



Review

SARS-CoV-2 and ACE2: The biology and clinical data settling the ARB and ACEI controversy



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ABSTRACT

Background: SARS-CoV-2 enters cells by binding of its spike protein to angiotensin-converting enzyme 2 (ACE2). Angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) have been reported to increase ACE2 expression in animal models, and worse outcomes are reported in patients with co-morbidities commonly treated with these agents, leading to controversy during the COVID-19 pandemic over whether these drugs might be helpful or harmful.

Methods: Animal, in vitro and clinical data relevant to the biology of the renin-angiotensin system (RAS), its interaction with the kallikrein-kinin system (KKS) and SARS-CoV-2, and clinical studies were reviewed.

Findings and Interpretation: SARS-CoV-2 hijacks ACE2 to invade and damage cells, downregulating ACE2, reducing its protective effects and exacerbating injurious Ang II effects. However, retrospective observational studies do not show higher risk of infection with ACEI or ARB use. Nevertheless, study of the RAS and KKS in the setting of coronaviral infection may yield therapeutic targets.

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1. Introduction

In the unprecedented crisis of the COVID-19 pandemic, we must define the epidemiology, predictors of complications and mortality, and potential modifiable risk factors that might prevent or decrease the severity of the disease. Recently there has been controversy over whether use of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) might be harmful in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in patients with cardiovascular disease, hypertension, or diabetes mellitus under treatment with these agents. In contrast, it has been suggested that ARBs could be protective in the setting of SARS-CoV-2 infection.

SARS-CoV-2, the coronavirus causing COVID-19, enters host cells via binding of the virus spike protein to angiotensin-converting enzyme 2 (ACE2). ACEIs or ARBs have been reported to increase expression of ACE2 in animal models [1]. This has led to speculation that use of ACEIs or ARBs might contribute to a higher risk of contracting the infection and worse outcomes of COVID-19 in patients with cardiovascular diseases, hypertension and diabetes [2], as these drugs are commonly used in these conditions. Moreover, these comorbid conditions are increased with age, which is itself also associated with worse outcomes. As we await evidence from and plan clinical studies, it is essential to understand the biology of the renin-angiotensin system (RAS) and its modulation by the SARS-CoV-2 virus.

2. Brief primer on ACE, ACE2 and the renin-angiotensin system (Fig. 1)

Angiotensin converting enzyme (ACE) catalyzes the removal of two residues from the decapeptide angiotensin I (Ang I) to form the

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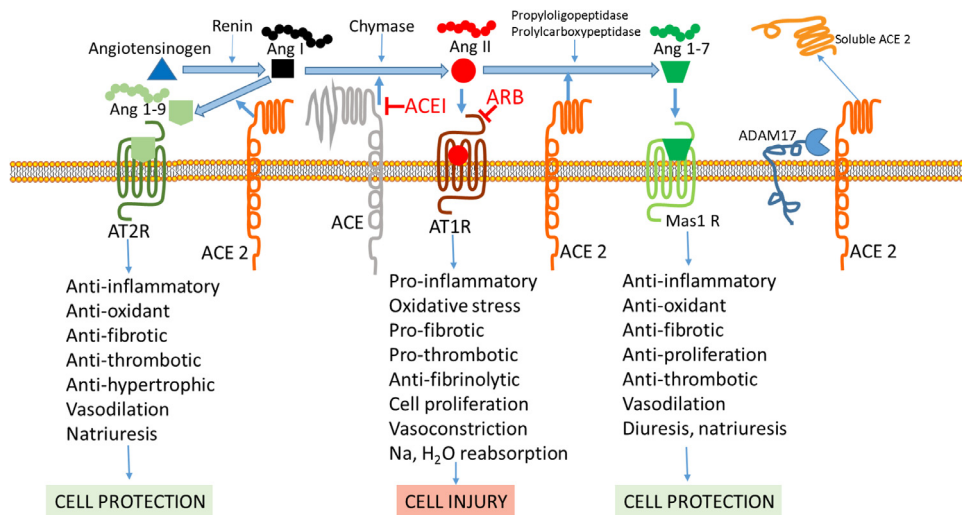


Fig. 1. ACE and ACE2 pathways. Renin from the kidneys converts angiotensinogen to Ang I. ACE, and to a lesser extent secreted proteases, such as chymase, catalyzes the conversion of Ang I to Ang II. Ang II binds to the angiotensin II receptor AT1R, leading to vasoconstriction and cellular injury pathways. ACEIs inhibit the conversion of Ang I to Ang II, reducing Ang II production, while ARBs block the Ang II receptor, AT1R. The ACE-Ang II-AT1R pathway is balanced by ACE2, which degrades Ang II and Ang I to produce Ang 1–7 and Ang 1–9. Ang 1–7 and Ang 1–9 pathways exert protective effects via the receptors Mas1 and AT2R, respectively. Downregulation of ACE2 is associated with an increase in Ang II and activation of the Ang II / AT1R pathway. Upregulation of ACE2 degrades Ang I, limiting the substrate for ACE, degrades Ang II, limiting its adverse effects, and generates Ang 1–7 and Ang 1–9, leading to protective effects. ACE2 is shed from the cell surface by the action of ADAM 17, which is dispensable, but which releases a soluble active form of ACE2 and reduces membrane-bound ACE2. Ang I - angiotensin I. Ang II - angiotensin II. Ang 1–7 - angiotensin 1–7. ACE - angiotensin converting enzyme. ACE 2 - angiotensin converting enzyme 2. AT1 R - angiotensin 1 receptor. AT2 R - angiotensin 2 receptor. Mas1 R - mitochondrial assembly receptor. ACEI - angiotensin converting enzyme inhibitor. ARB - angiotensin 1 receptor blocker.

octapeptide angiotensin II (Ang II). Secreted proteases, including mast cell chymase (human heart chymase), play a minor role in forming Ang II, but are more active when patients are on long-term ACEI therapy. Ang II binding to the type 1 angiotensin II receptor (AT1R) activates pro-inflammatory, vasoconstrictor, pro-oxidant, pro-thrombotic, anti-fibrinolytic, and pro-fibrotic signaling pathways. ACEIs inhibit conversion of Ang I to Ang II, thereby reducing Ang II production, while ARBs block the downstream effects of Ang II by blocking its receptor, AT1R. The functions of ACE are balanced by its homologue ACE2, which catalyzes the removal of one residue from Ang II to form Ang 1–7 and one residue from Ang I to form Ang 1–9. In doing so ACE2 reduces Ang II and its effects, while enabling the Ang 1–7 and Ang 1–9 pathways that protect against Ang II effects by promoting vasodilation, as well as anti-inflammatory, anti-oxidant, anti-thrombotic, and anti-fibrotic activity via the receptors Mas1 (a G-protein coupled receptor [GPCR]) and AT2R, respectively [3]. Ang 1–7 activated Mas1 receptors trigger downstream signaling pathways, including arachidonic acid release and activation of phospholipase A1, NOS, PI3K/Akt, MAP kinases, RhoA, and cAMP/PKA [4]. The Mas1 receptor also acts as an antagonist of the AT1R, and Ang 1–7/Mas1 signaling suppresses Ang II effects, including Ang II-induced reactive oxygen species (ROS) overproduction, apoptosis, phosphorylation of MAPKs and c-Src, leading to TGF- β 1 and collagen production, ICAM-1, VCAM-1, and MCP-1 expression [4]. Additionally, if expressed in molar excess to the AT1R, AT2R will directly bind Ang II, leading to its protective activity.

Supporting a complex balance of the ACE/ACE2 pathways in vivo, downregulation of ACE2 is associated with an increase in Ang II and activation of the Ang II / AT1R pathway. However, ACE2 upregulation increases Ang I degradation limiting the substrate for ACE, increases Ang II degradation limiting its adverse effects, and increases the production of Ang 1–7 and Ang 1–9, leading to their protective effects (Fig. 1) [5]. The mechanism of the reported increase of ACE2 expression by ACEIs and ARBs is not well understood, but may be mediated by increased Ang II metabolism by ACE2 and Ang 1–7 mediated mitogen-activated (MAP) kinase activity [1]. [6] [7] ACE2 is shed from the cell surface by the action of a disintegrin and metalloproteinase

(ADAM) 17, also called tumor necrosis factor- α converting enzyme, and ADAM10, which leads to release of a soluble active form of ACE2 and reduced membrane-bound ACE2 [3] [8]; elevated plasma ACE2 activity in heart failure is associated with worse prognosis [9].

3. ACE, ACE2, and Bradykinin (Fig. 2)

ACE and ACE2 also have intimate roles with the plasma kallikrein-kinin system (KKS), a hormonal pathway that modulates the intrinsic blood coagulation system, endothelial cell growth and angiogenesis, the complement pathway and RAS. The KKS consists of plasma and tissue kallikreins, plasma high (HK) and low (LK) molecular weight kininogens, their derivative kinin peptides, including bradykinin (BK) and des-Arg⁹-BK, and two G-protein-coupled bradykinin receptors (B2R and B1R) [10]. Plasma prekallikrein (PK) is activated by blood coagulation factor XII or an endothelial cell serine protease, prolylcarboxypeptidase, to preferentially cleave high molecular HK to liberate BK; the residual cleaved kininogen (CHK) is stable in plasma and may be used as a biomarker of KKS activation [11]. BK binds to its receptor B2R, which is constitutively expressed in the intravascular compartment, and des-Arg⁹-BK binds to the B1R, which arises in inflammatory states. Separately, tissue kallikreins have preference to cleave LK, releasing Lys-BK, which when acted on by several carboxypeptidases, generates des-Arg⁹-Lys-BK, which also activates B1R receptor.

The vasodilatory effects of BK are predominantly mediated through B2R, which is abundant in vascular endothelium and constitutively expressed in most tissues. B2R activation causes a cascade involving nitric oxide synthase (NOS), leading to synthesis of nitric oxide (NO) and cGMP [12]. BK and its active metabolite des-Arg⁹-bradykinin also agonise B1R, which is minimally expressed in healthy tissue, but induced by tissue injury and inflammatory stimuli, playing a role in chronic pain and inflammation [13,14]. Activation of both the B1 and B2 receptors mediate massive vascular permeability and inflammation, causing marked increases in the levels of inflammatory cytokines, such as IL-1, IL-2, IL-6, IL-8, and TNF- α that have been

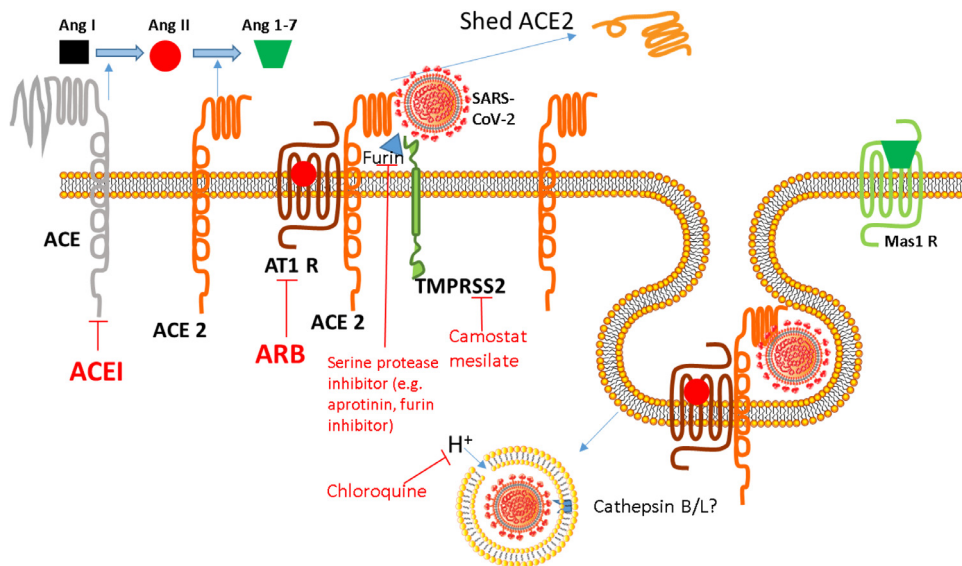


Fig. 3. SARS-CoV-2 interaction with ACE 2 and TMPRSS 2. The spike protein around SARS-CoV-2 binds to its receptor, ACE2, driving fusion of viral and host cell membranes. Viral entry is also dependent on spike protein priming at its S1/S2 cleavage site (e.g. by furin) and then at its S2' site by TMPRSS2, a process inhibited by camostat mesilate and serine protease / furin inhibition. Although SARS-CoV-2 fusion is thought to occur in the endosomes of target cells, the requirement of cathepsins B and L for optimal membrane fusion efficiency *in vivo* remains unclear. Chloroquine increases the pH of lysosomes and is thought to inhibit the activity of proteases that promote membrane fusion and viral release into the cell. Ang 1 - angiotensin I. Ang II - angiotensin II. Ang 1-7 - angiotensin 1-7. ACE - angiotensin converting enzyme. ACE 2 - angiotensin converting enzyme 2. AT1 R - angiotensin 1 receptor. Mas1 R - mitochondrial assembly receptor. ACEI - angiotensin converting enzyme inhibitor. ARB - angiotensin 1 receptor blocker. TMPRSS2 - transmembrane protein serine protease 2.

with renal disease [37] and post-mortem brain tissue in Alzheimer's disease [38].

6. The SARS-CoV-2 spike protein and ACE2

Given the novel emergence of SARS-CoV-2, studies on cellular and animal models are just emerging. Similar to SARS-CoV, the receptor for SARS CoV-2 is ACE2. The early availability of sequence information of virus isolates facilitated structural studies confirming the binding of SARS CoV-2 spike protein to ACE2. The SARS-CoV-2 spike protein has significant structural homology to the spike protein of SARS-CoV. Both spike proteins bind to ACE2, but SARS-CoV-2 spike protein has been reported to bind with tighter affinity than SARS-CoV [39], so the lessons from SARS-CoV are expected to apply to SARS-CoV-2, perhaps to an even higher degree. The contribution of the enhanced binding to ACE2 to the infectivity of SARS-CoV-2 is not well understood, and binding affinity may reflect genetic variation in ACE2, but the distribution of ACE2 in lung alveolar cells, mouth, intestines, heart, endothelium, kidneys, testes, and brain may explain effects on lung injury, gastrointestinal symptoms, cardiac damage, acute kidney injury, and reports of late potentially neurally mediated cardiorespiratory depression (Fig. 4). Like SARS-CoV, SARS-CoV-2 spike protein requires priming by the serine protease TMPRSS2 for optimal cell entry [40]. Lung and intestines show ACE2 and TMPRSS2 expression and are primary sites of viral entry. The heart shows high levels of ACE2, but low levels of TMPRSS2 expression, which calls into question the mechanism of injury and myocarditis observed in severe cases of COVID-19. However, a polybasic furin cleavage site has been recently identified in the SARS-CoV-2 spike protein [41]; furin-like proteases that may contribute to SARS-CoV-2 spike protein processing are more ubiquitously expressed and may explain an expanded cell and tissue tropism of SARS-CoV-2 compared to SARS-CoV, which lacks this site [41–43].

We now know that SARS-CoV-2 cell entry involves two spike protein subunits, which mediate distinct functions. The S1 subunit mediates ACE2 attachment through the receptor binding domain, whereas the S2 subunit, containing the fusion peptide and transmembrane

domains, drives fusion of viral and host cell membranes. In addition to attachment, viral entry is determined by spike protein cleavage at two proteolytic cleavage sites, termed S1/S2 and S2' subunits. Unlike SARS-CoV, the S1/S2 site of the SARS-CoV-2 spike protein is processed by the cellular protease furin [44]. Subsequently, processing of the S2' site by the cellular serine protease TMPRSS2 (transmembrane protein serine protease 2) occurs, and both furin and TMPRSS2 are required for entry into human lung cells [45]. Spike protein priming by TMPRSS2 was also shown to be essential for spread of SARS-CoV in infected mouse models [46–48]. Although SARS-CoV-2 fusion is thought to occur in the endosomes of target cells, the requirement of cathepsins B and L in acidic lysosomes for optimal membrane fusion efficiency *in vivo* remains unclear (Fig. 3) [40]. SARS-CoV-2 induced shedding of ACE2 may also reduce its ability to metabolise des-Arg-BK, which could contribute to pulmonary inflammation, vascular permeability, and cytokine generation via B1R, which is increased in the setting of tissue injury.

7. ACE2, platelets and thrombosis

Thrombotic disorders, including MI and stroke, are common features in patients with SARS-CoV-2 infection [49]. SARS-CoV-2 virus has been found in endothelium and leads to vessel apoptosis, a risk factor for thrombosis [50]. The coagulopathy associated with COVID-19 is like disseminated intravascular coagulation (DIC) with elevated d-dimer, but high fibrinogens and, in the majority of the patients in the USA, lacking strict criteria for DIC. The cross-talk between the KKS and coagulation system via the activation of Factor XII by kallikrein may contribute to the pro-coagulant state. Kallikrein has also been shown to stimulate activation of the complement system through C3 activation, which likely contributes to the associated coagulopathy [51]. In experimental models of thrombosis, ACE2 expression was detected in thrombus extract raising the possibility that ACE2 may play a role in the regulation of both thrombotic and hemostatic functions of circulating platelets [52]. Activation of the ACE2/Ang1-7/Mas pathway and/or reduction of Ang II by use of an ACE2 activator (XNT) demonstrated antithrombotic activity in an animal

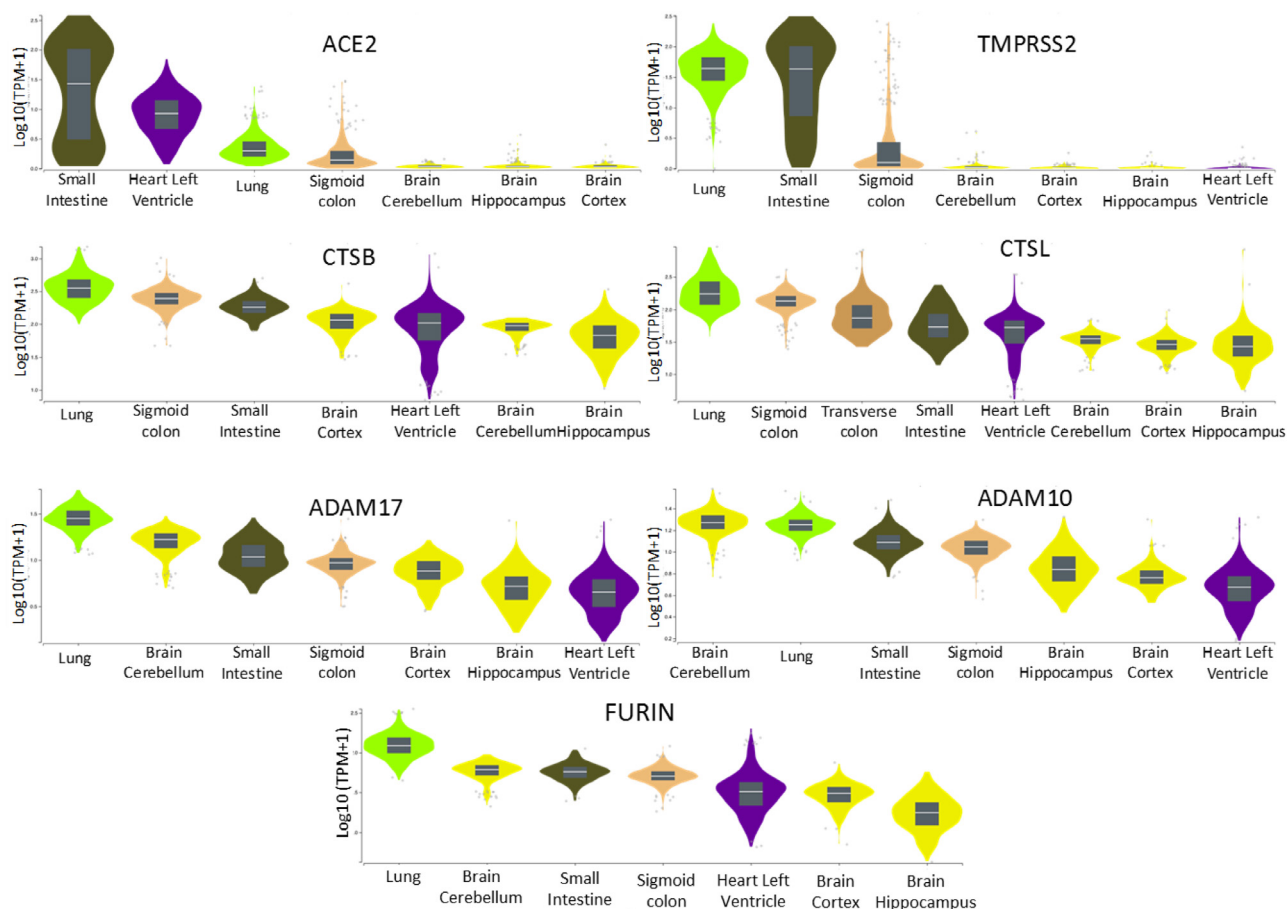


Fig. 4. Gene expression by tissue of potential genes encoding proteins that may interact with SARS-CoV-2 from GTEx. The lung and intestines express high levels of ACE2 and TMPRSS2, whereas the heart left ventricle expresses ACE2 at high levels, but TMPRSS2 at low levels. Brain tissues show low level expression of ACE2 and TMPRSS2. CTSL and CTSL (encoding cathepsins B and L, respectively), ADAM17, ADAM10, and FURIN show expression in all tissues shown. Obtained from the Genotype-Tissue Expression (GTEx) Portal, accessed on 04/06/2020 and 05/06/2020.

model [52]. SARS-CoV-2 replicates in lung tissue, and the lung is a major site for extra-medullary thrombopoiesis [53]. Single-stranded RNA (SSRNA) viruses, including influenza, were recently demonstrated to augment platelet activation and platelet-to-leukocyte recruitment through the platelet toll-like receptor 7 (TLR7) [54]. Since SARS-CoV-2, like influenza, is also a SSRNA virus, the possibility exists that SARS-CoV-2 may promote dysregulated platelet activity directly through surface receptor-mediated pathways or indirectly by secreting platelet-derived molecules that regulate the coagulation cascade. Lastly, angiotensin receptors are expressed on the surface of platelets, and medications inhibiting the RAS attenuate platelet activation [55,56]. Therefore, the impact of anti-platelet medications and ACEIs/ARBs on platelet function and thrombotic events in patients with SARS-CoV-2 needs further investigation.

8. ACEIs and ARBs

Helpful or harmful? Though ARBs and ACEIs may be associated with an increase in ACE2 expression, which theoretically may enhance viral infection, their inhibition of the RAS with increase in ACE2 expression, reduction of Ang II or Ang II effects, and increase in Ang1–7 and Ang 1–9 may have protective effects. ARBs may increase Ang II by competing with binding to AT1R, but this may create more available substrate for ACE2 and formation of Ang1–7 with its downstream protective effects. Binding of substrates to ACE2 may induce conformational changes in ACE2; it is unknown whether these interactions would enable or reduce SARS-CoV-2 spike protein binding.

Specific effects of ACEIs and ARBs on this process are not known. Genetic factors, including genetic variability in ACE2 polymorphisms, may also determine functional roles of ACE2 for ACEIs and ARBs, as well as in its interaction with the CoV-2 spike protein, and will be important to dissect in the future [57]. Finally, ACEI could reduce metabolism of BK, leading to B1R- and B2R-mediated inflammatory, vasodilatory, vascular permeability and fluid extravasation effects.

9. Clinical studies

Initial clinical data showing conflicting outcomes in COVID-19 associated with ARB or ACEI use were confounded by lack of adjustment for co-morbidities (Table). In a study of 187 COVID-19 patients from Wuhan, China, ACEI or ARB use was higher in patients with myocardial injury and elevated troponin T (TnT) levels (21•1%) compared to patients with normal TnT (5•9%), $p = 0•002$ [58]. In 42 patients with COVID-19 on antihypertensive therapy, severe disease was observed in 23•5% on and 48% not on an ACEI or ARB, but this was not significant due to the small sample size [59]. A pre-publication report of 78 COVID-19 + patients with hypertension reported ARB use ($n = 10$) was associated with lower occurrence of severe disease (OR 0•343, 95% CI 0•128–0•916, $p = 0•025$). In a study of 362 hospitalized COVID-19 patients with hypertension, ACEI and/or ARB use ($n = 115$) was not significantly different between patients with severe vs. non-severe illness or in non-survivors vs survivors [60].

In a report of 399 acute inpatients from the UK Acute Hospital Trust, 53 died or were transferred to a critical care unit within 21

Table
Studies of ACEIs and/or ARBs in COVID-19 patients.

Study	Population	N	Design	Outcome	P value
Guo, et al.[58]	COVID-19+ (Wuhan, China)	187	Retrospective; unadjusted	TnT on ACEI/ARB Normal Elevated 8/135 (5•9%) 11/52 (21•1%) Mortality ACEI/ARB + 7/19 (36•8%) - 43/168 (25•6%)	0•002
Meng, et al.[59]	COVID-19+ on antihypertensive therapy (Shenzhen, China)	42	Retrospective; unadjusted	Severe disease ACEI/ARB + 4/17 (23•5%) - 12/25 (48%)	NS
Liu, et al.[74]*	COVID-19+ hypertension (Shenzhen, Wuhan, Beijing, China)	78 HTN 10 ARB 2 ACEI	Meta-analysis unadjusted	Severe disease (Whole cohort NS) age > 65 (N = 46) ARB OR 0•343 95% CI 0•128–0•916 ACEI 0•571 0•139–2•342	0•025 0•378
Li, et al.[60]	COVID-19+ hypertension Hospitalized (Wuhan, China)	362 115 ACEI/ARB	Retrospective, unadjusted	Severe illness Non-severe illness ACEI use 9•2% 10•1% ARB use 24•9% 21•2% ACEI/ARB use 32•9% 30•7% Non-survivors Survivors ACEI use 9•1% 9•8% ARB use 19•5% 23•9% ACEI/ARB use 27•3% 33•0%	0•80 0•40 0•65 0•85 0•42 0•34
Bean, et al.[61]	UK Acute Hospital Trust acute inpatients (London, UK)	399	Retrospective; adjusted	No differences in ACEI/ARB use outcomes in patients with HTN and comorbidities Death or transfer to critical care unit for organ support within 21 days of symptom onset (n = 127) ACEI/ARB adjusted OR 0•63 95% CI 0•47–0•84#	<0•01
Rentsch, et al.[62]*	Veterans Administration Birth Cohort; veterans (USA)	3789 tested 585 COVID+ 40•5% ACEI/ARB	Retrospective cohort study; adjusted	Multivariable OR (95% CI) ARB/ACEI COVID+ test 0•98 (0•41–0•83) hospitalization 1•15 (0•71–1•87) ICU 1•66 (0•94–2•93)	NS NS NS
Zhang, et al.[75]	COVID-19+ hypertension (Hubei, China)	1128 HTN 188 ACEI/ARB (31 ACEI, 157 ARB)	Retrospective, adjusted and propensity score	Propensity score matched (1:2) ACEI/ARB (n = 174) vs non-ACEI/ARB (n = 522) HR 95% CI All-cause mortality 0•37 0•15–0•89 Septic shock 0•32 0•13–0•80 ARDS 0•65 0•41–1•04 AKI 0•78 0•37–1•65 Acute heart injury 0•76 0•44–1•32	0•03 0•01 0•07 0•52 0•33
Mehta, et al.[63]	Patients undergoing testing for COVID-19 (Ohio, Florida)	18,472 tested ACEI/ARB 2285 ACEI 1322 ARB 982 1735 positive ACEI 116 ARB 98	Retrospective cohort study; overlap propensity score weighted mean or proportion	SARS-CoV-2 test positivity: OR 95% CI ACEI 0•89 0•72–1•10 ARB 1•09 0•87–1•37 ACEI or ARB 0•97 0•81–1•15 Test positive patients ACEI ARB ACEI or ARB Admitted to hospital 1•84 (1•22–2•79) 1•61 (1•04–2•50) 1•93 (1•38–2•71) Admitted to ICU 1•77 (1•07–2•92) 1•16 (0•67–2•02) 1•64 (1•07–2•51) Mechanical ventilation 1•35 (0•74–2•47) 1•12 (0•59–2•12) 1•32 (0•80–2•18)	
Mancia, et al.[64]	Patients tested for SARS-CoV-2 vs Regional Health Service controls (Italy)	6272 SARS-CoV-2+ 30,759 controls Age ≥ 40 yrs	Population-based case-control, conditional logistic regression multivariate analysis	Adjusted OR, 95% CI cases vs. matched controls ARB ACEI Covid-19+ 0•95 (0•86–1•05) 0•96 (0•87–1•07) Mild to moderate disease 0•96 (0•87–1•07) 0•97 (0•88–1•07) Critical or fatal disease 0•83 (0•63–1•10) 0•91 (0•69–1•21) No significant differences in COVID-19 positivity or severity of disease	

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Table (Continued)

Study	Population	N	Design	Outcome	P value
Reynolds, et al.[65]	Patients tested for Covid-19 (New York City)	12,594 tested 5894 positive ACEI 1044 ARB 1328	Propensity score matched, Bayesian methods	All Matched patients Covid-19 test positivity ACEI ARB ACEI/ARB Severe Covid-19 ACEI ARB ACEI/ARB No differences in test positivity or severe disease with ARB and/or ACEI use Similar findings in matched patients with hypertension Mortality	Drug + 60•1% 62•5% 58•4% 56•2% 58•1% 57•7% 23•9% 25•9% 24•4% 25•8% 24•8% 24•9% 1•9 (-6•6 - 2•8) -1•4 (-6•1 - 3•3) -0•1 (-3•7 - 3•5) 95% CI 1•39–8•55 0•31–2•84 0•008 0•901 Median difference%(95% CI) -2•5 (-6•7 - 1•6) 2•2 (-1•9 - 6•3) -0•5 (-2•6 - 3•6)
Gao, et al.[66]	Patients admitted with COVID-19 (Wuhan, China)	2877 850 HTN RAASI 183	Propensity score adjusted	Mortality HTN RAASI RAASI – Severity of disease, mechanical ventilation - NS	0•09 0•61 0•15 0•67
Fosbal, et al.[67]	COVID-19 positive patients Danish national administrative registries	4480 with COVID-19 895 ACEI/ARB users 3585 nonusers	Cox regression model with nested case-control framework	30-day mortality Death or severe disease Severe disease Nested case control with HTN ACEI/ARB use	Fully adjusted HR (95% CI) 0•83 (0•67–1•03) 1•04 (0•89–1•23) 1•15 (0•95–1•41) Controls Adjusted HR (95%CI) 86•5% 1•05 (0•08–1•36)

ACEI = angiotensin converting enzyme inhibitor; AKI = acute kidney injury; ARB = angiotensin receptor blocker; ARDS = acute respiratory distress syndrome; HTN = hypertension; ICU = intensive care unit; OR = odds ratio; CI = confidence interval; NS = not significant; RAASI = renin-angiotensin-aldosterone system inhibitor.

* non-peer reviewed pre-print.

Adjusted for age, sex, hypertension, diabetes mellitus, chronic kidney disease, ischaemic heart disease, heart failure.

days of symptom onset. A lower rate of this endpoint occurred in patients on an ACEI or ARB (OR 0•63, CI 0•47–0•84, $p < 0•01$), adjusting for age, gender, hypertension, diabetes mellitus, chronic kidney disease, ischaemic heart disease and heart failure [61]. In a preprint from the Veterans Administration Birth Cohort, 585 patients tested positive for COVID-19 among 3789 tested, and 40•5% were on an ACEI or ARB; in adjusted analyses there were no differences in COVID-19 test positivity, hospitalisation, or intensive care in patients receiving or not receiving an ACEI or ARB [62].

Recently 6 studies using adjusted and/or propensity-score adjusted or matched analyses have been published (Table). Three tested associations of ACEI and/or ARB use on COVID-19 test positivity and found no significant differences. Disease severity and hospital outcomes were assessed in 4 studies. In a study of 1128 patients with hypertension, 188 were on an ACEI or ARB; propensity score matched cohorts showed a lower risk of all-cause mortality (HR 0•37, 95% CI 0•15–0•89, $p = 0•03$) and septic shock in patients on an ACEI or ARB. In a study of 18,472 patients tested, 1735 were positive; ACEI and/or ARB use was associated with a higher rate of hospital admission with ACEI and ACEI or ARB use was associated with a higher risk of admission to the ICU, but with no significant difference in need for mechanical ventilation [63]. In a study of 6272 COVID-19 positive patients, ARB or ACEI use was not associated with degree of disease [64]. Another study of 12,594 tested patients, including 5894 who tested positive, ACEI, ARB, or either were not associated with test positivity or severe COVID-19 disease [65]. In a study of 2877 hospitalized patients with COVID-19, 850 had hypertension of which 183 were treated with renin-angiotensin-aldosterone system inhibitors (RAASI) and 527 were not; RAASI use was not associated with severity of disease or mortality [66]. Lastly, in a study of 4480 patients with COVID-19 in Danish national administrative registries, prior ACEI or ARB use was not associated with death or severe COVID-19; in a nested case-control study of patients with hypertension, ACEI/ARB use was not significantly associated with COVID-19 diagnosis [67]. Taken together, it is now consistent clinical evidence that ACEI or ARB use does not appear to predispose to SARS-CoV-2 infection, which was the main concern raised due to postulated effects of ACEIs or ARBs in raising ACE2 expression. Recent studies have shown no association of ACEI or ARB use with SARS-CoV-2 test positivity. Studies do not indicate harm from ACEI or ARB use in terms of severity of disease but with some conflicting results regarding the benefit vs. risk of ACEIs. However, the overall the balance appears to be in favor of no significant harm from ACEI or ARB use in COVID-19, though larger studies are needed to assess the relative effects of ACEIs versus ARBs, whether continuation or withdrawal of these agents impact outcomes, or if ACEI/ARB use may actually be beneficial in alleviating lung or other organ injury in patients with COVID-19.

10. Recommendations regarding ACEIs and ARBs

Given the current reassuring data showing no significant association of ACEI or ARB use with test positivity, lack of consistent or convincing evidence as to the risk or benefit of an ACEI or ARB, as well as the potential harm that may occur with withdrawal of ACEIs or ARBs in patients with cardiovascular and other diseases [68], findings support the European Society of Cardiology, American Heart Association, American College of Cardiology, and the Heart Failure Society of America recommendations that patients on these therapies should be continued as clinically indicated. The recent data show consistent lack of an association with SARS-CoV-2 positivity. However, there remains a need to further assess impact of ACEIs and ARBs on severity of disease, potentially through larger or randomized studies.

11. Implications for novel and repurposable therapeutics

The spike protein is a target for drug discovery and vaccine development. Blocking the spike protein-ACE2 interaction sites may be targetable with antibodies or small molecules, and use of soluble ACE2 may competitively bind to the spike protein [69]. Strategies to increase ACE2 shedding from cells may be protective against viral infection [70]. Furin inhibitors or other serine protease inhibitors may inhibit SARS-CoV-2 replication via the S1/S2 cleavage site [45]. TMPRSS2 is dispensable for homeostatic function and blocked by the serine protease inhibitor camostat mesilate, a drug approved in Japan for unrelated conditions [40]. Our recent systems biology study suggested several repurposable drugs for potential treatment of COVID-19, including melatonin and ARBs (i.e., irbesartan) [71]. Melatonin regulates expression of several cellular targets of human CoV, including ACE2, Ang II and AT1R [72]. Hydroxychloroquine and chloroquine have been commonly tried for treatment of COVID-19. Besides inhibiting viral-endosomal fusion and release of viral particles to the cell by reducing endosomal acidification, chloroquine impairs terminal glycosylation of ACE2, which may have effects on binding affinity between ACE2 and Co-V spike protein [73]. However, efficacy remains to be established, and randomized trials are ongoing. Therapeutic application of Ang1–7 and Ang1–9 is limited because of the short half-life of these peptides and unavailability of FDA-approved drugs that can substitute for the potential benefits attributed to these peptides. Interventions directed at blocking BK and the pathways leading to its formation may also be of benefit. Hereditary angioedema, a rare genetic disorder causing predisposition to attacks of angioedema, is treated with medications to suppress activity in the KKS. These medications largely consist of direct kallikrein inhibitors, B2R antagonists, and replacement with C1 inhibitor. BK's role in COVID-19 is under investigation, and use of these suppressive medications is being explored. Until such time when there is a highly effective antiviral or a vaccine, these adjunctive approaches need to be developed.

12. Summary

In clinical practice the protective effects of ARBs and ACEIs are thought to be associated with an increase in ACE2 expression and their inhibition of the overactive renin-angiotensin system through reduction of Ang II effects. Coronavirus infection hijacks ACE2 expression to invade cells and spread infection-associated damage, downregulating ACE2 expression, reducing its protective effects and exacerbating the injurious Ang II effects. Retrospective observational studies do not show associations with higher risk of infection for persons receiving ACEIs or ARBs. However, controlled clinical trials would be needed to determine the risks or benefits of these agents in treating COVID-19. Studies in SARS-CoV-2 models and clinical retrospective and prospective studies in patients might further clarify these important questions. Such studies may also identify plausible therapeutic agents for targets within the RAS and KKS in the setting of coronaviral infection.

13. Outstanding Questions

Important questions remaining for future research include whether drugs targeting components of the RAS or the KKS might be helpful in the treatment of patients with COVID-19. Prospective controlled clinical trials are needed. Basic research on mechanisms to determine if ACE2 expression affects viral infectivity in vitro and expression of components of the RAS and KKS in infected tissues are needed to help clarify the role of cell-bound or shed ACE2 in COVID-19 pathophysiology. Investigations into specific cell types vulnerable to SARS-CoV-2 infection may help focus targeting of therapies.

Search Strategy and Selection Criteria. Data for this Review were identified by searches of PubMed with search terms including combinations of ACE inhibitors, ARBs, COVID-19, SARS-CoV-2, renin-angiotensin system, kallikrein-kinin system.

Authors' contributions

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 Jonathan D. Smith, PhD - revisions, insights in ACE2 shedding
 Ankur Kalra, MD - ACEI/ARB expertise in COVID-19, review and suggestions for manuscript
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 Edward S. Hawkins, MD - RAS revisions/suggestions for manuscript
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