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Renin–angiotensin system blockade in the COVID-19 pandemic

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ABSTRACT

In the early months of the coronavirus disease 2019 (COVID-19) pandemic, a hypothesis emerged suggesting that pharmacologic inhibitors of the renin–angiotensin system (RAS) may increase COVID-19 severity. This hypothesis was based on the role of angiotensin-converting enzyme 2 (ACE2), a counterregulatory component of the RAS, as the binding site for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), allowing viral entry into host cells. Extrapolations from prior evidence led to speculation that upregulation of ACE2 by RAS blockade may increase the risk of adverse outcomes from COVID-19. However, counterarguments pointed to evidence of potential protective effects of ACE2 and RAS blockade with regard to acute lung injury, as well as substantial risks from discontinuing these commonly used and important medications. Here we provide an overview of classic RAS physiology and the crucial role of ACE2 in systemic pathways affected by COVID-19. Additionally, we critically review the physiologic and epidemiologic evidence surrounding the interactions between RAS blockade and COVID-19. We review recently published trial evidence and propose important future directions to improve upon our understanding of these relationships.

Keywords: angiotensin-converting enzyme 2, angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, coronavirus, COVID-19, hypertension, renin–angiotensin system, SARS-CoV-2

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INTRODUCTION

Early in the coronavirus disease 2019 (COVID-19) pandemic, a hypothesis emerged proposing that angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), inhibitors of the renin–angiotensin system (RAS), may increase the risk of development and severity of COVID-19 [1]. This hypothesis was based on limited physiologic evidence coupled with initial clinical descriptions of individuals hospitalized with COVID-19. Considering that ACEIs/ARBs are among the most prescribed medications globally [2, 3], the hypothesis of a potential interaction between RAS blockade and COVID-19 quickly gained momentum in the lay press and medical community. Several medical societies quickly reacted, unanimously recommending that patients continue to take these medications until more information became available [4, 5].

The physiologic evidence for the hypothesis was based on the role of angiotensin-converting enzyme 2 (ACE2), a counterregulatory component of the RAS, as the primary binding site for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), facilitating SARS-CoV-2 entry into host cells [6–8]. Prior evidence suggested that ACEIs/ARBs upregulate ACE2 [9, 10], and individuals hypothesized that increased ACE2 expression may increase susceptibility to SARS-CoV-2 infection, increasing the risk of development and severity of COVID-19 (Figure 1) [1]. However, counterevidence supported a protective role of ACE2 as well as possible differential effects between ACEIs and ARBs on the risk of acute lung injury [11, 12]. Epidemiologically, the hypothesis was based on findings from initial case series, which showed that a high proportion of individuals hospitalized with COVID-19 had conditions commonly treated with RAS blockade, including hypertension, diabetes, and heart disease [13–15]. These initial case series did not provide information on the frequency of ACEI/ARB use nor could they be used to draw conclusions about the relationships between these risk factors with adverse outcomes [16].

The current review aims to provide an overview of classic RAS physiology and its interaction with SARS-CoV-2. We examine the role of ACE2 in hypertension; effects in the heart, kidneys, brain, lungs and thromboembolic pathways; and how ACE2 may impact host responses to SARS-CoV-2 infection. We also evaluate the strengths and limitations of the current physiologic and epidemiologic evidence of the relationships between RAS blockade and COVID-19 and propose approaches to improve our understanding of these relationships in future studies.

ROLE OF ACE2 IN RAS PHYSIOLOGY

ACE2 in hypertension

The RAS plays a crucial role in regulating blood pressure (BP), including fluid and electrolyte balance and vascular tone. Targeting the RAS with ACEIs/ARBs constitutes a very effective strategy to treat hypertension. Since the discovery of ACE in 1956 [17], our understanding of the RAS as an endocrine system has been focused primarily on studying the interaction of angiotensin II (Ang II) with the Ang II type 1 (AT_1) receptor to increase BP. However, our understanding of the RAS shifted dramatically with the identification of angiotensin 1–7 (Ang-1–Ang-7) in the late 1980s as the first amino-terminal angiotensin peptide possessing biological actions that oppose those of Ang II [18–22].

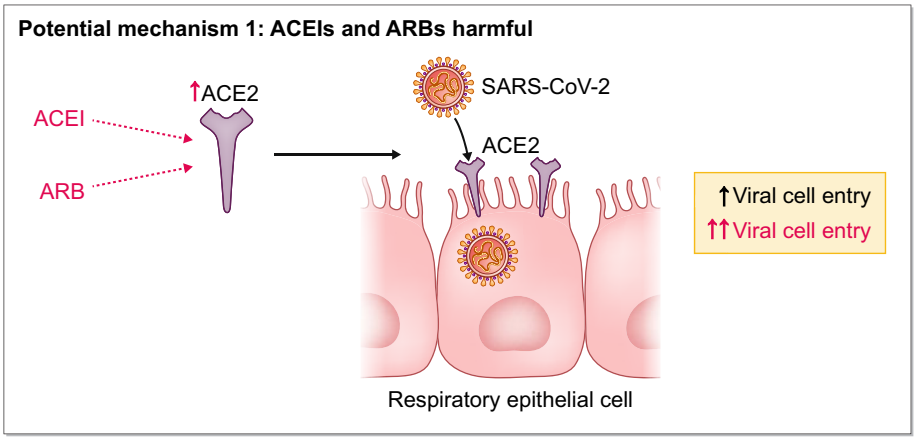
ACE2 catalyzes the formation of Ang-1–7 from Ang II [23, 24] and thus plays a critical role in RAS counterregulation by decreasing Ang II content and increasing Ang-1–7 content, among other roles [25]. Mice lacking the *ACE2* gene had an enhanced Ang II pressor response [26]. *ACE2*-deficient mice on the C57BL/6 background had modestly increased BP compared with controls at baseline, but acute Ang II infusion increased plasma Ang II concentrations and BP to a greater extent in the *ACE2*-deficient mice compared with controls [27]. In rat models of hypertension, renal ACE2 messenger ribonucleic acid (mRNA) and protein levels and expression are markedly lower than those observed in normotensive controls [28]. ACE2 enzymatic activity is not limited to the RAS; ACE2 can metabolize and inactivate other peptides such as pyr-apelin 13 and apelin 17, the dominant apelin peptides [29], which have hypotensive effects [30]. Indeed, *ACE2* knockout mice had potentiated hypotensive responses from pyr-apelin 13 that corresponded to greater plasma apelin levels and that the selective ACE2 inhibitor MLN-4760 blocked [31]. On the other hand, in select models, lisinopril may increase cardiac ACE2 mRNA expression but not activity, while losartan may increase both cardiac ACE2 mRNA expression and activity, in addition to their BP-lowering effects in Lewis rats [9].

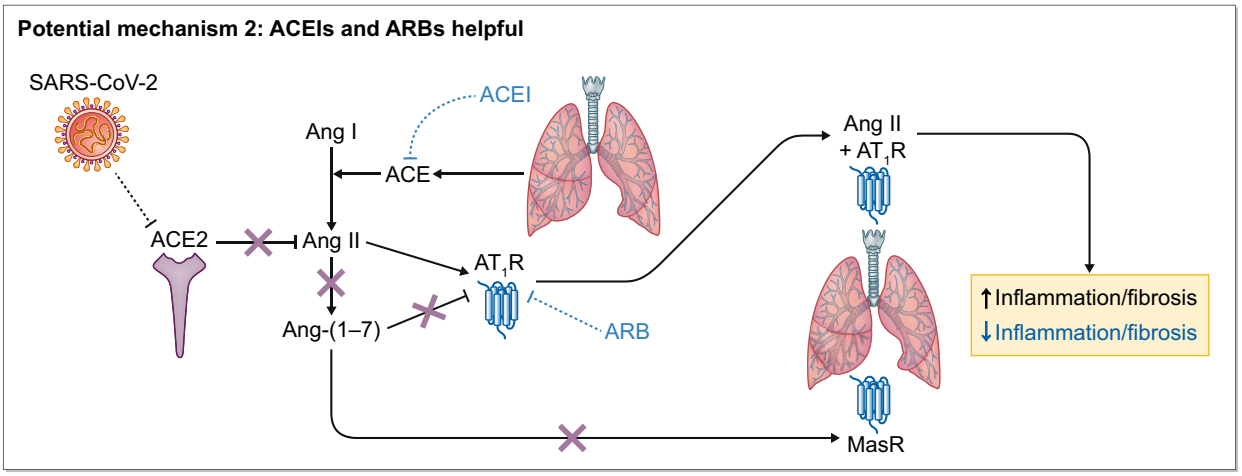
ACE2 in target organ disease. *Heart*. ACE2 plays a critical role in cardiovascular pathology development, especially heart failure. ACE2 is present in the membrane of most epithelial cells, cardiac myocytes, vascular smooth muscle and endothelial cells, the adventitia of large blood vessels and neural tissues [32]. *ACE2*-deficient mice exhibit severely reduced cardiac contractility [28]. Chronic ACE2 inhibition with MLN-4760 in transgenic rats increased cardiac Ang II content and significantly increased left ventricular anterior, posterior and relative wall thicknesses, as well as increased interstitial collagen fraction area and cardiomyocyte hypertrophy [33].

Furthermore, increasing Ang-1–7 content improves heart function, reverses cardiac remodeling and restores vascular responsiveness after myocardial infarction [34, 35], while recombinant human ACE2 successfully reduced cardiac hypertrophy and dysfunction in preclinical models of heart failure [36]. On the other hand, transgenic mice with increased cardiac ACE2 expression had a high incidence of sudden death due to progressive conduction and rhythm disturbances with sustained ventricular tachycardia and terminal ventricular fibrillation [37]. Thus the exact role of ACE2 in cardiovascular disease, and in particular heart disease, remains incompletely understood.

Kidney. The ACE/Ang II and ACE2/Ang-1–7 pathways are coexpressed throughout the kidney, including in the arterioles, epithelium, endothelium and podocytes of the glomerulus and throughout the tubular epithelium but particularly the proximal tubular brush border [10, 38, 39]. The ACE2/Ang-1–7 pathway generally opposes Ang II's actions to promote renal vasodilation and sodium and water excretion and to prevent or attenuate inflammation and fibrosis [40, 41]. In most experimental models and clinical studies, acute and chronic kidney injuries increase the ACE/Ang II pathway relative to decreasing the ACE2/Ang-1–7 pathway [42–44]. Furthermore, there are numerous feedback loops within the RAS. For example, Ang II downregulates ACE2 expression in kidney tubular cells via extracellular regulated 1/2 and p38 mitogen-activated protein kinases [45].

In rats, ischemia–reperfusion decreased renal ACE2 mRNA and protein expression and enzymatic activity, altered renal Ang-1–7 content and increased renal Mas receptor mRNA





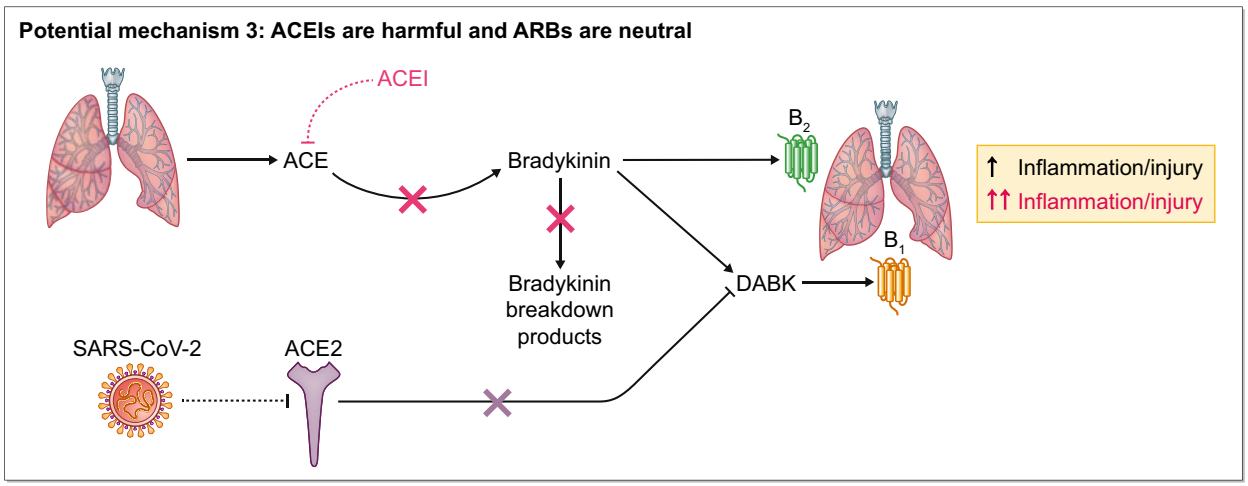


FIGURE 1: Three proposed mechanisms of ACEI and ARB effect in COVID-19. ACE, angiotensin-converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ACE2, angiotensin-converting enzyme 2; Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1–7), angiotensin-(1–7); MasR, mas receptor; AT_1R, angiotensin II type 1 receptor; DABK, [des-Arg^9]-bradykinin; B1, G-protein-coupled receptor for DABK; B2, G-protein-coupled receptor for bradykinin. Red dashed lines: harmful effects of ACEI/ARB; blue dashed lines: beneficial effects of ACEI/ARB; black dashed lines: effects of SARS-CoV-2; black solid lines: normal pathways; red Xs: downstream detrimental effects of ACEI on normal pathways; purple Xs: downstream detrimental effects of SARS-CoV-2 on normal pathways. This three-panel figure shows proposed mechanisms of ACEIs and ARBs in COVID-19 infection. Mechanism 1: ACEIs and ARBs are harmful. ACEIs and ARBs upregulate ACE2 expression on respiratory epithelial cells, thus increasing available receptors to bind SARS-CoV-2 and facilitate cell entry. Mechanism 2: ACEIs and ARBs are beneficial. ACEIs inhibit conversion of Ang I into Ang II, while ARBs inhibit Ang II binding to AT_1R, thereby both ACEIs and ARBs block Ang II-AT_1R–mediated deleterious effects in the lungs. Also shown are SARS-CoV-2-mediated ACE2 downregulation and subsequent Ang II cleavage into Ang-1–7 and Ang-1–7-MasR–mediated anti-inflammatory and anti-fibrotic effects. In the presence of SARS-CoV-2, there is less ACE2 available to cleave Ang II and hence more Ang II is available to bind to AT_1R. Additionally, less Ang-1–7 is available to bind to MasR, leading to increased inflammation and fibrosis. Mechanism 3: ACEIs are harmful and ARBs are neutral. ACEIs inhibit bradykinin breakdown into harmless products, thus increased bradykinin either binds to the B2 receptor or is converted to DABK that binds to the B1 receptor, leading to increased lung inflammation. ARBs play no role in the bradykinin cascade and are not pictured. Additionally, SARS-CoV-2 downregulates ACE2, which normally breaks down DABK. More DABK is then available to bind to the B1 receptor, further promoting lung inflammation.

expression [42, 44]. These effects were magnified in *ACE2* knockout mice and mitigated in *ACE2* transgenic mice [44]. Diabetes reduced renal ACE2 mRNA and protein expression and renal Ang-1–7 concentration despite increased renal and plasma ACE2 activity, effects that MLN-4760 enhanced [46]. Loss of ACE2 and the Mas receptor leads to Ang II–dependent glomerular injury and kidney dysfunction [47, 48]. Recombinant human ACE2 increased plasma ACE2 activity, increased Ang-1–7, decreased Ang II renal and plasma concentrations and improved kidney function and BP in diabetic mice [46]. Further, conditions of endogenous RAS activation likely enhance, or are required for, Ang-1–7's counterregulatory actions [49].

Brain. The role of the brain RAS in the central regulation of cardiovascular physiology and the autonomic nervous system is clear from studies of wild-type and transgenic animals. As in other tissues, the balance between the ACE/Ang II and ACE2/Ang-1–7 pathways in the brain is crucial for cardiovascular health [50]. Ang II AT_1 receptor signaling in the brain increases BP via enhanced sympathetic outflow [51, 52], vasopressin release [53] and cardiac baroreflex resetting [50, 54, 55]. In the spontaneously hypertensive rat, upregulation of brain RAS components (i.e. angiotensinogen, ACE, Ang II and AT_1 receptor) preceded and sustained hypertension development, which intracerebroventricular captopril injection reversed [56].

Meanwhile, the brain ACE2/Ang-1–7 pathway counterregulates Ang II's effects on cardiovascular function, autonomic function and cognition. ACE2 is expressed throughout the brain, including nuclei involved in central regulation of cardiovascular function in the brainstem and noncardiovascular areas such as the motor cortex and raphe [57]. In humans, ACE2 mRNA expression in the brain was at least 10-fold lower than ACE [58]. Ang-1–7 decreased BP and improved baroreflex function in animal models of hypertension [54, 59–61].

ACE2 knockout mice exhibited reduced cardiac baroreflex function compared with wild-type, which intracerebroventricular losartan injection restored [62]. ACE2 overexpression in the rostral ventrolateral medulla reduced BP in spontaneously hypertensive rats [63], which could be due to ACE2-induced Ang II degradation as well as AT_1 receptor downregulation in this region. In addition, ACE2 overexpression in the subfornical organ prevented Ang II–mediated pressor and thirst responses [64]. ACE2 overexpression in select areas of the brain in transgenic mice reduced sympathetic outflow and improved autonomic function [65]. Frontal cortex ACE2 activity was reduced in an Alzheimer's disease mouse model [66]. Intraperitoneal diminazene aceturate administration, an established ACE2 activator, lowered hippocampal Aβ and restored cognition in middle-aged symptomatic mice via increased ACE2 activity, restored Mas receptor levels and decreased extracellular regulated kinase 1/2 signaling [67].

ACE2 and the lung

The RAS regulates pulmonary vascular tone, the integrity of the alveolar capillaries and the immune system response to lung injury, among other functions. While several pulmonary cell types express the RAS, including alveolar epithelial cells, resident immune cells and pulmonary vascular endothelial and smooth muscle cells, ACE/Ang II pathway expression is typically much stronger than ACE2/Ang-1–7, which is predominantly limited to type II alveolar epithelial cells [32, 68]. Notably, lung injury increases ACE/Ang II but downregulates ACE2/Ang-1–7, further driving inflammation and fibrosis [69, 70]. Ang II–AT_1 receptor signaling increases pulmonary vasculature permeability, promotes immune system cell migration, activation and differentiation and induces alveolar epithelial cell apoptosis and fibroblast differentiation [71–74]. Ang-1–7 acts in part through the Mas receptor to block these Ang II–mediated effects [75].

In various models of acute lung injury (acid, sepsis, endotoxin, influenza), *ACE2* knockout mice had greater increases in inflammatory cell infiltration and lung edema that were associated with increased pulmonary vascular permeability, hyaline membrane formation and elastance and greater decreases in oxygenation compared with wild-type mice [76, 77]. Intraperitoneal recombinant human ACE2 administration improved elastance and edema and decreased Ang II content in acid-treated *ACE2* knockout and wild-type mice [76]. Similarly, acid exposure decreased ACE2 protein expression in wild-type mice and increased lung Ang II concentrations to a greater extent in *ACE2* knockout mice compared with wild-type mice [76]. SARS-CoV Spike protein binds to ACE2 on type II alveolar epithelial cells and subsequently downregulates ACE2 expression, increases lung Ang II concentration and enhances AT_1 receptor–mediated injury [6]. While this has been postulated to occur in SARS-CoV-2 as a mediating factor in COVID-19, to date there have been no studies to confirm this pathophysiologic process [7]. Mast cells recruited to areas of lung injury can release chymase, which could lead to further increases in Ang II [78, 79]. Loss of pulmonary ACE2 activity can also drive lung inflammation via increased [des-Arg^9]-bradykinin (DABK)/bradykinin receptor B1 activation and possibly via alterations to the apelin pathway [80, 81].

In rats exposed to lipopolysaccharide plus mechanical ventilation, treatment with losartan or cyclic Ang-1–7 increased ACE2 activity and Ang-1–7 concentrations in bronchoalveolar lavage fluid, improved oxygenation and reduced lung injury compared with placebo [69]. Pretreatment or treatment with losartan or Ang-1–7 reduced acute lung injury and fibrosis, while pretreatment or treatment with the Mas receptor antagonist A-779 not only reversed this protective effect, but enhanced progression to fibrosis [82, 83]. In humans, limited clinical evidence supports the experimental evidence. ACE and ACE2 activity in bronchoalveolar lavage fluid correlated weakly with inflammatory markers in patients with acute respiratory distress syndrome [84]. In a pilot study in patients with acute respiratory distress syndrome, intravenous recombinant human ACE2 decreased Ang II levels and increased Ang-1–7 levels in plasma but did not affect lung physiology or outcomes [85].

ACE2 and thrombosis

The ACE2/Ang-1–7 pathway is crucial to maintaining vascular endothelial integrity, in part via nitric oxide release, and is expressed by platelets and thus mediates vascular inflammation and the clotting cascade [32, 86–88]. Ang II–AT_1 receptor binding stimulates inflammatory cell recruitment and cytokine release to promote thrombosis, which ACE2/Ang-1–7 can mitigate in part via negatively modulating plasminogen activator inhibitor-1, extracellular signal-regulated kinase 1/2 and nicotinamide adenine dinucleotide phosphate oxidase and decreasing oxidative stress [89–93].

ACE2 knockout mice demonstrate increased expression of interleukin-6, monocyte chemoattractant protein-1 and vascular cell adhesion molecule-1, increased macrophage response

to Ang II and increased endothelial cell activation and responsiveness to tumor necrosis factor-α [94]. In a hypertension rat model, greater thrombus formation was associated with decreased ACE2 activity, and ACE2 inhibition increased thrombus weight while ACE2 activation attenuated thrombus formation and reduced platelet attachment [95]. Decreased ACE2 could also promote thrombosis via modulation of bradykinin/kallikrein [96]. SARS-CoV-2 decreased platelet ACE2 levels *in vitro* and patients with COVID-19 had lower platelet ACE2 levels compared with healthy controls [87]. Platelets express transmembrane protease serine 2, and SARS-CoV-2, via the Spike protein, was shown to bind directly to platelets, potentiate platelet activation, enhance platelet spread and stimulate coagulation factor and inflammatory cytokine release from platelets via mitogen-activated protein kinase pathways *in vitro* and *in vivo*, while recombinant human ACE2 suppressed these effects [87].

PROPOSED ROLE OF ACEIs AND ARBS ON ACE2 IN COVID-19. *Proposed mechanisms for adverse effects*. Despite the critical roles that ACEIs/ARBs have in diminishing adverse cardiovascular and kidney outcomes, concern emerged early in the pandemic that these drugs might be leading to adverse outcomes in patients with COVID-19. These concerns stemmed from the theory that ACEIs/ARBs may promote SARS-CoV-2 viral entry into cells by increasing ACE2 expression in respiratory epithelial cells (Figure 1) [8]. Some animal model studies have shown that ACEIs/ARBs can upregulate ACE2 expression and activity in heart and kidney tissue [9, 10]. Furthermore, a 2015 study in humans found that participants taking the ARB olmesartan had higher urinary ACE2 levels compared with control participants [97], while a recent study looking at ACEI/ARB use in patients with and without COVID-19 found that plasma ACE2 activity was higher in patients with COVID-19 who were taking ACEIs compared with those with COVID-19 who were not taking ACEIs/ARBs [98]. It was thus postulated that ACEIs/ARBs may upregulate ACE2 in the lungs.

While higher ACE2 mRNA expression in the lungs has been noted in patients who have comorbidities that are frequently found in patients with severe COVID-19 [99], such as diabetes mellitus, hypertension and chronic obstructive lung disease, there have yet to be any animal or human studies to definitively demonstrate that ACEIs/ARBs upregulate ACE2 in the lungs. In fact, Wysocki *et al.* [100] demonstrated in a mouse model that ACEI/ARB administration had no detectable effect on ACE2 levels or activity in the lungs. Additionally, conflicting evidence exists in human studies in that there is no consistent association between ACEIs/ARBs and circulating ACE2 concentration or activity [101–103]. Furthermore, it is unclear if the overexpression of ACE2 and its theoretical effect of SARS-CoV-2 viral entry in the lungs of patients with chronic illnesses is the cause of worse outcomes in COVID-19 or if patients with these comorbidities are simply more prone to adverse outcomes because of their underlying illnesses.

Proposed mechanisms for beneficial effects. Further complicating the issue is the fact that there exists limited evidence (in both animal models and human studies) that ACEIs/ARBs may actually be beneficial in patients with various viral pneumonias. Henry *et al.* [104] described a cohort of >500 patients with viral pneumonia in Texas from 2011 to 2014 in which patients who continued their ACEI during hospitalization had 75% lower odds of intubation and mortality. However, patients who were taking ACEIs prior to admission had 3-fold higher odds of death compared with those not taking ACEIs, raising the question of whether ACEIs may have been preferentially continued in healthier patients (i.e. implying several sources of bias including collider and selection bias [105]). A 2005 study using a mouse model examined the original SARS-CoV, which was responsible for the SARS epidemic in 2002 [6]. The authors found that in a mouse model of acid aspiration–induced acute lung injury who were treated *in vivo* with the SARS-CoV Spike protein, losartan attenuated pulmonary edema and lung injury severity. This was thought to be due to losartan's effect of blocking the AT_1 receptor and hence attenuating the effect of the excess Ang II generation and subsequent AT_1 receptor signaling from Spike protein-induced ACE2 downregulation (Figure 1) [6].

Potential differential roles of ACEIs *versus* ARBs in COVID-19. Given their differential effects on the RAS, it is possible that ACEIs may be detrimental in patients with COVID-19 while ARBs may be neutral or beneficial. ACE, in addition to converting Ang I to Ang II, breaks down bradykinin. Bradykinin is a vasoactive peptide that contributes to BP regulation and inflammation via its ability to increase vascular permeability and cause vasodilatation of blood vessels throughout the body. Bradykinin and its active metabolite, DABK, act on two G-protein-coupled receptors, B_1 and B_2; DABK binds mainly to B_1 while bradykinin binds mainly to B_2 [80, 106, 107]. The B_2 receptor is constitutively expressed in many cell lineages and is responsible for a number of bradykinin-mediated effects such as vasodilation, hypotension and increased vascular permeability. The B_1 receptor, however, is often absent in normal tissues and is instead expressed during inflammatory states such as septic shock and lung inflammation [108]. Furthermore, B_1 receptor activation can occur concomitantly with the pro-inflammatory chemokine C-X-C motif chemokine 5 (CXCL5) release and neutrophil recruitment to the lung [80]. Since ACEIs act by competitively inhibiting ACE, the addition of ACEIs can result in decreased bradykinin breakdown and elevated bradykinin levels, causing the cough and occasionally angioedema that can occur in patients taking ACEIs, along with possible lung injury [12, 109–112].

Roche *et al.* [113] discuss a separate bradykinin hypothesis that suggests that SARS-CoV-2-mediated ACE2 depletion may lead to bradykinin dysregulation, including increased bradykinin and DABK, leading to worsened acute lung injury and respiratory distress. Since ARBs do not competitively inhibit ACE, they have no known role in contributing to elevated bradykinin levels and thus should not precipitate further bradykinin-mediated lung injury. If dysregulated bradykinin signaling turns out to be a major mechanism by which patients with COVID-19 experience lung injury, it is plausible that concomitant ACEI use could be detrimental whereas ARB use would not (Figure 1).

CURRENT EPIDEMIOLOGIC EVIDENCE OF RAS BLOCKADE IN COVID-19

Summary of existing observational studies

The conflicting evidence and hypotheses regarding the relationship of RAS blockade with ACE2 expression and COVID-19-related outcomes resulted in a surge of studies aiming to assess the safety of continuing and the effectiveness of initiating ACEIs/ARBs in the management of COVID-19 [4, 11, 16]. In a relatively brief time frame, dozens of observational studies, systematic reviews and meta-analyses evaluating the association between RAS blockade, SARS-CoV-2 infection and development

and/or severity of COVID-19 were published and posted on preprint servers [114]. Notably, NephJC.com created a dynamic, open-access document that provided updates and critical reviews as new literature emerged related to the physiology and epidemiology of these relationships [4]. Similarly, Mackey *et al.* [115] developed a 'living' systematic review in the *Annals of Internal Medicine* that is continually updated as new epidemiologic evidence emerges and has identified >80 distinct observational studies at the time of submitting the current review. To date, the authors have repeatedly concluded that ACEIs/ARBs do not seem to be associated with testing positive for SARS-CoV-2 among symptomatic patients or patiients with more severe COVID-19. They have also concluded that evidence remains lacking on the effect of ACEIs/ARBs on the risk for mild or asymptomatic disease or for the treatment of COVID-19. Thus most observational studies support physiologic evidence suggesting that ACEIs/ARBs do not affect ACE2 expression, concentration or activity [100–103].

Strengths and limitations of existing observational studies

Given the enormous potential public health impact, the urgency of the study question yielded a paradigm for the utility of real-world evidence in identifying safety and effectiveness of pharmacologic therapies [116]. Observational evidence can be particularly helpful for answering study questions where randomized trials are infeasible, impractical or unethical, for example, due to the need for large sample sizes, long duration of follow-up or evaluation of the natural history of a disease process [117]. Observational study designs were best suited to evaluate the safety of prior ACEI/ARB use compared with other antihypertensive therapies with regard to the risk of developing symptomatic COVID-19. This question necessitated urgent answers to guide the ongoing care of patients during the pandemic and could not be readily or quickly answered with randomized controlled trials (RCTs). Accordingly, observational studies provided rapid, crucial evidence to support the safety of continuing ACEIs/ARBs during the COVID-19 pandemic among outpatients who have already been prescribed these medications [115, 118, 119].

However, understanding the rapid pace of implementation, many of the observational studies published early in the pandemic had important limitations [105]. Table 1 defines and describes common limitations of these studies that are essential to consider when interpreting the findings. For example, many studies did not address potential major confounders [117], such as confounding by indication for use of ACEIs/ARBs as opposed to other antihypertensive agents (e.g. due to proteinuric chronic kidney disease or heart failure with reduced ejection fraction) and unmeasured confounding due to inadequate access to healthcare or SARS-CoV-2 testing. Several studies may have introduced collider bias by restricting the study samples to individuals who were hospitalized or had symptomatic COVID-19, which can result in spurious associations between ACEIs/ARBs and COVID-19 [121, 122]. Additionally, several studies reported a lower risk of COVID-19 severity among hospitalized patients on ACEIs/ARBs compared with those not on these agents; however, a number of these studies introduced time-dependent bias, or failing to adequately account for the timing and duration of ACEIs/ARBs while hospitalized [124–126, 128].

Approaches to improve upon the epidemiologic evidence and future directions

Several of the limitations of existing observational evidence can be addressed with careful considerations about study design and the use of analytic methods that facilitate causal inference [105]. For example, directed acyclic graphs are diagrams that demonstrate the causal relationships between exposures and outcomes, as well as antecedents (causes) and descendants (effects) of these factors that can be sources of several types of bias [129]. Thus directed acyclic graphs are helpful tools in visualizing and identifying potential sources of bias, such as confounding, selection bias and collider bias, which can then guide the identification of an optimal data source, study sample, study design and analytic method to appropriately address these potential sources of bias. For example, in studies of hospitalized patients, the use of methods that address ACEI/ARB use as a time-varying exposure can mitigate time-dependent bias. Sensitivity analyses, such as evaluation of carefully selected negative controls, can also provide important insights into the potential effects of biases on the primary findings [130].

Ultimately, RCT evidence provides the highest-quality evidence to identify the safety of continuing RAS blockade and the potential efficacy of treatment with RAS blockade in COVID-19 (Table 2). Similar to observational evidence, early reports from trials demonstrate no elevated risk of COVID-19 incidence or severity in interim analyses of high-risk participants randomized to ACEI versus placebo [134] or from continuation versus withdrawal of ACEIs/ARBs in patients hospitalized with COVID-19 [133, 135]. The first peer-reviewed RCT results were published from the Randomized Elimination or ProLongation of ACEI/ARBs in COVID-19 (REPLACE COVID) trial in January 2021, corroborating early reports from other trials presented at scientific meetings or via preprint [131]. In this multicenter, international study, 152 patients hospitalized with COVID-19 were randomized to continue versus discontinue their ACEI/ARB upon admission. Patients were excluded with contraindications to continuing or discontinuing ACEI/ARB therapy, such as hyperkalemia or heart failure with reduced ejection fraction, respectively. The authors observed no difference between randomization arms with regard to the primary endpoint (a hierarchical global rank score in which patients were ranked by time to death and severity of illness), death, mechanical ventilation or intensive care unit admission, length of hospitalization, length of intensive care unit admission or multiorgan dysfunction measured by the area under the curve of a modified sequential organ failure assessment score [131]. Additionally, there were no differences in blood pressure, serum potassium and serum creatinine during hospitalization across the two randomization groups [131]. Strengths of the trial include its pragmatic approach and wide scope of participants enrolled from seven countries across three continents, making the findings generalizable to a broad range of hospitalized patients with COVID-19. The main limitation is the small sample size [136]; the authors noted that the small sample size was supported by the use of the global rank score as the primary endpoint, which allows for smaller sample sizes than other commonly used endpoints [131, 132].

While RCTs provide the highest quality evidence, they have important limitations to consider, some of which are amplified during a pandemic. RCTs are often not practical or feasible to answer important study questions due to issues of cost, ethical concerns and/or rapid pace of recruitment necessary to provide answers early in the course of a pandemic. For example, while a

Table 1. Common limitations of existing observational studies examining the association of ACEI or ARB therapy with development and severity of COVID-19

Limitation	Definition	Examples
Confounding [117]	Presence of a factor that is associated with the outcome, that is not on the causal path between the exposure and outcome, and is distributed unequally across exposure levels	• Different indications for use of ACEI or ARB therapy versus another antihypertensive class that may also be associated with worse outcomes (e.g. proteinuric chronic kidney disease and heart failure with reduced ejection fraction) • Unmeasured factors such as sociodemographics, access to healthcare, medication adherence
Selection bias [120]	Restriction of the study sample based on a confounding factor such that the sample is not representative of the population	• Restriction of the study sample to only individuals with diabetes mellitus, hypertension, chronic kidney disease, or heart failure
Collider bias [121, 122]	Restriction of the study sample based on a descendent factor that can induce a spurious association between the exposure and outcome	• Restriction of the study sample to only individuals with a positive COVID-19 test • Restriction of the study sample to only individuals hospitalized with COVID-19
Information bias [123]	Error in collecting or documenting information	• Non-differential: use of invalidated administrative codes in the electronic health record to identify past medical history • Differential: ACEI/ARB exposure history only reliably verified or updated in hospitalized individuals, in an electronic health record-based study evaluating COVID-19 hospitalization as the endpoint
Time-dependent bias [124–126]	Failure to appropriately account for the timing of the initial exposure or exposure during follow-up	• Immortal time bias: in a cohort of hospitalized patients, defining ACEI/ARB use at the time of ICU admission • Immeasurable time bias: in a cohort of hospitalized patients, defining exposure to ACEI/ARB use as having occurred at admission even among patients whose ACEI/ARB was held until they stabilized later in the admission/close to the time of discharge
Table 2 fallacy [127]	Evaluation of multiple effect estimates from the same multivariable model that confounds the interpretation of direct-effect and total-effect estimates	• Reporting the association of multiple different medications with COVID-19-related outcomes that were all analyzed in a single multivariable model

Table provides examples of common pitfalls of observational studies that were rapidly published to address concerns regarding the relationship of ACEI or ARB therapy with COVID-19-related outcomes. Most of these limitations can substantially alter the interpretability of the results but can be overcome or addressed with careful initial observational study design or ideally (but often not feasibly) by performing an RCT. Portions of the table were adapted from Cohen et al. [105].

randomized trial was initiated to evaluate the risk of continuing versus discontinuing ACEI/ARB use and testing positive for SARS-CoV-2 among outpatients (NCT04330300), the study required a very large sample size (>2400 participants) and was suspended, in part due to challenges with efficient enrollment. Additionally, randomized trials are unlikely to have adequate statistical power to be able to evaluate potentially high-risk subgroups and important effect modifiers. For example, the REPLACE COVID trial could not assess whether ACEIs have differential effects than ARBs with regard to COVID-19-related outcomes. High-quality observational studies that appropriately apply methods to facilitate causal inference have the potential to provide critical insights into these relationships.

CONCLUSION

In conclusion, the RAS plays a major role in mediating many physiological and inflammatory responses in a variety of important cardiovascular tissues. RAS blockade has an important role in treating hypertension, cardiovascular disease and kidney disease. However, mixed physiologic evidence exists regarding the role of ACEIs/ARBs in COVID-19. Observational evidence thus far has important limitations but suggests no elevated risk from continuing ACEIs/ARBs in patients already prescribed these medications. Randomized trials will provide important information on the safety and effectiveness of continuing and introducing RAS blockade in the treatment of COVID-19. Future observational studies, when carefully designed to appropriately address important sources of bias, can provide crucial information on potential differential effects of ACEIs and ARBs in acute lung injury and other adverse outcomes in COVID-19.

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Table 2. Registered RCTs evaluating the safety and effectiveness of ACEI or ARB therapy in COVID-19

ClinicalTrials.gov study identifier	Target *N* Country	Population	Interventions
Continuation versus withdrawal of ongoing ACEI or ARB therapy			
NCT04329195 (ACORES-2)	554 France	Hospitalized with COVID-19	Discontinue ACEI/ARB Comparator: continue ACEI/ARB (open-label)
NCT04338009 (REPLACE COVID) [131, 132]	152 USA/International	Hospitalized with COVID-19	Discontinue ACEI/ARB during hospitalization (resumed on discharge) Comparator: continue ACEI/ARB throughout hospitalization (open-label)
NCT04351581 (RASCOVID-19)	215 Denmark	Hospitalized with COVID-19	Continue ACEI/ARB at the same dose throughout hospitalization Comparator: discontinue ACEI/ARB during admission for up to 30 days (open-label)
NCT04364893 (BRACE-CORONA) [133]	500 Brazil	Hospitalized with COVID-19	Continue ACEI/ARB for 30 days Comparator: stop ACEI/ARB for 30 days (open-label)
NCT04353596 (ACEI-COVID)	208 Austria and Germany	Outpatient or hospitalized with COVID-19	Discontinue ACEI/ARB Comparator: continue ACEI/ARB (open-label)
Introduction of *de novo* ACEI or ARB therapy			
NCT04340557	200 USA	Hospitalized with COVID-19 requiring supplemental oxygen	Losartan 12.5 mg twice daily for up to 10 days Comparator: no losartan (open-label)
NCT04351724 (ACOVACT sub-study B)	500 Austria	Outpatient or hospitalized with COVID-19	Candesartan titrated to BP <120/80 mm Hg Comparator: non-ACEI/ARB antihypertensive medication (open-label)
NCT04311177	580 USA	Outpatient with COVID-19	Losartan 25 mg daily Comparator: placebo (blinded)
NCT04328012 (COVIDMED group 3)	4000 USA	Hospitalized with confirmed COVID-19	Losartan 25 mg daily for 5–14 days Comparator: placebo (blinded)
NCT04335786 (PRAETORIAN-COVID)	651 The Netherlands	Hospitalized with confirmed COVID-19	Valsartan 160 mg twice daily up to 14 days Comparator: placebo (blinded)

Table provides an overview of selected examples of registered RCTs evaluating the safety and effectiveness of continuation versus withdrawal and *de novo* introduction of ACEI or ARB therapy in both outpatients and inpatients with COVID-19.

Tomlinson, Vivek Bhalla, María José Soler, Michelle N. Rheault and Sundar Swaminathan.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts related to this review. J.B.C. is co-principal investigator of the REPLACE COVID trial described in this review, for which activities at the Data Coordinating Center were partially funded by the REPLACE COVID social media funding campaign. The information presented in this article has not been published previously in whole or part, except in abstract format.

DATA AVAILABILITY STATEMENT

There are no proprietary data presented in or related to this manuscript.

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