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PERSPECTIVE

Targeting the renin–angiotensin signaling pathway in COVID-19: Unanswered questions, opportunities, and challenges

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The role of the renin–angiotensin signaling (RAS) pathway in COVID-19 has received much attention. A central mechanism for COVID-19 pathophysiology has been proposed: imbalance of angiotensin converting enzymes (ACE)1 and ACE2 (ACE2 being the severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] virus "receptor") that results in tissue injury from angiotensin II (Ang II)-mediated signaling. This mechanism provides a rationale for multiple therapeutic approaches. In parallel, clinical data from retrospective analysis of COVID-19 cohorts has revealed that ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) may be beneficial in COVID-19. These findings have led to the initiation of clinical trials using approved drugs that target the generation (ACEIs) and actions (ARBs) of Ang II. However, treatment of COVID-19 with ACEIs/ARBs poses several challenges. These include choosing appropriate inclusion and exclusion criteria, dose optimization, risk of adverse effects and drug interactions, and verification of target engagement. Other approaches related to the RAS pathway might be considered, for example, inhalational administration of ACEIs/ARBs (to deliver drugs directly to the lungs) and use of compounds with other actions (e.g., activation of ACE2, agonism of MAS1 receptors, β-arrestin–based Angiotensin receptor agonists, and administration of soluble ACE2 or ACE2 peptides). Studies with animal models could test such approaches and assess therapeutic benefit. This Perspective highlights questions whose answers could advance RAS-targeting agents as mechanism-driven ways to blunt tissue injury, morbidity, and mortality of COVID-19.

ACE2 | SARS-CoV-2 | angiotensin | ACE inhibitor | angiotensin receptor blocker

The COVID-19 pandemic has motivated many efforts to identify effective therapies, including vaccines, antiviral drugs targeting the severe acute respiratory coronavirus 2 (SARS-CoV-2) virus, and approaches to mitigate disease pathology and complications resulting from interaction with host factors. Based on the key role of angiotensin converting enzyme 2 (ACE2), a zinc metalloprotease (carboxypeptidase), as the "receptor" for SARS-CoV-2 prior to viral entry into cells (1), much scrutiny has focused on this enzyme and its role in regulation of peptides in the renin–angiotensin signaling (RAS) pathway that mediate pathobiology of COVID-19 (2–4).

Debate has existed regarding whether drugs that target this pathway, in particular, inhibitors of ACE1, a dipeptide carboxypeptidase (ACEIs), or angiotensin receptor blockers (ARBs), are beneficial or harmful (5). The possibility that administration of ACEIs/ARBs might increase ACE2 expression, thereby perhaps increasing SARS-CoV-2 infectivity, has been a concern (6). However, the plurality of data from animal and human studies imply that this increase in ACE2

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expression likely does not occur (5). Moreover, much evidence supports a mechanistic hypothesis that defines a central role for imbalance in effects of ACE1 and ACE2 and dysregulation of the RAS pathway in COVID-19 pathobiology (2, 7) (Fig. 1). In addition, this framework can help explain increased thrombosis (8) and thrombin-mediated (protease-activated receptor) signaling in COVID-19 (7).

This model (2, 7) explains key features of COVID-19 and why certain patients (especially those with age-associated comorbidities) have greater morbidity and mortality, and predicts beneficial effects for drugs that target the RAS pathway by inhibiting the formation (ACEIs) or effects (ARBs) of angiotensin II (ANG II). Age and certain age-associated conditions (e.g., hypertension, diabetes, nonalcoholic fatty liver disease, chronic lung or kidney disease, and obesity) are comorbidities that enhance susceptibility for severe COVID-19 (2, 9–12). Such comorbidities may result from (and be associated with) "inflammaging": age-related elevation in systemic inflammation and concurrent weakening of the adaptive immune response to infection (13, 14). Increased activity of the RAS pathway prior to SARS-CoV-2 infection may also occur in such settings (e.g., cardiovascular disease and cardiac remodeling, discussed in ref. 2), thereby exacerbating dysregulation of the RAS pathway induced by SARS-CoV-2–mediated inhibition of ACE2. This mechanism may contribute to the increased severity in COVID-19 patients with comorbidities: Tissue injury and associated inflammatory processes in COVID-19 are accelerated by these comorbidities, driving pathology that leads to severe pulmonary injury before an effective adaptive immune response can be mounted (2). In effect, the comorbidities give the pathobiology of COVID-19 a "head start" versus the protective immune response. Such comorbidities may also relate to factors such as socioeconomic status, diet, and genetic predispositions, and potentially contribute to the association of such characteristics with disease severity and outcomes in COVID-19.

In addition to potential beneficial effects of RAS-targeting agents on pulmonary and cardiac pathology in COVID-19 (2), the association of kidney disease with COVID-19 morbidity provides added emphasis on RAS-targeting agents. Kidney disease enhances susceptibility to severity and adverse outcomes in COVID-19, including greater likelihood of developing acute kidney injury and increased mortality (15). ACEIs/ARBs, which blunt proteinuria and decrease blood pressure, are used to treat renal disease (16). Such effects may aid in treating COVID-19, perhaps mitigating renal pathology. It will thus be important to assess renal function in clinical trials of ACEIs/ARBs in COVID-19 patients, together with other biomarkers for target engagement (TE), which we discuss below.

In parallel to these mechanistic hypotheses, emerging data from retrospective clinical studies of COVID-19 patients receiving ACEIs/ARBs imply that these drugs may have a beneficial effect, including a reduction in mortality (17) and in markers of inflammation (18). Metaanalysis of multiple studies reveals that ACEI/ARB administration is not associated with poorer outcomes in COVID-19 but instead with reduced disease severity and improved survival (19, 20). A detailed summary of retrospective, observational studies on ACEI/ARB use in COVID-19 can be found at http://www.nephjc.com/news/covidace2 (21). Further, analysis of patient data for COVID-19 patients with hypertension showed that those prescribed ACEIs/ARBs who discontinued their use during hospitalization had higher rates of admission to intensive care units and greater mortality than did patients who continued using those drugs (22).

Such data imply the absence of harm and potential benefit from ACEI/ARB administration in COVID-19, including possible

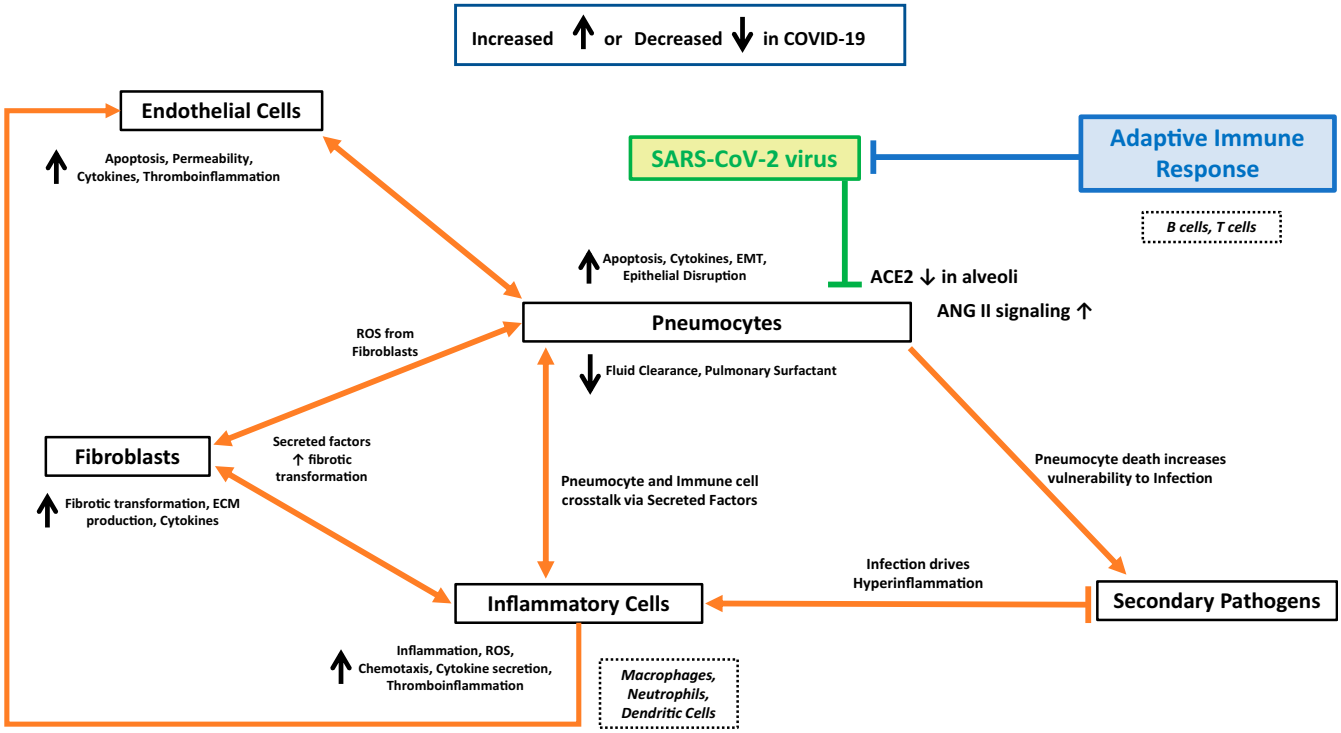

Fig. 1. A simplified model for COVID-19 pathobiology in the lung, driven by dysregulation of angiotensin II (ANG II) signaling and ACE1/ACE2 imbalance. EMT, epithelial-to-mesenchymal transition; ROS, reactive oxygen species; ECM, extracellular matrix.

Box 1.

Unanswered questions regarding the role of the renin–angiotensin signaling (RAS) pathway in COVID-19 and the targeting of this pathway for therapeutic effect are as follows: 1) ACEIs vs. ARBs: Which is preferable? 2) How can one validate the hypothesis for ACE1/ACE2 imbalance in animal models and ex vivo? 3) What are key unknowns regarding angiotensin II receptor (AGTR) and MAS1 physiology and pharmacology? 4) How can TE in the lungs be determined for RAS-targeting agents? 5) What are the challenges regarding clinical administration of ACEIs/ARBs? 6) Might ARBs/ACEIs be delivered by inhalation? 7) Might other RAS pathway components besides ACE and AGTR1 be targeted for COVID-19?

improvement in mortality. However, these studies have limitations, which include their retrospective nature, the contribution of comorbidities, the analytical methods used, and inadequate statistical power (21). An additional confounder relates to the high rate of discontinuation [~50% in a study of patients in New York (23)] of ACEIs/ARBs in hospitalized patients with COVID-19. Epidemiological studies often do not clarify whether ACEIs/ARBs were discontinued in the COVID-19 patient populations being analyzed.

These limitations highlight the need for prospective studies and randomized placebo-controlled trials (RCTs) to examine the efficacy of RAS inhibition versus placebo for the management of COVID-19 and its complications. In addition, populations with higher frequency of the D allele of ACE1 (which increases ACE1 activity and ANG II signaling) appear to experience higher rates of COVID-19 infection (24) and mortality (25). Such data imply a need for further studies to define the association between patient genotypes and COVID-19 outcomes.

The combination of a mechanistic hypothesis and epidemiologic data for the benefits of ACEIs/ARBs in COVID-19 have motivated the initiation of clinical trials to investigate the effects of ACEI/ARB treatment in COVID-19 patients, including double-blinded RCTs (e.g., NCT04366050, NCT04328012, NCT04312009). In addition, preclinical studies are needed to better define the utility of ACEIs/ARBs and other RAS-targeted drugs in COVID-19. Here, we discuss unanswered questions (Box 1) regarding the use of such drugs in COVID-19, the types of evidence needed to better understand their utility in this setting, challenges in using RAS-targeting drugs in COVID-19 patients, and opportunities for therapy using alternative delivery methods and novel RAS-targeting compounds. As widely prescribed agents with well-defined safety profiles, ACEIs/ARBs represent important candidates for repurposing for COVID-19, as a means to mitigate host pathobiology (26) and to potentially complement approaches that target viral replication. This Perspective thus seeks to help define directions for scientific inquiry in order to maximize understanding of RAS-targeting compounds as potentially useful therapeutics for COVID-19.

ACEIs vs. ARBs: Which Is Preferable? A key question in designing clinical trials of ACEIs/ARBs for the treatment of COVID-19 is which drug class might yield greater benefit. Logistical challenges, such as recruiting a sufficient number of patients for statistical power, make it easier to assess one type of drug at a time. This approach has been used thus far in RCTs that separately examine the effects of ARBs (e.g., NCT04312009) and ACEIs (e.g., NCT04366050).

Each drug class has advantages and disadvantages. ACEIs and ARBs generally have similar safety profiles. However, a concern with use of ACEIs, but not ARBs, is that 10 to 20% of patients receiving ACEIs develop cough, throat clearing, and vocal difficulties (27–29). Since dry cough is a common symptom in COVID-19, ACEIs may exacerbate this effect, worsening upper-respiratory symptoms and spread of infection.

An additional advantage of ARBs involves the potential role of tissue chymase in ANG signaling. Chymase can catalyze the conversion of ANG I to ANG II and thus is an alternative mechanism to increase ANG II signaling (17). Chymase is not inhibited by ACEIs. In healthy lungs, expression of chymase is lower than that of ACE1 [https://www.proteinatlas.org/ (30)], but a growing body of evidence indicates that chymase expression increases with lung and cardiac injury/disease and in other inflammatory conditions (31–34). It is important to define chymase expression in the lungs of COVID-19 patients and to determine whether its expression is induced by SARS-CoV-2. Analysis of cardiac tissue from COVID-19 patients may help clarify whether chymase expression and activity might limit the potential therapeutic efficacy of ACEIs in mitigating cardiac injury caused by COVID-19.

By contrast, an advantage of ACEIs in COVID-19 pathology stems from the ability of ANG II to signal via both AGTR1 and AGTR2. AGTR1 and AGTR2 are traditionally viewed as having effects that oppose one another (35). However, in lung injury, combined signaling by AGTR1 and AGTR2 may promote apoptotic cell death, implying that these receptors might both promote COVID-19 pathology (2). ACEIs reduce ANG II and signaling by AGTR1 and AGTR2, whereas ARBs, which target AGTR1 selectively, will not blunt AGTR2-mediated effects.

Defining differences between ACEIs and ARBs will likely require both clinical and preclinical studies. Retrospective studies in COVID-19 patients have attempted to evaluate ARBs and ACEIs separately and, in general, have not found significant differences in disease severity or mortality (21). However, firm conclusions are not yet possible. Parallel assessments of ARBs, ACEIs, and placebo in RCTs are the most rigorous approaches for this comparison but, to our knowledge, have not as yet been initiated.

Studies using ex vivo cell-based approaches and animal models may provide insight with respect to chymase and AGTR2. Limited information exists regarding AGTR2 function in key cell types that contribute to COVID-19 pathology (e.g., type II pneumocytes and lung fibroblasts [reviewed in brief in ref. 2]). Ex vivo studies that clarify cellular regulation by AGTR2 could provide insights regarding its role in COVID-19. Testing effects of AGTR2-selective agonists and antagonists [e.g., CGP42112, PD123319 (36)] and of chymase inhibitors [e.g., NK3201 (37)] using in vivo models of viral-induced lung injury may also provide useful information.

An additional issue requiring further study relates to the numerous ANG-derived peptides generated by ACE1, ACE2, and chymase and other peptidases involved in ANG metabolism [aminopeptidases, carboxypeptidase, etc. (38)]. Inhibition of ACE or blockade of AGTR1 by ARBs might alter the formation and concentration of ANG-derived peptides that can act via receptors whose activation may contribute to COVID-19 pathophysiology.

How Can One Validate the Hypothesis for ACE1/ACE2 Imbalance in Animal Models and Ex Vivo? A challenge in studying SARS-CoV-2 has been the search for appropriate animal models: animals susceptible to SARS-CoV-2 infection that show pathology akin to that in humans. SARS-CoV-2 infection has been shown in

ferrets (39, 40), cats (39), rhesus monkeys (41), and Syrian golden hamsters (42, 43). Rhesus monkeys and Syrian golden hamsters develop changes that recapitulate features of human COVID-19. Syrian hamsters show features (fever, pulmonary infection, and antibody response) similar to mild disease in humans (43), whereas rhesus monkeys have more severe pathology, including evidence of pneumonia (41). Mouse models have also been developed that use adenoviral transduction of human ACE2 (hACE2) (44, 45), resulting in hACE2 expression in the lungs and pulmonary pathology, representative of aspects of COVID-19 in humans. These models could be used to compare effects of ACEIs and ARBs on lung injury and mortality and to assess the efficacy of other RAS-targeting agents (discussed below). A primate model is likely best to predict responses in humans.

Animal models of SARS-1 are another option. ACEIs/ARBs (and other RAS-targeting agents) may be beneficial in this disease as well, due to the commonality of ACE2 as the viral receptor for SARS-1 and SARS-CoV-2 (46).

Ex vivo, cell-based models could be used to assess ACEIs/ARBs in studies with alveolar epithelial cells (AECs), in particular, type 2 pneumocytes, which express high levels of ACE2 (47) and are infected by SARS-CoV-2. Questions that such studies could address include the following: 1) What is the impact of SARS-CoV-2 on AEC function (proliferation, cell death, cytokine secretion, etc.)? 2) Can unbiased methods, such as transcriptomics and proteomics, provide insight into alterations in disease mechanisms in AECs infected with SARS-CoV-2? 3) Are physiological properties (e.g., alveolar permeability, sodium transport, cell adhesion) of AECs altered by SARS-CoV-2 infection? 4) Do SARS-CoV-2–infected AECs secrete moieties that alter the phenotypes of other cells, such as lung fibroblasts, endothelial cells (ECs), and immune cells, and, if so, can such cell–cell communication be assessed in organoids/spheroids and other types of three-dimensional cell cultures? 5) Do ACEIs/ARBs or other RAS-targeting agents blunt phenotypic changes induced by SARS-CoV-2 infection?

Limited data exist to answer such questions, although recent studies have shown that SARS-CoV-2 virus infects cells (48–51), including epithelial cell lines, and can alter cell viability (48). Such cell lines provide ex vivo model systems to address the questions above, in particular, the role of ANG signaling in mediating functional responses to infection. Work by Monteil et al. (52), provides support for the use of soluble, recombinant hACE2 as a decoy for SARS-CoV-2. Their data demonstrated that recombinant hACE2 prominently decreased SARS-CoV-2 infection of cultured Vero cells, and organoids of kidney and human blood vessels. Related findings were obtained in experiments that assessed inhibition of entry of a pseudotyped virus expressing the SARS-Cov-2 spike protein by an engineered protein that combined the extracellular domain of ACE2 with the Fc region of human IgG1 (53). Soluble ACE2 has been shown to have a similar effect as a decoy in vitro for SARS-CoV-1 (54).

Much of the available data on the interaction of ACE2 with SARS-CoV-2, including results discussed above that show efficacy of soluble ACE2, rely on ex vivo/in vitro models. Caution is needed in extrapolating such findings to the in vivo setting, underscoring the need for well-validated animal models that can provide evidence for the role of ACE2 and angiotensin signaling in COVID-19 pathobiology. Such in vivo data will also help support clinical trials.

What Are Key Unknowns Regarding AGTR and MAS1 Physiology and Pharmacology? The RAS pathway impacts on cells in lung alveoli via three G-protein–coupled receptors (GPCRs): AGTR1, AGTR2, and MAS1 (2). ANG II activates AGTR1 and AGTR2, while ANG (1–7) primarily activates MAS1 (36, 38, 55). AGTR1 signals via the Gq/G11 and Gi/Go pathways, (i.e., raising intracellular calcium and reducing cAMP, respectively), while AGTR2 primarily signals via Gi/Go (36). Signal transduction by AGTR1 and AGTR2 in AECs and other cell types in the alveolar environment has not been identified. Receptor-mediated functional data for such cells have been described, but mechanistic understanding of the relationship between signal transduction and functional effects is lacking. The signal transduction mechanisms of MAS1 are not clearly defined, with limited data for AECs, ECs, or lung fibroblasts. Thus, while RAS signaling is likely central to COVID-19 pathobiology in the lung, relatively little is known about the signaling and functional effects of peptide products in this pathway.

Open questions of high priority include the following:

1) What is the contribution of AGTR1 versus AGTR2 in ANG II-promoted responses in alveolar cells, for example, AEC, ECs, and fibroblasts, and does SARS-Cov-2 infection alter signaling by these receptors?
2) Which heterotrimeric G proteins (and G-protein subunits) mediate responses to AGTR1 (and perhaps AGTR2) in those cells, or do such responses occur via β-arrestin?
3) Which signal transduction mechanisms are regulated by MAS1 in alveolar cells?
4) What are the downstream transcriptional or posttranscription mechanisms that mediate particular functional responses of AGTR1, AGTR2, and MAS1 activation in alveolar cells?
5) Experiments in primary human cells are preferable for addressing these questions, but insights may be gained from studies with "standard" human cell lines (e.g., HEK cells, HELA cells etc.), in particular for fundamental questions, such as resolving the poorly characterized G-protein coupling of MAS1 and identifying a role for Gβ and Gγ proteins. In addition, a high degree of orthology exists among rodent and human GPCRs (56), implying that cells isolated from rodents might address these questions.

How Can TE in the Lungs Be Determined for RAS-Targeting Agents? In testing the efficacy of RAS-targeting agents in COVID-19 patients, it is important to verify TE along with response to treatment. A range of biomarker surrogates may aid in evaluating clinical characteristics in patients and in verifying RAS pathway modulation. Studies in animals may help validate such biomarkers as surrogates for TE in organs impacted by SARS-CoV-2.

Based on the notion that ACE1/ACE2 imbalance has a key role in SARS-CoV-2 pathobiology (2), such biomarkers might include immunoassays of circulating (and/or perhaps urinary) ACE1, ACE2, ANG II, and ANG 1-7. SARS-CoV-2 infection is expected to increase circulating ANG II and possibly reduce ACE2 and ANG 1-7, as the virus-mediated decrease in ACE2 in infected tissue reduces ANG 1-7 generation. ACE1 messenger RNA and protein expression increases in lung injury (57–59), which might be detectable in biological fluids, such as bronchoalveolar lavage fluid (BALF).

Treatment with soluble ACE2 (as a decoy for the virus) is predicted to increase circulating ANG 1-7 levels. Circulating levels of ANG II may also decrease if ACEIs/ARBs reduce lung injury and inflammation. BALF could be used to verify TE in the lung. Tissue sections from animals with COVID-19 treated with RAS-targeting

agents could be assayed for levels of RAS pathway-derived peptides. Radioactively labeled compounds that assess TE in tissues could be used in animal studies.

Questions associated with drug delivery and TE of RAS-targeting agents include the following: 1) Does oral administration of ACEIs/ARBs inhibit pulmonary ACE1/AGTR1, particularly in alveoli, the key sites of COVID-19 pathobiology? 2) Does systemic administration of soluble ACE2 impact pulmonary RAS and viral load in the lung? 3) Does systemic administration of ANG 1-7 and other MAS1 agonists increase their concentration in the lungs (and alveoli) and impact on readouts that reflect MAS1 actions? 4) What is the impact of ACEIs/ARBs on TMPRSS2 expression (discussed below) and viral entry via ACE2? 5) Do ACEIs/ARBs have a different impact on ACE2 expression and viral entry if their use precedes viral infection or if treatment with these agents precedes SARS-CoV-2 infection?

It will also be useful to assess indicators of disease mitigation, such as circulating markers of inflammation, especially as related to acute lung injury (ALI) by COVID-19. Such biomarkers include IL-6, IL-8, IL1-β, and RAGE, which are associated with ALI/lung inflammation and cytokine storm (2, 60–62). These biomarkers (among others) increase in COVID-19 (2, 62–64). Similarly, troponin I and T (65, 66) can be used as biomarkers to detect cardiac complications in COVID-19 and may aid in assessing efficacy of ACEIs/ARBs in preclinical and clinical studies.

What Are the Challenges Regarding Clinical Administration of ACEIs/ARBs? Numerous challenges exist related to the possible administration of ACEIs/ARBs in patients with COVID-19. Foremost among these is the timing when one should administer those drugs following diagnosis of COVID-19. Based on the potential central role of imbalance in ACE1/ACE2 function in cells and tissues infected with SARS-CoV-2 (2) and since ACEIs/ARBs may help mitigate disease progression and severity, it may be desirable to rapidly initiate treatment after diagnosis, that is, before extensive lung injury and evidence of severe hypoxia develop that may require placing a patient on a ventilator. This may not be possible if patients have conditions such as low blood pressure, abnormal serum potassium levels, or underlying renal disease. Another potential challenge is the possibility that patients with hypertension are being treated with other antihypertensive drugs to which ACEIs/ARBs may be additive or perhaps synergistic and excessively lower blood pressure. Rare adverse effects with these drugs include renal failure, which can also occur in COVID-19 patients, so it will be important to assess whether those agents increase the frequency or severity of renal failure. ACEIs/ARBs can also produce serious allergic reactions and angioedema, sequelae whose frequency or severity may be altered in COVID-19 patients with enhanced inflammation. The optimal doses of ACEIs and ARBs for effective treatment of COVID-19 might be suggested from preclinical studies but may require analyses of pharmacokinetics (PK) and pharmacodynamics (PD) in COVID-19 patients, including assessment of appropriate biomarkers that reflect TE.

ACEIs/ARBs may be particularly useful in patients who have mild to moderate COVID-19 but have not yet developed signs of circulatory dysfunction or cardiogenic shock that accompany severe disease (67), as the blood pressure lowering effects of these drugs will likely be contraindicated in patients who have hypotension. Once inflammation-induced cardiovascular instability occurs (as a consequence of hyperinflammation), ACEIs/ARBs are unlikely to be appropriate therapeutics, thus highlighting the need for early testing and rapid results to identify COVID-19 patients, so that treatment can begin soon after diagnosis.

Concerns have arisen regarding the possibility of long-term cardiac dysfunction following SARS-CoV-2 infection and associated myocardial injury, even after clearance of the virus (68). This sequela will likely necessitate monitoring of patients during convalescence, along with standard therapeutic approaches during recovery from myocardial injury. ACEIs/ARBs are commonly administered to patients after myocardial infarction, with heart failure and ventricular dysfunction (69). Thus these drugs are therapeutic candidates for convalescing patients.

An important concern with COVID-19 is the potential for complications arising from concurrent influenza infections. Analysis of electronic health care records for patients in the United Kingdom found that administration of ACEIs/ARBs does not increase susceptibility to influenza and may even reduce its incidence in those administered these medications for >2.5 y (70). Thus, ACEIs/ARBs are unlikely to increase susceptibility to SARS-CoV-2 or influenza but may confer a protective effect for both viruses.

Might ARBs/ACEIs Be Delivered by Inhalation? Inhaled delivery of ACEIs/ARBs is a possible alternative for treating RAS-mediated ALI in COVID-19. The benefits of Inhalational delivery include 1) increased drug delivery to the airway epithelium, including alveoli, thereby maximizing exposure of the infected, diseased cells, and 2) reduced systemic bioavailability/effects of ACEIs/ARBs—a critical issue in hospitalized patients on drugs (including ACEIs/ARBs) that lower blood pressure.

Certain ACEIs/ARBs are available for approved use in solution [e.g., lisinorpil and losartan; data at drugbank.ca (71)], allowing for their possible delivery via methods that include nebulizers or pressurized metered-dose inhalers. Limited preclinical data are available regarding this use of ACEIs/ARBs (72, 73). Whether drug delivery was adequate in those studies is unclear, nor can one draw conclusions regarding PK or PD. However, functional responses were observed in animal models, suggesting that inhalation can deliver therapeutic doses to the relevant sites. Nebulized delivery of radiolabeled ACEIs/ARBs in animals might help verify drug delivery to and TE in alveoli and aid in developing dosing regimens. In tandem, PK/PD measurements of drug levels in circulation are also necessary, in order to confirm that inhaled delivery results in localized or systemic effects.

Nanoparticles have been tested for inhaled delivery of ACEIs/ARBs (74) but the safety of such formulations presents additional challenges, making their use in the ongoing pandemic less feasible in the near term.

There are some potential drawbacks with the use of inhaled ACEIs/ARBs: 1) For ACEIs, cough and upper airway tract discomfort may be exacerbated. 2) Inhaled ACEIs/ARBs will likely not be beneficial in extrapulmonary tissues impacted by COVID-19. 3) Nebulizers could be associated with increased hazard of viral aerosolization and COVID-19 transmission (75); hence pressurized inhalers may be preferable, although ACEIs/ARBs have not been delivered in this manner. 4) If lung injury has developed, optimal delivery of inhaled drugs to collapsed alveoli may not be possible, that is, regions of the lung where drugs delivery is most needed may not be targeted.

Might Other RAS Pathway Components Be Targeted for COVID-19 besides ACE and AGTR1? Other approaches besides use of ACEIs and ARBs might involve the RAS signaling pathway for the treatment of COVID-19 (Fig. 2). Certain agents have been tested in experimental animals and, in some cases, clinical trials.

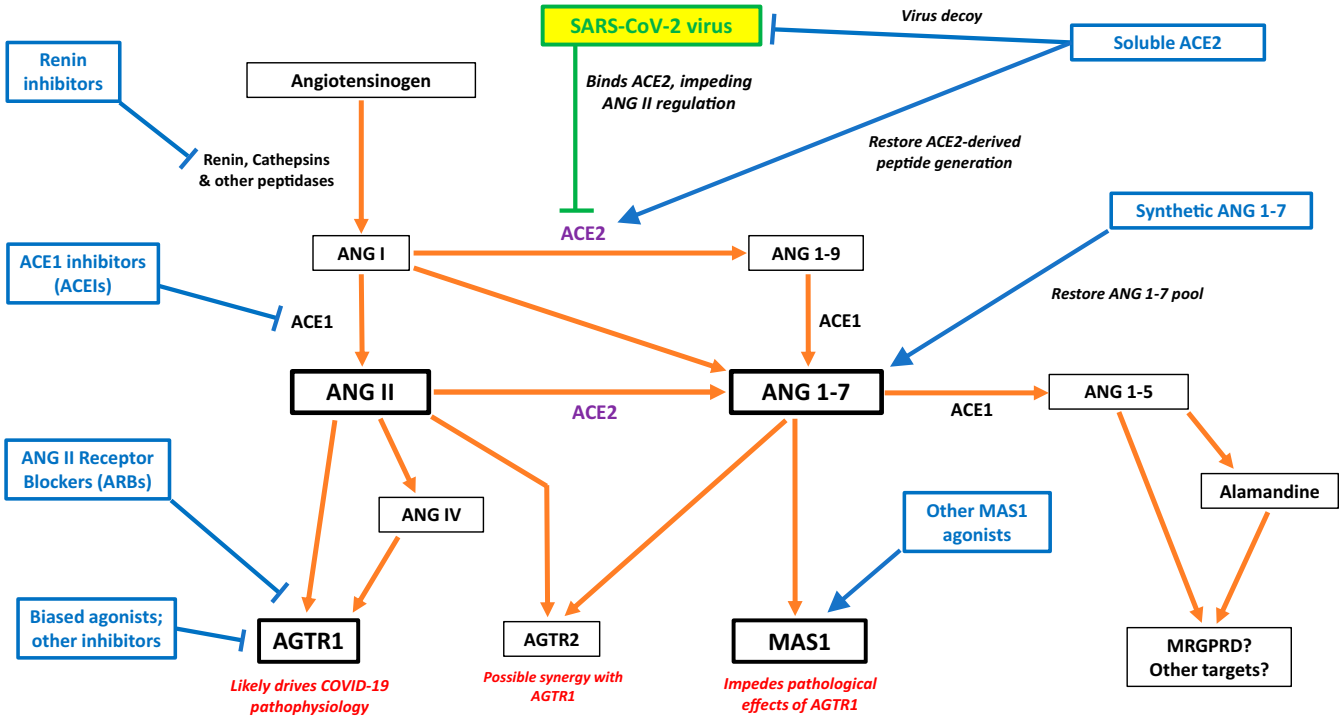

Fig. 2. A simplified schema illustrating the RAS pathway and available pharmacological agents to modulate the effects of this pathway. Besides ACE1 and ACE2, other enzymes (not shown) such as membrane metalloendopeptidase and prolyl endopeptidase also participate in this pathway. Ref. 2 illustrates these effects in more detail. MRGPRD, MAS related GPR family member D.

We identify candidate targets for which substantial data exist and note compounds that might be rapidly tested for efficacy in COVID-19 in preclinical models and perhaps early-phase clinical trials.

The MAS1 GPCR is considered the primary receptor by which ANG 1-7 exerts effects that oppose those of ANG II in driving pathology (38, 76). Decreased ACE2 activity in SARS-CoV-2 infection reduces ANG 1-7 generation (Fig. 2) in alveoli and other infected cells/tissues. Hence, the protective effects of ANG 1-7, via activation of MAS1, are suppressed by ACE1/ACE2 imbalance (2), providing a rationale for ANG 1-7 analogs and/or MAS1 agonists as possible therapeutics for COVID-19. Other ACE2-generated peptides, such as alamandine, can activate MAS1. These peptides promote G-protein signaling mechanisms that vary among cell types and agonist peptides (55). Details of MAS1 signaling in cell types relevant to COVID-19 are poorly understood; defining such effects requires preclinical research.

Administration of ANG 1-7 (or TXA127, a purified formulation of ANG 1-7) is a direct way to activate MAS1. The effects of ANG 1-7, largely from studies in rodents, have been discussed previously (38, 76), including its potential in COVID-19 (2). ANG 1-7/TXA127 has been tested in human studies and is in clinical trials for COVID-19: the ATCO trial (NCT04332666), a phase 2/phase 3 RCT testing whether TXA127 mitigates COVID-19 pulmonary manifestations, and NCT04401423, a phase 2 RCT assessing effects of TXA127 on outcomes related to kidney failure and pulmonary pathology. Of the ~30 other trials using ANG 1-7 registered on clinicaltrials.gov, only two seek to evaluate systemic administration of ANG 1-7, in both cases for treatment of cancer (NCT01553539 and NCT00771810). ANG 1-7 appears to be well tolerated, based on data from clinical trials in patients with sarcoma (77). In vivo effects of ANG 1-7 have been studied in animal models, and it is well tolerated in limited studies conducted in humans. Preliminary data (in mice) suggest the suitability of ANG 1-7 for delivery via inhalation (78). Hence, further studies with ANG 1-7, in particular, in contexts relevant to COVID-19, could help clarify the effects of MAS1/ANG 1-7 and how these may influence the course of the disease.

AVE 0991, a small-molecule, nonpeptide MAS1 agonist (79, 80), has been tested in rodent models but not in humans, nor are we aware of studies in higher organisms (e.g., nonhuman primates). Key results from studies using AVE 0991 of relevance to COVID-19 pathobiology include data showing a protective role for AVE 0991 in reducing vascular inflammation, for example, perivascular recruitment of monocytes and macrophages (80). Other studies have shown antiinflammatory effects in murine and rat models of arthritis, including reduction of leukocyte rolling and adhesion, neutrophil infiltration, edema, and inflammatory cytokine secretion—effects likely beneficial for COVID-19 pathobiology. Studies in a chronic lung inflammation model revealed a reduction in pulmonary pathology, including in cytokine secretion (79). Such data indicate promise for testing AVE 0991 in animal models of COVID-19 and studies with isolated cells relevant to pulmonary pathology (e.g., pneumocytes, fibroblasts, ECs).

Enhancement of ACE2 activity, via pharmacological activation of ACE2 or administration of exogenous ACE2, is another potential way to address dysregulation of ANG signaling and ACE1/ACE2 imbalance in COVID-19. Treatment of soluble ACE2 in animal models can mitigate lung injury in settings relevant to COVID-19 pathobiology (2). As discussed in earlier sections, administration of soluble, recombinant hACE2 (rhACE2) can be a scavenger of the SARS-CoV-2 virus (52). A synthetic form of human ACE2, GSK2586881, has been tested in clinical trials for pulmonary hypertension (NCT03177603) and ALI (NCT01597635) and was well tolerated (81). A phase-2 RCT is testing rhACE2 in

COVID-19 patients (NCT04335136). Studies using animal models of COVID-19 may help clarify the utility of rhACE2 in this setting. Small-molecule activators of ACE2 include diminazene aceturate and resorcinolnaphthalein; these compounds are not well characterized, especially in vivo. Hence, such agents are less likely to be candidates for clinical use than is rhACE2.

Another possible approach involves renin inhibitors, which reduce generation of ANG peptides and thereby ANG II signaling. One such inhibitor, aliskiren, is approved for treatment of hypertension, including in combination with ACEIs or ARBs (82). Aliskiren also generally appears to be well tolerated to a similar degree as ACEIs/ARBs (83, 84). In the context of COVID-19, inhibition of renin will reduce both beneficial and harmful angiotensin peptides, that is, products of ACE2 and ACE1, respectively. By contrast, ACEIs and ARBs, which selectively blunt ANG II/AGTR1 signaling, while largely preserving ACE2/ANG 1-7/MAS1 signaling, are more appealing as therapies for COVID-19.

In addition to traditional AGTR1 inhibitors (ARBs), compounds that induce biased signaling by AGTR1 may represent a novel strategy for mitigating ANG II-mediated pathology in COVID-19. As recently proposed by Manglik et al. (85), TRV027 is a biased AGTR1 agonist that selectively signals via β-arrestin. This biased agonism is postulated to be therapeutic, based on the idea that pathological effects of AGTR1 are mediated by Gq/G11 signaling, while effects of β-arrestin signaling may be protective (85). TRV027 has shown such effects in animal studies and was well tolerated in phase 2 trials in heart failure patients (86). Thus, TRV027 and perhaps other AGTR1 biased agonists are candidates for testing efficacy for COVID-19 in animal models and perhaps patients.

Other potential therapeutics have been suggested that involve the RAS pathway. These include the possibility that aldosterone (whose synthesis, primarily in the adrenal gland, and circulating levels are increased by ANG II/AGTR1), acting via mineralocorticoid receptors, can induce vascular damage, perhaps by cross-talk with ANG II. This response raises the possibility that mineralocorticoid receptor inhibitors (e.g., spironolactone, eplerenone) might be beneficial in COVID-19 (87). Another possible approach relates to neprilysin (also known as neutral endopepdiase, membrane metalloendopeptidase, CD10, and common acute lymphoblastic leukemia antigen), a zinc-dependent metalloprotease that can degrade a variety of peptides, including angiotensin peptides. Differing views have been expressed as to whether increased or decreased activity of neprilysin might be beneficial for treating COVID-19 (88, 89). Clarification of these alternative possibilities may have clinical impact, as drugs that combine an ARB with a neprilysin inhibitor (e.g., valsartan/sacubitril) are commonly prescribed for patients with low ejection fraction heart failure and are being evaluated in other settings.

In addition to ACE2, TMPRSS2 [a protease essential for internalization of SARS-Cov-2 (1)] is a potentially complementary target for inhibition of viral entry. Entry of SARS-CoV-2 into cells is reduced by camostat mesylate (1, 90), an inhibitor of serine proteases (including TMPRSS2) that is approved in Japan for the treatment of pancreatitis and other conditions (90). Clinical trials are underway to determine the efficacy of this drug in COVID-19 patients. Nafamostat mesylate, which inhibits entry of SARS-CoV-2 (and MERS-CoV), may have a similar action (91). Combinations of such drugs with soluble ACE2 might minimize viral entry; however, this idea will require in vitro and in vivo studies prior to clinical trials. It is also possible that combining camostat mesylate or nafamostat mesylate with an ACEI/ARB might simultaneously target viral entry as well as host pathobiology.

Attention has also focused on dipeptidyl peptidase-4, a cell surface endopeptidase that is targeted by numerous drugs administered to patients with type-2 diabetes mellitus (92). Such drugs are being tested for the treatment of COVID-19 (e.g., NCT04365517) and might be candidates for combination therapy with ACEIs/ARBs in managing COVID-19 pathobiology.

Conclusions

Data from preclinical studies and observational clinical reports have shifted thinking regarding ACEIs and ARBs from the idea that those drugs are harmful in COVID-19 patients to the view that such drugs and perhaps others in the RAS pathway may benefit those patients. Binding of the SARS-CoV-2 virus to its primary receptor, ACE2, appears to facilitate an imbalance in ANG signaling: a decrease in beneficial products derived from ACE2 relative to a maintained or perhaps increased role for harmful effects from ANG II/AGTR1 action. Such effects produce tissue injury, especially in the alveoli in the lungs and in the pulmonary vascular and other endothelia, along with damage in the heart, gastrointestinal tract, and other organs.

Current efforts to treat SARS-CoV-2 have emphasized approved or new antiviral agents, but COVID-19 patients also require therapeutics that target clinical manifestations resulting from tissue (especially lung) damage, cytokine storm/hyperinflammation, secondary infections, and thromboembolic and other complications. ACEIs and ARBs have the potential to treat and perhaps prevent a number of those features. Results from RCTs will be essential for assessing the benefit and risks of these drugs. In addition, answers to a number of questions identified above and addressed in preclinical studies can aid in understanding mechanisms that underlie the roles of the RAS pathway in COVID-19 pathobiology, in optimizing choices among various drugs, and in developing/testing other potential therapeutic strategies directed at this pathway.

A major challenge in the COVID-19 pandemic is the need to make decisions regarding therapeutic approaches in the absence of evidence or experience with this disease. Administration of approved drugs that target the RAS pathway may provide mechanism-based approaches that can be rapidly repurposed to treat patients. In addition, this pandemic crisis creates an opportunity and strong imperative to expand knowledge and the therapeutic potential of this pathway in this disease setting and its urgent need for safe and effective therapeutics.

Data Availability. There are no data underlying this work.

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