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Review Article

Angiotensin-converting enzyme 2 and COVID-19 in cardiorenal diseases

Ravindra K. Sharma[1], Jing Li[2], Suraj Krishnan[1], Elaine M. Richards[2], Mohan K. Raizada[2] and Rajesh Mohandas[1]

[1]Division of Nephrology, Hypertension and Renal Transplantation, Department of Medicine, University of Florida College of Medicine, Gainesville, FL 32610, U.S.A.; [2]Division of Nephrology, Hypertension and Renal Transplantation, Department of Physiology and Functional Genomics, University of Florida College of Medicine, Gainesville, FL 32610, U.S.A.

Correspondence: Mohan K. Raizada (mraizada@ufl.edu) or Rajesh Mohandas (rajesh.mohandas@medicine.ufl.edu)

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The rapid spread of the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has brought into focus the key role of angiotensin-converting enzyme 2 (ACE2), which serves as a cell surface receptor required for the virus to enter cells. SARS-CoV-2 can decrease cell surface ACE2 directly by internalization of ACE2 bound to the virus and indirectly by increased ADAM17 (a disintegrin and metalloproteinase 17)-mediated shedding of ACE2. ACE2 is widely expressed in the heart, lungs, vasculature, kidney and the gastrointestinal (GI) tract, where it counteracts the deleterious effects of angiotensin II (AngII) by catalyzing the conversion of AngII into the vasodilator peptide angiotensin-(1-7) (Ang-(1-7)). The down-regulation of ACE2 by SARS-CoV-2 can be detrimental to the cardiovascular system and kidneys. Further, decreased ACE2 can cause gut dysbiosis, inflammation and potentially worsen the systemic inflammatory response and coagulopathy associated with SARS-CoV-2. This review aims to elucidate the crucial role of ACE2 both as a regulator of the renin–angiotensin system and a receptor for SARS-CoV-2 as well as the implications for Coronavirus disease 19 and its associated cardiovascular and renal complications.

Introduction

Since its discovery in late 2000, angiotensin-converting enzyme 2 (ACE2) has garnered widespread attention for its multiple physiological roles: a negative regulator of renin–angiotensin system (RAS), a mediator of amino-acid transport and more recently a receptor for severe acute respiratory syndrome coronavirus (SARS-CoV) and the novel SARS-CoV-2, that has caused the Coronavirus disease 2019 (COVID-19). Over the past 20 years, numerous clinical and experimental interventions including recombinant ACE2, delivery of ACE2 gene, analogs of angiotensin-(1-7) (Ang-(1-7)), and Mas receptor agonists, have signified the vital role of ACE2 in ameliorating various cardiovascular diseases (CVDs) [1–10], and validated multiple promising drug targets for the treatment of various disorders associated with alterations in RAS [1,11,12].

ACE2 is a member of the angiotensin-converting enzyme (ACE) family. It is an ectoenzyme that cleaves the carboxyl-terminal amino acid phenylalanine from angiotensin II (AngII) and leucine from angiotensin I (AngI), hydrolyzing them into vasodilators Ang-(1-7) and angiotensin-(1-9) (Ang-(1-9)), respectively (Figure 1). Thus, ACE2 regulates RAS directly by down-regulating AngII/AT1R signaling and indirectly by activating the counterregulatory Ang-(1-7)/Mas receptor axis. The vasodeleterious AngII/AT1R signaling induces vasoconstriction, profibrotic and proinflammatory effects, whereas Ang-(1-7)/Mas receptor signaling elicits vasodilatory, antiproliferative and antiapoptotic effects that are beneficial in various CVDs [1,5,13,14]. Imbalance between vasodeleterious AngII/AT1R signaling and beneficial Ang (1-7)/Mas receptor signaling has been shown to underlie multiple CVDs as well as pathophysiological states that predispose to CVD such as obesity, diabetes and kidney disease. This view is further supported by the
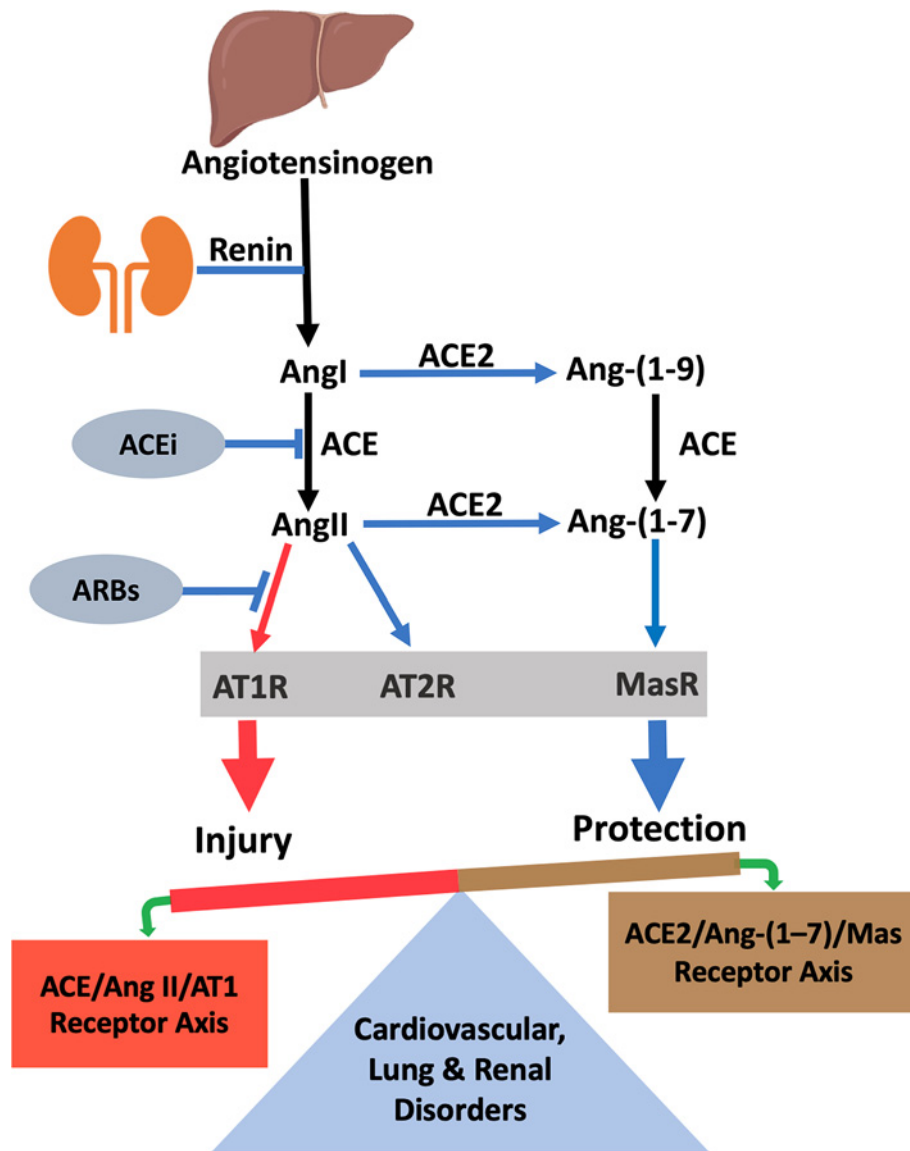
Figure 1. The renin–angiotensin system (RAS) with vasodeleterious axis (ACE/Ang II/AT1 receptor) and vasoprotective ACE2/Ang-(1-7)/Mas receptor axis

ACE2 is a major component of vasoprotective RAS which counterbalances the vasodeleterious effects of AngII activation. Abbreviations: ACEi, ACE inhibitor; ARB, angiotensin receptor blocker.

evidence that catalytically active ACE2 treatment ameliorates CVDs in various experimental models of human diseases [1,2,12,15].

Recent studies on SARS-CoV-2 infections indicate that individuals with pre-existing conditions such as obesity, diabetes, hypertension (HTN), lung and kidney disease, are at greater risk of severe manifestations of COVID-19. Since these pre-existing conditions are associated with abnormal RAS signaling, it is worth considering the influence of RAS on SARS-CoV-2 infection and COVID-19 disease. In a preliminary study of 12 patients with COVID-19, circulating AngII levels tended to correlate positively with lung injury and viral load [16]. In SARS-CoV infection, ACE2 expression is reduced in the heart and other tissues [1,17]. Many aspects of SARS-CoV and SARS-CoV-2 diseases are similar; this likely occurs in COVID-19, as well. Ongoing global efforts have focused on manipulating the ACE2/Ang-(1-7) axis to mitigate this deadly infection and attenuate lung and cardiovascular system damage from COVID-19. In this review, we summarize recent developments on ACE2 and its role as a receptor for SARS-CoV-2.

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We also discuss the implications of ACE2 on the pathophysiology of CVDs and potential therapeutic approaches for the treatment of COVID-19.

ACE2 structure and functions

In 2000, a gene encoding a protein with 42% homology in the catalytic domain to ACE, was discovered in a cDNA library prepared from the ventricle of a human heart failure (HF) patient and named ACE homolog or ACE2 [18]. Sequence comparison of ACE2 and ACE indicates that ACE2, like ACE, is an integral transmembrane protein tasked to metabolize circulating peptides with its extracellularly facing catalytic site. ACE has two such catalytic sites but ACE2 only one [2,19]. The ACE2 gene contains 18 exons located on chromosome X (band: Xp22.2) [1,20]. The translated protein comprised an extracellular N-terminal domain with 740 amino acids, a short single transmembrane domain and a short cytosolic C-terminal tail with 44 amino acids [1,20]. ACE2 is widely expressed throughout the body with highest expression in the gastrointestinal (GI) and oral epithelium and substantial levels in lungs, kidney and heart [1,2,18].

ACE2 as a receptor for SARS-CoV-2

Soon after the first outbreak of SARS in 2003, ACE2 was identified as a cell-surface receptor responsible for SARS-CoV entry into cells. ACE2 functions as a primary entry point for most coronaviruses including HCoV-NL63, SARS-CoV and SARS-CoV-2. However, the ACE2-binding domain has higher affinity for SARS-CoV-2 which may partly explain the markedly larger global impact of SARS-CoV-2 than the initial SARS outbreak [21].

SARS-CoV-2 is an enveloped, RNA virus with virions ranging in size from 55 to 180 nm [1,22]. Coronaviruses invade cells through their spike protein (S-protein), a glycoprotein that binds to the extracellular enzymatic domain of ACE2 located on the surface of the host cells, resulting in subsequent down-regulation of cell surface ACE2 expression [1,2,23]. Following entry into the cell, the virus replicates and induces cytotoxicity that may result in organ failure (Figure 2). The size of S-proteins has been reported to be ∼15.5 nm [1] and it may be found in either open or closed conformations [24]. In a recent study, Hoffmann et al., showed that entry of the SARS-CoV-2 depends on the priming of S-protein by the host cell serine protease TMPRSS2 (transmembrane protease serine 2) and cathepsin L/B [23]. Blocking of this priming by inhibitors of TMPRSS2, camostat mesilate, in combination with cathepsin L/B inhibitor E-64d [23] arrests SARS-CoV-2 infection. However, SARS-CoV-2 also utilizes multiple other host proteases including trypsin, elastase, factor X and furin to prime S-protein and facilitate cell entry following binding to the ACE2 receptor [1,2]. ACE2 and S-protein are both proteolytically modified during their interaction [1,2].

ADAM17 (a disintegrin and metalloproteinase domain-containing protein 17), a type I transmembrane protein, is also known to mediate the proteolysis and ectoderm shedding of ACE2 [2] (Figure 2) as well as other proteins including the precursor for TNF-α. Studies have shown that ACE2 shedding mediated by ADAM17 is enhanced upon binding of SARS-CoV S-protein to ACE2 while knocking down ADAM17 with siRNA prevents SARS-CoV entry into the cell [25,26]. Thus, it is reasonable to suggest that enhancement of ADAM17-mediated ACE2 shedding resulted in impaired ACE2-mediated conversion of AngII into Ang-(1-7) by the cells [2], while simultaneously catalyzing the production of TNF-α from its precursor, which damages tissue. However, this remains to be investigated in SARS-CoV-2. Additionally, inhibition of ADAM17 attenuates fibrosis and inflammation indicating its potential as a therapeutic agent for treatment of fibrotic kidney disease [27,28]. Overactivity of the vasodeleterious axis of the RAS predisposes to CVDs including HF, arterial fibrillation, HTN and coronary artery disease [2]. Moreover, loss of cellular ACE2 promotes accumulation of AngII and enhances ADAM17 activity leading to inflammation and other consequences of overactivation of the vasodeleterious RAS axis which may be relevant in SARS-CoV-2 infection.

GI ACE2 plays dual roles: the traditional RAS-dependent role and an RAS-independent role in the transport of amino acids [29]. ACE2 functions as a chaperone for B0AT1 (neutral amino acid transporter), a protein that is highly expressed in the brush border of the small intestine and kidney and tasked with absorbing neutral amino acids [30] such as leucine, tryptophan, glutamine and their metabolites [1]. The transporter activity of B0AT1 in the gut is dramatically augmented by ACE2 co-expression [1,31]. ACE2 in the gut also functions as a key regulator of innate immunity, gut microbial ecology and expression of antimicrobial peptides [29,32,33]. High expression of ACE2 throughout the luminal surface of the GI tract, particularly in enterocytes, suggests that the gut could serve as a secondary site for SARS-CoV-2 infection. Indeed, SARS-CoV particles were found within the surface microvilli of apical enterocytes of the ileum and colon [34]. In support of this concept, patients with COVID-19 show prominent GI manifestations, sometimes prior to the development of classic pulmonary symptoms [35]; Further, SARS-CoV-2 has been detected in the feces of COVID-19 patients, suggesting the possibility of fecal–oral transmission [36]. Importantly, decreased ACE2 activity after binding of SARS-CoV-2 with the intestinal apical membrane ACE2–B^{0}AT1 complex disrupts
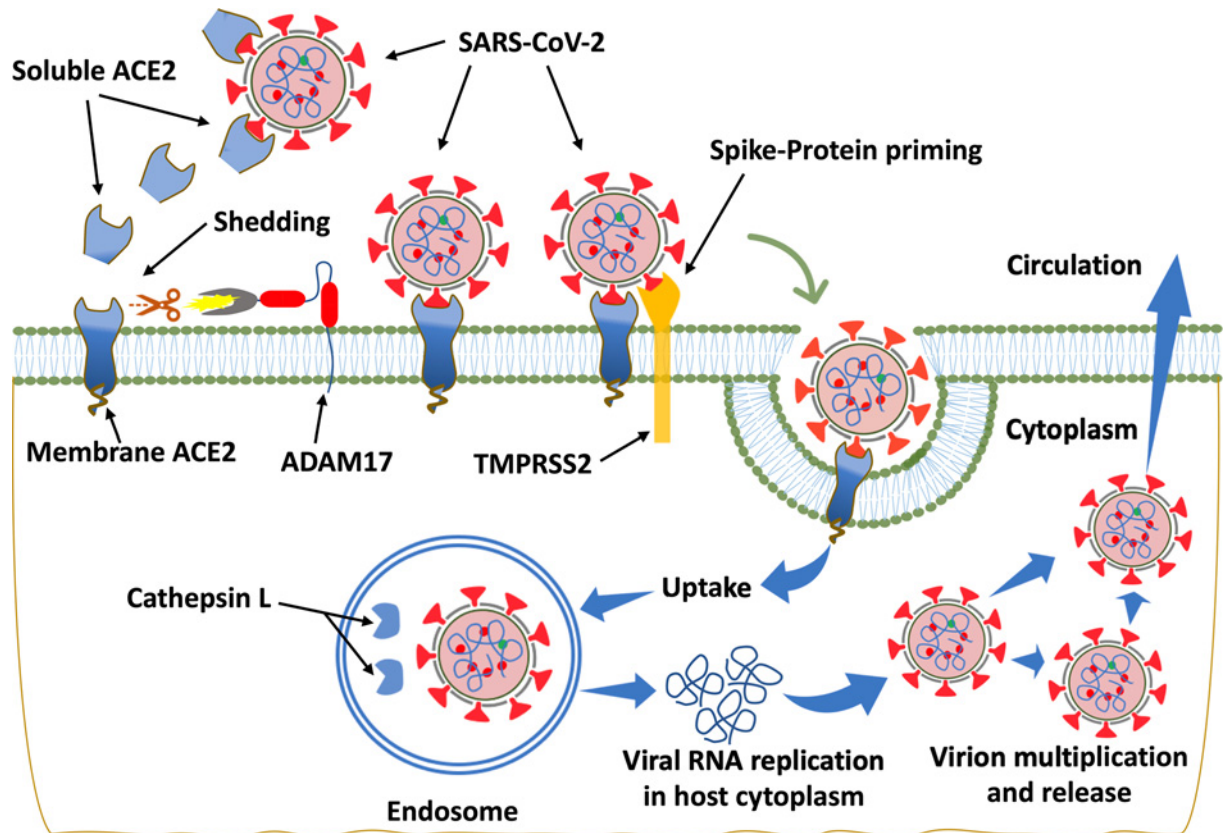
Figure 2. Diagrammatic representation of ACE2-mediated SARS-CoV-2 entry into the cell
Binding of SARS-CoV-2 with its cellular receptor, ACE2, results in loss of ACE2 and its cardioprotective effects. The entry of SARS-CoV-2 into the cell is facilitated by TMPRSS2 that primes S-protein. Virus may also enter through endocytosis and become activated by cathepsin L, although other proteases such as furin may also activate SARS-CoV-2. Following virus entry into host cells, SARS-CoV-2 undergoes RNA replication within endosomes. ADAM17 (a disintegrin and metalloproteinase 17) activity is up-regulated by the endocytosed S-proteins of SARS-CoV, and though not proven, likely to be by SARS-CoV-2 given the similarity of viral interactions with ACE2. ADAM17 mediates the proteolytic cleavage of ACE2, releasing soluble ACE2 (sACE2) into the circulation.

intestinal RAS, with consequent gut wall inflammation, compromised gut barrier integrity and microbial dysbiosis [2,37]. The impairment of the gut–blood barrier leads to systemic spread of bacteria, microbial metabolites and endotoxins [2,38]. These incidents alone or in combination could potentially affect the host's response to SARS-CoV-2 infection and contribute to multiorgan system dysfunction and septic shock [39–41].

The mechanism by which SARS-COV-2 interacts with ACE2 and its partners such as TMPRSS2 and B^0AT1 remains under extensive investigation. We have established an *ex vivo* 3D organoid system from rat and mouse colon for this purpose. These organoids have characteristics of colonic epithelium and express ACE2, ACE, TMPRSS2 and B^0AT1. These are essential components for SARS-CoV-2 infection (Figure 3). These rodent organoids and organoids from human subjects will be key in elucidating interactions among SARS-CoV-2, ACE2 and its partners.

ACE2 in cardiovascular system, implications in COVID-19
HTN
HTN is the one of the most prevalent risk factors for CVDs. The most recent American Heart Association report indicates that ∼49% of American adults have high blood pressure (BP) [42] and ∼20% of these patients have drug-resistant HTN (R-HTN) [43] because they do not have adequate BP control even when treated with three (or more) antihypertensive medications. Overactivity of the vasodeleterious axis (ACE/AngII/AT1R) and a decrease in the vasoprotective axis (ACE2/Ang-(1-7)/Mas receptor) of the RAS is a major contributor to HTN. There is ample evidence, particularly from animal studies, in support of this concept. This includes: (i) BP is elevated in ACE2 null
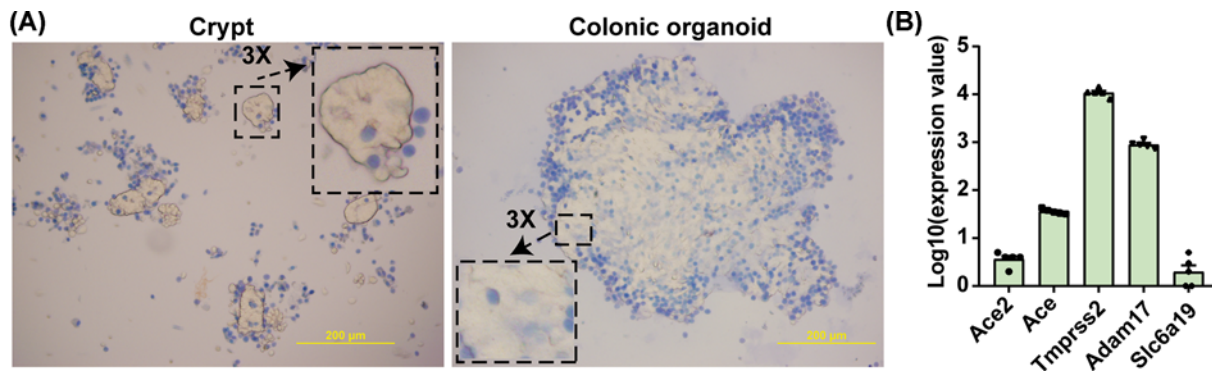
Figure 3. 3D colonic organoid cultures: an *ex vivo* model to study the interactions of ACE2, its partners and SARS-CoV-2
(**A**) Phase microscopic images documenting 7-day growth of organoids from isolated colonic crypts of WKY rats stained with Trypan Blue; dead cells are blue. Scale bar: 200 µM. (**B**) mRNA levels (by RNA-seq) of ACE2 and its partners in colonic organoids from WKY rats (*n*=5 per group). The detailed method for 3D colonic organoid cultures was published elsewhere [62]. Abbreviation: Slc6a19, solute carrier family 6 member 19, also known as B^0AT1.

mice on a C57BL/6 background [10]; AngII-induced HTN is more severe in these mice [10]. (ii) Lentiviral-mediated overexpression of ACE2 ameliorates high BP and HTN-associated pathophysiology in the spontaneously hypertensive rat (SHR), a rodent model of essential HTN [44,45]. Similarly, vascular ACE2 overexpression increased Ang-(1-7), restored endothelial function and decreased BP [46]. (iii) Renal ACE2 is inversely related to BP and is decreased in the SHR and the stroke-prone SHR [47]. On the other hand, a few studies have failed to find beneficial effects of ACE2 in BP control. For example, Crackower et al. found no change in BP in 3-month-old ACE2 null mice and unanticipated lower BP in the same mice at 6 months of age [48]. These ACE2 null mice do exhibit abnormal cardiac contractility that might have confounded effects on BP. Mas receptor null mice also had normal BP [49]. Administration of diminazene aceturate (DIZE), a putative ACE2 activator, caused an acute lowering of BP in the SHR [50], but it had no effect on BP in rats with kidney disease [51]. Importantly, in wild-type mice, rhACE2 treatment did not affect the baseline plasma AngII, Ang-(1-7) or BP suggesting that substrate availability is a key limiting factor in ACE2's enzymatic activity [52]. Finally, in a Phase I safety trial, rhACE2 administration to a small cohort of healthy human subjects had no effect on BP and heart rate [53]. These observations, taken together, suggest ambivalence about the effects of ACE2 on high BP in HTN and further studies are warranted to resolve this. It would also be prudent to consider alternate mechanisms for the role of ACE2/Ang-(1-7) in BP control. An attractive option is that this vasoprotective axis exerts direct local beneficial effects on cardiac, vascular, renal and neural signaling mechanisms that indirectly affect BP. The brain RAS and neurogenic HTN may be an example of such organ-specific effects of ACE2/Ang-(1-7).

The brain RAS is a major player in the regulation of autonomic function and has a role in control of BP. Components of the RAS, including ACE2, are widely expressed in many brain cells including neurons [54], microglia and astrocytes [55,56]. ACE2 is generally decreased in brains of animals with HTN and HF [2,48,52,57]. The decrease is suggested to be due to shedding of ACE2 into the CSF by ADAM17 [57]. This is consistent with an increased ACE2 in the CSF of hypertensive patients and a significant correlation of CSF ACE2 with systolic BP [58]. ACE2 gene deletion impairs autonomic and baroreceptor functions and disrupts the central regulation of BP [59,60]. Conversely, ACE2 overexpression is protective against neurogenic HTN [45,61]. Its beneficial effects involve increasing neuronal nitric oxide (NO), reducing proinflammatory cytokines and reactive oxygen species (ROS), and restoring cerebrovascular endothelial function [62]. Additional evidence of ACE2's involvement is derived from elegant studies with ADAM17 deletion [15]. Selective deletion of ACE2 and ADAM17 from neurons relevant to autonomic function decreases inhibitory inputs to pre-sympathetic neurons controlling BP [15]. Consistent with these observations, intracerebroventricular infusion of Ang-(1-7) into the nucleus tractus solitarius (NTS) [63], caudal ventrolateral medulla (CVLM) [64]), paraventricular nucleus (PVN) of hypothalamus [65] or anterior hypothalamus results in decreases in BP. This effect on BP appears to be specific to certain brain regions since administration of Ang-(1-7) into the rostral ventrolateral medulla (RVLM) increased BP [66,67]. Together, these studies strengthen the concept of a protective role for brain ACE2 in neurogenic HTN.

More than half the patients who are hospitalized with COVID-19 are hypertensive [68]. However, whether this high prevalence is due to more frequent or severe SARS-CoV-2 infection in hypertensive patients or confounding by

Table 1 Overview of current clinical trials evaluating various therapeutic agents on COVID-19

Clinical trial Identifier	Title of the clinical trial	Setting, severity	Active arm(s)	Status
NCT04364893	Angiotensin Receptor Blockers and Angiotensin-converting Enzyme Inhibitors and Adverse Outcomes in Patients With COVID-19 (BRACE-CORONA)	Hospitalized, COVID-19 (~700 subjects)	ACEi and ARBs	Recruiting
NCT04335136	Recombinant Human Angiotensin-converting Enzyme 2 (rhACE2) as a Treatment for Patients With COVID-19 (APN01-COVID-19)	Hospitalized, COVID-19 (200 subjects)	RhACE2, APN01	Recruiting
NCT04321096	The Impact of Camostat Mesilate on COVID-19 Infection (CamoCO-19)	Hospitalized, COVID-19 (580 subjects)	Camostat Mesilate	Recruiting
NCT04338009	Elimination or Prolongation of ACE Inhibitors and ARB in Coronavirus Disease 2019 (REPLACECOVID)	Hospitalized, COVID-19 (152 subjects)	ARB/ACEi	Recruiting
NCT04312009	Losartan for Patients With COVID-19 Requiring Hospitalization	Hospitalized, COVID-19 (200 subjects)	Losartan	Recruiting
NCT04311177	Losartan for Patients With COVID-19 Not Requiring Hospitalization	Patients with COVID-19 (580 subjects)	Losartan	Active, not Recruiting
NCT04318418	ACE Inhibitors, Angiotension II Type-I Receptor Blockers and Severity of COVID-19 (CODIV-ACE)	Hospitalized, COVID-19 (3400 subjects)	ACEi and ARBs	Completed
NCT04315298	Evaluation of the Efficacy and Safety of Sarilumab in Hospitalized Patients With COVID-19	Hospitalized, COVID-19 (1912 subjects)	Sarilumab	Completed
NCT04320615	A Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia (COVACTA)	Hospitalized, severe COVID-19 Pneumonia (450 subjects)	Tocilizumab (TCZ)	Completed
NCT04330638	Treatment of COVID-19 Patients With Anti-interleukin Drugs (COV-AID)	Hospitalized, CIVID-19 with systemic cytokine release syndrome (342 subjects)	Anakinra Siltuximab Tocilizumab	Recruiting
NCT04322188	An Observational study of the Use of Siltuximab (SYLVANT) in Patients Diagnosed With COVID-19 Infection Who Have Developed Serious Respiratory Complications (SISCO)	Hospitalized, ARDS, secondary to SARS-COV-2 Infection (220 subjects)	Siltuximab (SYLVANT)	Completed

age, obesity, kidney disease or other comorbidities remains unclear. Antihypertensive treatment with ACE inhibitors (ACEi) or Ang II receptor blockers (ARBs) is associated with increased ACE2, which could potentially increase the chances of SARS-CoV-2 infection or the severity of COVID-19. Lisinopril and losartan treatment increased ACE2 gene expression in the left ventricle of the normotensive Lewis rats [39] and ACE2 protein and activity are increased in the heart of rats treated with enalapril [40]. Similarly, ACE2 protein and mRNA increased following treatment with the ARB, Telmisartan [41]. However, studies from human subjects treated with ACEi or ARB demonstrate contradictory results on whether these medications increase ACE2, with some showing increased ACE2, while others showed no effect [2,44,45,69]. Studies in humans relied mostly on plasma levels of ACE2 to assess ACE2 activity and could account for some of these discrepancies. Retrospective studies examining the use of ACEi/ARB in hypertensive patients have either found improvement in mortality risk [70] or no effect on the incidence or severity of COVID-19 [71–73]. The preliminary results from the randomized controlled BRACE-CORONA trial suggests that continuing ACEi/ARB as opposed to withholding ACEi/ARB therapy does not change clinical outcomes in patients with COVID-19 (NCT04364893). Multiple randomized controlled trials are underway in the United States and elsewhere (Table 1) which should clarify this question.

HF

HF is a leading cause of deaths and a major public health problem. The prevalence of HF is 6.5 million in the U.S.A., over 25 million worldwide, and increasing. It is a complex, multifactorial syndrome consisting of many overlapping phenotypes. High BP, coronary artery diseases, cardiomyopathy and diabetes are major risk factors involved in the pathophysiology of HF. ACE2 is widely expressed in cardiac fibroblasts, cardiomyocytes and coronary endothelial cells where it negates the deleterious effects of AngII and activates Ang-(1-7)/Mas signaling [74]. Ang-(1-7) produced by ACE2 promotes endothelial NO production via Akt-dependent mechanisms [75], and ameliorates cardiac hypertrophy and fibrosis in a mouse model of insulin resistance [76]. Evidence indicates that many CVDs including HF are associated with relative ACE2 deficiency, and this loss of ACE2 reduces the homeostatic defensive mechanisms [19,77]. ACE2 overexpression prevents or even reverses the HF phenotype [76,78–80], whereas ACE2 deficiency may

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promote the development of HF [81]. Interestingly, HF patients show increased expression of ACE2 early in the disease, perhaps in an attempt to counter the deleterious effects of elevated AngII levels, but with progressing cardiac disease there is relative deficiency of ACE2 and a concomitant increase in AngII [5,81]. Further, the activity of circulating ACE2 is markedly higher in patients with decompensated HF compared with ambulatory HF patients, associated with poor prognosis [12,82].

Evidence indicates that COVID-19 is strongly associated with HF. In an early study from China involving 799 COVID-19 patients, HF was one of the major complications observed with a reported incidence of 24% in all patients and 49% in patients who died [83]. Of note, the median age of deceased patients (68 years) was significantly older than those who recovered (51 years) [83]. In a separate study from China involving 191 COVID-19 patients, HF was reported in 23% of all patients and 52% of patients who died [84]. Studies using cardiac magnetic resonance imaging, even in younger patients without comorbidities, have shown significant cardiac involvement in patients who had presumably recovered from COVID-19 [85]. Lending credence to direct myocardial involvement in COVID-19, the SARS-CoV genome was detected in the heart tissues in postmortem autopsy samples from SARS patients [17]. Tissue ACE2 is down-regulated while there is a concomitant increase in AngII in patients with COVID-19. The AngII levels correlate linearly with SARS-CoV-2 viral load [16]. Similarly, myocardial ACE2 expression is markedly reduced in postmortem autopsy samples from SARS-CoV-infected patients and was associated with concomitant increases in myocardial inflammation and fibrosis [17]. This decrease in ACE2 is thought to be due to increased endocytosis and proteolytic processing of membrane-bound ACE2 [2]. Importantly, cardiac pericytes with high expression of ACE2 have been shown to be a primary target in SARS-CoV-2 infection [86]. Injured pericytes due to virus infection may impair endothelial function and induce microvascular dysfunction. These adverse effects on endothelial and myocardial function might be compounded by circulating proinflammatory cytokines.

Since ACE2 is required for SARS-CoV-2 entry into cells, it has been suggested that ACE2 up-regulation may increase susceptibility to SARS-CoV-2 infection. On the other hand, increased ACE2 expression may also protect against AngII-induced vasoconstriction and inflammation. However, an association between ACE2 expression and susceptibility or severity of SARS-CoV-2 infection, is not evident [74]. In animal models of HF, use of ACEi/ARB increases ACE2 expression. For example, Losartan and Olmesartan increase ACE2 mRNA expression in the heart of rats after MI [87]. Similarly, Enalapril can normalize ACE2 expression in the left ventricle of rats with HF [88]. However, protective or ameliorating effects of ACEi/ARB use on myocardial function in patients with COVID-19 remain to be proven. In the absence of compelling clinical data, most professional organizations recommend continuing ACEi or ARB therapy in HF patients with or at risk of SARS-CoV-2 infection.

Vasculature/endothelium dysfunction/atherosclerosis

Recent evidence from clinical trials have confirmed the important role of inflammation in atherosclerosis. In healthy vessels the endothelium is important in modulating vascular tone by synthesizing and releasing a variety of endothelium-derived relaxing factors, vasodilator prostaglandins and most importantly NO [89]. Endothelial dysfunction precedes atherosclerosis and is thought to be an important pathogenic mechanism that contributes to the development of atherosclerosis and its complications [90,91]. Thus, endothelial dysfunction is often considered a marker for inherent atherosclerotic risk in an individual [92].

ACE2 expression is crucial for the healthy functioning of endothelial cells. Animal studies demonstrate that ACE2 deletion impairs endothelium-dependent vasodilatation [93] by decreasing bioavailability of NO [93]. ACE2 deficiency leads to a pro-oxidative state with increased reactive oxygen species (ROS) and concurrent decrease in NO bioavailability through formation of peroxynitrite ($ONOO^-$) free radicals [93–95]. ACE2 overexpression, on the other hand, promotes normal endothelial cell migration and tube formation favoring angiogenesis whereas silencing the ACE2 gene reverses these effects [93]. Importantly, ACE2 attenuates the formation of atherosclerotic plaques by regulating monocyte–endothelial cell interactions by decreasing endothelial expression of adhesion molecules (MCP-1, VCAM-1 and E-selectin) through production of Ang-(1-7), which curbs macrophage infiltration into the vessel wall [96]. Finally, ACE2 inhibits vascular smooth muscle cell proliferation and migration [97–99]. Pro-atherosclerotic effects of ACE2 deficiency are amplified in the presence of HTN or diabetes or both [100].

COVID-19 is emerging as a thrombotic and vascular disease affecting endothelial cells and is particularly noticeable in patients with cardiometabolic comorbidities such as HTN, diabetes and kidney disease [101]. Recent evidence indicates that SARS-CoV-2 directly infects blood vessels and initiates endothelial signaling associated with proinflammatory and pro-apoptotic mediators and cytokine dysfunction precipitating the thrombotic cascade [102]. Additionally, viral consumption of membrane ACE2 can also disrupt AngII metabolism leading to increased AngII and concomitant Ang-(1-7) deficiency, further augmenting inflammation, endothelium activation and recruitment of leukocytes and platelets [103]. All these events alone or in combination could induce a localized microvascular

inflammation, prothrombotic conditions and tissue damage [103]. Pulmonary embolism and deep vein thrombosis have been reported in one third of hospitalized COVID-19 patients [104] often associated with elevated D-dimer and fibrinogen levels [105].

Very recently in New York, a pediatric multi-inflammatory syndrome mimicking Kawasaki disease was described in children with COVID-19 [106]. Kawasaki disease is a rare acute pediatric vasculitis with coronary artery aneurysms as its main complication. These children have milder vasculitis than adults and better prognosis as well as survival. In Bergamo, Italy, several cases of pediatric vasculitis were also reported in the midst of the pandemic [107]. Vasculitis is suggested to be a direct consequence of SARS-CoV-2 infection [108]. The incidence of multiple vascular diseases point toward vascular complications as a major contributing factor to the pathogenesis of COVID-19. This emphasizes the notion that endothelial injury and inflammation occur due to either direct viral infection, through disordered cytokine release, or both, and might contribute to the systemic microcirculatory impairment in COVID -19 [101].

Renal diseases

Kidney works synchronously with the cardiovascular system to maintain BP and fluid balance [109,110]. These functions of kidney are vital for maintenance of normal cardiac ejection fraction [110,111] and tissue oxygen delivery [112]. In addition, kidney has important roles in timely clearance of uremic toxins [113], maintaining electrolyte and acid–base balance [114], erythropoiesis [115] and bone-mineral metabolism [116]. Progressive decline in renal function increases the risk of CVDs [117]. It is estimated that 37 million (one in seven) adults have chronic kidney disease (CKD), costing Medicare more than 84 billion dollars in the U.S.A. (CDC 2019).

Renal tissue is a major source of Ang-(1-7), a beneficial peptide derived by ACE2-mediated hydrolysis of AngII suggesting the presence of functional ACE2 in the kidney [PMIDs:19578709,14,146]. ACE2 is profoundly expressed in the brush border of proximal tubules and moderately in podocytes and parietal epithelial cells, whereas ACE2 expression is weak in glomerular endothelial cells and mesangial cells [118–120]. Growing evidence suggests that imbalance in the counterbalancing arms of the RAS is a fundamental driver of renal and systemic vascular dysfunction in various pathological states. Renal ACE2 activity and Ang-(1-7) were decreased after 4 h of reperfusion after ischemic injury and were negatively correlated with AngII [121]. Interestingly, the renal expression of Mas receptor was greatly increased after 4 h of reperfusion suggesting a critical role for the RAS components in acute kidney injury (AKI) [121]. Changes in kidney RAS expression has been validated in other animal models of kidney disease including subtotal nephrectomy and sepsis [121–123]. Further, patients with end stage renal disease on hemodialysis had lower plasma ACE2 activity compared with their pre-dialysis counterparts with CKD or renal transplant recipients [124]. In animal models of diabetes, studies have shown decreased glomerular ACE2 whereas intraglomerular ACE expression is increased [125,126]. Deficiency of ACE2 by either pharmacological inhibition or genetic knocking out of ACE2 exacerbates albuminuria, mesangial matrix expansion, glomerular basement membrane thickening and glomerulosclerosis, all of which are characteristic features of diabetic nephropathy [127,128]. Conversely, kidney-specific overexpression of ACE2 in diabetic animals (Akita mice) ameliorates glomerular injury and sclerosis, improves podocyte function and development of overt albuminuria [129,130]. The ratio of ACE to ACE2 positively correlates with mean BP, fasting blood glucose, proteinuria, as well as serum creatinine and inversely with glomerular filtration rate (GFR) [129]. Interestingly, endothelial ACE2 was found to be increased in the glomerular and interstitial capillaries in kidney diseases indicating ACE2 might be a marker of endothelial injury [119]. ACE2 activity is increased early in CKD but as renal function progressively deteriorates relative ACE2 deficiency follows [131]. Is the initial increase in ACE2 seen in earlier stages of CKD a compensatory mechanism to counterbalance the elevated circulating AngII? Understanding the temporal dynamics of these interactions might help identify the subset of patients who are vulnerable to develop progressive decline in renal functions, culminating in end stage renal disease. It could also help to develop novel strategies and the appropriate time of intervention to prevent AKI to CKD progression, which is currently a huge challenge. Therefore, the underlying change in ACE2, regulation of enzymes involved in ACE2 metabolism and its receptors as well as the consequences of these changes is still an area of active research.

Kidney is frequently involved in SARS-CoV infection. During the 2003 SARS outbreak in Hong Kong, 36 out of 536 patients showed elevated plasma creatinine levels during their clinical course [132]. Those who had renal injury tended to be older with high BP. In another study ∼6% of SARS-CoV patients developed AKI, and kidney injury was identified as a fatal complication of SARS-CoV as almost 92% of SARS-infected patients with AKI died [132]. COVID-19 shows a similar high incidence of AKI and poor prognosis. For example, more than 40% of patients have proteinuria at hospital admission, 20–40% of critically ill patients have AKI in Europe and U.S.A., which serves as a negative prognostic marker for the recovery [133]. An early report with 3235 patients indicated that 43% of all

hospitalized patients and 68% of those admitted to the intensive care unit developed AKI [134]. In a separate study of critically ill patients with COVID-19 in Wuhan, China, 29% of those who were admitted developed AKI [135]. Further, out of 113 patients who died from COVID-19, 28 of them had AKI during their hospitalization [83]. These data strongly suggest a significantly higher mortality rate in critically ill COVID-19 patients with AKI.

COVID-19-associated pathological changes in the kidney include characteristics of acute tubular necrosis such as diffuse tubular injury with loss of brush border integrity and necrotic debris composed of necrotic epithelium in tubular lumina. In some cases, presence of multiple inflammatory infiltrates is observed in the tubules [118]. In addition, capillary endothelial injury, vacuolar degeneration and erythrocyte aggregates occluding the capillary lumina are frequently seen [118]. The cause of AKI in SARS-CoV-2 infection appears to be multifactorial. Sepsis (or septic shock) is considered the major contributing factor of kidney injury in these patients. Increasing viral infection in alveolar cells (pneumonia) could lead to massive recruitment of immune cells to the kidney which produce high levels of cytokines escalating the systemic inflammatory response called cytokine storm [136]. This could also result in endothelial dysfunction, activation of coagulation cascade and microthrombi eventually resulting in multiple organ failure. This process is not specific to COVID-19 as cytokine-mediated inflammatory AKI has been observed in several clinical conditions [137]. SARS-CoV-2 viral load was quantified in autopsy tissue samples collected from 22 patients who died from COVID-19. Seventeen (77%) of these patients showed SARS-CoV-2 in the kidney [138]. Presence of more than two coexisting conditions is associated with SARS-CoV-2 tropism for the kidneys, even in patients without a history of CKD [134,138,139]. Although these findings have been disputed, electron microscopic ultrastructural studies showed viral particles in the renal tubular epithelium morphologically identical with SARS-CoV-2, and presence of coronavirus-like particles with distinctive spikes in the tubular epithelium and podocytes of infected individuals indicating a direct infection (or circulating viremia) of the kidney in setting of AKI in COVID-19 [140]. Examination of single cell databases from 15 human subjects showed that ACE2 and TMPRSS2 are highly co-expressed in podocytes and proximal convoluted tubules of the kidney samples [141]. Since viral entry depends on the activity of protease TMPRSS2 with ACE2, these observations would suggest that kidneys with high co-expression of ACE2 and TMPRSS2 could amplify SARS-CoV-2 infection. Presence of high numbers of SARS-CoV-2 copies per cell in the kidney supports this view [138]. Finally, the loss of ACE2 via ADAM17-mediated proteolytic cleavage, which is activated in COVID-19 [26,142], might promote further injury to the kidney and cardiovascular system, particularly in patients with diabetes [2,142,143]. This loss of ACE2 leads to accumulation of renal AngII resulting in activation of vasodeleterious axis of RAS. Systemic and glomerular capillary HTN ensue from the increased intrarenal AngII, eventually causing endothelial injury and kidney dysfunction. Further, in a healthy kidney, ACE2 degrades AngII to Ang-(1-7) activating the vasoprotective axis. Ang-(1-7) dilates pre-constricted renal afferent arterioles and increases renal blood flow [144]. Ang-(1-7) also increases the production of renal atrial natriuretic peptide which reduces oxidative stress and fibrotic, proliferative and inflammatory effects of AngII in the kidney [145,146]. Thus Ang-(1-7) is contemplated to be an important physiological regulator opposing the harmful effects of disproportionate AngII production [146].

ACE2 targeted therapeutic strategies against SARS-CoV-2 infection

While research on developing a vaccine/treatment is being pursued aggressively, currently there are a few ACE2 based therapeutic approaches that could be effective during SARS-COV-2 infection that could be available sooner. For instance, up-regulating the function and expression of ACE2 by increasing gene transcription, translation or its catalytic activity has been shown to have beneficial outcomes in various cardiopulmonary disorders characterized by dysregulated RAS. In pulmonary arterial hypertension (PAH), rhACE2 ameliorates vascular damage, lung injury and fibrosis, arterial remodeling and improves right ventricular performance [1,2]. In an experimental model of HTN, rhACE2 treatment produced decreased left ventricular hypertrophy, myocardial fibrosis and improved cardiac function [5]. Recently, Penninger et al. showed that clinical-grade human recombinant soluble (rhACE2) can reduce viral load and block entry of SARS-CoV-2 infection into Vero E6 cells [147]. Further, they showed that SARS-CoV-2 could directly infect human organoids such as kidney and this infection can be inhibited by rhACE2 in earlier stages of SARS-CoV-2 infection [147]. Excessive soluble ACE2 (sACE2) binding with SARS-CoV-2 decreases infectivity up to 5000-fold in blood vessels and kidney organoids [147]. In two Phase II clinical trials, rhACE2 administration was well tolerated in patients with PAH and acute respiratory distress syndrome (ARDS) with no apparent side effects [148,149]. More definitive clinical trials are underway to determine the effectiveness of rhACE2 to block viral entry and decrease replication by regulating systemic RAS (NCT04335136). However, there might be a few inherent barriers that challenge the therapeutic use of ACE2 including its restricted penetrance, lack of stability *in vivo* (short half-life ~3.5 h) and high cost associated with the manufacturing of recombinant protein. However, an effort has been made

to change the stability of ACE2 via modulating Ser^{680} phosphorylation which could have significant translational relevance of ACE2 in treating lung disorders [150]. Oral delivery is the preferred route of drug administration. Oral administration of rhACE2 protein has limitations because it is degraded by the acidic environment of the GI tract. To circumvent this problem, our group has developed an oral delivery system which bioencapsulates ACE2 utilizing transplastomic technology. Oral delivery of bioencapsulated ACE2 ameliorated monocrotaline-induced PAH and improved right heart functions in rodents [11]. This was accompanied by increased pulmonary ACE2 and angiotensin type 2 receptors (AT2Rs) and decreased proinflammatory cytokines [11]. Our group also developed an oral delivery system for Ang-(1-7). Moreover, the oral delivery of ACE2 therapeutics is more cost-effective, convenient and likely to be accepted by patients.

Decreased plasma membrane ACE2 due to internalization of SARS-CoV-2 shifts the RAS toward the vasodeleterious axis potentiating the inflammatory, hypertrophic and fibrotic actions of AngII and AT1R that are often observed in SARS-CoV-2 patients. Therefore, increasing membrane expression of ACE2 could be beneficial, as it might reduce the systemic inflammation and shift the RAS balance toward the beneficial side. There could be many potential mechanisms for the beneficial effects of increased ACE2. Increased sACE2 can bind with SARS-CoV-2 and may remove it from the circulation that may be helpful in reducing the infectious load [1]. Finally, increased ACE2 may foster self-dimerization in the cell membrane thereby diminishing affinity for virus, internalization and spread. Proteases such as ADAM17 and TMPRSS2 have been implicated in regulation of plasma membrane ACE2 and therefore may serve as potential therapeutic targets. ADAM17 inhibitors such as paricalcitol and synthetic vitamin D analogs, might be beneficial in COVID-19 and need to be tested for clinical efficacy. A clinical trial is underway to determine the efficacy in SARS-CoV-2 positive patients (NCT04321096). On the other hand, ADAM17 increases sACE2 by cleaving the extracellular domain of ACE2. The resultant sACE2 has enzymatic activity and the ability to bind SARS-CoV [26]. Thus, the final effect of ADAM17 on SARS-CoV2 infection and complications remains to be determined.

Several small molecule activators have been synthesized to enhance the activity of endogenous ACE2 by utilizing structure-based drug design. DIZE, the most potent activator, increases ACE2 activity in plasma, heart, lung, kidney and retina [151–154] and has been shown to have protective effects in several animal models of heart and lung disease [151,153,154]. DIZE was effective in attenuating ischemia-induced pathology in a murine model of myocardial infarction [151], as well as pulmonary HTN and fibrosis [154]. Additionally, Ang-(1-7) receptor agonist AVE0991 has been shown to have beneficial effects in lung and CVD, by stimulating ACE2/Ang-(1-7)/Mas receptor signaling [155]. Therefore, the ACE2/Ang-(1-7) axis together with its widely expressed Mas receptor, may play a dynamic role in maintaining ACE2 or Ang-(1-7) levels and could be a target to decrease severity or prevent complications in patients infected with SARS-CoV-2.

Non-ACE2-targeted RAS inhibition-based therapeutic approaches against SARS-CoV-2 infection

Present therapies aim to inhibit RAS in numerous ways. ARBs and ACEi, in addition to their primary pharmacological function to block AT1 receptor activity or inhibit ACE respectively, up-regulate ACE2 expression and activity in experimental models. The direct implications of increased ACE2 in COVID-19 patients with HTN is not clear yet. Theoretically, an increase in ACE2 expression by ARBs and ACEi could increase the risk for or severity of the SARS-CoV-2 infection. This has led to some controversy around the world whether these inhibitors should be discontinued. Some recent studies have brought some clarity to this issue. For instance, a retrospective study in New York city of 12594 patients, 46.8% of whom were COVID-19 positive, found no association between the use of ARBs or ACEi and COVID-19 infection, severity or mortality [72]. A Chinese study of 362 COVID-19 patients found no difference in severity or mortality compared with the use of ACEi/ARB [156]. Finally, an Italian population-based cohort of 6272 case patients found no evidence ARBs or ACEi increased the risk of COVID-19 [71]. However, a retrospective multicenter study of 1128 patients showed significantly lower mortality with the use of ACEi/ARB [157]. Although increased ACE2 could facilitate cellular entry of the virus, ACE2 could increase the conversion of vasodeleterious AngII into Ang-(1-7), which has known vasodilatory and anti-inflammatory effects. The Randomized Elimination or Prolongation of ARB and ACEi in COVID-19 (REPLACE) trial is a prospective randomized controlled trial underway to examine whether discontinuation of these drugs would ameliorate the severity of the disease (NCT04338009). Several other clinical trials (ClinicalTrial.gov numbers NCT04312009, NCT04311177, and NCT04318418) (Table 1) are underway addressing viral-mediated RAS imbalance and the risks and benefits associated with the use of ARBs and ACEi in COVID-19 patients.

Sepsis and inflammation are characteristic features of SARS-CoV-2 infection. In addition, neurological abnormalities were evident in COVID-19 patients suggesting neuroinflammation could be a key factor in the pathogenesis

of COVID-19 [1,158,159]. However, the mechanisms that mediate neuroinflammation and any long-term consequences and management of these neurological symptoms remain to be clarified. Studies have examined the effect of non-steroidal anti-inflammatory drugs in COVID-19, but a clear benefit on outcomes was not discernable. It might be worthwhile to examine if anti-inflammatory drugs like minocycline, an anti-inflammatory antibiotic that crosses the blood–brain barrier and ameliorates neuroinflammation-driven HTN, pulmonary HTN and lung inflammation in animal models [160–162] could be beneficial in COVID-19.

Recent investigations found increased IL-6 in patients with confirmed COVID-19 pneumonia that positively correlated with severity of disease [163]. Elevated levels of some other inflammatory cytokines (IFN-γ, TNF-α etc.) are also found in COVID-19 patients [40] indicative of cytokine storm/sepsis. This overproduction of cytokines might cause more damage to the host cell than the SARS-CoV-2. Interestingly, in a pilot study, rhACE2 infusion decreased IL-6 in patients with ARDS [149]. Therefore, considering the critical role of cytokines in severe COVID-19, rhACE2 targeted to decrease IL-6 could be considered as a potential therapeutic approach. Finally, FDA-approved IL-6 inhibitors and anti-IL-6 monoclonal antibodies are under clinical trial currently for their use in COVID-19 patients (Sarilumab: NCT04315298; Tocilizumab: NCT04320615, Siltuximab: NCT04330638, NCT04322188) (Table 1).

Concluding remarks

SARS-CoV-2 requires membrane-bound ACE2 for entry into cells. ACE2 can be down-regulated directly by binding and internalization of SARS-CoV-2 or indirectly via proteolytic processing, autophagy and increased shedding. Decreased ACE2 or increased circulating sACE2 can bind SARS-CoV-2 and potentially decrease SARS-CoV-2 infection or disease severity. On the other hand, decreased ACE2 can cause microbial dysbiosis and inflammation, that can worsen the systemic response to SARS-CoV-2. Studies are ongoing to clarify the effects of modulating ACE2 to mitigate COVID-19. Apart from the effects on SARS-CoV-2 infection, decreased ACE2 can have profound effects on the cardiovascular and pulmonary systems. Multiple lines of incontrovertible evidence have proven the pivotal role of ACE2 in down-regulating detrimental AngII signaling and up-regulating the beneficial Ang-(1-7)/Mas receptor signaling. Thus, altered ACE2 signaling can have detrimental effects on BP, cardiac function, vasculature, kidney and the brain. The long-term consequences of these effects are yet to be determined and could inform us on the relevance of ACE2 signaling to human health and disease.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ACE2, angiotensin-converting enzyme 2; ACEi, ACE inhibitor; ADAM17, a disintegrin and metalloproteinase 17; AKI, acute kidney injury; AngII, angiotensin II; Ang-(1-7), angiotensin-(1-7); Ang-(1-9), angiotensin-(1-9); ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; BP, blood pressure; CKD, chronic kidney disease; COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; DIZE, diminazene aceturate; GI, gastrointestinal; HF, heart failure; HTN, hypertension; NO, nitric oxide; PAH, pulmonary arterial hypertension; sACE2, soluble ACE2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SHR, spontaneously hypertensive rat; S-protein, spike protein; TMPRSS2, transmembrane protease serine 2.

References

1   Sharma, R.K., Stevens, B.R., Obukhov, A.G., Grant, M.B., Oudit, G.Y., Li, Q. et al. (2020) ACE2 (Angiotensin-Converting Enzyme 2) in cardiopulmonary diseases: ramifications for the control of SARS-CoV-2. *Hypertension* **76**, 651–661, https://doi.org/10.1161/HYPERTENSIONAHA.120.15595

2   Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J.C., Turner, A.J. et al. (2020) Angiotensin converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system. *Circ. Res.* **126**, 1456–1474, https://doi.org/10.1161/CIRCRESAHA.120.317015

3   Yamazato, Y., Ferreira, A.J., Hong, K.H., Sriramula, S., Francis, J., Yamazato, M. et al. (2009) Prevention of pulmonary hypertension by Angiotensin-converting enzyme 2 gene transfer. *Hypertension* **54**, 365–371, https://doi.org/10.1161/HYPERTENSIONAHA.108.125468

4   Treml, B., Neu, N., Kleinsasser, A., Gritsch, C., Finsterwalder, T., Geiger, R. et al. (2010) Recombinant angiotensin-converting enzyme 2 improves pulmonary blood flow and oxygenation in lipopolysaccharide-induced lung injury in piglets. *Crit. Care Med.* **38**, 596–601, https://doi.org/10.1097/CCM.0b013e3181c03009

5   Zhong, J., Basu, R., Guo, D., Chow, F.L., Byrns, S., Schuster, M. et al. (2010) Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation* **122**, 717–728, 718 p following 728, https://doi.org/10.1161/CIRCULATIONAHA.110.955369

6   Ferreira, A.J., Shenoy, V., Yamazato, Y., Sriramula, S., Francis, J., Yuan, L. et al. (2009) Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **179**, 1048–1054, https://doi.org/10.1164/rccm.200811-1678OC

7   Ferreira, A.J., Jacoby, B.A., Araújo, C.A., Macedo, F.A., Silva, G.A., Almeida, A.P. et al. (2007) The nonpeptide angiotensin-(1-7) receptor Mas agonist AVE-0991 attenuates heart failure induced by myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.* **292**, H1113–H1119, https://doi.org/10.1152/ajpheart.00828.2006

8   Patel, V.B., Bodiga, S., Basu, R., Das, S.K., Wang, W., Wang, Z. et al. (2012) Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the angiotensin II/AT1 receptor axis. *Circ. Res.* **110**, 1322–1335, https://doi.org/10.1161/CIRCRESAHA.112.268029

9   Tikellis, C., Pickering, R., Tsorotes, D., Du, X.J., Kiriazis, H., Nguyen-Huu, T.P. et al. (2012) Interaction of diabetes and ACE2 in the pathogenesis of cardiovascular disease in experimental diabetes. *Clin. Sci. (Lond.)* **123**, 519–529, https://doi.org/10.1042/CS20110668

10   Gurley, S.B., Allred, A., Le, T.H., Griffiths, R., Mao, L., Philip, N. et al. (2006) Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. *J. Clin. Invest.* **116**, 2218–2225, https://doi.org/10.1172/JCI16980

11   Shenoy, V., Kwon, K.C., Rathinasabapathy, A., Lin, S., Jin, G., Song, C. et al. (2014) Oral delivery of Angiotensin-converting enzyme 2 and Angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension. *Hypertension* **64**, 1248–1259, https://doi.org/10.1161/HYPERTENSIONAHA.114.03871

12   Basu, R., Poglitsch, M., Yogasundaram, H., Thomas, J., Rowe, B.H. and Oudit, G.Y. (2017) Roles of angiotensin peptides and recombinant human ACE2 in heart failure. *J. Am. Coll. Cardiol.* **69**, 805–819, https://doi.org/10.1016/j.jacc.2016.11.064

13   Vickers, C., Hales, P., Kaushik, V., Dick, L., Gavin, J., Tang, J. et al. (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J. Biol. Chem.* **277**, 14838–14843, https://doi.org/10.1074/jbc.M200581200

14   Simões e Silva, A.C., Silveira, K.D., Ferreira, A.J. and Teixeira, M.M. (2013) ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis. *Br. J. Pharmacol.* **169**, 477–492, https://doi.org/10.1111/bph.12159

15   Mukerjee, S., Gao, H., Xu, J., Sato, R., Zsombok, A. and Lazartigues, E. (2019) ACE2 and ADAM17 interaction regulates the activity of presympathetic neurons. *Hypertension* **74**, 1181–1191, https://doi.org/10.1161/HYPERTENSIONAHA.119.13133

16   Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J. et al. (2020) Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci. China Life Sci.* **63**, 364–374, https://doi.org/10.1007/s11427-020-1643-8

17   Oudit, G.Y., Kassiri, Z., Jiang, C., Liu, P.P., Poutanen, S.M., Penninger, J.M. et al. (2009) SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur. J. Clin. Invest.* **39**, 618–625, https://doi.org/10.1111/j.1365-2362.2009.02153.x

18   Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N. et al. (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* **87**, E1–E9, https://doi.org/10.1161/01.RES.87.5.e1

19   Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J.C., Turner, A.J. et al. (2020) Response by Gheblawi et al to Letter Regarding Article, "Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2". *Circ. Res.* **127**, e46–e47, https://doi.org/10.1161/CIRCRESAHA.120.317332

20   Tipnis, S.R., Hooper, N.M., Hyde, R., Karran, E., Christie, G. and Turner, A.J. (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* **275**, 33238–33243, https://doi.org/10.1074/jbc.M002615200

21   Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H. et al. (2020) Structural basis of receptor recognition by SARS-CoV-2. *Nature* **581**, 221–224, https://doi.org/10.1038/s41586-020-2179-y

22   Bar-On, Y.M., Flamholz, A., Phillips, R. and Milo, R. (2020) SARS-CoV-2 (COVID-19) by the numbers. *Elife* **9**, e57309, https://doi.org/10.7554/eLife.57309

23   Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S. et al. (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **181**, 271.e278–280.e278, https://doi.org/10.1016/j.cell.2020.02.052

24   Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T. and Veesler, D. (2020) Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* **181**, 281.e286–292.e286, https://doi.org/10.1016/j.cell.2020.02.058

25   Haga, S., Yamamoto, N., Nakai-Murakami, C., Osawa, Y., Tokunaga, K., Sata, T. et al. (2008) Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7809–7814, https://doi.org/10.1073/pnas.0711241105

26   Lambert, D.W., Yarski, M., Warner, F.J., Thornhill, P., Parkin, E.T., Smith, A.I. et al. (2005) 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.* **280**, 30113–30119, https://doi.org/10.1074/jbc.M505111200

27   Kefaloyianni, E., Muthu, M.L., Kaeppler, J., Sun, X., Sabbisetti, V., Chalaris, A. et al. (2016) ADAM17 substrate release in proximal tubule drives kidney fibrosis. *JCI Insight* **1**, e87023, https://doi.org/10.1172/jci.insight.87023

28   Kato, T., Hagiyama, M. and Ito, A. (2018) Renal ADAM10 and 17: their physiological and medical meanings. *Front. Cell Dev. Biol.* **6**, 153, https://doi.org/10.3389/fcell.2018.00153

29   Hashimoto, T., Perlot, T., Rehman, A., Trichereau, J., Ishiguro, H., Paolino, M. et al. (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **487**, 477–481, https://doi.org/10.1038/nature11228

30   Camargo, S.M., Singer, D., Makrides, V., Huggel, K., Pos, K.M., Wagner, C.A. et al. (2009) Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. *Gastroenterology* **136**, 872–882, https://doi.org/10.1053/j.gastro.2008.10.055

31   Jando, J., Camargo, S.M.R., Herzog, B. and Verrey, F. (2017) Expression and regulation of the neutral amino acid transporter B0AT1 in rat small intestine. *PLoS ONE* **12**, e0184845, https://doi.org/10.1371/journal.pone.0184845

32   Sharma, R.K., Oliveira, A.C., Yang, T., Karas, M.M., Li, J., Lobaton, G.O. et al. (2020) Gut pathology and its rescue by ACE2 (Angiotensin-Converting Enzyme 2) in hypoxia-induced pulmonary hypertension. *Hypertension* **76**, 206–216, https://doi.org/10.1161/HYPERTENSIONAHA.120.14931

33 Sharma, R.K., Oliveira, A.C., Yang, T., Kim, S., Zubcevic, J., Aquino, V. et al. (2020) Pulmonary arterial hypertension-associated changes in gut pathology and microbiota. *ERJ Open Res.* **6**, 00253–02019, https://doi.org/10.1183/23120541.00253-2019

34 Leung, W.K., To, K.F., Chan, P.K., Chan, H.L., Wu, A.K., Lee, N. et al. (2003) Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* **125**, 1011–1017, https://doi.org/10.1016/j.gastro.2003.08.001

35 Tian, Y., Rong, L., Nian, W. and He, Y. (2020) Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. *Aliment. Pharmacol. Ther.* **51**, 843–851, https://doi.org/10.1111/apt.15731

36 Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X. et al. (2020) Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol. Hepatol.* **5**, 434–435, https://doi.org/10.1016/S2468-1253(20)30083-2

37 Zuo, T., Zhang, F., Lui, G.C.Y., Yeoh, Y.K., Li, A.Y.L., Zhan, H. et al. (2020) Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology* **159**, 944–955, https://doi.org/10.1053/j.gastro.2020.05.048

38 Logsdon, A.F., Erickson, M.A., Rhea, E.M., Salameh, T.S. and Banks, W.A. (2018) Gut reactions: How the blood-brain barrier connects the microbiome and the brain. *Exp. Biol. Med. (Maywood)* **243**, 159–165, https://doi.org/10.1177/1535370217743766

39 Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J. et al. (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* **323**, 1061–1069, https://doi.org/10.1001/jama.2020.1585

40 Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y. et al. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506, https://doi.org/10.1016/S0140-6736(20)30183-5

41 Guan, W.J., Ni, Z.Y., Hu, Y., Liang, W.H., Ou, C.Q., He, J.X. et al. (2020) Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* **382**, 1708–1720, https://doi.org/10.1056/NEJMoa2002032

42 Whelton, P.K., Carey, R.M., Aronow, W.S., Casey, D.E., Collins, K.J., Dennison Himmelfarb, C. et al. (2018) 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* **71**, 1269–1324

43 Benjamin, E.J., Muntner, P., Alonso, A., Bittencourt, M.S., Callaway, C.W., Carson, A.P. et al. (2019) Heart Disease and Stroke Statistics-2019 Update: a report from the American Heart Association. *Circulation* **139**, e56–e528, https://doi.org/10.1161/CIR.0000000000000659

44 Díez-Freire, C., Vázquez, J., Correa de Adjounian, M.F., Ferrari, M.F., Yuan, L., Silver, X. et al. (2006) ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR. *Physiol. Genomics* **27**, 12–19, https://doi.org/10.1152/physiolgenomics.00312.2005

45 Yamazato, M., Yamazato, Y., Sun, C., Diez-Freire, C. and Raizada, M.K. (2007) Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. *Hypertension* **49**, 926–931, https://doi.org/10.1161/01.HYP.0000259942.38108.20

46 Rentzsch, B., Todiras, M., Iliescu, R., Popova, E., Campos, L.A., Oliveira, M.L. et al. (2008) Transgenic angiotensin-converting enzyme 2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function. *Hypertension* **52**, 967–973, https://doi.org/10.1161/HYPERTENSIONAHA.108.114322

47 Tikellis, C., Johnston, C.I., Forbes, J.M., Burns, W.C., Burrell, L.M., Risvanis, J. et al. (2003) Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension* **41**, 392–397, https://doi.org/10.1161/01.HYP.0000060689.38912.CB

48 Crackower, M.A., Sarao, R., Oudit, G.Y., Yagil, C., Kozieradzki, I., Scanga, S.E. et al. (2002) Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* **417**, 822–828, https://doi.org/10.1038/nature00786

49 Walther, T., Wessel, N., Kang, N., Sander, A., Tschöpe, C., Malberg, H. et al. (2000) Altered heart rate and blood pressure variability in mice lacking the Mas protooncogene. *Braz. J. Med. Biol. Res.* **33**, 1–9, https://doi.org/10.1590/S0100-879X2000000100001

50 Gjymishka, A., Kulemina, L.V., Shenoy, V., Katovich, M.J., Ostrov, D.A. and Raizada, M.K. (2010) Diminazene aceturate is an ACE2 activator and a novel antihypertensive drug. *FASEB J.* **24**, 1032.3–1032.3

51 Velkoska, E., Patel, S.K., Griggs, K., Pickering, R.J., Tikellis, C. and Burrell, L.M. (2015) Short-term treatment with diminazene aceturate ameliorates the reduction in kidney ACE2 activity in rats with subtotal nephrectomy. *PLoS ONE* **10**, e0118758, https://doi.org/10.1371/journal.pone.0118758

52 Patel, V.B., Zhong, J.C., Grant, M.B. and Oudit, G.Y. (2016) Role of the ACE2/Angiotensin 1-7 Axis of the renin-angiotensin system in heart failure. *Circ. Res.* **118**, 1313–1326, https://doi.org/10.1161/CIRCRESAHA.116.307708

53 Haschke, M., Schuster, M., Poglitsch, M., Loibner, H., Salzberg, M., Bruggisser, M. et al. (2013) Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. *Clin. Pharmacokinet.* **52**, 783–792, https://doi.org/10.1007/s40262-013-0072-7

54 Doobay, M.F., Talman, L.S., Obr, T.D., Tian, X., Davisson, R.L. and Lazartigues, E. (2007) Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R373–R381, https://doi.org/10.1152/ajpregu.00292.2006

55 Gowrisankar, Y.V. and Clark, M.A. (2016) Angiotensin II regulation of angiotensin-converting enzymes in spontaneously hypertensive rat primary astrocyte cultures. *J. Neurochem.* **138**, 74–85, https://doi.org/10.1111/jnc.13641

56 Zhu, Y., Jiang, M., Gao, L. and Huang, X. (2020) Single cell analysis of ACE2 expression reveals the potential targets for 2019-nCoV. *Preprints* 2020020221, https://doi.org/10.20944/preprints202002.0221.v1

57 Xia, H., Sriramula, S., Chhabra, K.H. and Lazartigues, E. (2013) Brain angiotensin-converting enzyme type 2 shedding contributes to the development of neurogenic hypertension. *Circ. Res.* **113**, 1087–1096, https://doi.org/10.1161/CIRCRESAHA.113.301811

58 Xu, J., Sriramula, S., Xia, H., Moreno-Walton, L., Culicchia, F., Domenig, O. et al. (2017) Clinical relevance and role of neuronal AT. *Circ. Res.* **121**, 43–55, https://doi.org/10.1161/CIRCRESAHA.116.310509

59 Xia, H., Feng, Y., Obr, T.D., Hickman, P.J. and Lazartigues, E. (2009) Angiotensin II type 1 receptor-mediated reduction of angiotensin-converting enzyme 2 activity in the brain impairs baroreflex function in hypertensive mice. *Hypertension* **53**, 210–216, https://doi.org/10.1161/HYPERTENSIONAHA.108.123844

60 Xia, H. and Lazartigues, E. (2010) Angiotensin-converting enzyme 2: central regulator for cardiovascular function. *Curr. Hypertens. Rep.* **12**, 170–175, https://doi.org/10.1007/s11906-010-0105-7

61 Sriramula, S., Cardinale, J.P., Lazartigues, E. and Francis, J. (2011) ACE2 overexpression in the paraventricular nucleus attenuates angiotensin II-induced hypertension. *Cardiovasc. Res.* **92**, 401–408, https://doi.org/10.1093/cvr/cvr242

62 Sriramula, S., Xia, H., Xu, P. and Lazartigues, E. (2015) Brain-targeted angiotensin-converting enzyme 2 overexpression attenuates neurogenic hypertension by inhibiting cyclooxygenase-mediated inflammation. *Hypertension* **65**, 577–586, https://doi.org/10.1161/HYPERTENSIONAHA.114.04691

63 Chaves, G.Z., Caligiorne, S.M., Santos, R.A., Khosla, M.C. and Campagnole-Santos, M.J. (2000) Modulation of the baroreflex control of heart rate by angiotensin-(1-7) at the nucleus tractus solitarii of normotensive and spontaneously hypertensive rats. *J. Hypertens.* **18**, 1841–1848, https://doi.org/10.1097/00004872-200018120-00019

64 Alzamora, A.C., Santos, R.A. and Campagnole-Santos, M.J. (2002) Hypotensive effect of ANG II and ANG-(1-7) at the caudal ventrolateral medulla involves different mechanisms. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R1187–R1195, https://doi.org/10.1152/ajpregu.00580.2001

65 Whitaker, A.M. and Molina, P.E. (2013) Angiotensin (1-7) contributes to nitric oxide tonic inhibition of vasopressin release during hemorrhagic shock in acute ethanol intoxicated rodents. *Life Sci.* **93**, 623–629, https://doi.org/10.1016/j.lfs.2013.08.020

66 Nakagaki, T., Hirooka, Y., Ito, K., Kishi, T., Hoka, S. and Sunagawa, K. (2011) Role of angiotensin-(1-7) in rostral ventrolateral medulla in blood pressure regulation via sympathetic nerve activity in Wistar-Kyoto and spontaneous hypertensive rats. *Clin. Exp. Hypertens.* **33**, 223–230, https://doi.org/10.3109/10641963.2011.583967

67 Fontes, M.A., Baltatu, O., Caligiorne, S.M., Campagnole-Santos, M.J., Ganten, D., Bader, M. et al. (2000) Angiotensin peptides acting at rostral ventrolateral medulla contribute to hypertension of TGR(mREN2)27 rats. *Physiol. Genomics* **2**, 137–142, https://doi.org/10.1152/physiolgenomics.2000.2.3.137

68 Richardson, S., Hirsch, J.S., Narasimhan, M., Crawford, J.M., McGinn, T., Davidson, K.W. et al. (2020) Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA* **323**, 2052–2059, https://doi.org/10.1001/jama.2020.6775

69 Ferrario, CM., Jessup, J., Chappell, MC., Averill, DB., Brosnihan, KB., Tallant, EA., Diz, DI., Gallagher, PE. et al. (2005) Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2.. *Circulation* **111**, 2605–10, https://doi.org/10.1161/CIRCULATIONAHA.104.510461

70 Zhang, , P., Zhu,, L., Cai,, J., Lei,, F., Qin,, J.-J., Xie,, J. et al. (2020) Correction to: Association of inpatient use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ. Res.* **127**, e147, https://doi.org/10.1161/CIRCRESAHA.120.317134

71 Mancia, G., Rea, F., Ludergnani, M., Apolone, G. and Corrao, G. (2020) Renin-angiotensin-aldosterone system blockers and the risk of Covid-19. *N. Engl. J. Med.* **382**, 2431–2440, https://doi.org/10.1056/NEJMoa2006923

72 Reynolds, H.R., Adhikari, S., Pulgarin, C., Troxel, A.B., Iturrate, E., Johnson, S.B. et al. (2020) Renin-angiotensin-aldosterone system inhibitors and risk of Covid-19. *N. Engl. J. Med.* **382**, 2441–2448, https://doi.org/10.1056/NEJMoa2008975

73 Fosbøl, E.L., Butt, J.H., Østergaard, L., Andersson, C., Selmer, C., Kragholm, K. et al. (2020) Association of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use with COVID-19 diagnosis and mortality. *JAMA* **324**, 168–177, https://doi.org/10.1001/jama.2020.11301

74 Guo, J., Huang, Z., Lin, L. and Lv, J. (2020) Coronavirus Disease 2019 (COVID-19) and cardiovascular disease: a viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome coronavirus 2 infection. *J. Am. Heart Assoc.* **9**, e016219

75 Sampaio, W.O., Souza dos Santos, R.A., Faria-Silva, R., da Mata Machado, L.T., Schiffrin, E.L. and Touyz, R.M. (2007) Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. *Hypertension* **49**, 185–192, https://doi.org/10.1161/01.HYP.0000251865.35728.2f

76 Mori, J., Patel, V.B., Abo Alrob, O., Basu, R., Altamimi, T., Desaulniers, J. et al. (2014) Angiotensin 1-7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. *Circ. Heart Fail.* **7**, 327–339, https://doi.org/10.1161/CIRCHEARTFAILURE.113.000672

77 Wang, W., Bodiga, S., Das, S.K., Lo, J., Patel, V. and Oudit, G.Y. (2012) Role of ACE2 in diastolic and systolic heart failure. *Heart Fail. Rev.* **17**, 683–691, https://doi.org/10.1007/s10741-011-9259-x

78 Jia, G., Hill, M.A. and Sowers, J.R. (2018) Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ. Res.* **122**, 624–638, https://doi.org/10.1161/CIRCRESAHA.117.311586

79 Wong, C. and Marwick, T.H. (2007) Obesity cardiomyopathy: pathogenesis and pathophysiology. *Nat. Clin. Pract. Cardiovasc. Med.* **4**, 436–443, https://doi.org/10.1038/ncpcardio0943

80 Patel, V.B., Mori, J., McLean, B.A., Basu, R., Das, S.K., Ramprasath, T. et al. (2016) ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes* **65**, 85–95

81 Oudit, G.Y., Kassiri, Z., Patel, M.P., Chappell, M., Butany, J., Backx, P.H. et al. (2007) Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. *Cardiovasc. Res.* **75**, 29–39, https://doi.org/10.1016/j.cardiores.2007.04.007

82 Epelman, S., Tang, W.H., Chen, S.Y., Van Lente, F., Francis, G.S. and Sen, S. (2008) 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.* **52**, 750–754, https://doi.org/10.1016/j.jacc.2008.02.088

83 Chen, T., Wu, D., Chen, H., Yan, W., Yang, D., Chen, G. et al. (2020) Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* **368**, m1091, https://doi.org/10.1136/bmj.m1091

84 Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z. et al. (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* **395**, 1054–1062, https://doi.org/10.1016/S0140-6736(20)30566-3

85 Puntmann, V.O., Carerj, M.L., Wieters, I., Fahim, M., Arendt, C., Hoffmann, J. et al. (2020) Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol.*, https://doi.org/10.1001/jamacardio.2020.3557

86 Chen, L., Li, X., Chen, M., Feng, Y. and Xiong, C. (2020) The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc. Res.* **116**, 1097–1100, https://doi.org/10.1093/cvr/cvaa078

87 Ishiyama, Y., Gallagher, P.E., Averill, D.B., Tallant, E.A., Brosnihan, K.B. and Ferrario, C.M. (2004) Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* **43**, 970–976, https://doi.org/10.1161/01.HYP.0000124667.34652.1a

88 Ocaranza, M.P., Godoy, I., Jalil, J.E., Varas, M., Collantes, P., Pinto, M. et al. (2006) Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* **48**, 572–578, https://doi.org/10.1161/01.HYP.0000237862.94083.45

89 Godo, S. and Shimokawa, H. (2017) Endothelial functions. *Arterioscler. Thromb. Vasc. Biol.* **37**, e108–e114, https://doi.org/10.1161/ATVBAHA.117.309813

90 Ross, R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **362**, 801–809, https://doi.org/10.1038/362801a0

91 Deanfield, J.E., Halcox, J.P. and Rabelink, T.J. (2007) Endothelial function and dysfunction: testing and clinical relevance. *Circulation* **115**, 1285–1295, https://doi.org/10.1161/CIRCULATIONAHA.106.652859

92 Bonetti, P.O., Lerman, L.O. and Lerman, A. (2003) Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler. Thromb. Vasc. Biol.* **23**, 168–175, https://doi.org/10.1161/01.ATV.0000051384.43104.FC

93 Lovren, F., Pan, Y., Quan, A., Teoh, H., Wang, G., Shukla, P.C. et al. (2008) Angiotensin converting enzyme-2 confers endothelial protection and attenuates atherosclerosis. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H1377–H1384, https://doi.org/10.1152/ajpheart.00331.2008

94 Rabelo, L.A., Todiras, M., Nunes-Souza, V., Qadri, F., Szijártó, I.A., Gollasch, M. et al. (2016) Genetic deletion of ACE2 induces vascular dysfunction in C57BL/6 mice: role of nitric oxide imbalance and oxidative stress. *PLoS ONE* **11**, e0150255, https://doi.org/10.1371/journal.pone.0150255

95 Rabelo, L.A., Alenina, N. and Bader, M. (2011) ACE2-angiotensin-(1-7)-Mas axis and oxidative stress in cardiovascular disease. *Hypertens. Res.* **34**, 154–160, https://doi.org/10.1038/hr.2010.235

96 Zhang, Y.H., Dong, X.F., Hao, Q.Q., Zhou, X.M., Yu, Q.T., Li, S.Y. et al. (2015) ACE2 and Ang-(1-7) protect endothelial cell function and prevent early atherosclerosis by inhibiting inflammatory response. *Inflamm. Res.* **64**, 253–260, https://doi.org/10.1007/s00011-015-0805-1

97 Zhang, C., Zhao, Y.X., Zhang, Y.H., Zhu, L., Deng, B.P., Zhou, Z.L. et al. (2010) Angiotensin-converting enzyme 2 attenuates atherosclerotic lesions by targeting vascular cells. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15886–15891, https://doi.org/10.1073/pnas.1001253107

98 Hayashi, N., Yamamoto, K., Ohishi, M., Tatara, Y., Takeya, Y., Shiota, A. et al. (2010) The counterregulating role of ACE2 and ACE2-mediated angiotensin 1-7 signaling against angiotensin II stimulation in vascular cells. *Hypertens. Res.* **33**, 1182–1185, https://doi.org/10.1038/hr.2010.147

99 Koka, V., Huang, X.R., Chung, A.C., Wang, W., Truong, L.D. and Lan, H.Y. (2008) Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. *Am. J. Pathol.* **172**, 1174–1183, https://doi.org/10.2353/ajpath.2008.070762

100 Srivastava, P., Badhwar, S., Chandran, D.S., Jaryal, A.K., Jyotsna, V.P. and Deepak, K.K. (2019) Imbalance between Angiotensin II - Angiotensin (1-7) system is associated with vascular endothelial dysfunction and inflammation in type 2 diabetes with newly diagnosed hypertension. *Diabetes Metab. Syndr.* **13**, 2061–2068, https://doi.org/10.1016/j.dsx.2019.04.042

101 Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A.S. et al. (2020) Endothelial cell infection and endotheliitis in COVID-19. *Lancet* **395**, 1417–1418, https://doi.org/10.1016/S0140-6736(20)30937-5

102 Labò, N., Ohnuki, H. and Tosato, G. (2020) Vasculopathy and coagulopathy associated with SARS-CoV-2 infection. *Cells* **9**, 1583, https://doi.org/10.3390/cells9071583

103 Leisman, D.E., Deutschman, C.S. and Legrand, M. (2020) Facing COVID-19 in the ICU: vascular dysfunction, thrombosis, and dysregulated inflammation. *Intensive Care Med.* **46**, 1105–1108, https://doi.org/10.1007/s00134-020-06059-6

104 Bilaloglu, S., Aphinyanaphongs, Y., Jones, S., Iturrate, E., Hochman, J. and Berger, J.S. (2020) Thrombosis in hospitalized patients with COVID-19 in a New York City health system. *JAMA* **324**, 799–801, https://doi.org/10.1001/jama.2020.13372

105 Fogarty, H., Townsend, L., Ni Cheallaigh, C., Bergin, C., Martin-Loeches, I., Browne, P. et al. (2020) COVID19 coagulopathy in Caucasian patients. *Br. J. Haematol.* **189**, 1044–1049, https://doi.org/10.1111/bjh.16749

106 Team, C. C.-R. (2020) Coronavirus disease 2019 in children - United States, February 12-April 2, 2020. *MMWR Morb. Mortal. Wkly. Rep.* **69**, 422–426, https://doi.org/10.15585/mmwr.mm6914e4

107 Verdoni, L., Mazza, A., Gervasoni, A., Martelli, L., Ruggeri, M., Ciuffreda, M. et al. (2020) An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* **395**, 1771–1778, https://doi.org/10.1016/S0140-6736(20)31103-X

108 Becker, R.C. (2020) COVID-19-associated vasculitis and vasculopathy. *J. Thromb. Thrombolysis* **50**, 499–511, https://doi.org/10.1007/s11239-020-02230-4

109 Roumelioti, M.E., Glew, R.H., Khitan, Z.J., Rondon-Berrios, H., Argyropoulos, C.P., Malhotra, D. et al. (2018) Fluid balance concepts in medicine: principles and practice. *World J. Nephrol.* **7**, 1–28, https://doi.org/10.5527/wjn.v7.i1.1

110 Coffman, T.M. (2014) The inextricable role of the kidney in hypertension. *J. Clin. Invest.* **124**, 2341–2347, https://doi.org/10.1172/JCI72274

111 Mascarenhas, J., Laszczynska, O., Severo, M., Friões, F., Alvelos, M., Bettencourt, P. et al. (2018) Prognostic effect of renal function in ambulatory patients with heart failure and reduced ejection fraction: the kidney is a marker of cardiac function. *Can. J. Cardiol.* **34**, 1325–1332, https://doi.org/10.1016/j.cjca.2018.06.006

112 Haase, V.H. (2013) Mechanisms of hypoxia responses in renal tissue. *J. Am. Soc. Nephrol.* **24**, 537–541, https://doi.org/10.1681/ASN.2012080855

113 Masereeuw, R., Mutsaers, H.A., Toyohara, T., Abe, T., Jhawar, S., Sweet, D.H. et al. (2014) The kidney and uremic toxin removal: glomerulus or tubule? *Semin. Nephrol.* **34**, 191–208, https://doi.org/10.1016/j.semnephrol.2014.02.010

114 Nagami, G.T. and Hamm, L.L. (2017) Regulation of acid-base balance in chronic kidney disease. *Adv. Chronic Kidney Dis.* **24**, 274–279, https://doi.org/10.1053/j.ackd.2017.07.004

115 Donnelly, S. (2003) Why is erythropoietin made in the kidney? The kidney functions as a 'critmeter' to regulate the hematocrit. *Adv. Exp. Med. Biol.* **543**, 73–87, https://doi.org/10.1007/978-1-4419-8997-0˙6

116 Williams, M.E. (2009) Chronic kidney disease/bone and mineral metabolism: the imperfect storm. *Semin. Nephrol.* **29**, 97–104, https://doi.org/10.1016/j.semnephrol.2009.01.002

117 Go, A.S., Chertow, G.M., Fan, D., McCulloch, C.E. and Hsu, C.Y. (2004) Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N. Engl. J. Med.* **351**, 1296–1305, https://doi.org/10.1056/NEJMoa041031

118 Bourgonje, A.R., Abdulle, A.E., Timens, W., Hillebrands, J.L., Navis, G.J., Gordijn, S.J. et al. (2020) Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J. Pathol.* **251**, 228–248, https://doi.org/10.1002/path.5471

119 Lely, A.T., Hamming, I., van Goor, H. and Navis, G.J. (2004) Renal ACE2 expression in human kidney disease. *J. Pathol.* **204**, 587–593, https://doi.org/10.1002/path.1670

120 Ye, M., Wysocki, J., William, J., Soler, M.J., Cokic, I. and Batlle, D. (2006) Glomerular localization and expression of Angiotensin-converting enzyme 2 and Angiotensin-converting enzyme: implications for albuminuria in diabetes. *J. Am. Soc. Nephrol.* **17**, 3067–3075, https://doi.org/10.1681/ASN.2006050423

121 da Silveira, K.D., Pompermayer Bosco, K.S., Diniz, L.R., Carmona, A.K., Cassali, G.D., Bruna-Romero, O. et al. (2010) ACE2-angiotensin-(1-7)-Mas axis in renal ischaemia/reperfusion injury in rats. *Clin. Sci. (Lond.)* **119**, 385–394, https://doi.org/10.1042/CS20090554

122 Velkoska, E., Dean, R.G., Burchill, L., Levidiotis, V. and Burrell, L.M. (2010) Reduction in renal ACE2 expression in subtotal nephrectomy in rats is ameliorated with ACE inhibition. *Clin. Sci. (Lond.)* **118**, 269–279, https://doi.org/10.1042/CS20090318

123 Gupta, A., Rhodes, G.J., Berg, D.T., Gerlitz, B., Molitoris, B.A. and Grinnell, B.W. (2007) Activated protein C ameliorates LPS-induced acute kidney injury and downregulates renal INOS and angiotensin 2. *Am. J. Physiol. Renal Physiol.* **293**, F245–F254, https://doi.org/10.1152/ajprenal.00477.2006

124 Roberts, M.A., Velkoska, E., Ierino, F.L. and Burrell, L.M. (2013) Angiotensin-converting enzyme 2 activity in patients with chronic kidney disease. *Nephrol. Dial. Transplant.* **28**, 2287–2294, https://doi.org/10.1093/ndt/gft038

125 Chodavarapu, H., Grobe, N., Somineni, H.K., Salem, E.S., Madhu, M. and Elased, K.M. (2013) Rosiglitazone treatment of type 2 diabetic db/db mice attenuates urinary albumin and angiotensin converting enzyme 2 excretion. *PLoS ONE* **8**, e62833, https://doi.org/10.1371/journal.pone.0062833

126 Reich, H.N., Oudit, G.Y., Penninger, J.M., Scholey, J.W. and Herzenberg, A.M. (2008) Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. *Kidney Int.* **74**, 1610–1616, https://doi.org/10.1038/ki.2008.497

127 Soler, M.J., Wysocki, J., Ye, M., Lloveras, J., Kanwar, Y. and Batlle, D. (2007) ACE2 inhibition worsens glomerular injury in association with increased ACE expression in streptozotocin-induced diabetic mice. *Kidney Int.* **72**, 614–623, https://doi.org/10.1038/sj.ki.5002373

128 Wong, D.W., Oudit, G.Y., Reich, H., Kassiri, Z., Zhou, J., Liu, Q.C. et al. (2007) Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury. *Am. J. Pathol.* **171**, 438–451, https://doi.org/10.2353/ajpath.2007.060977

129 Nadarajah, R., Milagres, R., Dilauro, M., Gutsol, A., Xiao, F., Zimpelmann, J. et al. (2012) Podocyte-specific overexpression of human angiotensin-converting enzyme 2 attenuates diabetic nephropathy in mice. *Kidney Int.* **82**, 292–303, https://doi.org/10.1038/ki.2012.83

130 Liu, C.X., Hu, Q., Wang, Y., Zhang, W., Ma, Z.Y., Feng, J.B. et al. (2011) Angiotensin-converting enzyme (ACE) 2 overexpression ameliorates glomerular injury in a rat model of diabetic nephropathy: a comparison with ACE inhibition. *Mol. Med.* **17**, 59–69, https://doi.org/10.2119/molmed.2010.00111

131 Wysocki, J. and Batlle, D. (2013) Reduced plasma ACE2 activity in dialysis patients: another piece in the conundrum of factors involved in hypertension and cardiovascular morbidity? *Nephrol. Dial. Transplant.* **28**, 2200–2202, https://doi.org/10.1093/ndt/gft240

132 Chu, K.H., Tsang, W.K., Tang, C.S., Lam, M.F., Lai, F.M., To, K.F. et al. (2005) Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. *Kidney Int.* **67**, 698–705, https://doi.org/10.1111/j.1523-1755.2005.67130.x

133 Ronco, C., Reis, T. and Husain-Syed, F. (2020) Management of acute kidney injury in patients with COVID-19. *Lancet Respir. Med.* **8**, 738–742, https://doi.org/10.1016/S2213-2600(20)30229-0

134 Hardenberg, J.B. and Luft, F.C. (2020) Covid-19, ACE2 and the kidney. *Acta Physiol. (Oxf.)* **230**, e13539, https://doi.org/10.1111/apha.13539

135 Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H. et al. (2020) Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir. Med.* **8**, 475–481, https://doi.org/10.1016/S2213-2600(20)30079-5

136 Mehta, P., McAuley, D.F., Brown, M., Sanchez, E., Tattersall, R.S., Manson, J.J. et al. (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* **395**, 1033–1034, https://doi.org/10.1016/S0140-6736(20)30628-0

137 Cantaluppi, V., Quercia, A.D., Dellepiane, S., Ferrario, S., Camussi, G. and Biancone, L. (2014) Interaction between systemic inflammation and renal tubular epithelial cells. *Nephrol. Dial. Transplant.* **29**, 2004–2011, https://doi.org/10.1093/ndt/gfu046

138 Puelles, V.G., Lütgehetmann, M., Lindenmeyer, M.T., Sperhake, J.P., Wong, M.N., Allweiss, L. et al. (2020) Multiorgan and renal tropism of SARS-CoV-2. *N. Engl. J. Med.* **383**, 590–592, https://doi.org/10.1056/NEJMc2011400

139 Braun, F., Lütgehetmann, M., Pfefferle, S., Wong, M.N., Carsten, A., Lindenmeyer, M.T. et al. (2020) SARS-CoV-2 renal tropism associates with acute kidney injury. *Lancet* **396**, 597–598, https://doi.org/10.1016/S0140-6736(20)31759-1

140 Farkash, E.A., Wilson, A.M. and Jentzen, J.M. (2020) Ultrastructural Evidence for Direct Renal Infection with SARS-CoV-2. *J. Am. Soc. Nephrol.* **31**, 1683–1687, https://doi.org/10.1681/ASN.2020040432

141 Pan, X.W., Xu, D., Zhang, H., Zhou, W., Wang, L.H. and Cui, X.G. (2020) Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Med.* **46**, 1114–1116, https://doi.org/10.1007/s00134-020-06026-1

142 Obukhov, A.G., Stevens, B.R., Prasad, R., Li Calzi, S., Boulton, M.E., Raizada, M.K. et al. (2020) SARS-CoV-2 infections and ACE2: clinical outcomes linked with increased morbidity and mortality in individuals with diabetes. *Diabetes* **69**, 1875–1886, https://doi.org/10.2337/dbi20-0019

143 Wang, K., Gheblawi, M. and Oudit, G.Y. (2020) Angiotensin converting enzyme 2: a double-edged sword. *Circulation* **142**, 426–428, https://doi.org/10.1161/CIRCULATIONAHA.120.047049

144 Sampaio, W.O., Nascimento, A.A. and Santos, R.A. (2003) Systemic and regional hemodynamic effects of angiotensin-(1-7) in rats. *Am. J. Physiol. Heart Circ. Physiol.* **284**, H1985–H1994, https://doi.org/10.1152/ajpheart.01145.2002

145 Bernardi, S., Burns, W.C., Toffoli, B., Pickering, R., Sakoda, M., Tsorotes, D. et al. (2012) Angiotensin-converting enzyme 2 regulates renal atrial natriuretic peptide through angiotensin-(1-7). *Clin. Sci. (Lond.)* **123**, 29–37, https://doi.org/10.1042/CS20110403

146 Pinheiro, S.V. and Simões E Silva, A.C. (2012) Angiotensin converting enzyme 2, Angiotensin-(1-7), and receptor MAS axis in the kidney. *Int. J. Hypertens.* **2012**, 414128, https://doi.org/10.1155/2012/414128

147 Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R.A., Stahl, M. et al. (2020) Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* **181**, 905.e907–913.e907, https://doi.org/10.1016/j.cell.2020.04.004

148 Hemnes, A.R., Rathinasabapathy, A., Austin, E.A., Brittain, E.L., Carrier, E.J., Chen, X. et al. (2018) A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur. Respir. J.* **51**, 1702638, https://doi.org/10.1183/13993003.02638-2017

149 Khan, A., Benthin, C., Zeno, B., Albertson, T.E., Boyd, J., Christie, J.D. et al. (2017) A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit. Care* **21**, 234, https://doi.org/10.1186/s13054-017-1823-x

150 Zhang, J., Dong, J., Martin, M., He, M., Gongol, B., Marin, T.L. et al. (2018) AMP-activated protein kinase phosphorylation of angiotensin-converting enzyme 2 in endothelium mitigates pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **198**, 509–520, https://doi.org/10.1164/rccm.201712-2570OC

151 Qi, Y., Zhang, J., Cole-Jeffrey, C.T., Shenoy, V., Espejo, A., Hanna, M. et al. (2013) Diminazene aceturate enhances angiotensin-converting enzyme 2 activity and attenuates ischemia-induced cardiac pathophysiology. *Hypertension* **62**, 746–752, https://doi.org/10.1161/HYPERTENSIONAHA.113.01337

152 Qiu, Y., Shil, P.K., Zhu, P., Yang, H., Verma, A., Lei, B. et al. (2014) Angiotensin-converting enzyme 2 (ACE2) activator diminazene aceturate ameliorates endotoxin-induced uveitis in mice. *Invest. Ophthalmol. Vis. Sci.* **55**, 3809–3818, https://doi.org/10.1167/iovs.14-13883

153 Thatcher, S.E., Zhang, X., Howatt, D.A., Yiannikouris, F., Gurley, S.B., Ennis, T. et al. (2014) Angiotensin-converting enzyme 2 decreases formation and severity of angiotensin II-induced abdominal aortic aneurysms. *Arterioscler. Thromb. Vasc. Biol.* **34**, 2617–2623, https://doi.org/10.1161/ATVBAHA.114.304613

154 Shenoy, V., Gjymishka, A., Jarajapu, Y.P., Qi, Y., Afzal, A., Rigatto, K. et al. (2013) Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models. *Am. J. Respir. Crit. Care Med.* **187**, 648–657, https://doi.org/10.1164/rccm.201205-0880OC

155 Ferreira, A.J., Murça, T.M., Fraga-Silva, R.A., Castro, C.H., Raizada, M.K. and Santos, R.A. (2012) New cardiovascular and pulmonary therapeutic strategies based on the Angiotensin-converting enzyme 2/angiotensin-(1-7)/mas receptor axis. *Int. J. Hypertens.* **2012**, 147825

156 Li, J., Wang, X., Chen, J., Zhang, H. and Deng, A. (2020) Association of renin-angiotensin system inhibitors with severity or risk of death in patients with hypertension hospitalized for coronavirus disease 2019 (COVID-19) infection in Wuhan, China. *JAMA Cardiol.* **5**, 825–830, https://doi.org/10.1001/jamacardio.2020.1624

157 Zhang, P., Zhu, L., Cai, J., Lei, F., Qin, J.J., Xie, J. et al. (2020) Association of inpatient use of angiotensin converting enzyme inhibitors and angiotensin ii receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ. Res.* **2020**, 1–12

158 Montalvan, V., Lee, J., Bueso, T., De Toledo, J. and Rivas, K. (2020) Neurological manifestations of COVID-19 and other coronavirus infections: a systematic review. *Clin. Neurol. Neurosurg.* **194**, 105921, https://doi.org/10.1016/j.clineuro.2020.105921

159 Ellul, M.A., Benjamin, L., Singh, B., Lant, S., Michael, B.D., Easton, A. et al. (2020) Neurological associations of COVID-19. *Lancet Neurol.* **19**, 767–783, https://doi.org/10.1016/S1474-4422(20)30221-0

160 Sharma, R.K., Oliveira, A.C., Kim, S., Rigatto, K., Zubcevic, J., Rathinasabapathy, A. et al. (2018) Involvement of neuroinflammation in the pathogenesis of monocrotaline-induced pulmonary hypertension. *Hypertension* **71**, 1156–1163, https://doi.org/10.1161/HYPERTENSIONAHA.118.10934

161 Santisteban, M.M., Ahmari, N., Carvajal, J.M., Zingler, M.B., Qi, Y., Kim, S. et al. (2015) Involvement of bone marrow cells and neuroinflammation in hypertension. *Circ. Res.* **117**, 178–191, https://doi.org/10.1161/CIRCRESAHA.117.305853

162 Shi, P., Diez-Freire, C., Jun, J.Y., Qi, Y., Katovich, M.J., Li, Q. et al. (2010) Brain microglial cytokines in neurogenic hypertension. *Hypertension* **56**, 297–303, https://doi.org/10.1161/HYPERTENSIONAHA.110.150409

163 Chen, L.D., Zhang, Z.Y., Wei, X.J., Cai, Y.Q., Yao, W.Z., Wang, M.H. et al. (2020) Association between cytokine profiles and lung injury in COVID-19 pneumonia. *Respir. Res.* **21**, 201, https://doi.org/10.1186/s12931-020-01465-2