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REVIEW | *The Pathophysiology of COVID-19 and SARS-CoV-2 Infection*

Disequilibrium between the classic renin-angiotensin system and its opposing arm in SARS-CoV-2-related lung injury

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Sarzani R, Giulietti F, Di Pentima C, Giordano P, Spannella F. Disequilibrium between the classic renin-angiotensin system and its opposing arm in SARS-CoV-2-related lung injury. *Am J Physiol Lung Cell Mol Physiol* 319: L325–L336, 2020. First published July 8, 2020; doi:10.1152/ajplung.00189.2020.—A dysregulation of the renin-angiotensin system (RAS) has been involved in the genesis of lung injury and acute respiratory distress syndrome from different causes, including several viral infections. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection of pneumocytes, the hallmark of the pandemic coronavirus disease 2019 (COVID-19) involving both alveolar interstitium and capillaries, is linked to angiotensin-converting enzyme 2 (ACE2) binding and its functional downregulation. ACE2 is a key enzyme for the balance between the two main arms of the RAS: the ACE/angiotensin (Ang) II/Ang II type 1 receptor axis ("classic RAS") and the ACE2/Ang(1–7)/Mas receptor (MasR) axis ("anti-RAS"). The ACE2 downregulation, as a result of SARS-coronaviruses binding, enhances the classic RAS, leading to lung damage and inflammation with leaky pulmonary blood vessels and fibrosis, when the attenuation mediated by the anti-RAS arm is reduced. ACE inhibitors (ACE-I) and Ang II type 1 receptor blockers (ARB), effective in cardiovascular diseases, were found to prevent and counteract acute lung injury in several experimental models by restoring the balance between these two opposing arms. The evidence of RAS arm disequilibrium in COVID-19 and the hypothesis of a beneficial role of RAS modulation supported by preclinical and clinical studies are the focus of the present review. Preclinical and clinical studies on drugs balancing RAS arms might be the right way to counter COVID-19.

ACE2; acute respiratory distress syndrome; COVID-19; renin-angiotensin system; SARS-CoV-2

INTRODUCTION

The coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been declared a pandemic by the World Health Organization on March 11, 2020. Children and younger healthy adults usually have mild symptoms or may even be asymptomatic, whereas older adults are at higher risk of severe complications and death (25, 63). Furthermore, increasing data have linked obesity to a more severe course of COVID-19 and death even in younger people (82). Next to aging and obesity, cardiovascular (CV) comorbidities are likely to play a key role in the prognosis of COVID-19. It is not surprising that hypertension and other CV diseases (CVD) are so common among patients who died from COVID-19 (112). Hypertension is highly prevalent in the general population, being the first CV risk factor in older people, while CVD are known to be the leading cause of death worldwide (99). High prevalence of hypertension has also been found in patients who died after Middle East Respiratory Syndrome (MERS)-CoV and influenza viruses infections (2), indicating its nonspecificity for COVID-19, but most likely representing a confounder, linked to age. Heart failure (HF) is also likely to be highly prevalent among patients deceased for COVID-19, even though published data on this association are still scarce (71).

Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for SARS-CoV-2, as it was for SARS-CoV (33, 104). ACE inhibitors (ACE-I) and angiotensin II type 1 receptor blockers (ARB) are cornerstone drugs for the management of both hypertension and CVD, and their use has been previously found to be associated with increased ACE2 levels in some

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tissues or fluids. On this basis, some authors hypothesized a greater susceptibility to SARS-CoV-2 infection and a higher risk for severe disease associated with these drugs (18, 110), despite the absence of solid experimental evidence. Promptly, almost all major international scientific societies and drug authorities clarified that no experimental data support these speculations, stressing the risks due to withdrawal, especially in patients at higher CV risk. Furthermore, several clinical observational studies recently published have largely refuted this hypothesis (50). Actually, the exact opposite appears to be more likely from previous experimental studies on virus-mediated lung injury. Indeed, a dysregulation of the renin-angiotensin system (RAS) plays a key role in the genesis of lung damage after SARS-CoV-2 infection, and RAS inhibitors may restore the equilibrium between the "classic RAS" arm and its counterregulatory arm, here defined as "anti-RAS," of which ACE2 is the cornerstone. These two pathways and the possible role of a "pathophysiological" drug therapy based on RAS inhibitors, aiming at restoring equilibrium and avoiding severe lung damage and death in COVID-19, are described in depth below (Fig. 1).

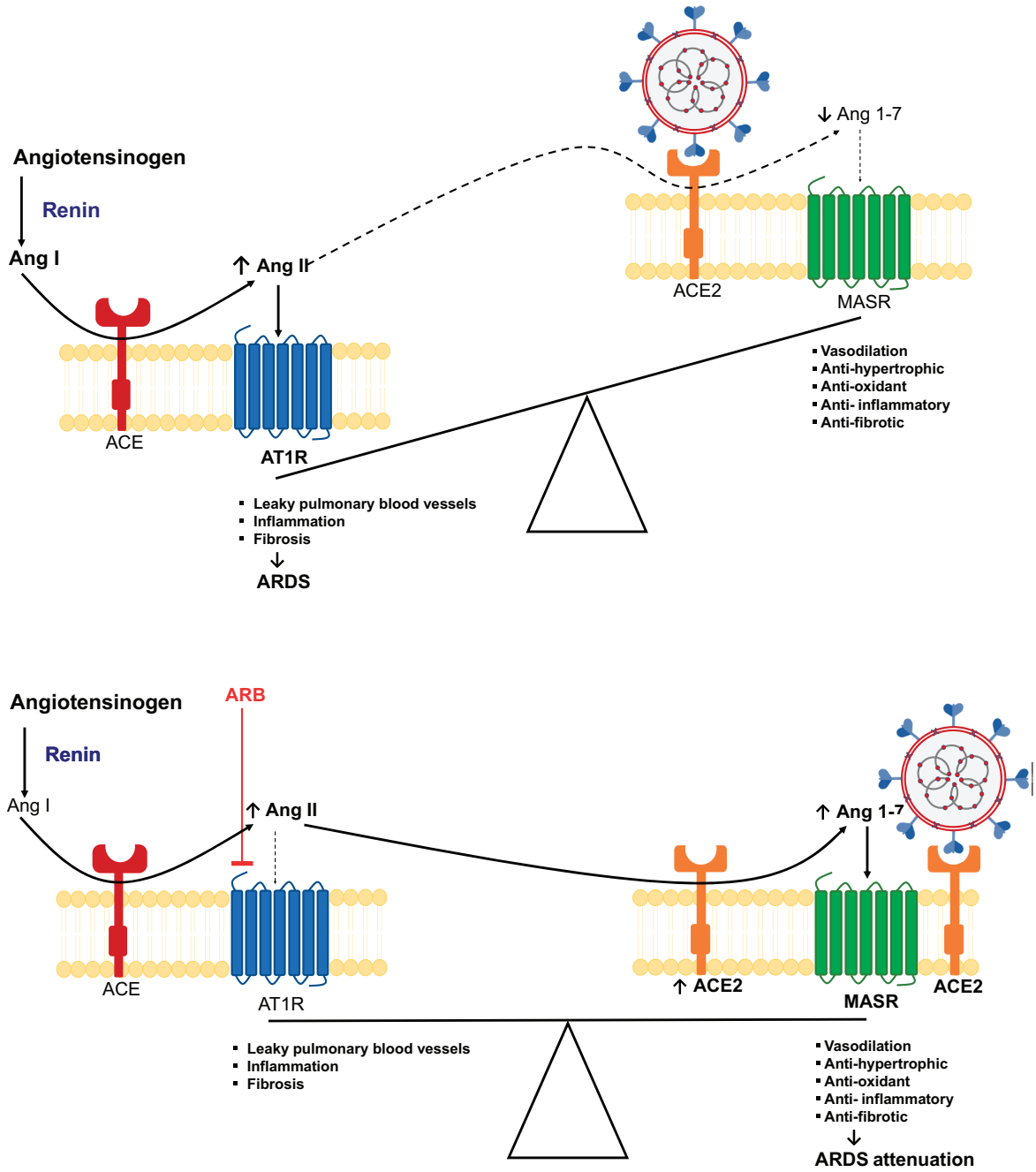

Fig. 1. Graphic synthesis of the proposed disequilibrium caused by severe acute respiratory syndrome coronavirus 2 infection in lung tissue (*top*) restored by angiotensin II type 1 receptor blocker (ARB) therapy (*bottom*). Ang II, angiotensin II; ACE, angiotensin-converting enzyme; AT_1R, angiotensin II type 1 receptor; ARDS, acute respiratory distress syndrome; MasR, Mas receptor; MrgD, Mas-related G protein-coupled receptor D.

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PHYSIOLOGY OF RAS PATHWAYS IN CARDIOVASCULAR AND PULMONARY HOMEOSTASIS

The Classic RAS and the Anti-RAS Arms

The RAS plays a central role in both the homeostatic control of CV and renal systems and the regulation of both fluid volume and blood pressure, but also exerts trophic and pathomorphological effects on several tissues, including lungs, mainly acting on the microvasculature and mesenchymal cells. The RAS blockade, or better, the RAS modulation, represents a therapeutic cornerstone of both arterial hypertension and its related CV sequelae, such as ischemic heart disease and HF (67).

The classic RAS consists in a cascade of both systemic and tissue enzymatic reactions, mainly resulting in generation of angiotensin (Ang) II (Fig. 2*A*, *top*). Renin, a protease released by the kidneys in its active form, cleaves angiotensinogen, produced by the liver in large quantities, but also by the adipose tissue (79), to form Ang I, both locally and systemically, which is then hydrolyzed by the widespread endothelial ACE, producing the octapeptide Ang II. The ACE is very abundant in microvasculature-rich tissues, such as lungs, being highly expressed in the endothelium. Therefore, the pulmonary vascular bed is capable to rapidly convert Ang I to Ang II. It is important to note that this ability may play a key role in the overproduction of Ang II in conditions characterized by increased circulating levels of Ang I, such as obesity or overweight with central (visceral) obesity (79). This biologically active peptide binds Ang II type 1 and type 2 receptors (AT_1R and AT_2R, respectively). The AT_1R binding induces vasoconstriction, but also direct vascular damage when excessively activated (66). Furthermore, in the adrenals, Ang II stimulates aldosterone secretion, promoting sodium reabsorption and blood pressure increase.

The dominant physiological and pathophysiological role of the classic RAS pathway was rethought 20 years ago, after the discovery of ACE2 (17). ACE2 is a type I transmembrane metallocarboxypeptidase, with homology to ACE and, just like

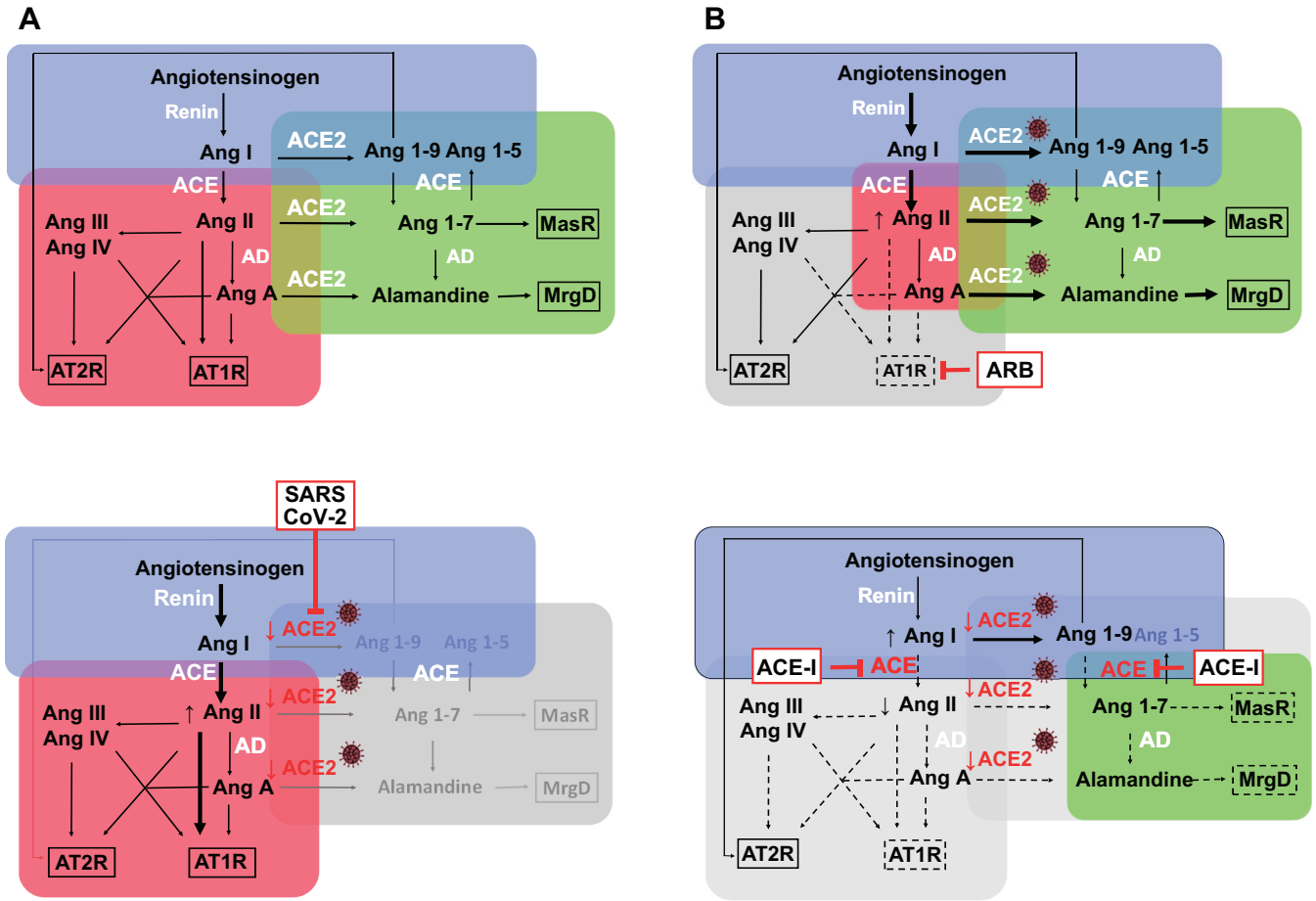

Fig. 2. Schematic of the "classic renin-angiotensin system (RAS)" and "anti-RAS" arms in different conditions as follows: physiology (*A*, *top*), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (*A*, *bottom*), angiotensin II type 1 receptor blocker (ARB) therapy (*B*, *top*), and angiotensin-converting enzyme inhibitor (ACE-I) therapy (*B*, *bottom*). The schematic includes circulating factors and local synthesis by the main cells involved (pneumocytes type 1 and 2, interstitial mesenchymal cells, and capillary endothelium). The thickness of the connecting lines and arrows or dashed arrows represents proportional increases or reductions of the pathways. SARS-CoV-2 infection might lead to a downregulation of ACE2 with a consequent decrease of the anti-RAS arm, mainly Ang(1–7) and alamandine. Treatment with ARB could counteract this imbalance, through angiotensin II type 1 receptor (AT_1R) blockade and the angiotensin II (Ang II) increase, moving the pathway toward anti-RAS prevalence. The reequilibrium of the anti-RAS arm could be done also likely by treatment with ACE-I through a different mechanism [increased availability of Ang(1–9), decreased degradation of Ang(1–7)] that also involves angiotensin II type 2 receptor (AT_2R) stimulation. ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; AD, aspartate decarboxylase; MasR, Mas receptor; MrgD, Mas-related G protein-coupled receptor D; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor.

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ACE, abundant in lung tissue, consisting of an extracellular NH_2-terminal domain containing a catalytic site and intracellular COOH-terminal tail. ACE2 has many peptides as substrates, but mainly cleaves Ang I into a nonapeptide [Ang(1–9)] that binds AT_2R and Ang II into a heptapeptide [Ang(1–7)] that binds an endogenous orphan receptor (Mas receptor, MasR) (Fig. 2*A*, *top*) (73). The catalytic efficiency of ACE2 is ~400-fold higher for Ang II than Ang I (95). Ang(1–9) can also be rapidly converted in Ang(1–7) by ACE. Therefore, ACE2 mainly produces Ang(1–7) from Ang II, unless ACE is inhibited, leading to decreased Ang II and increased Ang I levels so that ACE2 can switch to more Ang(1–9) being produced.

Differences in the modulation of ACE2 and RAS by ACE-I and ARB are then expected. ACE-I reduce the Ang II-AT_1R pathway activity by reducing Ang II synthesis with increased Ang I substrate for ACE2 and a reduced conversion of Ang(1–9) to Ang(1–7). Therefore, ACE-I can specifically enhance the activity of Ang(1–9) on AT_2R, especially when Ang II is reduced (Fig. 2*B*, *bottom*). At the same time, given that ACE also cleaves Ang(1–7) to the inactive Ang(1–5), ACE-I may also increase the lifespan of Ang(1–7) (Fig. 2*B*, *bottom*) (9). Differently, ARB compete with Ang II at the AT_1R level, leading to a secondary increase of Ang II due to the reduced negative feedback on renin release and thus increasing Ang II substrate for ACE2 (Fig. 2*B*, *top*).

ACE2 also interacts with other angiotensin peptides (called alatensins) in which the NH_2-terminal aspartate is replaced by alanine, leading to Ala-Ang 1–7 (called alamandine) that has been found to bind the Mas-related G protein-coupled receptor D (MrgD) (74), showing similar effects to Ang(1–7) stimulating MasR (Fig. 2*A*, *top*). The largest part of ACE2 is membrane bound, whereas its shorter soluble form circulates in blood at very low levels but can be increased through cleavage of full-length ACE2 membrane-anchor by the disintegrin and metalloprotease 17, which is directly upregulated by the Ang II-AT_1R axis (102). The "shedding" of ACE2, induced by the classic RAS effector Ang II/AT_1R, is likely to result in local downregulation of the tissue enzymatic activity, similarly from what is observed following SARS-CoV infection (24, 39).

Role of ACE2 in Cardiovascular and Pulmonary Homeostasis

Initially thought to be expressed only in limited organs (mainly heart and kidney), ACE2 was later found to be ubiquitous, and highly expressed in the lungs (98), exerting its key roles in maintaining tissues and body homeostasis by counterbalancing classic RAS activity. ACE2 is abundantly expressed on the cell surface of alveolar type II epithelial cells (type II pneumocytes), which are small cylindrical cells very close to pulmonary capillaries and responsible for the synthesis of alveolar surfactant. ACE2 is also widely expressed in other airway cells, from the epithelial cells of oral mucosa (101) to the bronchial transient secretory cells (49). Furthermore, it is also well detectable on the enterocytes of the small intestine, as well as on vascular endothelium of several human organs and systems, including cardiovascular system, kidneys, lungs, and brain (31, 74), thus probably explaining the ability of SARS-CoV-2 to affect so many organs.

The two main catalytic roles of ACE2 in human CV homeostasis, in the absence of RAS-interfering drugs, are the degradation of Ang II, which is the major effector of the classic RAS arm that promotes vasoconstriction, sodium retention, oxidative stress, inflammation, and fibrosis, and the sharp increase of bioactive peptide Ang(1–7), which binds MasR and counterregulates the Ang II-AT_1R axis through its vasodilating, anti-inflammatory, and antifibrotic effects (Fig. 2*A*, *top*) (66). ACE2 could mediate both blood pressure reduction and protection from CV damage in preclinical studies (14, 47). According to experimental models, the activation of the Ang(1–7)-MasR axis attenuates inflammation and fibrosis in several tissues. The ACE2-Ang(1–7) pathway also plays a key protective role against HF and coronary heart disease (4). Treatment with the Ang(1–7)/MasR antagonist D-Ala7-Ang-(1–7) ("DALA") worsened Ang II-induced cardiac fibrosis and increased the expression of multiple cytokines with profibrotic actions (58). In ACE2 knockout mice, Ang II infusion induces hypertension, myocardial hypertrophy, fibrosis, and diastolic dysfunction, whereas recombinant ACE2 (rhACE2) attenuates myocardial remodeling, which is the main feature of HF with preserved ejection fraction (HFpEF) (66, 111).

The ACE2-driven anti-RAS arm is also potentiated by alamandine and its specific receptor that, reinforcing the MasR-dependent effects, may protect against inflammation and fibrosis by improving the endothelial dysfunction and mitigating vascular remodeling also in the lung (16, 74). Moreover, alamandine counteracts cardiac hypertrophy induced by Ang II and left ventricular dysfunction (38). Although AT_2R is believed to be the anti-AT_1R by some authors, the role of the ACE2-Ang(1–9)-AT_2R pathway is still less clear, mostly because the postreceptorial cascade after AT_2R activation is still not fully understood and because it is likely to play a role in pathological conditions only (74). AT_2R activation likely leads to both vasodilation and a general attenuated response to Ang II/AT_1R (34). Administration of Ang(1–9) reduced blood pressure in hypertensive rats through AT_2R (62).

In synthesis, many well-conducted studies in different animal models and humans lead to the solid knowledge that a decrease of ACE2 activity leaves unopposed the effects of the Ang II-AT_1R axis, contributing to progression of microvascular damage and CVD, whereas increased ACE2 activity leads to the activation of both Ang(1–7)-MasR and alamandine-MrgD pathways and maybe also the Ang(1–9)-AT_2R axis, contributing to protect against microvascular damage and CVD due to its antihypertrophic, antifibrotic, and anti-inflammatory activities (66).

SPECIFIC ROLE OF RAS PATHWAYS IN LUNG DISEASE

Viral Infection and Lung Damage

The coronaviruses involved in severe acute respiratory syndromes, such as SARS-CoV-2, can frequently cause interstitial pneumonia. Alveolar epithelial injury is the first crucial step for the development of interstitial pneumonia in humans, especially when SARS coronaviruses are involved. Indeed, these features are shared by SARS-CoV, SARS-CoV-2, and MERS-CoV (103). Even if too much attention has been given to ancillary direct viral damage of other tissues, infection of the lung respiratory epithelium and alveolar damage are the hallmark of COVID-19 (65). This damage is rapidly associated with the involvement of both capillaries and lung interstitium.

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High-resolution chest computed tomography (HR-CT) well reflects the evolution of pathological lung changes and the course of COVID-19 (72). In the early stage, radiological images are characterized by multilobar ground-glass opacities (GGO) with a peripheral or posterior distribution. GGO are commonly seen also in other lung diseases characterized by microvascular transudate or exudate filling alveolar spaces. Next, maybe depending also on the viral load, there is an increase in both number and size of GGO and progressive transformation into bilateral multifocal consolidative opacities and septal thickening, with development of a "crazy paving" pattern and a possible evolution in fibrosis (72).

In the early phases, typical pathological findings are enlarged microvessels, alveolar exudation of plasma, and hyaline membrane formation, in the absence of significant inflammatory cell infiltration (93, 103). The very few anatomical and histological studies in humans (93, 103) and macaques (70) published to date found pneumocyte necrosis (involving not only type II but also type I pneumocytes) with reparative replication and vascular (capillary) leakage of protein and fibrin-rich edema filling patchy lung areas. Neutrophils and lymphocytes are not prominent (70), and the subsequent cytokine production after lung damage is likely part of the reparative response. The evolution of alveolar exudation from damaged and leaky capillaries can be toward extensive fibrosis due to secondary hyperstimulation of local fibroblasts, which is believed to be driven by Ang II/AT_1R. The clinical picture is finally ascribable to the acute respiratory distress syndrome (ARDS), a common arrival point for many acute viral respiratory diseases in which the dysregulation of the local RAS pathways has been found to exert a key role (44).

Disequilibrium Between Classic RAS and Anti-RAS Arms in Lung Diseases

An imbalance between the classic RAS and the ACE2-driven anti-RAS arms is very likely to promote and accelerate lung injury. The ACE-Ang II-AT_1R pathway, especially when inappropriately activated, or in the context of ACE2 downregulation, induces pulmonary vasoconstriction and microvascular damage with increased vascular permeability, resulting in enlarged and "leaky" pulmonary microvessels and damaged alveoli filled with plasma proteins. Furthermore, secondary production of inflammatory cytokines, accelerated apoptosis in alveolar epithelial cells, and promotion of both mesenchymal cell growth and extracellular matrix synthesis leading to lung fibrosis have been demonstrated (94). All of these features are well-documented in coronavirus-mediated lung injury, as described above, and suggest an active role of the Ang II-AT_1R axis. On the contrary, Ang(1–7) was found to reduce inflammation, attenuate lung fibrosis, and improve oxygenation in acute lung injury, a noteworthy factor in protecting against ARDS-related poor prognosis (108). The use of different ARB or the less studied ACE-I, through reduction of Ang II/AT_1R stimulation and likely induction of ACE2 activity, has been effective in decreasing lung injury in animal models of ARDS (84).

A brief history on the topic tells us that ACE2 returned to the limelight in 2003, following the discovery that it serves as a receptor for the binding and entry of SARS-CoV (44). Similarly, SARS-CoV-2 recognizes ACE2 as its host receptor, binding to viral Spike protein, with a 10- to 20-fold higher affinity compared with SARS-CoV (100). After binding to ACE2, SARS-CoV-2 Spike protein conformational change triggers a proteolytic digestion mediated by host cell transmembrane protease serine 2 (TMPRSS2), allowing the virus entry into the cell (33). ACE2, besides being the gateway for the virus, exerts many favorable actions in lung. In fact, previous well-conducted studies found that ACE2 can protect lungs from severe acute injury in mice through different mechanisms, while the Ang II-AT_1R pathway promotes lung disease, leading to leaky pulmonary blood vessels, and impairs lung function (35). Ye and Liu showed how ACE2 injection can attenuate inflammatory response, by inhibiting the lipopolysaccharide (LPS)-TLR4 pathway in mice with LPS-induced acute lung injury (107). Furthermore, previous studies on SARS and MERS showed that the binding of the viral surface Spike protein to ACE2 leads to its downregulation, through its internalization and probably its shedding (35). Importantly, ACE2 downregulation itself results in a hypereffective Ang II-AT_1R pathway, promoting ARDS development. It has also been hypothesized that ACE2 downregulation could be linked to the systemic endothelial damage and coagulopathy observed in several COVID-19 cases, through an abnormal secretion of sFlt-1, a decoy receptor of placental growth factor (PLGF), mediated by Ang II, such as in preeclampsia (23). A study conducted in Shenzhen on 12 COVID-19 patients found that plasma samples from infected patients had significantly increased levels of Ang II compared with healthy individuals, and these levels were strongly associated with viral load and lung injury severity (48). Also, in H5N1 influenza, another viral infection that can lead to ARDS, a marked elevation of Ang II levels was linked to disease severity and fatal outcomes in humans (113). This suggests that Ang II, although not always easy to assay, is likely a good biomarker with a predictive value for lung injury.

Experimental SARS-CoV infection of wild-type mice in vivo led to reduced ACE2 expression in lungs, suggesting that reduced ACE2 expression might have a role in SARS-CoV-mediated severe acute lung disease (44). In a mouse model of acid aspiration-induced lung injury, the addition of SARS-CoV Spike protein decreased ACE2 and increased Ang II levels. After the AT_1R blockade with losartan, ACE2 increased, and the acute severe lung injury in Spike-Fc-treated mice was attenuated. More importantly, even pulmonary edema was attenuated after AT_1R blockade (44, 84). Therefore, SARS-CoV infections are likely to induce acute lung injury and failure through an imbalance between the classic RAS and the ACE2-driven anti-RAS arm, a process that could be attenuated by the AT_1R blockade (35). Further insights of this mechanism were elucidated in a cellular and rodent model, where ACE2 was found to physically interact with AT_1R on the cell membrane, and, through ACE2-AT_1R stabilization, AT_1R blockade with ARB prevented the internalization and lysosomal degradation of ACE2 mediated by Ang II (15). In addition to SARS coronaviruses, ACE2 also mediated the severe acute lung injury induced by respiratory syncytial virus infection in an experimental mouse model in which ACE2 deficiency worsened the disease pathogenesis mainly by its action on the AT_1R (26). The potential benefit of ACE2 in viral-mediated lung injury was also observed in mice infected by H7N9 influenza virus (106) in which worse lung injury and survival were

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observed in ACE2 knockout mice. Moreover, in three different acute lung injury models, loss of ACE2 expression precipitated severe acute lung failure, whereas treatment with rhACE2 attenuated ARDS and further reduced Ang II levels in lungs (35). The severity of acid-induced lung injury in ACE2 knockout mice was also reduced through pharmacological AT_1R blockade, whereas inhibition of AT_2R had no apparent effect on the acute lung injury phenotypes, confirming the causative role of Ang II in acute lung failure mediated mainly by AT_1R activation, instead of AT_2R (35).

All of these findings suggest an important protective role of AT_1R blockade in virus-related lung injury, in which ACE2 downregulation promotes a dangerous imbalance between the RAS pathways (Fig. 2*A*, *bottom*), subverting the alveolar-capillary homeostasis through what we like to define an "Ang II/AT_1R storm." The acute increase in the local ratio of Ang II/Ang(1–7) following ACE2 internalization is likely to exacerbate the pulmonary tissue damage initially provoked by pneumocyte viral infection in SARS disease, and most likely also in COVID-19. However, while a reduced ACE2 expression after SARS-CoV infection has been demonstrated (64), whether SARS-CoV-2 also induces ACE2 downregulation in humans is yet to be fully determined, although it can be extrapolated.

At the same time, the ACE2 overexpression following ACE-I and ARB administration is not fully consistent among studies and may differ between the two drug classes and the organs taken into account. Among ARB, olmesartan, losartan, valsartan, candesartan, telmisartan, and irbesartan all increased ACE2 levels in a mouse model, mainly referred to heart tissue (96), while there is very limited data for ACE-I showing upregulation of ACE2. ARB are likely to upregulate ACE2 activity because of the AT_1R blockade and the increased substrate load of Ang II (Fig. 2*B*, *top*). On the other hand, ACE-I enhance the Ang(1–9) stimulation of AT_2R (Fig. 2*B*, *bottom*) with specific protective consequences, including reduction of inflammatory cytokines in a rat model of induced lung injury and pulmonary hypertension (8). Moreover, a distinction should be made between membrane-bound and soluble ACE2 because an increase in soluble ACE2 might imply a decrease in membrane-bound ACE2 even with opposite consequences. In several animal models, both ACE-I and ARB could upregulate ACE2, especially in heart tissue (19, 37, 61), but it is not known to happen in human lungs. Human studies reporting ACE2 upregulation following ARB treatment took into account only soluble ACE2 levels (1, 22, 68), as long as in vivo membrane-bound ACE2 measuring in human tissues is technically challenging. Urinary ACE2 was found to increase after treatment with ARB in hypertensive and nephropathic patients, but it is not possible to draw conclusions given the scarce evidence and the complexity of the measurement (1, 22). We do not know if ACE2 plasma levels reflect its tissue expression or if upregulation would occur in all tissues in the same way. Further evidence is needed to clarify this topic. In any case, it is necessary to distinguish between the ability of these drugs to increase ACE2 expression, yet to be demonstrated, and their ability to modulate/increase the ACE2 activity, for example, through the regulation of substrates, which instead appears more conceivable by the different experimental models (Fig. 2*B*).

As mentioned above, the lung disease following SARS-CoV-2 infection can evolve into pulmonary fibrosis. In lung tissues, the activation of the Ang(1–7)-MasR axis attenuates inflammation, endothelial dysfunction, tissue damage, and fibrosis in different pathological models, such as pulmonary hypertension, asthma, pulmonary emphysema, and idiopathic pulmonary fibrosis (6, 51, 52, 85–87). The attenuation of key signaling events in the initiation and progression of cellular fibrosis, such as MAP kinases ERK1/2, p38, and JNK, and the reduced Smad phosphorylation, may be involved in the antifibrotic actions of Ang(1–7), counteracting Ang II-induced migration and both transforming growth factor-β1 (TGF-β1) and collagen expression of pulmonary myofibroblasts (59). TGF-β1 is a key factor in vascular-related fibrosis induction (76). Ang(1–7) effectively inhibits epithelial-mesenchymal transition induced by TGF-β1 via disruption of the TGF-β1-Smad signaling pathway in alveolar epithelial cells (83). Nevertheless, Ang(1–9) was found to activate AT_2R in monocrotaline-induced pulmonary hypertensive rats, ameliorating both lung injury and pulmonary vascular remodeling together with a reduction of apoptosis genes and inflammatory cytokine levels (8).

The pathophysiological potential benefits of RAS inhibitors in acute lung injury based on experimental studies are described in Table 1.

Evidence of RAS Modulation Efficacy in Lung Injury: From Experimental Animal Models to Clinical Data

Some clinical studies support the concept that ACE-I and ARB may be useful in patients with acute lung injuries. However, evidence in humans regarding RAS modulation and lung disease are limited to small pilot studies or retrospective studies, although all agree on favorable outcome. On the other hand, clinical data on the possible direct benefits of ACE2/Ang(1–7)/MasR arm overstimulation in humans are still lacking to date. A meta-analysis of 37 studies showed reduced risk of pneumonia-related mortality in patients treated with ACE-I or ARB compared with control treatments (7). Patients with ARDS taking ACE-I or ARB showed better survival compared with controls in a retrospective case-control study (42). Another retrospective study on 539 patients with viral pneumonia showed that ACE-I use was associated with lower rates of intubation and death (32). The rhACE2 infusion in a randomized double-blind pilot trial on ARDS patients decreased Ang II and increased Ang(1–7) with a decrease in interleukin-6, although it had no power to detect changes in acute physiology or clinical outcomes (41).

Regarding COVID-19, several observational retrospective studies on different populations have been published recently. All studies found neutral or even beneficial effects of RAS inhibitors in COVID-19, regarding both the risk of getting infected and the risk of greater disease severity or death (50). Patients taking RAS inhibitors certainly do not have a higher risk of SARS-CoV-2 infection (3, 20, 53, 56, 69). Furthermore, treatment with ACE-I/ARB does not affect hospitalization for COVID-19 (13) or disease severity (28, 69). A retrospective analysis on 1,128 Chinese hypertensive patients found a significant lower risk of death in patients taking ACE-I/ARB compared with other antihypertensive drugs after adjustment for confounders (109). In small samples of COVID-19, ACE-

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Table 1. *Pathophysiological and clinical potential benefits of RAS inhibitors in COVID-19, based on experimental and clinical studies*

Pathophysiological Benefits	Clinical Implications
Direct effects on the lung	
Less injury to lung alveolar cells and capillary endothelial cells and enhanced repair by MSCs	Preservation of functioning alveolo-capillary membrane for proper gases exchanges
Less vascular permeability	Reduced alveolar/interstitial transudation of fluids and exudation of proteins and cells
Less proinflammatory cytokines production	Reduced inflammation
Less interstitial-mesenchymal cell proliferation and less production of TGF-β	Reduced pulmonary fibrosis and preserved lung compliance
Less coagulation cascade activation within the pulmonary vasculature	Reduced pulmonary vascular thrombosis
Less lung oxidative stress (less ROS) and vasodilation	Reduced pulmonary arterial pressure
Indirect effects on the lung (cardiovascular, renal, and metabolic effects)	
Less angiotensin II and aldosterone production with less sodium reabsorption and vasodilation	Reduced blood pressure and HMOD
Less VSMC and myofibroblast proliferation and migration	Reduced myocardial, glomerular, and systemic vascular fibrosis
Less oxidative stress (less ROS) with improved endothelial function and less inflammation	Reduced atherosclerosis and proteinuria
Less coagulation cascade activation within the systemic vasculature	Reduced risk of systemic thromboembolism
Less mitochondrial dysfunction	Reduced insulin resistance and balanced energy metabolism

TGF-β, transforming growth factor-β; MSCs, mesoderm-derived mesenchymal stem cells; ROS, reactive oxygen species; HMOD, hypertension-mediated organ damage; VSMC, vascular smooth muscle cells.

I/ARB therapy affected both IL-6 and peripheral T cell levels (57), being associated with lower levels of inflammatory indices (105). Other observational studies found no association between COVID-19 mortality and ACE-I/ARB treatment (20, 40, 45). Nine observational studies on COVID-19 hypertensive patients have been recently included in a meta-analysis showing that patients taking ACE-I/ARB treatment had a 43% lower risk of mortality for COVID-19 compared with patients not taking ACE-I/ARB (28).

The consistent favorable data on both in vitro and in vivo studies on animals regarding the imbalance of RAS arms and virus-mediated lung injury are leading to randomized controlled trials (RCTs) aimed at obtaining consistent clinical data in COVID-19 patients. One of these RCTs regards the use of rhACE2, aiming at evaluating viral load over time (NCT04287686), while two other RCTs with losartan are ongoing to investigate the possible benefits in COVID-19 patients requiring or not requiring hospitalization (NCT04312009; NCT04311177).

Independent from the mechanisms in acute lung injury, RAS inhibitor withdrawal may cause severe clinical decompensation in high-risk CV patients (30) in which lung infection, per se, is associated with increased risk of CV events (12). This should be a fundamental warning for patients with COVID-19, which is likely to result in a RAS overactivation mediated by Ang II activity, thus exacerbating the cardiac damage that may occur in critically ill patients (48), such as myocardial injury (27, 48, 88), myocardial stress, and cardiomyopathy (5). Indeed, a possible direct cardiac involvement of SARS-CoV-2 has been demonstrated (36). Furthermore, prevalence of HF has not been adequately assessed in the first reports from China, but it is likely to be very common, especially in older COVID-19 patients. Up to 32% of deaths for COVID-19 are due to a failing heart after myocardial injury in a Chinese case series (71). Older inpatients with multiple comorbidities often have an underlying HF (81). Previous studies found that ACE-I/ARB therapy was associated with lower risk of death, likely due to a better CV protection and a higher CV resistance to acute illness, in older patients hospitalized for medical conditions, including infections (91). This association could be theoretically true even for COVID-19 patients. Therefore, it is important to stress the concept that patients affected by HF or other CVD taking ACE-I and ARB as per indication still continue to adhere to their prescribed regimens, even in the COVID-19 era (77). The main potential clinical benefits of RAS inhibitors in COVID-19 are described in Table 1.

Possible Role of RAS in the Severity of COVID-19 According to Sex and Obesity

Based on currently available data, especially in U.S. and European cohorts, obesity is one of the main risk factors for severe COVID-19 course and death, particularly among younger patients without comorbidities (82). Obese patients aged <60 yr have a double risk of critical care admission compared with normal-weight patients that become seven-fold higher in patients with a body mass index (BMI) ≥ 35 kg/m^2, and obesity is associated with higher need of invasive mechanical ventilation (46, 89). Obesity, per se, is related to the main CV risk factors and CVD, such as coronary heart disease, chronic kidney disease, and HF, predisposing to adverse outcomes (75, 92). The RAS dysregulation in obese patients is well known, as well as the role of adipose tissue in upregulating RAS activity, both locally and systemically (79). Furthermore, food intake plays a key role (21). Indeed, upon overfeeding stress, almost 20–30% of circulating angiotensinogen is produced by white adipose tissue, favoring obesity-related hypertension (54), with Ang II acting as a "growth factor" for adipocytes. In obese hypertensive patients, "inappropriately normal" levels of plasma renin activity and aldosterone have been documented, even in patients taking ACE-I or ARB, with consequent vascular damage (78, 90). It is reasonable to speculate that the Ang II/AT_1R storm promoted at the lung level by the SARS-CoV-2-induced ACE2 downregulation, might be even much worse in overweight/obese patients, who

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have both Ang I and Ang II increased levels coming from visceral adipose tissue to the systemic venous circulation, and then to the pulmonary microcirculation (82).

Near obesity, male sex has been associated with a worse COVID-19 course and a greater need for intensive care compared with female sex (60). An Italian report found that 82% of COVID-19 patients referred to intensive care units were males (25). Animal models of obesity have shown sex differences in expression, activity, and tissue responsiveness of RAS components. Importantly and differently from males, obese females were likely protected from hypertension and metabolic complications induced by Ang II-AT_1R pathway activation, through upregulation of the anti-RAS pathway, such as ACE2/Ang(1–7)/MasR (97). Estrogenic activity decreases the vascular response [vasoconstriction and NAD(P)H oxidase activation] to Ang II and facilitates the action of ACE2/Ang(1–7) in animal models (43). Moreover, obese women have more subcutaneous than proinflammatory visceral adipose tissue, and adipocytes are smaller, more lipogenic, and insulin sensitive than obese males (97). Finally, the ACE2 gene is X linked, and females have higher ACE2/Ang(1–7) levels at least in animal models (29, 43). All of these findings could affect the clinical course of COVID-19.

Potential Role of Angiotensin Receptor-Neprilysin Inhibitor in Lung Disease and COVID-19

All of the pharmacological strategies able to prevent CV complications should be implemented in COVID-19 patients, given their relevance on disease prognosis (11). ACE-I and ARB are cornerstone not only for hypertension but also for HF treatment. An emerging topic of interest is the potential role of angiotensin receptor-neprilysin inhibitor (ARNI) sacubitril/valsartan in patients with pulmonary vascular remodeling, pulmonary hypertension, and right ventricular overload related to lung diseases (10). The synergistic effects of RAS and neprilysin inhibition, versus RAS inhibition alone, may magnify the protective benefits of natriuretic peptides by reducing fibrosis and vascular remodeling and promoting vasodilation and natriuresis (80). Given the prevalence and the negative prognostic role of myocardial injury in COVID-19 patients (11), ARNI may not only be effective in reducing morbidity and mortality in patients with chronic symptomatic HF with reduced ejection fraction (HFrEF) (55) but may also counterbalance the Ang II-AT_1R pathway, which has been implicated in maladaptive right ventricular hypertrophy and fibrosis associated with pulmonary hypertension (10). Indeed, neprilysin inhibition is intimately related to RAS activation, but it is unclear whether ARNI has any impact on ACE2 or in COVID-19 patients. Caution is needed because circulating neprilysin can convert Ang I in the lung-protective Ang(1–7) (66). Therefore, sacubitril could partially mitigate this conversion. ACE-I could amplify the attenuation of this pathway by increasing Ang I levels, and the association with neprilysin inhibitors is contraindicated, while the concomitant use of ARB could theoretically counteract this negative effect by increasing Ang II levels and bypassing the direct role of neprilysin in the RAS pathway.

CONCLUSION AND PERSPECTIVES

The balance between RAS pathways in the lung is likely to be of fundamental importance in the genesis and promotion of acute lung injury, but it is complex, and more data on humans are needed. Hasty speculations on a negative relationship between RAS blockers and COVID-19 are not justified by our present knowledge. Hypertensive patients, especially if having CVD such as HF, should continue to take these drugs with high adherence to the prescribed regimens. On the other hand, the administration of ACE-I or ARB aiming at preventing COVID-19 or reducing the disease severity is still not supported by conclusive data, although preclinical evidence and several observational findings are encouraging. Therefore, if on one hand there is solid evidence of the benefits of RAS modulation in CVD, on the other hand caution and clinical equipoise must be maintained regarding RAS modulation in COVID-19. There is a clinical rush toward several COVID-19 "therapies," such as old antimalarial drugs with possible serious cardiac side effects or low-molecular-weight heparin, assuming that COVID-19 is primarily a coagulation disorder of the pulmonary circulation, or monoclonal antibodies against many different interleukins to counter the cytokine "storm" caused by the direct virus-driven lung damage. In this chaos, research on drugs that interact with RAS pathways is probably the most founded on a solid pathophysiological basis. Further evidence is urgently needed to discover more effective therapies for COVID-19.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.D.P. prepared figures; R.S., F.G., C.D.P., and F.S. drafted manuscript; R.S., F.G., and P.G. edited and revised manuscript; R.S., F.G., C.D.P., P.G., and F.S. approved final version of manuscript.

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