

Renin Angiotensin Axis, Angiotensin Converting Enzyme 2 and Coronavirus

Eje Renina Angiotensina, Enzima Convertidora de Angiotensina 2 y Coronavirus

F. Cano^{a,b}, M. Gajardo^{b,c}, M. Freundlich^d

^aPediatric Nephrology Unit, Luis Calvo Mackenna Hospital. Santiago, Chile

^bMedical School, University of Chile. Santiago, Chile

^cPediatric Nephrology Unit, Roberto del Rio Hospital. Santiago, Chile

^dDivision of Pediatric Nephrology. Miller School of Medicine. Miami, Florida, United States

Received: May 20, 2020 Accepted: May 31, 2020

What do we know about the subject matter of this study?

The SARS-CoV-2 pandemic has highlighted the important role of the Renin Angiotensin System (RAAS) in viral infection at both the pulmonary and systemic levels, in particular the role played by Angiotensin Converting Enzyme 2 as a receptor for the Coronavirus.

What does this study contribute to what is already known?

This article reviews previous experiences on Coronavirus and SARS infection, and provides a physiopathological view of SARS-CoV-2 infection that helps to understand the relationship between ACE2 as a viral receptor and at the same time as a stabilizer of the Angiotensin 2-mediated inflammatory cascade activated by SARS-CoV-2.

Abstract

The renin-angiotensin-aldosterone system (RAAS) is the main plasma volume regulator, which maintains cardiovascular and hydrosaline homeostasis. In the classical pathway, the angiotensin-converting enzyme (ACE) generates Angiotensin II (AngII), which is powerfully inflammatory and vasoconstrictive. This classical pathway is also regulated by ACE2, which converts AngI to Ang 1-9, and degrades AngII to Ang 1-7, whose vasodilatory and anti-inflammatory functions balance out the effects of AngII. ACE2 has been associated with the pathogenesis of respiratory infections such as RSV and severe acute respiratory syndrome coronavirus (SARS-CoV and SARS-CoV-2). Recent studies have shown that ACE2 corresponds to the main SARS-CoV-2 receptor, which together with other receptors such as the TMPRSS2, allows the virus to attach, fuse, and enter the host cell. These studies have shown that in animals infected with coronavirus there is a drop in tissue concentration of ACE2 and Ang 1-7, leading to overexpression of AngII and its vasoconstrictive and inflammatory effects. Experiments with recombinant ACE2 have shown a protective effect against overexpression of RAAS

Keywords:

Renin; Angiotensin; Converting Enzyme; ACE2; Coronavirus; AT1 Blockers; COVIR

Correspondence:
Francisco Cano S.
fcanosch@gmail.com

in coronavirus-infected animals, which is similar to that demonstrated with the use of AngII receptor blockers (AT1). Evidence on the protective role of ACE2 seems to support the recommendations regarding not discontinuing these drugs in COVID-19 infection. In this article, we present the current knowledge about the role of RAAS in coronavirus infection, based on physiopathological concepts, molecular bases, and experimental and clinical evidence.

Abbreviations

RAAS:	Renin-Angiotensin-Aldosterone-System
AngI:	Angiotensin I
AngII:	Angiotensin II
ACE:	Angiotensin converting enzyme
ACE2:	Angiotensin converting enzyme 2
AT1:	Angiotensin II type 1 receptor
AT2:	Angiotensin II type 2 receptor
Ang 1-7:	Angiotensin 1-7
Ang 1-9:	Angiotensin 1-9
RSV:	Respiratory Syncytial Virus
SARS-CoV:	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2:	Coronavirus 2 COVID19
TMPRSS2:	Serine Transmembrane Protease 2 Associated with the Host Surface
RNA:	Ribonucleic Acid
rACE2:	Recombinant Angiotensin 2 Converting Enzyme
KO:	Knock Out
ICU:	Intensive Care Unit
AKI:	Acute Kidney Injury
RRT:	Renal Replacement Therapy
FGF23:	<i>Fibroblast Growth Factor 23</i>

Key Concepts

- The RAAS is closely linked to the body's response to SARS-CoV infection.
- ACE2 corresponds to the critical coronavirus receptor, which acts in conjunction with the TMPRSS2 protease
- Increased AngII and decreased ACE2 are characteristic of RSV and SARS-CoV infections.
- Deficiency of primary (ACE2 knockout animal model) or secondary (SARS-CoV and SARS-CoV-2 infection) aggravates acute lung damage.
- ACE2 plays an important protective role against SARS-CoV infection.
- The use of ACE2 has been shown to be protective against inflammatory changes mediated by AngII.
- Blocking of the AT1 receptor by blockers such as Losartan inhibits the inflammatory response to SARS-CoV infection

Introduction

The renin-angiotensin-aldosterone system (RAAS) is the main regulator of plasma volume in mammals and plays a central role in sodium and cardiovascular homeostasis. The RAAS exerts vasoconstrictor and water/sodium retentive actions, therefore derangements in these physiological functions may result in hypertension and cardiovascular diseases¹.

Activation of the so-called “classical pathway” of the RAAS begins in the juxtaglomerular apparatus with the release of preformed renin from its precursor prorenin, secondary to baroreflexes, beta-adrenergic or molecular stimuli in the macula dense. Renin cleaves the angiotensinogen precursor in the liver into AngI². This decapeptide, without specific biological action, is converted to the octapeptide AngII (Ang 1-8) by the effect of ACE, a metalloproteinase mostly located in lung endothelial cells, as well as in other tissues. AngII exerts effects via the AT1 receptor producing systemic vasoconstriction, increasing oxidative stress with subsequent inflammatory and pro-fibrotic phenomena, and releasing aldosterone from the adrenal cortex to promote reabsorption of sodium and water in aldosterone-sensitive segments of the nephron.

In recent years, it has become clear the existence of an alternative arm within the RAAS to counterbalance the classical pathway, as well as local RAAS pathways in various organs that would function independently, via autocrine and paracrine mechanisms⁴.

The RAAS and ACE2

The discovery and characterization of the Ang 1-7 peptide almost three decades ago, prompted the discovery of the “non-classical” counter-regulatory system of AngII actions. This heptapeptide is generated by cleavage of AngI by a variant of ACE called ACE2⁵. ACE is an essential component of the RAAS, produced by the endothelium of various tissues, and constitutes a transmembrane protein with two active catalytic domains of the N and C type terminals. The cleavage of the terminal C domain generates the soluble carboxypeptidase that removes the carboxyterminal di-peptide from AngI, generating AngII, while the hydrolysis of

the vasodilator peptides bradykinins is produced by the enzymatic action of both domains⁶. ACE2, identified simultaneously by Donoghue and Tipnis in 2000^{7,8}, is a monocarboxypeptidase homologue of ACE, with a single transmembrane helix, with an intracellular segment with N and C terminal domains, but with a single active enzymatic site that provides different characteristics from ACE. Its synthesis is regulated by the ACE2 gene located on the X chromosome (Xp22) which codes for its 805 amino acids⁹. When initially described, its effect was to convert Ang I into Ang 1-9 by removing the terminal C residue^{7,8}, which led through an enzymatic process to the production of Ang 1-7. Later studies^{10,11} emphasized the important action in the degradation of Ang II towards Ang 1-7, which acts through the transmembrane receptor coupled to a G protein ‘MAS’, and exerts the vasodilatory, anti-inflammatory and cardioprotective actions mediated by nitric oxide (NO) that limit the vasoconstrictive effect of Ang II (Figure 1). The distribution of ACE2 is more restricted than ACE, mostly located in heart, kidney and testes, but Harmer et al. in 2002 demonstrated their transcriptional expression in the lung (type 2 alveolar epithelial cells and pulmonary blood

vessels), and in the gastrointestinal system^{12,13}. The demonstration of pulmonary expression was followed by studies describing the associations and role of ACE2 in the pathogenesis of respiratory infections such as RSV, Influenza A (H1N1), SARS-CoV and recently SARS-CoV-2¹⁴.

Coronavirus and ACE2

More than a decade ago several authors¹⁵⁻¹⁷ studied the ACE2 receptor using Vero E6 African monkey cells that allowed replication of SARS-CoV naturally. By infecting 293T host cells transfected with a plasmid expressing ACE2 with SARS-CoV, they observed that the SARS-CoV spike protein was specifically associated with cells expressing the ACE2 receptor but did not with those transfected with a control vector. Furthermore, the authors observed that ACE2 interacted with the S1 domain of the spike protein, but not with ACE, and that the virus replication on Vero E6 cells was specifically inhibited by anti-ACE2 antibodies. Numerous publications have shown that the entry of SARS-CoV-2 into the host cell depends on the interaction between the spike protein and the ACE2 receptor¹⁷⁻²⁰. There is

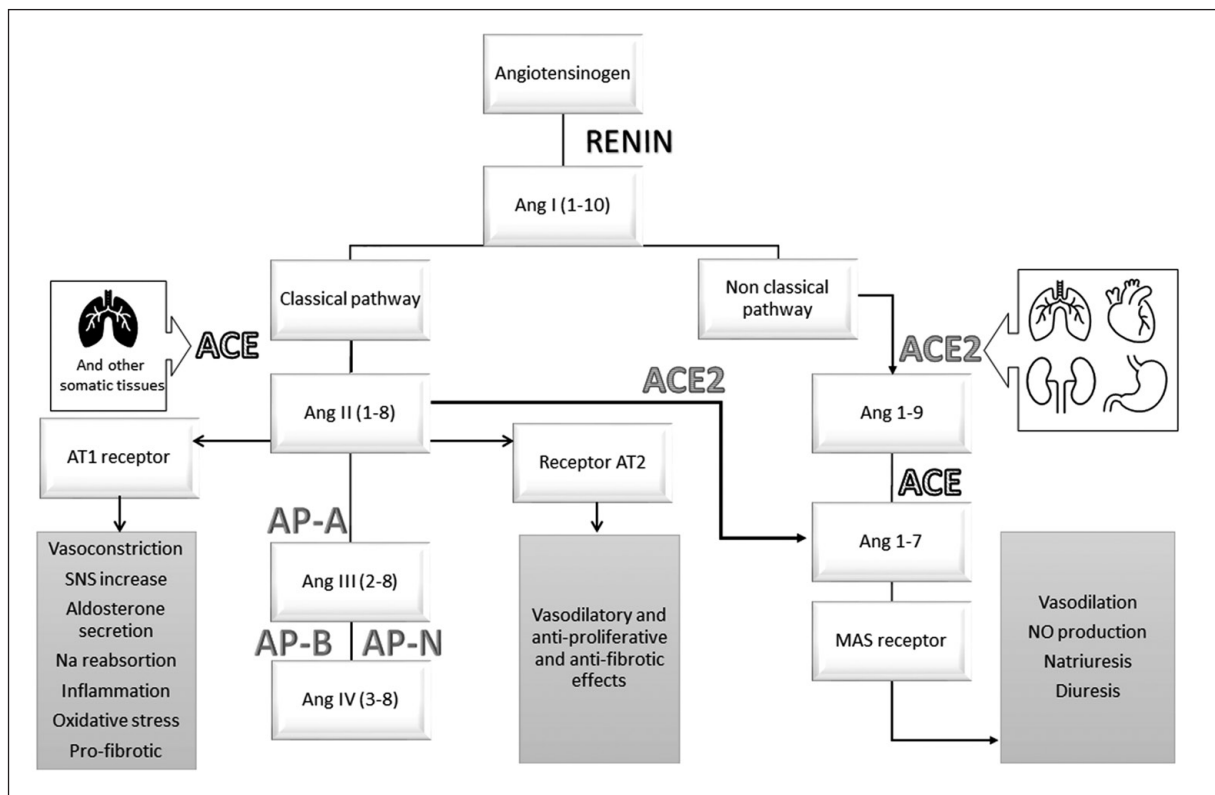


Figure 1. Renin angiotensin aldosterone system, classical and non-classical pathways and their principal actions. Ang: angiotensin. SNS: sympathetic nervous system. Na: sodium. ACE: angiotensin converting enzyme. AP: antipeptidase. ACE2: angiotensin converting enzyme 2. NO: nitric oxide.

evidence that there are also other equally important receptors for the entry of SARS-CoV-2, in particular the TMPRSS2 protein which has been identified as a second receptor necessary for this process. Its interaction is essential for the priming of SARS-CoV-2 in the cell, and its inhibition suppresses the entry of the virus into the alveolar cell¹⁸.

The first step in the viral invasion is the binding of the virus spike protein to a high affinity receptor in the cell, the ACE2 protein. A characteristic of the surface proteins in SARS-CoV is their structure formed by an N-terminal surface unit (S1), which houses the receptor-binding domain, and a C-terminal transmembrane unit (S2), which corresponds to the domain required for fusion to the cell membrane. Proteolytic

cleavage of both S1 and S2 units by the ACE2 and TM-PRSS2 proteases is critical for virus fusion to the cell membrane, since the fixation segment of the S1 unit is located at the C-terminal end, and the fusion segment of the S2 unit is located at the N-terminal end. After cleavage, the fusion segment of the S2 unit adheres to the cell membrane allowing entry of viral RNA. The location of proteases in the cell membrane determines whether this fusion and entry is via endosomes, with release of viral genetic material mediated by L-Cathepsin, or directly by interaction of the TMPRSS2 protease with ACE2¹⁵⁻²¹ (Figures 2 and 3). The integrated genomic study by Guzzi et al.¹⁷ showed that the pathogenic effect of the virus depended fundamentally on the ACE2 receptor, which decreases its expression

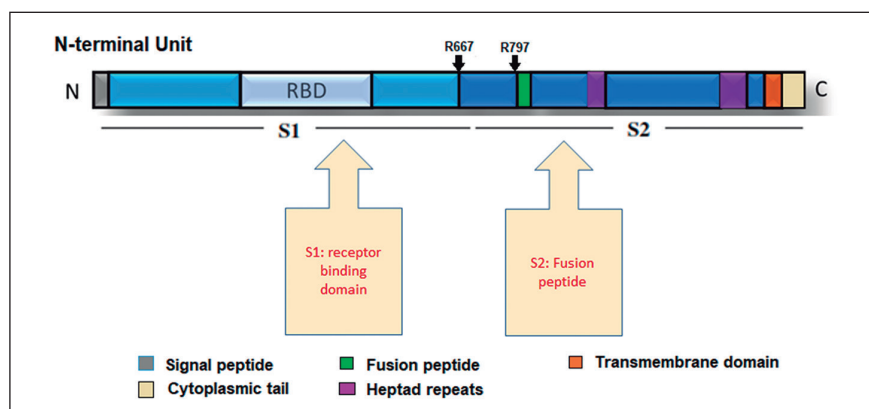


Figure 2. Domain organization of the SARS-CoV-2 spike protein. The split of S1 surface unit and S2 transmembrane unit because of host cell proteases is prerequisite for fusion to the membrane cell. Modified from²¹. N y C: amin and carboxylate terminal Unit. RBD: receptor binding domain. R667 y R797: position of the amino acids cleaved by cell proteases.

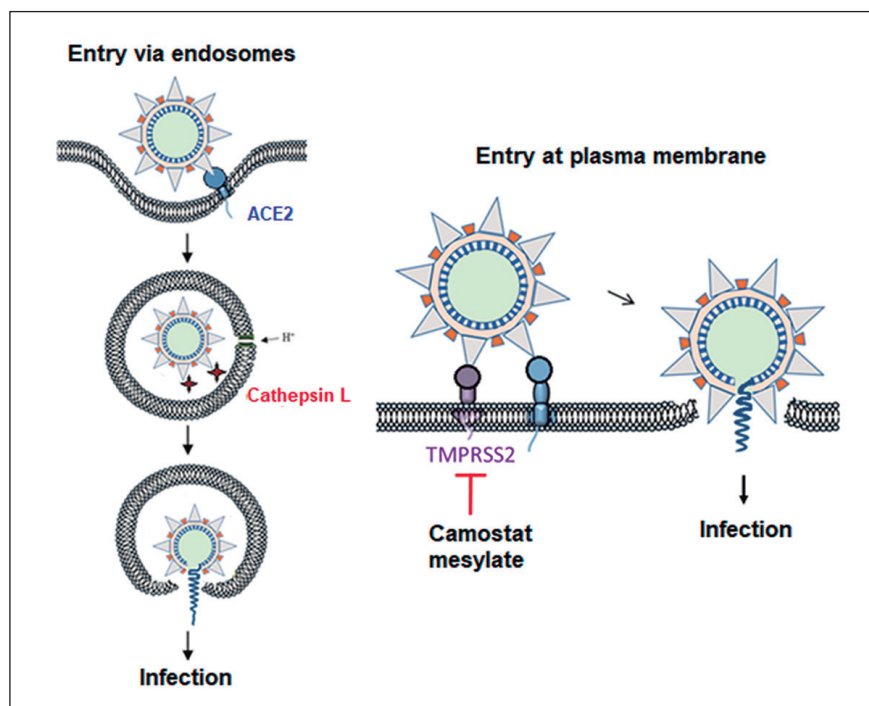


Figure 3. Routes employed by SARS-CoV-2 for entry into host cells. Entry by ACE2 endosomes and by proteases TMPRSS2-ACE2. Modified from²¹. ACE2: angiotensin converting enzyme 2.

due to the effect of SARS-CoV-2, in agreement with preclinical studies in which recombinant (r) rACE2 was administered to compensate for the loss of the enzyme, resulting in an attenuation of the inflammatory effects associated with the viral infection²². Functionally there are 2 forms of ACE2, a complete amino acid sequence enzyme with an extracellular domain that functions as the spike protein receptor, and a soluble form that lacks the domain of S1 binding to the cell membrane and exists in small circulating quantities²³. This soluble form can act as a competitor-interceptor of the virus, preventing the fixation of the spike protein to the cell membrane. The extracellular domain of the enzyme fused to the Fc segment of immunoglobulin has demonstrated neutralization capacity of SARS-CoV-2 *in vitro*²⁴.

These findings raise the following question: what is the evidence linking a system that regulates salt and water balance and exerts vasopressor properties with the pathophysiology of SARS-CoV-2 infection? A recent study published by Gu et al.²⁵ contributed to the understanding of the role of SARS and ACE2 in a RSV model. Based on the hypothesis that SARS and ACE2 mediated RSV-induced lung damage, they evaluated RAAS levels and the inflammatory response in a preclinical model of ACE2 KO neonatal rats and studied children with RSV. They showed that in 34 RSV-infected children the plasma concentration of AngII significantly exceeded that of 20 controls, a difference that disappeared in the regression phase of the disease, suggesting that AngII may be involved in the virus-induced inflammatory response. Following the intranasal injection of RSV into healthy rats, they observed similar peak levels of AngII as previously noted in children on the third day of infection, concluding that the RAAS plays a pathogenic role in the lung infection by RSV. To determine the possible role of ACE2 as a mechanism responsible for the observed rise in AngII, they measured ACE2 levels obtained from pulmonary homogenates of RSV-infected rats and showed significantly decreased ACE2 levels coinciding with the rise in circulating AngII in the infected rats, strengthening the pathophysiologic links associating ACE2 with viral pathogens.

The molecular mechanisms underlying the role of ACE2 as a viral receptor for SARS-CoV had already been confirmed by different authors. Kuba et al.¹⁶ studied ACE2 KO rats and healthy controls, which were infected with SARS-CoV, subsequently quantifying the viral load and the number of copies of the spike molecules in lung tissue. Both viral load and spike molecule copy numbers were significantly decreased in the group of mutant rats lacking ACE2, concluding that the presence of ACE2 is essential for SARS-CoV to invade and replicate in alveolar epithelial cells. Downre-

gulation of the ACE2 receptor has been associated with a more severe course of the acute respiratory condition²⁶, leading to the hypothesis of a protective role of ACE2 on lung damage. This hypothesis has been confirmed in experimental models in ACE2 KO rats exposed to acute lung damage induced by acid aspiration, a classic model of acute lung injury, that received rACE2. The observed decrease in lung damage in this model confirmed the protective effects of ACE2 *in vivo*. Significant increases of lung inflammation markers such as edema due to increased vascular permeability and leakage of albumin, water and electrolytes, were observed in ACE2 KO rats by measuring vascular leakage with IV Evans blue and Dextran markers in acute lung damage models. These effects were significantly attenuated in AT1 -/- strains of rats, which cannot express the effects of AngII. Of note, similarly to other observations²⁵, a significant increase in the expression of pulmonary tissue AngII was observed by inducing lung inflammation. The observed AngII increase in models of lung inflammation, suggested a dysregulation of the normal balance between both RAAS pathways as a result of the lung injury, linked to the fall in the levels of ACE2. These authors demonstrated that, during the acute inflammation phase, ACE2 levels decrease while those of ACE remain unchanged, suggesting that the AngII pathway is enhanced. These observations as well as the reduced ANGII levels following genetic inactivation of ACE in both control and ACE2 KO mice, have served as the basis for therapies that block the RAAS in acute lung injury.

ACE2 as a protector of lung injury

The role of the enzyme ACE2 as a protective factor against lung damage has been suggested by several studies^{25,26} based on the demonstration that a deficit of ACE2 increases the severity of RSV-induced lung damage²⁵. In KO rats for ACE2 compared to normal RSV-infected rats, lung tissue inflammation and leukocyte infiltration were significantly elevated, while survival was found to be significantly decreased in those rats lacking ACE2 compared to controls. In KO rats for ACE2, lung tissue viral load and AngII expression were increased by up to five times compared with RSV-infected control rats. As in previous experiments, treatment of rats with rACE2 pre and post viral infection significantly attenuated, alveolar inflammatory changes, lung tissue viral load, and the rise in AngII, compared with control rats treated with placebo.

Recent reviews have attempted to bring together theories and explain the controversy that may be generated by considering ACE2 as a receptor and gateway to viral infection, and ACE2 as a potential protective

mechanism^{27,28}. SARS-CoV-2 would use the ACE2 receptor to enter and infect the cell, increasing pro-inflammatory cytokines, developing a cytokine storm and increasing viral replication, helped in the entry process by TMPRSS2, but at the same time the cellular infection would generate an internalization and decrease of the available ACE2 receptors, triggering the decrease of the soluble enzyme ACE2, with dysregulating of the RAAS leading to overexpression of the AngII pathway, generating the cascade of severe acute lung damage as demonstrated in several studies.

Angiotensin II receptor blockade and lung injury

A question of great clinical importance is whether ACE-AngII blockade can be protective against virus-induced lung damage, and how AT1 blockers affect the expression of ACE2²⁹. Kuba et al.¹⁶ evaluated the role of ACE2 in SARS-CoV infection by intraperitoneal injection of the purified Fc spike fraction of the virus, which was associated with a significant rise in AngII in lung tissue of control rats. Therefore, to confirm whether lung inflammation was dependent on SARS-CoV-induced rise in AngII, an AT1 receptor blocker was administered to previously treated animals, observing a significant decrease in pulmonary edema and inflammation in treated rats, suggesting that the inflammation and increase in AngII produced by the virus was directly related to the drop in ACE2 levels and overexpression of RAAS via ACE, with an increase in AngII-mediated effects. Since the decrease in ACE2 was related to an increase in mortality, with higher AngII levels and lung inflammation in ACE2 KO rats²⁵, the effect of AT1 blockade was investigated. Control rats infected with RSV displayed overexpression of AngII and lung inflammation as previously described. Losartan was administered at a dose of 15 mg/kg to one group of animals, while another group, equally infected, was treated with a placebo vehicle. The rise in AngII, inflammatory changes and lung parenchymal viral load were significantly decreased in rats treated with Losartan versus those treated with placebo. When repeating this experiment in ACE2 KO rats, the Losartan-treated group at day -1, 1 and 3 post-RSV infection showed decreased viral loads, decreased AngII levels, and milder inflammatory changes in lung tissue, in contrast to the severe damage documented in the placebo animals, suggesting a strong protective effect of AT1 blockade against viral infection²⁵. Blocking the TMPRSS2 receptor with the drug Camostat Mesylate (Figure 3), a serine protease inhibitor approved for the treatment of some gastrointestinal diseases, has emerged as a therapeutic option in SARS-CoV-2 infection¹⁸.

Recently Khan et al.³⁰ reported a phase 2 clinical trial evaluating the efficacy of GSK258688, a human rACE2 drug in patients with SARS-CoV, demonstrating that when applied 2 daily doses a rapid decrease in AngII levels was observed, along with an increase in Ang 1-7 in the patients' plasma. These initial studies should be corroborated, particularly concerning safety issues. There is a warning regarding the use of ACE2 as a potential cardioprotective agent after myocardial injury, because of the high incidence of arrhythmia and sudden death in treated transgenic animals with overexpression of ACE2^{10,31}.

Coronavirus, ACE2 and kidney

Recent studies suggest that up to 10% of confirmed cases for SARS-CoV-2 require hospitalization in the ICU, and of this percentage 60% have evidence of AKI, 20-30% requiring RRT³², a statistic confirmed by reports from Italy where 40% of hospitalized patients have required ICU, and 20% of them RRT. Recent reports of the current SARS-CoV-2 pandemic have shown variable but significant percentages of renal involvement^{33,35}. Naicker et al. reported 34% nephrotic range albuminuria in a cohort of 59 patients infected with SARS-CoV-2 on admission to hospital, 63% of them with massive proteinuria during hospitalization³³. Cheng et al. in a pre-print communication, reported 701 patients hospitalized with SARS-CoV-2, of which 43.9% presented proteinuria and 26.7% hematuria on admission to the hospital, 15% of this group presented increased blood urea nitrogen, with acute renal failure being an independent predictor of in-hospital mortality, reaching 12%³⁶.

A series of etiopathogenic mechanisms have been associated with AKI, hypovolemia, sepsis, cytokine storm, hypoxia, cardiogenic shock, acute tubular necrosis and direct renal damage caused by the virus. ACE2 is highly expressed in the tubular, glomerular and vascular epithelial cells of the kidney, where the downregulation of ACE2 balance determines the overexpression of AngII, resulting in tissue inflammation, and in this particular organ, through various possible mechanisms of AKI such as ischemia-reperfusion, endotoxemia and shock³⁷⁻³⁹. Changes in the ACE/ACE2 ratio, where their concentrations evolve in opposite directions after viral infection, have been used as a marker for the degree of tissue injury, suggesting that an increase in the ACE/ACE2 ratio may contribute to AKI as a result of AngII accumulation⁴⁰. In the context of chronic kidney disease, it has been shown that there are factors that can decrease the intrarenal concentration of ACE2. The phosphaturic hormone FGF23, which is elevated in AKI and associated in multiple studies

with cardiovascular mortality, directly suppresses the expression of ACE2 in the distal tubule and may thus contribute to the activation of AngII and increased renal damage⁴¹. Therapies aimed at increasing the renal concentration of ACE2 favoring the expression of Ang 1-7, are being evaluated through the use of ACE2 in different models of nephropathy, including AKI⁴². These experiences are based on previous studies with soluble rECA2 in kidney tissues of SARS-CoV-2 infected monkeys, which have shown inhibition of viral replication⁴³, as well as previous communications where treatment with rACE2 in RSV infected control rats showed a significant decrease in alveolar inflammatory changes, pulmonary tissue viral load, and increased AngII, with respect to control rats treated with placebo²⁵.

Unfortunately, there is currently no specific antiviral therapy or vaccine developed for SARS-CoV-2, therefore intensive research is underway on different therapies to control the infection. Patients recovering from previous outbreaks of the SARS-CoV family have a neutralizing antibody that can be detected up to 24 months post-infection⁴⁴ specifically directed at

the spike protein, inhibiting its ability to recognize the host cell receptor, or directly producing lysis of the viral particle. The importance and mechanisms of action of neutralizing antibodies in SARS-CoV infection have been the subject of multiple publications that discuss this therapy in detail⁴⁵⁻⁴⁹. Knowledge of the pathogenic mechanisms of SARS-CoV infection, its close relationship with RAAS and ACE2 -described in this review, will allow us to evaluate in the future the rationale behind the proposed therapies.

In the course of the current pandemic, concerns have been raised about the risk that ACE inhibitor and AT1 blocker therapies may represent a risk for more severe disease progression. The accumulated evidence on the protective role of ACE2 in slowing down the overexpression of the classical pathway and the increase of AngII seems to support the current recommendations of the scientific community regarding the non-suspension of these drugs against SARS-CoV-2 infection⁵⁰. Future lines of research on the different therapies mentioned in this article will help clinicians chart the optimal therapeutic strategies based on the best available evidence.

References

- Sparks M, Crowley S, Gurley S, et al. Classical renin-angiotensin system in kidney physiology. *Compr Physiol*. 2014;4:1201-28.
- Atlas S. The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. *J Manag Care Pharm*. 2007;13(8)(suppl S-b):S9-S20.
- Fournier D, Luft F, Bader M, et al. Emergence and evolution of the renin-angiotensin-aldosterone system. *J Mol Med*. 2012;90(5):495-508.
- Zhuo J, Ferrao F, Zheng Y, Li X. New frontiers in the intrarenal renin-angiotensin system: a critical review of classical and new paradigms. *Front endocrinol*. 2013;4:166.
- Ferrario C, Chappell M, Tallant A, et al. Counterregulatory actions of angiotensin-(1-7). *Hypertension*. 1997;30:535-41.
- Sturrock E, Lubbe L, Cozier G, et al. Structural basis for the C-domain-selective angiotensin-converting enzyme inhibition by bradykinin-potentiating peptide b (BPPb). *Biochem J*. 2019;476(10):1553-70.
- Donoghue M, Hsieh, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res*. 2000;87:e1-e9.
- Tipnis SR, Hooper NM, Hyde R, et al. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem*. 2000;275(43):33238-43.
- Soler M, Lloveras J, Batlle D. Enzima conversiva de la angiotensina 2 y su papel emergente en la regulación del sistema renina-angiotensina. *Med Clin (Barc)*. 2008;131(6):230-6.
- Burrell L, Johnston C, Tikellis C, Cooper M. ACE2, a new regulator of the renin-angiotensin system. *Trends Endocrinol Metab*. 2004;15(4):166-9.
- Bitker L, Burrell L. Classic and nonclassic renin-angiotensin systems in the critically ill. *Crit Care Clin*. 2019;35(2):213-27.
- Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett*. 2002;532(1-2):107-10.
- Hamming I, Timens W, Bulthuis M, et al. 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.
- Zhou P, Yang X, Wang X, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579:270-3.
- Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003;426(6965):450-4.
- Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. 2005;11(8):875-9.
- Guzzi P, Mercatelli D, Ceraolo C, Giorgi F. Master Regulator Analysis of the SARS-CoV-2/Human Interactome. *J Clin Med*. 2020;9(4):E982.
- Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181(2):271-80.
- Hofmann H, Pöhlmann S. Cellular entry of the SARS coronavirus. *Trends Microbiol*. 2004;12(10):466-72.
- Walls A, Park Y, Tortorici M, et al. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*. 2020;181(2):281-92.
- Simmons G, Zmora P, Gierer S, et al. Proteolytic activation of the SARS-coronavirus spike protein: Cutting enzymes at the cutting edge of antiviral research. *Antiviral Res*. 2013;100(3):605-14.
- Zhang H, Baker A. Recombinant human ACE2: acing out angiotensin II in ARDS therapy. *Crit Care*. 2017;21(1):305.

23. Wysocki J, Ye M, Rodríguez E, et al. Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: Prevention of angiotensin II-dependent hypertension. *Hypertension*. 2010;55(1):90-8.
24. Lei C, Fu W, Qian K, et al. Potent neutralization of 2019 novel coronavirus by recombinant ACE2-Ig. *Nat Commun*. 2020;11(1):2070.
25. Gu H, Xie Z, Li T, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. *Sci Rep*. 2016;6:19840.
26. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005;436(7047):112-6.
27. Li G, He X, Zhang L, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. *J Autoimmun*. 2020: 102463.
28. South A, Tomlinson L, Edmonston D, et al. Controversies of renin-angiotensin system inhibition during the COVID-19 pandemic. *Nat Rev Nephrol*. 2020:1-3.
29. Perico L, Benigni A, Remuzzi G. Should COVID-19 Concern Nephrologists? Why and to What Extent? The Emerging Impasse of Angiotensin Blockade. *Nephron*. 2020:1-9.
30. Khan A, Benthin C, Zeno B, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. 2017;21(1):234.
31. Donoghue M, Wakimoto H, Maguire C, et al. Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with downregulated connexins. *J Mol Cell Cardiol*. 2003;35(9):1043-53.
32. Data from Emory in Atlanta, presented April 21, 2020, ASN webcast-seminar.
33. Naicker S, Yang C, Hwang S, et al. The Novel Coronavirus 2019 epidemic and kidneys. *Kidney Int*. 2020;97(5):824-28.
34. Li Z, Wu M, Guo J, et al. Caution on kidney dysfunctions of 2019-nCoV patients. *MedRxiv*. 2020. doi: <https://doi.org/10.1101/2020.02.08.20021212>.
35. National Kidney Foundation, at <https://www.kidney.org/contents/be-prepared-kidney-patient-prep-coronavirus#>, last visited April 29, 2020.
36. Cheng Y, Luo R, Wang K, et al. Kidney impairment is associated with in-hospital death of COVID-19 patients. *Kidney Int* 2020;97:829-38.
37. Da Silveira K, Bosco K, Diniz L, et al. ACE2-angiotensin-(1-7)-mas axis in renal ischaemia/reperfusion injury in rats. *Clin Sci*. 2010;119(9):385-94.
38. Gupta A, Rhodes G, Berg D, et al. Activated protein C ameliorates LPS-induced acute kidney injury and downregulates renal INOS and angiotensin 2. *Am J Physiol Renal Physiol*. 2007;293:F245-54.
39. Yang X, Wang Y, Wang J, et al. Role of angiotensin-converting enzyme (ACE and ACE2) imbalance on tourniquet-induced remote kidney injury in a mouse hindlimb ischemia-reperfusion model. *Peptides* 2012;36:60-70.
40. Mizuiri S, Hemmi H, Arita M, et al. Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls. *Am J Kidney Dis*. 2008;51(4):613-23.
41. Dai B, David V, Martin A, et al. A comparative transcriptome analysis identifying FGF23 regulated genes in the kidney of a mouse CKD model. *PLoS One*. 2012;7(9):e44161.
42. Wysocki J, Schulze A, Batlle D. Novel Variants of Angiotensin Converting Enzyme-2 of Shorter Molecular Size to Target the Kidney Renin Angiotensin System. *Biomolecules*. 2019;9(12):886.
43. Monteil V, Kwon H, Prado P, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell*. 2020;181:1-9.
44. Zhou G, Zhao Q. Perspectives on therapeutic neutralizing antibodies against the Novel Coronavirus SARS-CoV-2. *Int J Biol Sci*. 2020;16(10):1718-23.
45. Jin Y, Lei C, Hu D, et al. Human monoclonal antibodies as candidate therapeutics against emerging viruses. *Front Med*. 2017;11(4):462-70.
46. Coughlin M, Prabhakar B. Neutralizing human monoclonal antibodies to severe acute respiratory syndrome coronavirus: target, mechanism of action, and therapeutic potential. *Rev Med Virol*. 2012;22(1):2-17.
47. Prabakaran P, Zhu Z, Xiao X, et al. Potent human monoclonal antibodies against SARS CoV, Nipah and Hendra viruses. *Expert Opin Biol Ther*. 2009;9(3):355-68.
48. Zhu Z, Prabakaran P, Chen W, et al. Human monoclonal antibodies as candidate therapeutics against emerging viruses and HIV-1. *Virology*. 2013;28(2):71-80.
49. Zhang M, Choudhry V, Xiao X, Dimitrov DS. Human monoclonal antibodies to the S glycoprotein and related proteins as potential therapeutics for SARS. *Curr Opin Mol Ther*. 2005;7(2):151-6.
50. Sparks M, South A, Welling P, et al. Sound Science Before Quick Judgement Regarding RAS Blockade in COVID-19. *Clin J Am Soc Nephrol*. 2020;CJN.03530320. doi:10.2215/CJN.03530320.

