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THE PRESENT AND FUTURE

JACC REVIEW TOPIC OF THE WEEK

Clinical Implications of SARS-CoV-2 Interaction With Renin Angiotensin System

JACC Review Topic of the Week

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ABSTRACT

Severe acute respiratory-syndrome coronavirus-2 (SARS-CoV-2) host cell infection is mediated by binding to angiotensin-converting enzyme 2 (ACE2). Systemic dysregulation observed in SARS-CoV was previously postulated to be due to ACE2/angiotensin 1-7 (Ang1-7)/Mas axis downregulation; increased ACE2 activity was shown to mediate disease protection. Because angiotensin II receptor blockers, ACE inhibitors, and mineralocorticoid receptor antagonists increase ACE2 receptor expression, it has been tacitly believed that the use of these agents may facilitate viral disease; thus, they should not be used in high-risk patients with cardiovascular disease. Based on the anti-inflammatory benefits of the upregulation of the ACE2/Ang1-7/Mas axis and previously demonstrated benefits of lung function improvement in SARS-CoV infections, it has been hypothesized that the benefits of treatment with renin-angiotensin system inhibitors in SARS-CoV-2 may outweigh the risks and at the very least should not be withheld. (J Am Coll Cardiol 2020;75:3085–95)

The novel coronavirus disease-2019 (COVID-19) outbreak, caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), originated from the Wuhan, Hubei providence in central China in December 2019 and was declared a pandemic by the World Health Organization on March 11, 2020 (1). Compared with SARS-CoV, which caused the 2002 to 2003 outbreak, SARS-CoV-2 appears to have a stronger rate of transmission. Although the SARS infection exhibits a prolonged clinical course predominantly involving respiratory manifestations, the clinical course of the novel coronavirus is unclear. Further clinical insights from Wuhan suggest that some patients with COVID-19 exhibit severe cardiovascular damage, and those with underlying cardiovascular disease appear to have an increased risk of death (1,2). Both SARS-CoV and CoV2 belong to the beta-coronavirus phylogeny (3). Although bats may be natural reservoirs for SARS-like coronavirus (3), interspecies transfer of SARS-CoV and SARS-CoV-2 could have occurred through civets (4) and pangolins (5), respectively. Both SARS-CoV strains have been identified to use the angiotensin-converting enzyme 2 (ACE2) receptor as the portal of entry into the affected cell (1,4). ACE2 is a key modulator of the renin-angiotensin system (RAS), which is a signaling pathway involved in the regulation of vascular function, including the regulation of blood pressure, natriuresis, and blood volume control (6). Normally, the RAS involves the formation of angiotensin II (Ang II) through ACE, which contributes to multiple cardiovascular physiological and pathophysiological functions, including hypertension, myocardial hypertrophy, cardiac fibrosis, inflammation, vascular remodeling, and atherosclerosis (7–9). Because of the adverse

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ABBREVIATIONS AND ACRONYMS

ACE = angiotensin-converting enzyme

ACE2 = angiotensin-converting enzyme 2

ADAM 17 = disintegrin and metalloprotease 17

Ang 1-7 = angiotensin 1-7

Ang I = angiotensin I

Ang II = angiotensin II

ARB = angiotensin II receptor blocker

ARDS = acute respiratory distress syndrome

AT1 = angiotensin II type I receptor

AT2 = angiotensin II type II receptor

ERK = extracellular signal-regulated kinase

ET = endothelin

IL = interleukin

MAPK1 = mitogen-activated protein kinase 1

MRAs = mineralocorticoid receptor antagonists

RAS = renin-angiotensin system

rhACE2 = recombinant angiotensin-converting enzyme 2

SARS-CoV-2 = severe acute respiratory-syndrome coronavirus-2

TACE = tumor necrosis factor α-converting enzyme

TNF = tumor necrosis factor

cardiovascular effects of RAS upregulation, its inhibition through ACE inhibitors, angiotensin II receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs) has been critical for the management of various cardiovascular diseases. In the last 2 decades, the identification of ACE2 and its involvement in the counter regulation of the classic RAS has offered a potentially new therapeutic target (10-12). ACE2 exists both as membrane-bound and soluble forms, the former of which mediates SARS-CoV-2 infection via S-protein binding (13,14). It is unclear whether SARS-CoV-2 interferes with ACE2 in a manner that contributes to the pathogenesis of SARS or the cardiovascular damage observed (1,2). This raises the question of whether RAS inhibition in cardiovascular patients should be reassessed in the setting of this novel coronavirus.

ANGIOTENSIN-CONVERTING ENZYME 2

Classically, the RAS involves the conversion of angiotensinogen by renin into angiotensin I (Ang I). Ang I is metabolized to Ang II via the dipeptide carboxypeptidase ACE. The pro-inflammatory effects of Ang II (7-9) are mediated through Ang II type I (AT1) receptors. Recently, the ACE2 receptor and its signaling pathway were identified as an important counter regulatory mechanism to the classic RAS.

ACE2 is a type I integral membrane glycoprotein (15) expressed predominantly in the bronchus, lung parenchyma, heart, endothelium, kidneys, duodenum, and small intestine (16). ACE2 is a monocarboxypeptidase, unlike its homolog, ACE, which is a dipeptidase; ACE2 is not antagonized by ACE inhibitors (17). Although ACE contains 2 active catalytic domains, ACE2 has a single catalytic domain with 42% identical residues (18,19). The major substrate of ACE2 is Ang II, which upon C-terminus cleavage, produces angiotensin 1-7 (Ang1-7) and L-phenylalanine (20). Other substrates for ACE2 include Ang I, apelin-13, and dynorphin-13, which are catalyzed at much lower affinities (21). The non-catalytic C-terminal domain of ACE2 shares a 48% sequence homology with collectrin, a protein involved in neutral amino acid reabsorption from the intestine and the kidney (22,23). In the presence of a disintegrin and metalloproteinase 17 (ADAM17), also known as tumor necrosis factor (TNF)-α–converting enzyme (TACE), ACE2 exhibits ectodomain shedding (24), which results in the formation of a soluble enzyme. ACE2 also contains a calmodulin domain on its cytoplasmic tail that influences ectodomain shedding (25).

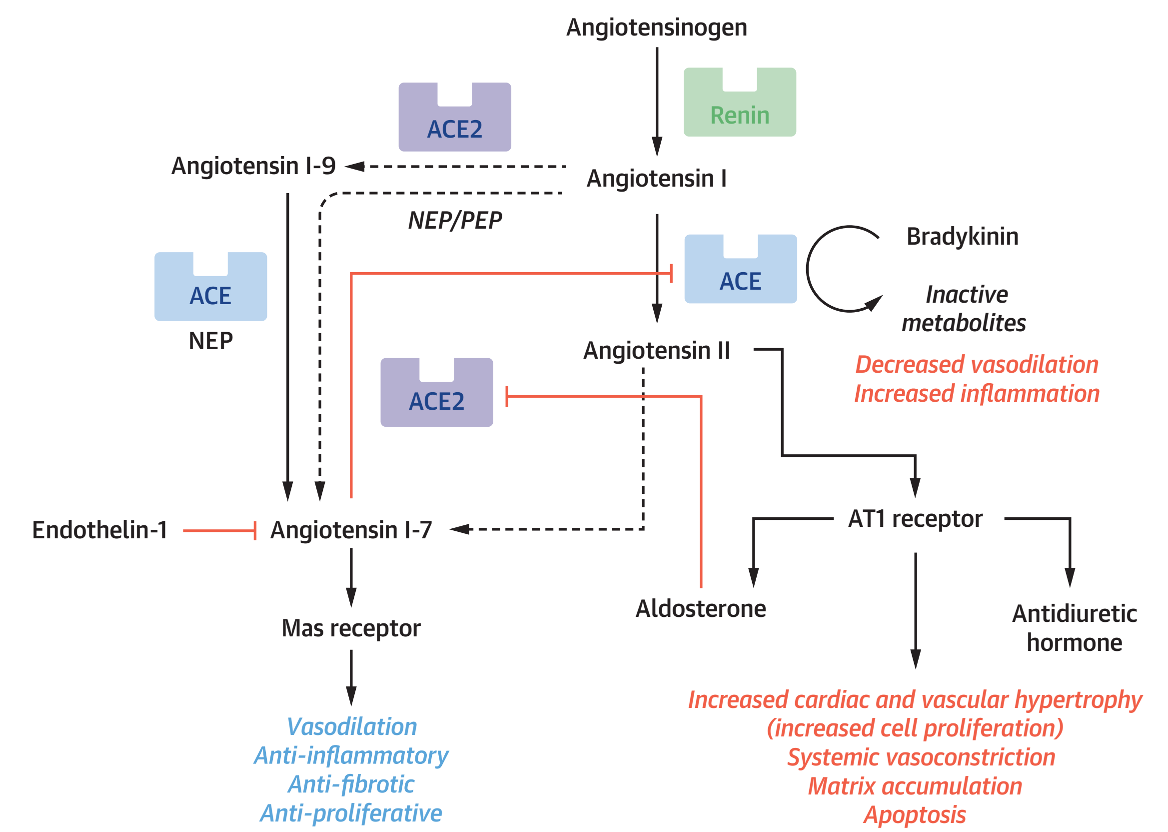
HIGHLIGHTS

- COVID-19 has been associated with cardiac involvement. SARS-CoV-2 requires binding to ACE2 in the RAS.
- The ACE2/Ang1-7/Mas pathway counterbalances the RAS, which results in activation of anti-inflammatory pathways.
- ACE inhibitors, ARBs, and MRAs upregulate ACE2 activity and expression.
- More data are required to determine if regulation of ACE2 in patients with cardiovascular disease and COVID-19 would help improve clinical outcomes.

ACE2/Ang1-7/MAS AXIS REGULATION

Ang I is a decapeptide that is converted into the octapeptide Ang II by ACE. Unlike ACE, Ang I can be converted to Ang1-9 by ACE2, and, more importantly, Ang II is converted to Ang 1-7 through ACE2 (17). Ang1-7 has a range of anti-inflammatory, antioxidant, vasodilatory, and natriuretic effects that are mediated by the G protein coupled receptor (GPCR) Mas receptor (11,26,27). Ang1-7 may be produced directly from Ang I through the alternative pathways involving a zinc metallopeptidase neprilysin or conversion of Ang1-9 to Ang1-7 via ACE, although at a significantly lower efficiency (17). Genetic deletion studies have established ACE2 as an essential regulator of cardiovascular function (28). Studies focused on the regulation of ACE2 in cardiac myocytes and cardiac fibroblasts have demonstrated that although Ang II significantly reduced ACE2 activity and downregulated *ACE2* mRNA in cardiac myocytes, it only reduced ACE2 activity in fibroblasts (29). In myocytes, endothelin (ET)-1 also significantly decreased *ACE2* mRNA production (29). This reduction in *ACE2* mRNA by Ang II or ET-1 was blocked by inhibitors of mitogen-activated protein kinase 1 (MAPK1), which suggested that Ang II and ET-1 activate extracellular signal-regulated kinase (ERK)1/ERK2 to reduce ACE2 (29). Furthermore, in vivo murine studies showed Ang II–mediated loss of membrane-bound cardiomyocyte ACE2 correlated

FIGURE 1 RAS and ACE2/Ang1-7/Mas Axis Regulation



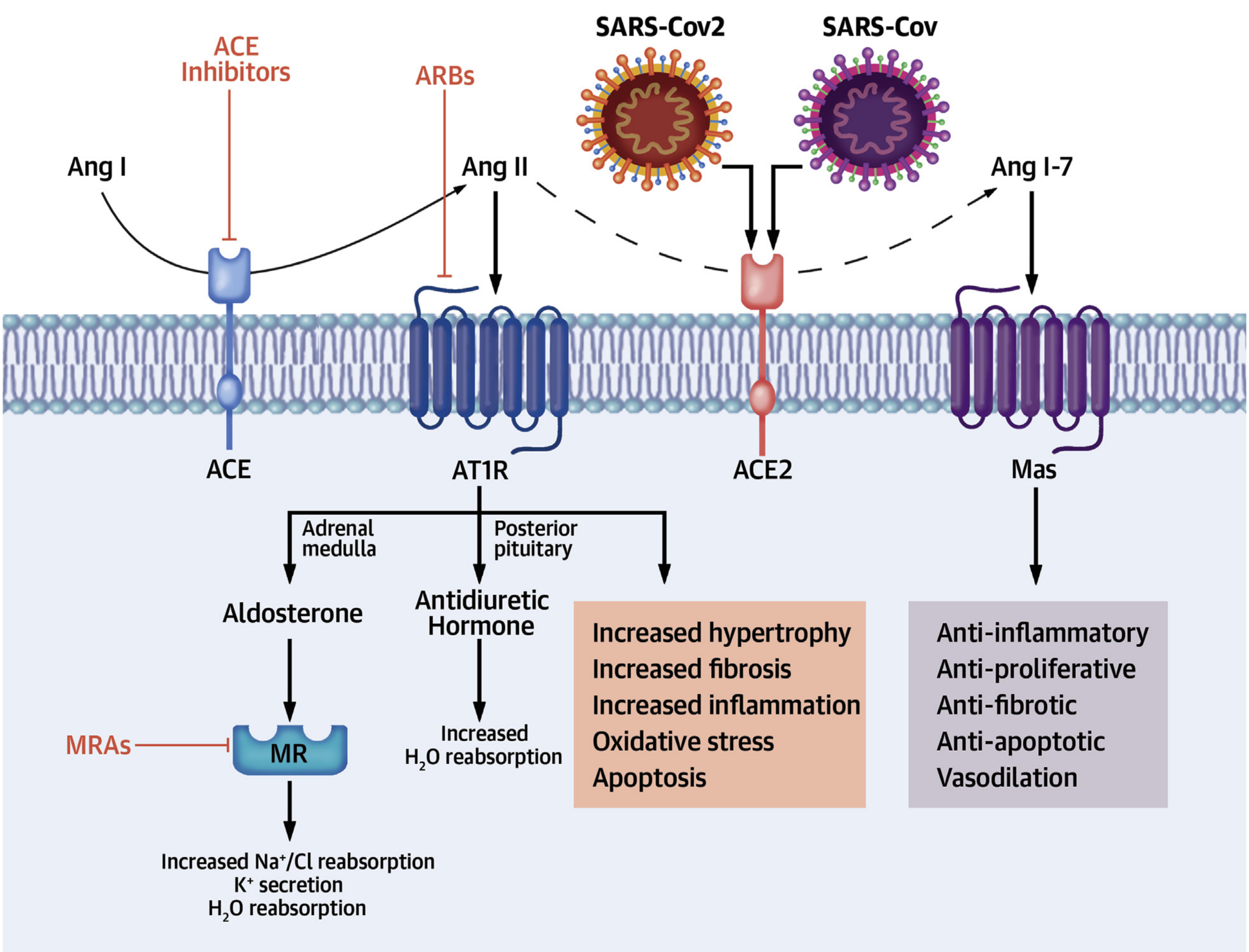
Angiotensinogen is converted to angiotensin I (Ang I) via renin. Ang I is converted to Ang II via angiotensin-converting enzyme (ACE), which also hydrolyzes bradykinin into its inactive metabolites, promoting inflammation. The pro-inflammatory effects of Ang II are mediated by Ang II type I receptor (AT1), which stimulates aldosterone secretion from the adrenal medulla and antidiuretic hormone from the posterior pituitary. Aldosterone decreases membrane ACE2 expression. Endothelin-1 inhibits angiotensin 1-7 (Ang1-7) via extracellular signal-regulated kinase (ERK)1/ERK2 pathways. Ang II, under favorable conditions **(dashed line)**, can be converted to Ang1-7 via ACE2, whose counter regulatory effects are mediated by the Mas receptor. Ang1-7 can also be formed via conversion of Ang I to an intermediate Ang1-9 or directly via zinc metallopeptidase neprilysin/prolyl endopeptidase (PEP). RAS = renin-angiotensin system.

with the upregulation of TACE/ADAM17 activity, which was prevented with AT1 receptor blockade (30). Cardiac fibroblasts and coronary endothelial cells also express ACE2 and TACE, and this reciprocal relationship extends to these cell types as well (31,32). Ang II activates several other signaling cascades, such as the PKC and JAK2-STAT3 signaling pathways, which results in myocardial hypertrophy and increased fibrosis (33). The binding of Ang1-7 to the C-terminal domain also inhibits the proteolytic function of the ACE enzyme and promotes bradykinin function (34). Studies in human vascular and cardiac tissue and plasma showed Ang1-7 has a higher affinity to ACE than Ang I, which suggests the inhibitory effects of Ang1-7 on ACE may contribute to its protective effects (35). The treatment of ACE2 knockout mice with Ang II infusion and recombinant ACE2 (rhACE2) eliminated ERK1/2, JAK2-STAT3, and PKC signaling by rhACE2 and was at least partially responsible for attenuation of Ang II–induced myocardial hypertrophy and fibrosis and improvement in diastolic dysfunction (33). Other studies highlighted the role of the ACE2/Ang1-7/Mas axis in modulating the expression of pro-inflammatory cytokines, such as TNF-α, interleukin (IL)-1β, IL-6, monocyte chemoattractant protein-1, and transforming growth factor-β in cardiac and/or lung fibrosis, pulmonary hypertension, and vascular remodeling (36–41) (**Figure 1**).

ACE2 REGULATION AND CARDIOVASCULAR DISEASE

Because of the importance of the RAS in cardiovascular disease, its regulation via ACE inhibitors, ARBs, and MRAs has played an essential role in the

CENTRAL ILLUSTRATION The Renin-Angiotensin System Interaction With COVID-19



Brojakowska, A. et al. J Am Coll Cardiol. 2020;75(24):3085–95.

Normally, angiotensin I (Ang I) is converted to Ang II via angiotensin-converting enzyme (ACE), which could be inhibited by ACE inhibitors. The pro-inflammatory effects of Ang II are mediated through AT1R in several ways: 1) in the zona glomerulosa of the adrenal medulla, it stimulates aldosterone secretion and binding to mineralocorticoid receptors to promote water reabsorption and to increase salt retention; it is inhibited by mineralocorticoid receptor antagonists (MRAs); 2) in the posterior pituitary, Ang II stimulates antidiuretic hormone secretion to promote water retention; and 3) in other tissues, it stimulates pathways responsible for hypertrophy, fibrosis, oxidative stress, and apoptosis. These effects are attenuated by angiotensin receptor blockers (ARBs), which block Ang II binding to AT1R. Ang II can also be converted to angiotensin 1-7 (Ang 1-7) via ACE2, which stimulates the Mas receptor promoting anti-inflammatory benefits. The ACE2/Ang1-7/Mas axis acts as a counter regulatory pathway to the traditional renin-angiotensin system (RAS). AT1R and ACE2 are coupled. Ang II binding to AT1R allows dissociation of ACE2 and subsequent degradation. ARB prevents dissociation of ACE2 and renders it availability for unused Ang II conversion to Ang 1-7. ACE2 has been identified as the targeted receptor for both the severe acute respiratory syndrome coronavirus (SARS-CoV) 2 and SARS-CoV. ACE2 mediates S protein binding that stimulates viral entry into the host cytosol that results in infection and viral replication. Diversion of Ang II towards ACE2 could competitively inhibit viral binding and also counter regulate the adverse effects caused by AT1R and improve outcomes by Mas R–based favorable effects.

management of cardiovascular diseases (**Central Illustration**).

Several studies have elucidated the role of these drug classes on the modulation of the ACE2/Ang1-7/Mas axis. Mouse peritoneal macrophages treated in vitro with aldosterone, demonstrated significantly increased ACE activity as well as *ACE* mRNA and significantly reduced *ACE2*. However, in mouse peritoneal macrophages treated with nicotinamide adenine dinucleotide phosphate oxidase inhibitor, aldosterone could not increase *ACE* or decrease *ACE2*, which suggested these effects were mediated in part by nicotinamide adenine dinucleotide phosphate oxidase (42). These effects were also attenuated with treatment with an MRA (eplerenone) (42). Human monocyte-derived macrophages obtained from patients with heart failure before and after 1 month of treatment with another MRA (spironolactone; 25 mg/day) showed 47% reduction in ACE activity and 53% reduction in *ACE* mRNA expression. At the same time, ACE2 activity increased by 300% and *ACE2* mRNA expression increased by 654% (42). In mice treated for 2 weeks with eplerenone, cardiac ACE2 activity increased 2-fold and was paralleled by increased ACE2 activity in macrophages (42). This study demonstrated that the MRA reduced oxidative stress, decreased ACE activity, and increased ACE2 activity and/or expression, which suggested the protective role played by increased generation of Ang 1-7 and decreased formation of Ang II. Overall, aldosterone decreased *ACE2* transcription through a nicotinamide adenine dinucleotide phosphate oxidase–mediated pathway (42), and in vascular smooth muscle cells, potentiated Ang II signaling with increased phosphorylation of ERK1/2 and c-Jun kinase, which are also dependent on reactive oxygen species generation (43). Thus, the beneficial effects of MRAs are likely associated with reduction of oxidative stress and differential control of these angiotensinases. MRAs appeared to promote membrane ACE2 expression and suppress the peripheral effects of Ang II; however, the effect of MRAs on soluble ACE2 remains unclear.

Similar upregulation of ACE2 was observed in studies focused on the effects of ARB treatment. Spontaneously hypertensive rats treated with olmesartan demonstrated a 5-fold greater expression of *ACE2* mRNA and increased Ang1-7 in their thoracic aortas, whereas those treated with atenolol and hydralazine exhibited no change in ACE2 expression or Ang1-7 (44). Comparison of vessel wall dimensions showed that olmesartan selectively reduced the thoracic aorta media-to-lumen ratio, whereas vascular hypertrophy was unchanged in spontaneously hypertensive rats given atenolol or hydralazine (44). There was no change in ACE2/Ang1-7 expression and/or activity in the carotid arteries of the treated animals. The possibility that the effects of olmesartan on vascular ACE2 gene and protein expression were the result of reduced arterial blood pressure was ruled out because of the comparative effect observed in mice treated with atenolol or hydralazine (44).

Sprague-Dawley rats treated with a 4-week course of Ang II infusion showed Ang II upregulated AT1 receptor, downregulated AT2 receptor, ACE2 activity, endothelial nitric oxide synthase expression, as well as increased CD44 expression and hyaluronidase (45). However, rats treated with telmisartan exhibited significantly increased ACE2 activity and endothelial nitric oxide synthase expression in intracardiac vessels and intermyocardium, as well as downregulated local expression of the AT1 receptor. Treatment with telmisartan also inhibited membrane CD44 expression and reduced transforming growth factor–β and Smad expression (45). Studies in normotensive rats with post-coronary artery ligation left ventricular remodeling and dysfunction exhibited partial resolution following losartan and olmesartan treatment while augmenting plasma concentrations of the angiotensins (46). This was associated with recovery of cardiac AT1 receptor mRNA and increased *ACE2* mRNA post-myocardial infarction, which implied the beneficial effects of ARBs on cardiac remodeling were accompanied by direct blockade of AT1 receptors and increased ACE2 expression and/or activity (46). The literature offers conflicting results pertaining to ARB use and the level of ACE2 expression on the myocardium; most of the controversy arises from the difference in ACE2 cell surface expression and plasma ACE2 levels. In the Sprague-Dawley rats with left coronary artery ligation and myocardial infarction, plasma Ang II and Ang1-7 were not elevated, but plasma ACE2 was elevated, along with enhanced cardiac *ACE2* and AT1 receptor mRNA at the infarct border (47). Receptor upregulation was not observed in the remote myocardium (47). Treatment with ramipril and valsartan resulted in increased plasma Ang I and Ang II and suppression in plasma ACE and ACE2 activity; however, neither monotherapy nor combination therapy affected ACE2 or AT1 receptor expression, both of which remained at levels comparable to non-myocardial infarction control (47). However, a previous study in the same murine model showed ACE and ACE2 upregulation in the border, infarct zones, and in viable myocardium after myocardial infarction. Treatment with ramipril reduced ACE expression, whereas ACE2 remained elevated compared with the noninfarcted control subject (48). A recent study in the same murine model demonstrated that treatment with olmesartan or telmisartan increased both cardiac *ACE2* mRNA and protein expression while augmenting plasma Ang1-7/Ang II ratios, which resulted in improved cardiac function and alleviated collagen disposition (49). These experiments suggested that both ACE inhibitors and ARBs variably upregulated ACE2 expression (49). ARBs inhibited binding of Ang II to the AT1 receptor, which permitted circulating Ang II to be shunted to ACE2 for conversion to Ang1-7. These studies suggest that the ACE2/Ang1-7 axis collaborates with or is regulated by the AT1 receptor and may be important in mediating the vascular and cardiac remodeling effects of Ang II.

The mechanisms by which ACE inhibitors act are complex. Although ACE2 is not inhibited by ACE inhibitors (19), an increase in Ang1-7 suggests their clinical effects are partly mediated by the angiotensinases. ACE inhibitors inhibit the conversion of Ang I to Ang II and inhibit the hydrolysis of bradykinin. ACE inhibition promotes the vasodilatory effects of bradykinin, improved endothelium-dependent vasodilation through increased prostaglandin and nitric oxide production, and down regulation of the AT1 receptor (50–52). Studies that elucidated the effect of ACE inhibition on the *ACE2* gene showed that inhibition of Ang II synthesis regulated *ACE2* mRNA but not ACE2 activity (53). However, ACE inhibition alone or in combination with losartan was demonstrated to increase plasma Ang1-7 while reducing plasma Ang II (53). Compared with the degree of *ACE2* mRNA upregulation seen with post-losartan monotherapy, combination of losartan and lisinopril resulted in suppressed upregulation of *ACE2* mRNA, which suggested ACE inhibitors might override a signal that regulates *ACE2* transcription (53). Although Ang II is the predominant substrate, ACE2 can also convert Ang I into Ang 1-9, which, in turn, could be converted to Ang 1-7 via ACE; Ang I can be directly converted into Ang 1-7 via zinc metallopeptidase neprilysin (17), although with less favorable kinetics at baseline. Thus, it can be assumed ACE inhibitors disrupt the balance between catalytically active ACE and ACE2, resulting in favored activation of the ACE2/Ang1-7/Mas axis.

Overall, because of the demonstrated anti-inflammatory, antifibrotic, and antithrombotic effects associated with the ACE2/Ang1-7/Mas axis, upregulation could serve as a valuable therapeutic target.

ACE2 AND ANG1-7: CLINICAL TRIALS

ACE2 regulates RAS signaling by reducing Ang II/AT1 receptor signaling and by activating the ACE2/Ang1-7/Mas counterregulatory pathway. Thus far, only a few pilot clinical studies have been conducted using rhACE2 in acute respiratory distress syndrome (ARDS), sepsis, and pulmonary arterial hypertension.

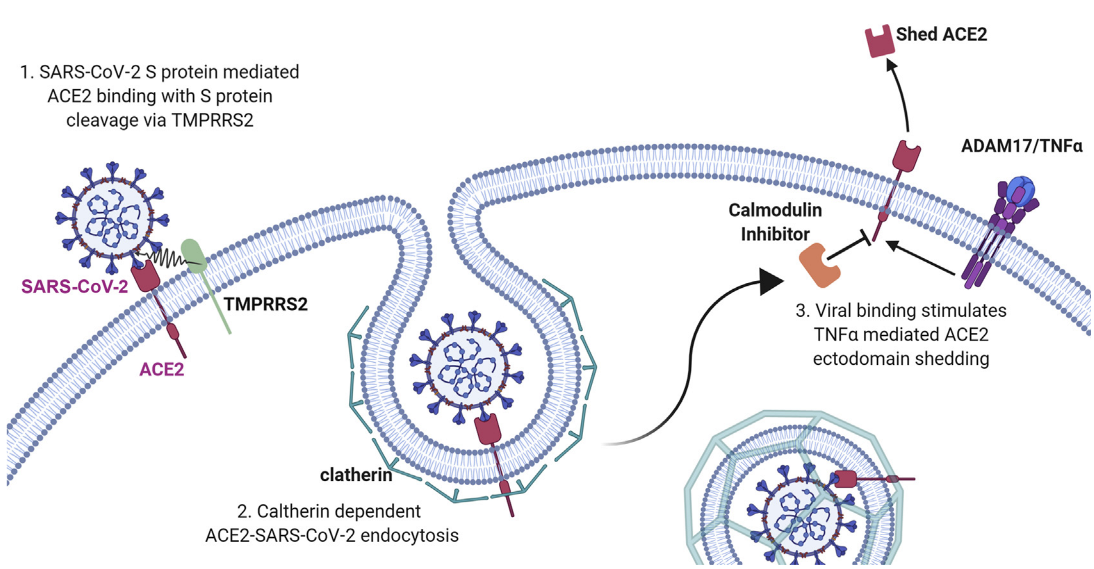
Multiple murine studies demonstrated rhACE2 modulates the RAS pathway, although it is unclear if these effects translate to humans. A clinical study (Safety and Tolerability Study of APN01 [Recombinant Human Angiotensin Converting Enzyme 2]; NCT00886353) that assessed the pharmacokinetics and pharmacodynamics of soluble rhACE2 treatment in healthy volunteers with no known comorbidities showed a decrease in plasma Ang1-8 and increased Ang1-7 and Ang1-5 with no effect on blood pressure and heart rate (54). Common side effects included diarrhea and headache. No antibodies to rhACE2 developed, which suggested there was no elicit immune response to single or repeated dosing (54). Further studies investigating the immunogenicity of rhACE are required.

Serum levels of ACE and Ang II are elevated in patients with ARDS and sepsis (55,56). Studies focused on microvascular dysfunction in sepsis showed that the degree of elevation in plasma renin and Ang II were correlated with the extent of organ failure and the degree of microvascular dysfunction, especially in patients who received exogenous vasoconstrictors (56); there was also a negative correlation between re-oxygenation rates and both concentrations of plasma renin and Ang II (56). In a pilot clinical trial (Safety, Tolerability, PK and PD of GSK2586881 in Patients With Acute Lung Injury; NCT01597635), patients with ARDS who were treated with rhACE2 exhibited decreased plasma Ang II and elevated plasma Ang 1-7 and surfactant protein-D, which is involved in innate immunity (57). IL-6 concentrations in treated patients were also reduced, albeit statistically insignificantly, which was due to intrasubject variability and baseline imbalance. Although rhACE2 attenuated RAS mediators, infusions of the medication did not show improvement in physiological or clinical measures of ARDs in this study (57).

An additional pilot study (NCT101884051) investigated the effects of rhACE2 in human pulmonary arterial hypertension, which is characterized by reduced ACE2 activity (58). Treatment with rhACE2 showed improved cardiac output that coincided with maximum suppression of plasma cytokines and reduction in nitrotyrosine levels, improved peripheral vascular resistance, and improved renal perfusion (58).

Ongoing clinical studies assessing the modulation of RAS axis include: 1) the assessment of the relative activity of ACE and ACE2 in patients with diabetes following treatment with candesartan (Non-Insulin Dependent Diabetes Mellitus [NIDDM] and Angiotensin Converting Enzyme 2 [ACE2]: Diabetic Patients Treated With Antihypertensive Drugs; NCT00192803); and 2) the overexpression of ACE2/Ang 1-7 in cardiac progenitor cells to assess for enhancement in reparative function and the potential to attenuate myocardial ischemia–induced cardiac damage (Cardiovascular Disease Protection Tissue; NCT02348515). It is evident that targeting the

FIGURE 2 **Proposed Mechanism of Entry for SARS-CoV-2 Via ACE2**



Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to the catalytic site of ACE2 via its S protein. To gain access to the host cytosol, the S protein undergoes acid dependent proteolytic cleavage by ACE2, tumor necrosis factor (TNF)-α, transmembrane protease serine 2 (TMPRRS2), which results in the formation of an S1 and S2 subunit. S2 fusion peptide inserts into the membrane, which allows for clatherin-dependent endocytosis of ACE2-SARS-CoV-2. Viral binding stimulates TNF-α and calmodulin inhibitors to promote ectodomain cleavage of ACE2. ADAM 17 = disintegrin and metalloprotease 17; other abbreviations as in Figure 1.

ACE2/Ang1-7/Mas axis is going to be interesting in clinical settings because of the observed cardioprotective effects in the in vivo murine and in vitro cell culture models. However, further investigation is required to demonstrate whether these favorable experimental effects could be translated into clinical benefit.

ACE2, COVID-19, AND CARDIOVASCULAR DAMAGE

Several reports have noted COVID-19 is associated with cardiac involvement. In cohort studies of hospitalized patients with confirmed COVID-19, several patients presented with elevated troponin I, C-reactive protein, and N-terminal pro-B-type natriuretic peptide suggestive of myocardial injury (2,59,60). Anecdotal studies have reported patients presenting with cardiac magnetic resonance imaging–verified acute myopericarditis with systolic dysfunction masquerading as diffuse ST-segment elevation myocardial infarction with elevated cardiac markers in the absence of obstructive coronary disease (59). In a cohort study of 139 patients with COVID-19 hospitalized in Wuhan, China, 7.2% had acute myocardial injury, 8.7% had shock, and 16.7% had an arrhythmia (61). Of the observed patients, those with cardiac injury were found to have a high risk of death both from time of symptom onset and time of admission (60). As more epidemiological studies emerge from China, Italy, and other affected areas, more data will be available to elucidate the clinical presentation of patients and the cardiovascular damage associated with this novel coronavirus.

With regard to COVID-19, there are currently several clinical studies investigating the effects of RAS inhibition and ACE2 regulation. An ongoing study will assess the impact of ACE inhibitor and ARB treatment on the severity and prognosis of patients with COVID-19 (Hypertension in Patients Hospitalized With COVID-19 [HT-COVID19], NCT04318301; ACE Inhibitors, Angiotensin II Type-I Receptor Blockers and Severity of COVID-19 [CODIV-ACE], NCT04318418). Along these lines, there are 2 recently launched trials testing the effects of losartan among patients hospitalized with COVID-19 (Losartan for Patients With COVID-19 Requiring Hospitalization; NCT04312009) and those who are ambulatory (Losartan for Patients With COVID-19 Not Requiring Hospitalization; NCT04311177). Further studies have been launched to evaluate the effect of continuation versus replacement (Coronavirus [COVID-19] ACEi/ARB Investigation [CORONACION]; NCT04330300) or withdrawal (ACE Inhibitors or ARBs Discontinuation in Context of SARS-CoV-2 Pandemic [ACORES-2]; NCT04329195) of RAS inhibitors on the clinical outcomes in patients with cardiovascular disease and COVID-19. There is also an ongoing pilot study assessing the effects of rhACE2 treatment in patients with COVID-19 (Recombinant Human Angiotensin-converting Enzyme 2 [rhACE2] as a Treatment for Patients With COVID-19; NCT04287686). Currently, there is no data to support any conclusive effects of the use of RAS inhibitors in patients with COVID-19.

ACE2 AND SARS-CoV-2

SARS-CoV, which emerged in the Guangdong province, China, and SARS-CoV-2, which emerged in Wuhan, China are closely related beta-coronaviruses whose affected receptor is ACE2 (1,3,4). At this time, it is unknown if the approximate 76% sequence similarity between these strains of viruses translates into similar biological properties (14). Recent studies have confirmed COVID-19 exploits ACE2 for entry and thus may target a similar spectrum of cells as SARS-CoV (14). SARS-CoV-2 binds to ACE2 via its spike (S) protein (13,14). The surface unit S1, of the S protein binds to ACE2, which facilitates viral attachment to target cells. Following receptor binding, the virus must gain access to host cytosol, which is accomplished by acid-dependent proteolytic cleavage of the S protein by cellular serine protease TMPRSS2, which is similar to S protein priming in SARS-CoV (14) (**Figure 2**).

Because of the sequence similarity between SARS-CoV and SARS-CoV-2, their affected receptor, and recently confirmed TMPRSS2-mediated viral entry, it is reasonable to hypothesize that SARS-CoV-2 may act similarly with respect to using host endocytosis machinery, subsequent virus propagation, and further infection. Upon binding to ACE2, cleavage of the S protein at the S1/S2 sites and S2 allows for fusion of viral and cellular membranes. SARS-CoV is then internalized and penetrates early endosomes in a clathrin-dependent manner (62). Viral binding to ACE2 appears to affect TNF-α activity, which in the presence of calmodulin inhibitors promotes ectodomain cleavage (63). In the case of SARS-CoV-2, it is possible this shedding is also mediated by TNF-α because 1 of the clinical features noted in patients with COVID-19 has been the presence of a cytokine storm with increased plasma concentrations of IL-2, IL-7, IL-10, granulocyte-colony stimulating factor (GCSF), interferon gamma-induced protein 10 (IP10), monocyte chemoattractant protein 1, macrophage inflammatory protein 1 alpha (MIP1A), and TNF-α (2). It is also possible that ACE2 shedding may be mediated by other cytokines dysregulated in COVID-19. This shedding contributes to the down-regulation of membrane-bound ACE2 observed in severe acute lung injury (64). Ectodomain shedding increases the concentration of plasma ACE2, which remains catalytically active, although the function of soluble ACE2 remains unclear. In patients with advanced heart failure, plasma ACE2 activity is increased in direct proportion with worsening clinical status and reduction in ejection fraction and correlates with adverse clinical outcomes (65). Because down-regulation of bound ACE2 is observed in severe acute lung injury (65) and after myocardial infarction (46), and concentrations of soluble ACE2 appear to correlate with clinical outcomes of patients with heart failure (30), it is possible to suggest that concentrations of soluble ACE2 may correlate to the extent of tissue damage sustained and may correlate to the degree by which systemic inflammatory pathways are upregulated. There is some evidence to suggest soluble ACE2 is able to regulate systemic Ang II. Clinical trials have shown rhACE2 could convert systemic Ang II to Ang 1-7 (57,58) and play some pathological, compensatory, or counter regulatory roles.

If SARS-CoV-2 does induce ACE2 ectodomain shedding, which results in the reduction of ACE2 entry sites on the infected cell, it is possible that following transcription S proteins fuse directly at the host cell membrane and directly promote infection of neighboring cells, which results in the formation of multinucleated syncytia (66). Formation of multinucleated cells would allow the virus to spread without being detected or neutralized by virus-specific antibodies (66). Otherwise, following replication and transcription, complete virion assembly in the Golgi would result in transportation of the virus in vesicles and release by exocytosis (66). In the setting of full virion assembly and exocytosis, it is unclear if ACE2 ectodomain shedding would be favorable for further propagation and infection.

MEDICAL MANAGEMENT IN CARDIOVASCULAR PATIENTS WITH SARS-CoV-2

Regardless of how SARS-CoV-2 completes virion assembly, it is clear that membrane-bound ACE2 would play a physiological role in the replication of the novel virus. The question remains whether the use of ACE inhibitors, ARBs, and MRAs should be avoided in the setting of SARS-CoV infection because each agent (42-46,53) upregulates ACE2 expression and activity.

Lipopolysaccharide-induced acute lung injury mouse models exhibited decreased expression of ACE2, lung and inflammatory injury; however, this was ameliorated by the injection of cells transfected with ACE2 and resulted in the improvement of lung function and lung injury. Treatment of these mice with ACE inhibitors and ARBs also alleviated lipopolysaccharide-induced pneumonic injury (67). Previous studies showed the SARS-CoV S protein can exaggerate acute lung failure through dysregulation of the RAS. However, SARS-CoV Spike-mediated lung failure could be rescued by inhibition of the AT1 receptor (67). Again, adequate data on the effects of RAS inhibition in patients with COVID-19 is not available, and ongoing clinical and/or observations studies are being conducted (see previous mentions of NCT04318301, NCT04318418, NCT04312009, NCT04311177, NCT04287686, NCT04330300, NCT04329195).

If SARS-CoV-2 down-regulates membrane-bound ACE2 by promoting the ADAM17–mediated ectodomain shedding, resulting in increased concentrations of soluble ACE2 without compromising viral propagation, we hypothesize this would result in the overall down regulation of the ACE2/Ang1-7/Mas pathway, which would contribute to the severity of inflammation and systemic dysregulation observed in SARS-CoV-2. Thus, in patients with cardiovascular disease and SARS-CoV-2, the use of ACE inhibitors, ARBs, or MRAs may be favorable as a method to endogenously upregulate ACE2 as a compensatory mechanism that provides anti-inflammatory, antifibrotic, and antithrombotic support as well as reduction in progression of vascular and/or cardiac remodeling and heart failure. Several societies, including the American College of Cardiology, American Heart Association, Heart Failure Society of America (68), and European Society of Cardiology (69) have recommended continuing RAS antagonists because of the lack of conclusive data on a link between upregulation of systemic or tissue ACE2 and the increased susceptibility to COVID-19 in patients with cardiovascular disease. Based on our review, we hypothesize cardiovascular patients with COVID-19 should remain on RAS inhibitors because of the protective effects of the ACE2 pathway until RAS blockade is proven to increase the risk of COVID-19.

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REFERENCES

1. 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.

2. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.

3. Lecis R, Mucedda M, Pidinchedda E, et al. Molecular identification of Betacoronavirus in bats from Sardinia (Italy): first detection and phylogeny. Virus Genes 2019;55:60–7.

4. Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev 2005;69:635–64.

5. Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr Biol 2020;30:1346–51.e2.

6. Crowley SD, Gurley SB, Oliverio MI, et al. Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. J Clin Invest 2005;115:1092–9.

7. Holmberg J, Bhattachariya A, Alajbegovic A, et al. Loss of vascular myogenic tone in miR 143/145 knockout mice is associated with hypertension-induced vascular lesions in small mesenteric arteries. Arterioscler Thromb Vasc Biol 2018;38:414–24.

8. Schnee JM, Hsueh WA. Angiotensin II, adhesion, and cardiac fibrosis. Cardiovasc Res 2000;46:264–8.

9. Johnston CI. Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling. Hypertension 1994;23:258–68.

10. Thomas MC, Pickering RJ, Tsorotes D, et al. Genetic Ace2 deficiency accentuates vascular inflammation and atherosclerosis in the ApoE knockout mouse. Circ Res 2010;107:888–97.

11. Ferrario CM, Trask AJ, Jessup JA. Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. Am J Physiol 2005;289:H2281–90.

12. Gurley SB, Allred A, Le TH, et al. Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. J Clin Invest 2006;116:2218–25.

13. 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;11:875–9.

14. Hoffmann M, Keline-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 March 4 [E-pub ahead of print].

15. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. Chem Biol Chem 2000;275:33238–43.

16. Fagerberg L, Hallström BM, Oksvold P, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 2014;13:397–406.

17. Rice GI, Thomas DA, Grant PJ, et al. Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. Biochem J 2004;383:45–51.

18. Ehlers MRW, Riordan JF. Angiotensin-converting enzyme: zinc- and inhibitor-binding stoichiometries of the somatic and testis isozymes. Biochemistry 1991;30:7118–26.

19. Soubrier F, Alhenc-Gelas F, Hubert C, et al. Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. Proc Natl Acad Sci U S A 1988;85:9386–90.

20. Turner AJ, Hooper NM. The angiotensin-converting enzyme gene family: genomics and pharmacology. Trends Pharmacol Sci 2002;23:177–83.

21. Vickers C, Hales P, Kaushik V, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 2002;277:14838–43.

22. Zhang H, Wada J, Hida K, et al. Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. J Biol Chem 2001;273:17132–9.

23. Kowalczuk S, Broer A, Tietze N, et al. A protein complex in the brush-border membrane explains a Hartnup disorder allele. FASEB J 2008;22:2880–7.

24. Lambert DW, Yarski M, Warner FJ, et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). J Biol Chem 2005;280:30113–9.

25. Lambert DW, Clarke NE, Hooper NM, et al. Calmodulin interacts with angiotensin-converting enzyme-2 (ACE2) and inhibits shedding of its ectodomain. FEBS Lett 2008;582(2):385–90.

26. Probstfield JL, O'Brien KB. Progression of cardiovascular damage: the role of renin-angiotensin system blockade. Am J Cardiol 2010; 105:10A–20A.

27. Simões-Silva AC, Silveira KD, Ferreira AJ, et al. ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis. Br J Pharmacol 2003; 169:477–92.

28. Crackower M, Sarao R, Oudit G, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002;417:822–8.

29. Gallagher PE, Ferrario CM, Tallant EA. Regulation of ACE2 in cardiac myocytes and fibroblasts. Am J Physiol 2009;295:H2373–9.

30. Patel VB, Clarke N, Wang Z, et al. Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. J Mol Cell Cardiol 2014;66:167–76.

31. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991;83:1849–65.

32. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol 2007; 292:C82–97.

33. Zhong J, et al. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. Circulation 2010;122:717–28.

34. Tom B, de Vries R, Saxena PR, et al. Bradykinin potentiation by angiotensin-(1-7) and ACE inhibitors correlates with ACE C- and N-domain blockade. Hypertension 2000;38:95–9.

35. Iusuf D, Henning RH, Wiek HG. Angiotensin-(1-7): pharmacological properties and pharmacotherapeutic perspectives. Eur J Pharmacol 2008; 585:303–12.

36. Iwata N, Cowling RT, Gurantz D, et al. Angiotensin-(1-7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. Am J Physiol Heart Circ Physiol 2005;289: H2356–63.

37. Zeng W, Chen W, Leng X, et al. Chronic angiotensin-(1-7) administration improves vascular remodeling after angioplasty through the regulation of the TGF-beta/Smad signaling pathway in rabbits. Biochem Biophys Res Commun 2009;389:138–44.

38. Grobe JL, Mecca AP, Lingis M, et al. Prevention of angiotensin II-induced cardiac remodeling by angiotensin-(1-7). Am J Physiol Heart Circ Physiol 2007;292:H736–42.

39. Sriramula S, Cardinale JP, Lazartigues E, Francis J. ACE2 overexpression in the paraventricular nucleus attenuates angiotensin II-induced hypertension. Cardiovasc Res 2011;92: 401–8.

40. Ferreira AJ, Shenoy V, Yamazato Y, et al. Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. Am J Respir Crit Care Med 2009;179:1048–54.

41. Yamazato Y, Ferreira AJ, Hong KH, et al. Prevention of pulmonary hypertension by angiotensin-converting enzyme 2 gene transfer. Hypertension 2009;54:365–71.

42. Keidar S, Gamliel-Lazarovich A, Kaplan M, et al. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. Circ Res 2005; 97:946–53.

43. Mazak I, Fiebeler A, Muller DN, et al. Aldosterone potentiates angiotensin II-induced signaling in vascular smooth muscle cells. Circulation 2004;109:2792–800.

44. Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM. Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1-7) expression in the aorta of spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 2005;289: H1013–9.

45. Bai F, Xue-Fen P, Li-Hui Zhang, et al. Angiotensin II AT1 receptor alters ACE2 activity, eNOS expression and CD44-hylauronan interaction in rats with hypertension and myocardial fibrosis. Life Sciences 2016;153:141–52.

46. Ishyama Y, Gallagher PE, Averill DB, et al. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension 2004;43: 970–6.

47. Burchill LJ, Velkoska E, Dean RG, Griggs K, Patel SK, Burrell LM. Combination renin-angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. Clin Sci (Lond) 2012;123:649–58.

48. Burrell LM, Gayed D, Griggs K, et al. Adverse cardiac effects of exogenous angiotensin 1-7 in rats with subtotal nephrectomy are prevented by ACE inhibition. PLoS One 2017;12: e0171975.

49. Wang J, He W, Guo L, et al. The ACE2-Ang (1-7)-Mas receptor axis attenuates cardiac remodeling and fibrosis in post-myocardial infarction. Mol Med Rep 2017;16:1973–81.

50. Iyer SN, Yamada K, Diz DI, et al. Evidence that prostaglandins mediate the antihypertensive actions of angiotensin-(1-7) during chronic blockade of the renin-angiotensin system. J Cardiovasc Pharmacol 2000;36:109–17.

51. Luque M, Martin P, Martell N, et al. Effects of captopril related to increased levels of prostacyclin and angiotensin-(1-7) in essential hypertension. J Hypertens 1996;14:799–805.

52. Li P, Chappell MC, Ferrario CM, et al. Angiotensin-(1-7) augments bradykinin-induced vasodilation by competing with ACE and releasing nitric oxide. Hypertension 1996;29:394–400.

53. Iyer SN, Chappell MC, Averill DB, Diz DI, Ferrario CM. Vasodepressor actions of angiotensin-(1-7) unmasked during combined treatment with lisinopril and losartan. Hypertension 1998;31: 699–705.

54. Haschke M, Schuster M, Poglitsch M, et al. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. Clin Pharmacokinet 2013;52:783–92.

55. Wenz M, Hoffmann B, Bohlender J, et al. Angiotensin II formation and endothelin clearance in ARDS patients in supine and prone positions. Intensive Care Med 2000;26:292–8.

56. Doerschug KC, Delsing AS, Schmidt GA, et al. Renin-angiotensin system activation correlates with microvascular dysfunction in a prospective cohort study of clinical sepsis. Crit Care 2010;14: R24.

57. 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;21:234.

58. Hemnes AR, Rathinasabapathy A, Austin EA, et al. A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. Eur Respir J 2018; 51:1702638.

59. Inciardi RM, Lupi L, Zaccone G, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). JAMA Cardiol 2020 Mar 27 [E-pub ahead of print].

60. Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020 Mar 25 [E-pub ahead of print].

61. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061–9.

62. Inoue Y, Tanaka N, Tanaka Y, et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. J Virol 2007;81:8722–9.

63. Haga S, Yamamoto N, Nakai-Murakami CN, et al. Modulation of TNFα converting enzyme by spike protein of SARS-CoV and ACE2 induces TNFα production and facilitates viral entry. PNAS 2008; 105:7809–14.

64. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005;436:112–6.

65. Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. J Am Coll Cardiol 2008;52:750–4.

66. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 2015;1282:1–23.

67. Ye R, Liu Z. ACE2 exhibits protective effects against LPS-induced acute lung injury in mice by inhibiting the LPS-TLR4 pathway. Exp Mol Pathol 2019;113:104350.

68. Bozkurt B, Kovacs R, Harrington B. HFSA/ACC/AHA statement addresses concerns re: using RAAS antagonists in COVID-19. American College of Cardiology 2020. Available at: https://www.acc.org/latest-in-cardiology/articles/2020/03/17/08/59/hfsa-acc-aha-statement-addresses-concerns-re-using-raas-antagonists-in-covid-19. Accessed March 29, 2020.

69. Kuster GM, Pfister O, Burkard T, et al. SARS-CoV2: should inhibitors of the renin-angiotensin system be withdrawn in patients with COVID-19? Eur Heart J 2020 Mar 20 [E-pub ahead of print].

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