreover, it can reduce organ remodeling by limiting hypertrophy and collagen deposition, and thus fibrosis, with subsequent improvement in organ functions [41]. A recent study on experimental models of arthritis [44] showed the anti-inflammatory effects of the Ang-(1-7) Mas receptor. The activation of Mas, through the use of a Mas agonist, decreased the release of cytokines (TNF-α and IL-1β), and the rolling and adhesion of leukocytes to the endothelium at the injured site. It also decreased histological abnormalities and classical signs of inflammation, such as pain and edema. The authors suggested that Mas receptor agonists may be a novel therapeutic approach for the treatment of arthritis in humans.

Limited information is available concerning the role of Ang-(1-7) and its receptor in healthy or diseased lungs. Uhal et al. [45] recently demonstrated the protective effects of Ang-(1-7) on alveolar epithelial cell survival via the Mas-mediated inhibition of apoptosis. In fact, Ang-(1-7) inhibits JNK phosphorylation, caspase activation and nuclear fragmentation, acting through Mas receptors, blockade of which, conversely, favors apoptosis.

Therapeutic Strategies

The previously reviewed data show that RAS has a role in ALI/ARDS pathogenesis. For this reason, several experimental studies have been performed to evaluate the therapeutic role of RAS modulation in this setting. For example, Hagiwara et al. [46] developed a model of LPS-induced ALI in rats and showed that therapy with ACE inhibitors could diminish interstitial edema, alveolar cellular infiltration and cytokine release, probably via a reduction of Ang II levels and inhibition of the NF-κB pathway. In a different model of ALI [47], induced by the association of chronic ethanol ingestion and endotoxemia, alveolar epithelial permeability was improved by therapy with lisinopril or losartan. The same drugs were able to reduce TGF-β levels in lung tissue and avoid glutathione depletion, thus limiting epithelial dysfunction and oxidative stress. Finally, an experimental model of oleic acid-induced ALI [48] confirmed that captopril could attenuate pulmonary injury by protecting the vascular endothelium: The number of circulating endothelial cells in the captopril-treated group was reduced as compared to the control group, in addition to a decreased expression of intercellular adhesion molecule (ICAM)-1 and activation of the NF-κB pathway. Captopril treated rats also had a better PaO_2/FiO_2 ratio and less edema.

No clinical trial has been performed to investigate the effects of RAS modulation in ARDS patients. However, a retrospective cohort study [49] has been con-

ducted to assess the effects on survival of prior use of ACE inhibitors in patients hospitalized with community acquired pneumonia. Prior use of ACE inhibitors was associated with a lower 30-day mortality.

As far as the ACE2-Ang(1-7)-Mas arm is concerned, the anti-inflammatory properties of ACE2 have been proposed as therapy for ALI/ARDS. The injection of recombinant human ACE2 (rhACE2) in *Ace2* knockout mice (rescue of phenotype) decreased the severity of ALI, and improved ALI/ARDS symptoms in wild type mice, suggesting that rhACE2 administration may be a novel potential therapeutic strategy for ARDS [30]. A study evaluating the therapeutic potential of rhACE2 in an animal model of LPS-induced lung injury [50] showed that treatment with rhACE2 reduced plasma TNF-α and AngII levels, improving the inflammatory response and pulmonary edema. Control animals receiving LPS infusion developed pulmonary hypertension, whereas in the treatment group, mean pulmonary arterial pressure was reduced (without changes in pulmonary artery occlusion pressure), indicating a pulmonary vasodilatory function of ACE2. In addition, rhACE2 treatment improved LPS-induced arterial hypoxemia, probably because of a more homogeneous perfusion in ACE2 treated animals.

Conclusion

In this chapter, we have reviewed the main evidence concerning the role of RAS components in ALI/ARDS. In addition to its well known cardiovascular and renal regulatory properties, the RAS participates in the different phases of inflammation, from the release of cytokines and the extravasation of leukocytes to matrix deposition and tissue repair. ALI/ARDS is characterized by a powerful inflammatory response that persists over time and, while attempting to repair the injury, results in the formation of scar tissue. Although RAS modulation appears a promising therapeutic strategy, more data need to be collected to elucidate whether it would be a feasible and clinically effective means of attenuating lung injury. Enhancement of the effects of the recently discovered ACE-AngII-AT1R arm may be a potential strategy to decrease the deleterious effects of AngII.

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