STATE OF THE ART
INFECTIOUS DISEASE

Understanding the renin–angiotensin–aldosterone–SARS-CoV axis: a comprehensive review

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The interplay of SARS-CoV-2 with the renin–angiotensin–aldosterone system probably accounts for much of its unique pathology. Appreciating the degree and mechanism of this interaction highlights potential therapeutic options, including blockade (ARBs). https://bit.ly/3aue4tS

Cite this article as: Ingraham NE, Barakat AG, Reilkoff R, *et al*. Understanding the renin–angiotensin–aldosterone–SARS-CoV axis: a comprehensive review. *Eur Respir J* 2020; 56: 2000912 [https://doi.org/10.1183/13993003.00912-2020].

ABSTRACT

Importance: Coronavirus disease 2019 (COVID-19), the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been declared a global pandemic with significant morbidity and mortality since first appearing in Wuhan, China, in late 2019. As many countries are grappling with the onset of their epidemics, pharmacotherapeutics remain lacking. The window of opportunity to mitigate downstream morbidity and mortality is narrow but remains open. The renin–angiotensin–aldosterone system (RAAS) is crucial to the homeostasis of both the cardiovascular and respiratory systems. Importantly, SARS-CoV-2 utilises and interrupts this pathway directly, which could be described as the renin–angiotensin–aldosterone–SARS-CoV (RAAS–SCoV) axis. There exists significant controversy and confusion surrounding how anti-hypertensive agents might function along this pathway. This review explores the current state of knowledge regarding the RAAS–SCoV axis (informed by prior studies of SARS-CoV), how this relates to our currently evolving pandemic, and how these insights might guide our next steps in an evidence-based manner.

Observations: This review discusses the role of the RAAS–SCoV axis in acute lung injury and the effects, risks and benefits of pharmacological modification of this axis. There may be an opportunity to leverage the different aspects of RAAS inhibitors to mitigate indirect viral-induced lung injury. Concerns have been raised that such modulation might exacerbate the disease. While relevant preclinical, experimental models to date favour a protective effect of RAAS–SCoV axis inhibition on both lung injury and survival, clinical data related to the role of RAAS modulation in the setting of SARS-CoV-2 remain limited.

Conclusion: Proposed interventions for SARS-CoV-2 predominantly focus on viral microbiology and aim to inhibit viral cellular injury. While these therapies are promising, immediate use may not be feasible, and the time window of their efficacy remains a major unanswered question. An alternative approach is the modulation of the specific downstream pathophysiological effects caused by the virus that lead to morbidity and mortality. We propose a preponderance of evidence that supports clinical equipoise regarding the efficacy of RAAS-based interventions, and the imminent need for a multisite randomised controlled clinical trial to evaluate the inhibition of the RAAS–SCoV axis on acute lung injury in COVID-19.

Received: 30 March 2020 | Accepted after revision: 18 April 2020

Introduction

Coronavirus disease 2019 (COVID-19), the infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has left over 180 countries and territories grappling with a devastating pandemic. In December 2019, Wuhan, China, was identified as the epicentre of this outbreak. At the time of writing, reported COVID-19 cases exceeded 700 000, with more than 30 000 deaths [1–3]. While early estimates vary and true values remain uncertain, mortality is estimated between 0.4% and 3.4% [4, 5] with initial morbidity and mortality disproportionally affecting older patients [6]. Infectivity (R_0) is estimated at 2.24 to 3.58 [4, 7]. COVID-19-related hospitalisations are mostly due to the need for respiratory support and progressively higher levels of care, with respiratory failure being the underlying aetiology of COVID-19-related deaths [8, 9]. As many countries are struggling with the onset of their epidemics, pharmacotherapeutics remain lacking [10, 11]. Learning from prior pandemics and related viruses can focus our efforts to control spread and treat those infected [12].

This novel coronavirus is closely related to the severe acute respiratory syndrome coronavirus (SARS-CoV), which caused an outbreak of disease (SARS) in 2003. Genetic studies found that SARS-CoV-2 shares almost 80% and 50% sequence identity with SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), respectively [13]. The genetic variations between SARS-CoV and SARS-CoV-2 translate into differences in infectivity [14] and immune response [15]. Despite these differences, the shared genome translates into common clinical, microbiological and biochemical phenotypes. SARS-CoV-2 mimics SARS-CoV's mechanism of infection, which utilises angiotensin-converting enzyme (ACE) 2, part of the renin–angiotensin–aldosterone system (RAAS). Following virus binding, ACE2 activity is downregulated through multiple mechanisms, preventing it from performing its usual function in states of health, as will be further discussed later in this review [13, 16–18]. The association between ACE2 and COVID-19 is rooted in two concepts: the mechanism of SARS-CoV-2 infection and the regulatory role of ACE2 during RAAS overactivation. Following the SARS outbreak, extensive research advanced our knowledge of highly morbid coronavirus infections [16–25]. The similar renin–angiotensin–aldosterone–SARS-CoV (RAAS–SCoV) axis interactions shared by the two coronaviruses provide an opportunity to further our understanding of this unique interplay in our pursuit of treatment options.

Early attempts to develop safe and effective vaccines for SARS or MERS have been unsuccessful. It is hypothesised that current efforts will take time to develop, and may or may not be efficacious for this or future coronavirus pandemics [10, 26–28]. Many potential therapies have been proposed, with a subset currently undergoing investigation [29, 30]. Meanwhile, extensive efforts to date have been appropriately focused on screening, identification and containment, through the collaboration and coordination between global and local health and governmental agencies [31]. Unfortunately, these public health efforts have had, at best, mixed success in curbing the spread of the pandemic [32]. Given the rapid spread of SARS-CoV-2 and the significant morbidity and mortality associated with infection, it behoves the medical community to evaluate and leverage novel treatments for efficacy, especially if they are already available and have an established safety profile.

RAAS blockade has been proposed as a potential treatment for SARS-CoV-2 [33–36]. This hypothesis was initially published by Sun *et al.* [35] on 4 February 2020 and reinforced in a publication in *Drug Development Research* on 4 March 2020 [33]. Other reviews have voiced concern regarding the association between COVID-19 and cardiovascular disease [37], going so far as to postulate that continued RAAS blockade may cause harm and to recommend considering discontinuation [38]. The latter argument is based on the observation that pharmacological blockers of the RAAS can upregulate ACE2 expression, which might increase viral entry into the cell [38]. Evidence from human subjects to support such an assertation is scant, and, as we will see in this review, preclinical and current observational COVID-19 evidence would support the contrary hypothesis, that discontinuation of RAAS blockade may prove harmful. These contrasting hypotheses underscore the dire need to evaluate potential mechanisms, if any, through which RAAS modulation would have an impact on the pathophysiology of COVID-19 [35, 37, 39].

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In this review, we intend to compile the existing evidence in order to discuss how we might bridge knowledge gaps regarding the interplay between SARS-CoV-2, ACE2 and the RAAS.

The RAAS in states of health

Overview

Renin, angiotensin and aldosterone represent the core of a complex hormonal axis, referred to as the RAAS, which contributes to blood pressure control, sodium reabsorption, inflammation and fibrosis [40]. RAAS imbalance or modification can cause or treat many diseases, including heart failure, hypotension, diabetes and atherosclerosis [41]. This review focuses on several physiological and pathological effects of angiotensin II (Ang II) cell signalling (figure 1).

The Ang II/AT_1 receptor relationship

Ang II, the primary physiological product of the RAAS, is a potent vasoconstrictor. As illustrated in figure 1, ACE catalyses the transformation of angiotensin I (Ang I) to Ang II. Ang II elicits its effects by activating two receptors: the type 1 angiotensin II (AT_1) receptor and the type 2 angiotensin II (AT_2) receptor [42]. Ang II action through the AT_1 receptor causes a cascade with resultant inflammation, vasoconstriction and atherogenesis [43]. These effects also promote insulin resistance and thrombosis [44]. In contrast, AT_2 receptor stimulation causes vasodilation, decreased platelet aggregation and the promotion of insulin action. However, the expression of AT_2 receptor is low in healthy adults [44]. Thus, Ang II's effects in adults are modulated and balanced indirectly by ACE2, which converts Ang II into lung-protective Angiotensin-(1–7) (Ang-(1–7)), which gives effects similar to those seen from AT_2 receptor stimulation [40, 45]. Conceptually grouping pathways into an Ang II/AT_1 receptor pathway and its opposing ACE2/Ang-(1–7) pathway helps with understanding the countering forces and the sequelae when an imbalance occurs (figure 1).

The consequences of excessive Ang II include pulmonary vasoconstriction, inflammatory and cytokine-induced organ damage [46] secondary to increased membrane permeability [47], and increased epithelial cell apoptosis [48]. The effects are predominantly mediated through the unopposed AT_1 receptor activation secondary to decreased ACE2 levels [49]. Furthermore, the pro-inflammatory cascade [50] and increased vascular permeability [47] caused by over-activation of the AT_1 receptor in the lungs directly induce acute lung injury and acute respiratory distress syndrome (ARDS), and lead to death [10, 33].

Angiotensin-converting enzyme 2

ACE2 is expressed in many tissue types, including respiratory epithelial cells [51]. ACE2 is predominantly expressed on the apical surface and converts Ang I and Ang II into lung-protective Ang-(1–9) and Ang-(1–7), respectively, by catalysing a pathway that prevents unopposed AT_1 receptor activation. Therefore, ACE2 functions as a counterbalance to its structurally similar counterpart ACE [52], through the conversion of Ang II into Ang-(1–7), which, in contrast to ACE, promotes vasodilation, reduces proliferation and prevents apoptosis [52]. ACE2 levels are known to be low in healthy individuals [53] and

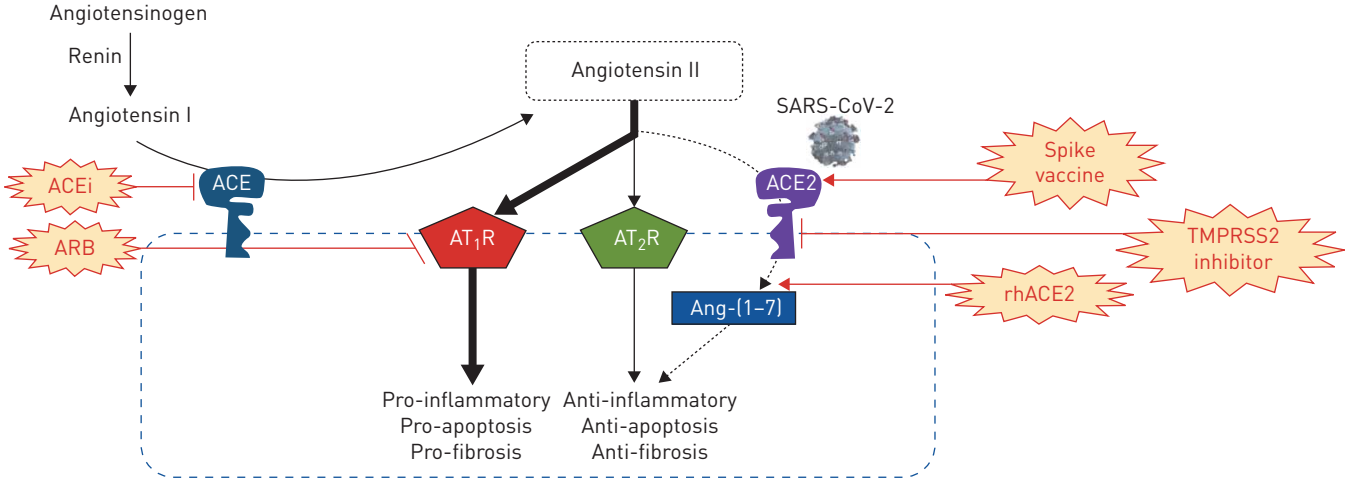

FIGURE 1 The renin–angiotensin–aldosterone system with COVID-19. The thicker arrows show an increase in the degree of pathway activation; dotted arrows show a decrease in pathway activation. ACE: angiotensin-converting enzyme; ACEi: ACE inhibitors; ARB: angiotensin receptor blocker; AT_1R: type 1 angiotensin II receptor; AT_2R: type 2 angiotensin II receptor; Ang-(1–7): angiotensin-(1–7); rhACE2: recombinant human ACE2; TMPRSS2: transmembrane serine protease 2.

ACE2 levels decrease with age [54]. Chronically elevated levels of ACE2 are an independent predictor of disease progression, but evidence suggests these changes are compensatory rather than causal [53].

ACE inhibitors and angiotensin receptor blockers

Both ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs) increase tissue and plasma levels of renin and Ang I [55]. ACEi inhibit Ang I to Ang II conversion, decreasing serum Ang II levels to baseline, and have little to no affinity for ACE2 [55]. At clinical doses, ACEi only partially affect this conversion because 40% of Ang II formation occurs outside the ACE pathway [56]. ACE levels are in turn decreased through a negative feedback in states of high Ang II levels [57]. Ang II levels increase in response to ARBs at all levels, except in the kidney. The consequence of this increase is a redirection of Ang II activity through the anti-inflammatory and vasodilatory ACE2 and AT_2 receptor pathways [55]. As a result, ACE2, compared to ACE, regulates local levels of both Ang II and Ang-(1–7) to a greater degree [41]. Tikellis and Thomas [41] suggest that ARBs, therefore, have a higher potential to favourably rebalance the RAAS pathway compared to ACEi. Furthermore, the kallikrein–kinin system is regulated by ACE and ACE2. Increased activity of the kallikrein–kinin system occurs under pro-inflammatory conditions [58], specifically bradykinin and des-Arg^9-bradykinin, which can lead to vascular leakage when unchecked by ACE and ACE2, respectively. This mechanism has been posited to be, at least in part, a cause of cough [59] and pulmonary oedema [60], independent of proposed Ang II-induced hydrostatic pressure. The dysregulated kallikrein–kinin system's role remains theoretical in COVID-19; however, if it is involved, AT_1 receptor blockade may be favoured (over ACE inhibition) in an attempt to preserve the regulatory mechanisms of this system [60]. While there may still be a key role for ACE inhibition in the context of the current disease, as we will elaborate in the following sections, these observations support our decision to narrow the focus of this review to the downstream RAAS modifiers, ARBs. A summary of key points is given in table 1.

The RAAS in cardiovascular disease

Modulation of the RAAS axis is central to the management of cardiovascular disease [61]. Cardiovascular disease, heart failure, atrial fibrillation and kidney disease are associated with elevated levels of ACE2 [59]. It is critical to discern whether these elevations are a culprit or sequelae of disease in order to separate association from causation [62]. More recent opinions suggest that ACE2 represents a biomarker, rather than a culprit, of cardiovascular disease [63].

Ramchand *et al.* [53] demonstrated elevated ACE2 levels to be an independent risk factor of major adverse cardiac events in patients with known coronary artery disease, although, as noted by the authors, the data do not differentiate association *versus* causation. To begin to dissect this interplay, we turn to preclinical models, specifically ACE2 knockout mice, which are characterised by severe cardiac defects. Critically, these defects are notably absent in double ACE and ACE2 knockout models, which highlights the highly balanced interplay between ACE/Ang II/AT_1 and ACE2/Ang-(1–7) [52, 64]. Similar protective effects to prevent cardiovascular events and strokes have been demonstrated in mouse models with overexpressed ACE2 [65]. Ramchand *et al.* [53] concluded that patients with elevated ACE2 might "reflect a persistent albeit insufficient counter-regulatory process to shift the balance away from the deleterious effects of sustained Ang II activation." As such, ACE2 overexpression may be a compensatory mechanism to mitigate the effect of unopposed Ang II stimulation through increased degradation into protective Ang-(1–7) [66].

It is important to note that preclinical animal model treatment with losartan upregulates cardiac ACE2 mRNA expression and increases ACE2 activity [67, 68]. However, human studies found no significant difference in ACE2 plasma levels in patients treated with ACEi or ARBs and thus have not confirmed such an effect, although local tissue ACE2 levels were not measured [53]. Through anecdotal reports, COVID-19 may be associated with high rates of myocarditis and heart failure in the critically ill. While

TABLE 1 Summary of the renin–angiotensin–aldosterone system (RAAS) and its relation to COVID-19

ACE2 levels are high in diseased states, which is likely to be secondary to an insufficient compensatory response to overactive RAAS activity
In COVID-19, high rates of pulmonary oedema and cough may be due, in part, to reduced breakdown of bradykinin from decreased ACE activity
ARBs may provide crucial regulation to the overactive RAAS–SCoV axis

ACE: angiotensin-converting enzyme; ARBs: angiotensin receptor blockers; SCoV: SARS-CoV.

TABLE 2 Summary of renin–angiotensin–aldosterone system (RAAS) blockade in cardiovascular disease states

ACEi and ARBs interrupt maladaptive pathophysiological responses to heightened activation of the RAAS
ARBs and ACEi mitigate the deleterious effects of unopposed AT_1 receptor pathway activation, which in turn decreases inflammation, insulin resistance and lung injury

ACEi: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; AT_1: type 1 angiotensin II.

beyond the scope of this article, we will note that increased Ang II and decreased ACE2 are also associated with impaired cardiac contractility, which can be rescued with losartan [69]. More observational data are needed to assess whether other signs of RAAS overdrive (e.g. hypokalaemia or hypertension) portend poor outcomes and may be associated with arrhythmias that have been noted in the diseases [70]. Nevertheless, this could potentially underscore potential benefits of RAAS modulation that extend beyond the attenuation of lung injury. A further summary of key points is given in table 2.

The RAAS in pulmonary disease

Chronic lung disease

In COPD, patients treated with ARBs (compared to those on ACEi) had less severe exacerbations, fewer exacerbations overall, lower mortality, lower mechanical ventilation requirements, and fewer hospitalisations [71]. Moreover, patients aged >65 years who were on ARBs before and during their hospitalisation for pneumonia had decreased mortality when compared to patients without such treatment [72].

Acute lung injury

Unopposed RAAS activation *via* the Ang II/AT_1 receptor pathway causes inflammation [50], increased vascular permeability [47] and severe lung injury [10, 33], while ARBs significantly attenuate these changes [47–50]. Importantly, the mere presence of high concentrations of Ang II can further regulate the expression of ACE2, leading to dysregulated Ang II/AT_1 receptor activity [73]. In mice, losartan reduced mortality by blunting Ang II-associated increases in soluble epoxide hydrolase, a promoter of lung injury [74]. Animal models of ventilator-associated lung injury have demonstrated benefit from losartan, mitigating Ang II activity and AT_1 receptor expression [75–77]. While most studies include pre-treated animal models, rescue models also demonstrate efficacy, with the restoration of ACE2 levels, blunting of arterial oxygen tension (P_{aO_2}) decline, and attenuation of lung injury [49].

In human patients, genetic cohort studies yield further insights into the relationship between the RAAS and acute lung injury. Jerng *et al.* [78] found that polymorphisms in the ACE gene are associated with outcomes in ARDS. These findings were corroborated by Adamzik *et al.* [79], who identified patients with the ACE DD genotype (associated with increased ACE activity) to have the highest risk of ARDS-related death (hazard ratio 5.7). Other human studies evaluating the association between RAAS inhibition and ARDS remain observational. Kim *et al.* [80] found that ARDS patients that were taking ACEi or ARBs had better survival rates when compared to those without RAAS inhibition. A secondary analysis of a 2010 randomised control trial in patients with acute respiratory failure suggested that treatment with ACEi/ARB at discharge following an episode of acute respiratory failure was associated with a 44% reduction in 1-year mortality [81]. More recently, Hsieh *et al.* [82] observed lower adjusted odds of hospital mortality in patients with sepsis (with and without shock) who were on ARB or ACEi therapy. Mortensen *et al.* [83] also found a 58% decrease in the odds of hospital mortality in patients taking ARBs before admission. Based on these data, there have been calls to further elucidate the potential benefits of ACEi and ARBs in ARDS [84]. However, randomised control trials around this topic were not identified in the peer-reviewed literature or ClinicalTrials.gov registry, at the time of writing this article.

Pneumonia

While influenza and other types of pneumonia may interact with the RAAS axis, both animal and human studies illustrate clear indirect effects on the RAAS in the setting of certain influenza strains in particular. Previous studies suggest that Ang II levels predict mortality in undifferentiated patients with influenza [85], and continuation of RAAS inhibitor therapy during admission is associated with decreased hospital mortality and odds of intubation in viral cases of pneumonia [86]. The RAAS may have implications for other viral pneumonias as well, as Gu *et al.* [87] found that children with respiratory syncytial virus tend to have higher Ang II levels compared to healthy children. Based on this observation, they demonstrated

the benefit of recombinant ACE2 therapy on respiratory syncytial virus infection in a preclinical mouse model.

Critically, both H7N9 and H5N1 influenza have been shown to cause lung injury through ACE2 downregulation, upregulation of Ang II, and AT_1 receptor-induced lung injury [88, 89]. Mouse models of H5N1 and H7N9 demonstrated decreased interleukin (IL)-6, lung oedema, lung injury and mortality if treated with losartan [89, 90]. The mechanism by which losartan prevents lung injury may not reside solely within the RAAS pathway, however [50]. Liu *et al.* [91] suggest that losartan inhibits the activation of pulmonary dendritic cells. In a study of rats with *Pseudomonas* pneumonia, AT_1 receptor blockers suppressed the activation of neutrophils through a mechanism that does not involve AT_1 receptor downregulation [92]. Such studies have raised concern that losartan treatment might decrease microbial clearance. In sharp contrast, study results actually demonstrate reduced viral loads [90] and increased bacterial clearance [50] in lung injury models treated with losartan. Given the complexity of these interactions, future investigations remain necessary to further elucidate these relationships.

ACE2 binding by SARS-CoV

In 2003, ACE2 was identified as the binding protein for SARS-CoV. In the years to follow, studies demonstrating the downstream consequences of downregulation of ACE2 with resultant increases in Ang II concentration have illustrated the vicious cycle caused by the disruption of the RAAS, which we refer to in this article as the RAAS–SCoV axis [18]. Of relevance to the current pandemic, Wrapp *et al.* [14] found SARS-CoV-2 to have a 10- to 20-fold higher affinity for ACE2 than SARS-CoV. The authors postulate that this may cause increased infectivity, which may help explain the differences in the evolution of the two epidemics.

Both SARS coronaviruses contain four structural proteins: the nucleocapsid, membrane, envelope, and spike protein [93]. The nucleocapsid, membrane and envelope proteins have roles in viral replication, structural integrity and host response, among other mechanisms [93]. The spike protein, known as the S-protein, is integral in attaching to and infecting host cells [14, 93]. Phan [15] identified specific deletions that increase the potential of the SARS-CoV-2 spike to evade immune responses over time. These changes have implications for both initial and subsequent infection severity, immunity, and the likelihood of developing an efficacious vaccine. More relevant to the current review, however, is the potential effect such changes might have on the RAAS–SCoV axis, which could serve to explain not only their predilection for causing respiratory failure but possibly other commonalities between these infections.

SARS-CoV infection downregulates the surface expression of the binding protein, ACE2, a pivotal component for host cell entry [17]. Notably, low ACE2 expression is associated with increased phenotype severity in human airway epithelial cell *in vitro* studies [24]. Imai *et al.* [23] validated these findings in a whole animal model by demonstrating that loss of ACE2 in knockout mice leads to impairments in oxygenation, increased inflammation, and worsening of oedema compared to wildtype mice after acid-induced lung injury. Pre-treatment with AT_1 receptor inhibitors before acid-induced ARDS showed significantly lower lung injury, confirming the AT_1 receptor's role in the physiological response [23]. Subsequently and most critically, Kuba *et al.* [17] demonstrated that SARS-CoV spike protein augments Ang II increase and ACE2 downregulation, with resultant lung injury. In this model, losartan can attenuate the severity of acute lung injury due to SARS-CoV infection in both a pre-treatment model and a more clinically relevant post-infection (rescue) model [17].

Ensuing studies have confirmed and built upon these findings [19]. AT_1 receptor blockade upregulates ACE2 through a negative feedback mechanism, and serves as a lung-protective mechanism through the increased conversion of Ang II to Ang-(1–7), effectively blunting pulmonary injury from the virus [49]. These findings have led investigators to posit that the ACE2/Ang-(1–7) pathway may serve as a therapeutic target to combat the pathological effects of Ang II [73]. To that end, there is already at least one trial of recombinant human ACE2 therapy for the treatment of COVID-19 that attempts to leverage this relationship and mitigate downstream lung injury [23, 35].

The mechanism by which SARS-CoV downregulates ACE2 may also be relevant to the development of novel therapeutics. Haga *et al.* [20] demonstrated that SARS-CoV binding to ACE2 induces ACE2 shedding in a soluble form into the serum, and further studies validated those findings [19]. In addition, endocytosis of the ACE2 protein occurs after binding to SARS-CoV, further decreasing ACE2 activity [94], with potential implications for the Ang II/Ang-(1–7) balance. A final critical mechanism that cannot be overlooked includes the role of the AT_1 receptor in the downregulation of ACE2. Deshotels *et al.* [73] found that ACE2 downregulation induced persistent elevation of Ang II through local interaction with the AT_1 receptor, which triggers a vicious cycle where Ang II downregulates ACE2 leading to further increases in local tissue levels of Ang II. These mechanisms promote an unopposed Ang II/AT_1 receptor axis (figure 1).

TABLE 3 Summary of renin–angiotensin–aldosterone system interplay with lung injury and disease

SARS-CoV decreases surface expression of ACE2 during infection
Decreased ACE2 activity leads to increased Ang II and further downregulation of ACE2 in a vicious cycle, driving acute lung injury
While the main point of entry involves ACE2, other receptors can independently mediate SARS-CoV infection

ACE: angiotensin-converting enzyme; Ang II: angiotensin II.

The ACE2–SARS/COVID-19 interplay has led to controversy with clinical implications. The finding that ACE2 knockout mice are protected against SARS-CoV infection [17] raises a concern that an upregulated ACE2 is harmful in the setting of SARS-CoV and COVID-19 infections, as the virus cannot enter the cell. Conversely, ACE2 downregulation following infection is concerning considering the preclinical data summarised to this point, which instead suggest a putative causative mechanism of Ang II/Ang-(1–7) in the evolution of SARS-CoV-2-associated acute lung injury. Even if abolishing ACE2 were feasible, Han *et al.* [21] detected alternative receptors, DC/L-SIGN, which mediated SARS-CoV entry independently of ACE2. A further summary of key points is given in table 3.

Controversies regarding the causative role of ACE2 in COVID-19

Limited mechanistic investigations regarding COVID-19 currently exist [78]. Early Chinese data illustrate a progressive increase in Ang II levels among patients hospitalised in the setting of confirmed COVID-19 [95], along with concomitant increasing levels of IL-6, with the highest levels in nonsurvivors [96]. Similarly, an analysis in 2014 of H7N9 patients showed progressively increasing levels of Ang II, up to 4 weeks following infection, a finding associated with a worse prognosis [89]. In a recent COVID-19 patient study, quantified levels of Ang II were positively correlated with viral titres and closely associated with P_{aO_2}/inspiratory oxygen fraction measures, suggesting a relationship between infection, the RAAS and lung injury. Accordingly, the study investigators postulated that angiotensin receptor blockade might represent a promising therapeutic target [95].

As noted previously, ACE2 levels are known to be low in healthy individuals [53] and to decrease with age [54]. They are also lower in men than in women [97]. Age is an independent risk factor for mortality in patients admitted with COVID-19 [96]. The data also suggest a disproportionate rate of disease occurrence, greater severity, and worse outcomes, in males compared to females during this pandemic [98]. These associations might be interpreted to indicate that low ACE2 during times of health are appropriate, but the ability to regulate an overactive RAAS or RAAS–SCoV axis during illness is, at least in part, dependent on ACE2. For instance, one study identified a higher expression of the lung-protective AT_2 receptor among women, which could explain the disproportionately lower mortality in female COVID-19 subjects [99]. Interestingly, ACE2 is located on the X chromosome, which has been hypothesised to affect the ability of men to maintain adequate ACE2 production in situations of severe illness (*i.e.* ARDS or COVID-19) [39].

Perhaps most importantly, the most common comorbidities of severely ill COVID-19 patients have included hypertension, diabetes mellitus and coronary heart diseases [9]. These comorbidities are associated with either an overactivation of Ang II/AT_1 or a deficiency of ACE2 [41, 44], and all have shown benefit from RAAS blockade in chronic conditions [100], including an attenuation in AT_1 receptor expression with losartan [101]. Furthermore, smoking tends to increase AT_1 receptor expression [101] along with ACE2 expression in human and mouse epithelial cells [102, 103]. RAAS activation in states of stress with high circulating Ang II levels may be particularly harmful for such patients, and could explain at least some of the observed clinical outcomes, *e.g.* data from China found that the odds of disease progression in those with COVID-19 are 14 times higher in smokers [8]. However, we acknowledge that misinterpreting association for causation can be dangerous and that the pathology underlying acute and chronic conditions may not be equivalent [104]. Nevertheless, RAAS manipulation remains an attractive therapeutic target, not to treat or prevent infection with COVID-19, but rather to mitigate its downstream consequences.

Side-effects of RAAS manipulation are understandably a cause for concern, specifically hypotension and acute kidney injury in the setting of acute illness. The most severe risks of ARBs include teratogenicity in the setting of pregnancy, hypersensitivity including angioedema, symptomatic hypotension, worsening of renal function, and electrolyte abnormalities; however, the most common side-effects include fatigue,

weakness, diarrhoea, chest pain and anaemia. Clinical trial and post-marketing surveillance data of losartan demonstrate an excellent safety profile. In over 4000 patients, there was a low incidence of adverse events (2.1%) comparable to placebo (3.7%) [105]. The effects more common in losartan than placebo were clinically minor, including dizziness (3% *versus* 2%), upper respiratory infection (8% *versus* 7%), nasal congestion (2% *versus* 1%) and back pain (2% *versus* 1%). While losartan has the potential to cause symptomatic hypotension, four studies have identified that losartan has a negligible effect on resting blood pressure and heart rate in healthy volunteers [106–109]. These reassuring data cannot be extrapolated to COVID-19 patients; however, when assessing the risk/benefit balance for RAAS modulation, the baseline risk appears low in the healthy population whereas repurposing of other medications may not have the same favourable profile. There are also concerns that the upregulation of ACE2 by ARBs could accelerate SARS-CoV-2 entry in host cells. Such concerns become more relevant when one considers the observation that patients with hypertension tend to have worse outcomes, and many patients with hypertension are on agents that might upregulate ACE2 expression. Consequently, some clinicians have argued for discontinuing ACEi and ARBs and switching their patients to different anti-hypertensive classes. However, to our knowledge, there are no data to suggest such a change is beneficial, nor are there well-controlled, risk-adjusted analyses demonstrating that these medications are harmful.

On the contrary, the data summarised in this review actually suggest that discontinuation of ACEi or ARBs could pose a serious risk of harm through the propagation of excessive Ang II-mediated acute lung injury and downregulation of protective ACE2. In a recent preprint, Liu *et al.* [110] retrospectively studied 511 COVID-19 patients (age >65 years) with hypertension. Patients were categorised based on home anti-hypertensive regimen. Patients on ARBs had significantly decreased odds of developing severe COVID-19 disease on univariate analysis (OR 0.34, p=0.025) and multivariable analysis (OR 0.25, p=0.046), while none of the other medications demonstrated improved outcomes. Two subsequent retrospective studies have demonstrated a protective effect of RAAS blockade, including significant reductions in viral load [111, 112]. Finally, in addition to the inhibitory effects on the AT_1 receptor, a preprint publication from Iran identified losartan as a potential candidate treatment based on molecular docking and dynamic simulation [113]. Therefore, in addition to the effects on angiotensin homeostasis, losartan may directly inhibit the viral macrodomain and cell cycle [113]. Even prior to these observational data, based on preclinical evidence and non-COVID-19 studies, the International Society of Hypertension, the European Society of Cardiology and other international committees have strongly recommended that physicians and patients continue treatment with their usual anti-hypertensive therapy as there is no clinical or scientific evidence to suggest that treatment with ACEi or ARBs should be discontinued in the setting of the SARS-CoV-2 pandemic at the current time [114–116].

Conclusion

Current clinical evidence is insufficient to recommend treating with or withholding RAAS blockade during this pandemic. Despite elevated ACE2 levels being found in chronic disease states, the upregulation of ACE2 seems more likely to be an adaptive response rather than a culprit [66]. Significant preclinical data and retrospective human data suggest RAAS inhibition decreases lung injury and improves survival, while simultaneously decreasing viral load in animal models with viral infections that utilise the ACE2 receptor. Human clinical data regarding the effects of RAAS inhibitors on outcomes remain modest and an area of active investigation. While the association between hypertension and poor outcomes might be mediated through ACE2 upregulation and increasing viral susceptibility, these differential outcomes are at least as likely to be a result of dysregulated Ang II-mediated lung injury. We believe this represents a state of clinical equipoise and hypothesise that RAAS inhibition may improve outcomes in patients infected with COVID-19. Furthermore, the wide spectrum of severity during the pandemic highlights the heterogeneity in the patients with COVID-19. As different phenotypes emerge, certain subgroups (*i.e.* those with overactive RAAS) may differ in their response to therapy. Time will also need to be considered. RAAS modulation may have significantly different effects early in the disease process compared to later, given the complexity of the RAAS–SCoV axis. This underscores the exigency for high-quality evidence to investigate any significant association between RAAS modulation and pulmonary pathophysiology, with a focus on ACE2-mediated viral infections, particularly SARS-CoV-2.

Acknowledgements: We gratefully acknowledge the editorial assistance of Michael Lotti, Senior Editor of the University of Minnesota's Institute for Engineering in Medicine.

Conflict of interest: None declared.

Support statement: This work was funded by the National Heart, Lung, and Blood Institute, grant T32HL07741. Funding information for this article has been deposited with the Crossref Funder Registry.

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