Received: 27 March 2020 | Revised: 6 April 2020 | Accepted: 7 April 2020

DOI: 10.1002/ddr.21672

COMMENTARY

DDR WILEY

Response to recent commentaries regarding the involvement of angiotensin-converting enzyme 2 (ACE2) and renin-angiotensin system blockers in SARS-CoV-2 infections

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Recent commentary, correspondence and a Science Translational Medicine Blog (Fang, Karakiulakis, & Roth, 2020; Gurwitz, 2020) https://blogs.sciencemag.org/pipeline/archives/2020/03/17/angiotensin-and-the-coronavirus have addressed the involvement of ACE2 as the receptor for the SARS viruses, raising concerns about the use of angiotensin-converting enzyme inhibitors, (ACE inhibitors, for example, captopril, lisinopril, enalapril, as well as angiotensin receptor blockers (ARBs, for example, losartan, valsartan, telmisartan) in individuals who have hypertension, cardiovascular or renovascular diseases.

All three of these publications report that ARBs increase ACE2, either based upon an observation of increased urinary ACE2 (Furuhashi et al., 2015), a misquote of a recent report (Wan, Shang, Graham, Baric, & Li, 2020) of the interactions of SARS CoV with ACE2 (Fang et al., 2020), and amplification of this misquote https://blogs.sciencemag.org/pipeline/archives/2020/03/17/angiotensin-and-the-coronavirus.

The interpretation of the Furuhashi et al., 2015 (Furuhashi et al., 2015) report, that increased urinary ACE2 indicates an increase in ACE2 synthesis is likely incorrect for three reasons: (a) the increase in urinary ACE2 was only seen with olmesartan. Losartan, candesartan, valsartan and telmisartan did not increase urinary ACE2. (b) Soluble ACE2 is ACE2 that is shed from membranes, reflecting a distinctly different process than ACE2 synthesis. Increased urinary ACE2 suggests a reduction in membrane bound ACE2, indeed, it has been suggested that increased urinary ACE2 is a marker for and might be a causal factor for diseases associated with hyperactivity of the renin-angiotensin system (RAS), such as chronic kidney disease (Palau, Pascual, Soler, & Riera, 2019). (c) Urinary ACE2 likely reflects proximal tubule ACE2 expressed on the apical side of tubular epithelial cells that has been shed by the actions of ADAM17 (also known as TNFα converting enzyme, TACE, and TNFα convertase) (Palau et al., 2019; Wysocki et al., 2013; Xiao et al., 2014). The molecular weight of shed ACE2 arising from the cell membranes from healthy individuals is ~90 and ~ 120 kDa (Mizuiri et al., 2011), well above the glomerular filtration limit, so urinary ACE2 would not reflect ACE2 levels outside of the kidney (Wysocki et al., 2013) unless smaller fragments of shed ACE2 that retain immunoreactivity to the ACE2 antibody used in the ELISA assay are present.

At this time the effects of ATR1 blockers and ACE inhibitors on membrane bound ACE2 in human lung is unknown, so any suggestion that they increase SARS-CoV-2 infectivity currently lacks a sound rationale (Danser, Epstein, & Batlle, 2020; Vaduganathan et al., 2020). ACE2 plays an important role in inactivating angiotensin (Ang) II as well as to generate Ang 1–7 (Lazartigues, Feng, & Lavoie, 2007; Warner, Smith, Hooper, & Turner, 2004), and any reduction in its activity could increase the ability of Ang II to stimulate AT_1 receptors, which mediate both the pressor and proinflammatory actions of Ang II (Forrester et al., 2018; Piqueras & Sanz, 2020; Ranjbar et al., 2019; Zhou, Ando, Macova, Dou, & Saavedra, 2005) as well as reduce formation of Ang 1–7 which is reported to have cytoprotective properties in the lung and its vasculature (Y. Li et al., 2016; Ye & Liu, 2020). Moreover, it is unlikely that inhibitors of ACE2 activity would significantly compete for the same or overlapping binding sites on ACE2, as the active site of ACE2 is the HEMGH domain at amino acids 374–378, while the putative SARS-CoV-2 spike protein binding domains are amino acids 30–41, 82–84, and 353–357 https://www-ncbi-nlm-nih-gov.ezproxylocal.library.nova.edu/protein/NP_001358344.1. Moreover, the binding sites for SARS-CoV-1 on ACE2, which are the same as those reported for SARS-CoV-2 (Walls et al., 2020) are reported to not overlap with the substrate binding site of ACE2 based upon the inability of the ACE2 inhibitor MLN-4760 (Dales et al., 2002) to inhibit SARS-CoV-1 binding to ACE2 (F. Li, Li, Farzan, & Harrison, 2005). Of interest however, is the promising concept of administering exogenous ACE2 coupled with the Fc region of an immunoglobulin as a neutralizing antibody to serve as a decoy receptor and inactivator of SARS-Cov-2 (Kruse, 2020) thereby preventing it from binding to membrane bound

TABLE 1 Clinical trials of renin-angiotensin system-based therapies (including therapies targeting SARS-Cov-2 ACE2 interactions) listed in clinicaltrials.gov as of April 6, 2020

NCT number type of trial	Trial title	Listing date	Description (excerpted from listing)
NCT04287686 withdrawn as of 3/17/20 Viral decoy soluble ACE2	Recombinant Human Angiotensin-converting Enzyme 2 (rhACE2) as a Treatment for Patients With COVID-19	2/27/20	An open label, randomized, controlled, pilot clinical study in patients with COVID-19, to obtain preliminary biologic, physiologic, and clinical data in patients with COVID-19 treated with rhACE2 or control patients, to help determine whether a subsequent phase 2B trial is warranted.
NCT04324996 Viral decoy circulating ACE2 with virus immuno-neutralizing capability	A phase I/II study of universal off-the-shelf NKG2D-ACE2 CAR-NK cells for therapy of COVID-19	3/27/20	This construct is a universal off-the-shelf IL15 superagonist- and GM-CSF neutralizing scFv-secreting NKG2D-CAR-NK expressing ACE2 on its cell surface to attract SARS-CoV-2. NKG2D is an activating receptor of NK cells, which can recognize and thus clear unbound SARS-CoV-2 virus particles as well as SARS-CoV-2 infected cells.
NCT04335136 Viral decoy soluble ACE2	Phase II clinical trial of APN01 (recombinant human ACE2 to treat COVID-19)	4/6/20	Randomized double blind study of patients with severe COVID-19 infections, to determine the efficacy of rhACE2 administration.
NCT04312009 Angiotensin II AT_1 receptor blockade (ARB) to reduce COVID 19 pathology	Losartan for patients with COVID-19 requiring hospitalization	3/17/20	This is a multicenter, double-blinded study of COVID-19 infected patients requiring inpatient hospital admission randomized 1:1 to daily losartan or placebo for 7 days or hospital discharge.
NCT04311177 Angiotensin II AT_1 receptor blockade (ARB) to reduce COVID 19 pathology	Losartan for patients with COVID-19 not requiring hospitalization	3/17/20	This is a multicenter, double-blinded study of COVID-19 infected patients randomized 1:1 to daily losartan or placebo for 10 days or treatment failure (hospital admission).
NCT04328012 Angiotensin II AT_1 receptor blockade (ARB) to reduce COVID 19 morbidity	Comparison of therapeutics for hospitalized patients infected with SARS-CoV-2 in a pragmatic aDaptive randoMizED Clinical Trial During the COVID-19 Pandemic (COVID MED Trial)	3/31/20	Hospitalized patients: Group 1 standard care and lopinavir/ritonavir, Group 2 standard care and hydroxychloroquine, Group 3 standard care and losartan, or Group 4 standard care and placebo, will be followed for up to 60 days, with data collected to quantify the NIAID COVID-19 ordinal severity scale (NCOSS) over time (the primary objective). Secondary objectives (a) length of hospital stay, (b) level of ICU care, (c) length of use of mechanical ventilation, (d) survival.
NCT04335786 Angiotensin II AT_1 receptor blockade (ARB) to reduce lung pathology	Valsartan for prevention of acute respiratory distress syndrome in hospitalized patients with SARS-COV-2 infection disease	4/6/20	Currently available AT1R blockers (ARBs) such as valsartan, have the potential to block pathological increases in pulmonary vascular permeability mediated by angiotensin (Ang) II through its Ang II Type 1 receptor (AT1R), when activity of ACE2 to metabolize ang II is decreased by SARS-Cov-2.
NCT04335123 Assess safety of angiotensin II AT_1 receptor blockade (ARB)	Study of open label losartan in COVID-19	4/6/20	Open label, phase 1 clinical trial to evaluate the safety of losartan in respiratory failure due to COVID-19
NCT04332666 Proactive study of Ang 1–7 to treat severe SARS-CoV-2 infections	Angiotensin-(1,7) treatment in COVID-19: the ATCO Trial	4/3/20	To test the safety, efficacy and clinical impact of the infusion of angiotensin-(1–7), the product of ACE2, in COVID-19 patients with respiratory failure requiring mechanical ventilation.
NCT04329195 Discontinuation of angiotensin II AT_1 receptor blockade (ARB) therapy	ACE inhibitors or ARBs discontinuation in context of SARS-CoV-2 pandemia	4/1/20	Compare continuation of RAS blocker therapy with discontinuation of RAS blocker therapy
NCT04330300 Discontinuation or continuation of angiotensin II AT_1 receptor blockade (ARB) therapy in COVID 19 patients	Coronavirus ACEi/ARB Investigation	4/1/20	Continue ACEi/ARB antihypertensive or switch to an alternative BP medication (specifically a calcium channel blocker [CCB] or thiazide/thiazide-like diuretic at an equipotent blood pressure lowering dose).

TABLE 1 (Continued)

NCT number type of trial	Trial title	Listing date	Description (excerpted from listing)
NCT04318301 Retrospective study of ACE inhibitor or ARB use to assess benefits or harms in SARS-CoV-2 infections	Hypertension in patients hospitalized with COVID-19	3/23/20	There is a large number of patients with SARS-CoV-2 that have hypertension, suggesting that patients with hypertension may be more susceptible to covid19. This retrospective study will follow-up patients admitted to Hankou hospital to explore the impact of hypertension and hypertension treatment on severity and prognosis of patients with SARS-CoV-2,
NCT04318418 Retrospective study of ACE inhibitor and ARB use to assess benefits or harms in SARS-CoV-2 infections	ACE inhibitors, angiotensin II Type-I receptor blockers and severity of COVID-19	3/24/20	To retrospectively test whether 2019-nCoV patients treated with ACE-I or ARB, compared with patients who not treated with ACE-I or ARB, are at higher risk of having severe COVID-19 (including death).
NCT04322786 Retrospective study of ACE inhibitor use relationship to the incidence of influenza	The use of angiotensin-converting enzyme inhibitors and incident respiratory infections, are they harmful or protective?	3/26/20	The study use UK based linked electronic health records from the clinical research datalink (CALIBER) of 5.6 million individuals to conduct a matched case–control study to investigate the incidence of influenza in individuals prescribed ACEI compared to those not prescribed ACEI.
NCT04327479 Retrospective study of antihypertensive medications and COVID 19 infection outcomes	Comparison of therapeutics for hospitalized patients infected with SARS-CoV-2 in a Pragmatic aDaptive randoMizED Clinical Trial During the COVID-19 Pandemic (COVID MED Trial)	3/31/20	Assessment of cardiovascular diseases and cardiovascular risk factors At inclusion, patients will be screened for preexisting cardiovascular diseases and cardiovascular risk factors, as well as medication.
NCT04331574 Retrospective study of ARB and ACE inhibitor use relationship to the incidence of COVID-19 infection	Renin-angiotensin system inhibitors and COVID-19	4/2/20	Using anamnestic data collected from the health record of the hospital or of the general practitioner, we will count the number of COVID-19 patients enrolled that were treated with ACE inhibitors or ARBs to observe whether antihypertensive ACE inhibitors or ARBs increases the severity of the clinical manifestation of COVID19.

Note: As of April 6, 2020 there are 16 trials listed with Clinicaltrials.gov: Three viral decoy studies, one of which has been withdrawn, five ARB therapy studies, one Ang 1–7 (product of ACE2) study, two withdrawal of ACE inhibitor or ARB therapy with or without antihypertensive drug replacement studies, and five retrospective studies of ARB or ACE inhibitor use relationship to COVID infection incidence and morbidity. Different types of studies are separated by shading differences.

ACE2. A similar strategy using ACE2 expressing CAR T cells as a decoy with cytotoxic targeting of the SARS-CoV-2 virus has recently been registered on ClinicalTrials.gov (Table 1).

Since inflammation is one of the major causes of morbidity of SARS-CoV-2 infection, and AT_1 receptors are known to cause inflammation (Forrester et al., 2018; Piqueras & Sanz, 2020; Ranjbar et al., 2019; Zhou et al., 2005), AT_1 receptor blockers (ARBs) present an additional therapeutic modality to minimize complications of the respiratory impairments caused by this virus. While ACE inhibitors present an equivalent therapeutic option to ARBs for treatment of hypertension and cardiovascular and renovascular disease, their ability to protect bradykinin from degradation, manifested as the ACE inhibitor cough, as well as the increased risk of angioedema could be a cause for concern (Messerli, Bangalore, Bavishi, & Rimoldi, 2018). However, ACE inhibitors can increase Ang 1–7 production directly from Ang I via endopeptidase activity (Karamyan & Speth, 2007) and they also protect Ang 1–7 from degradation to the inactive metabolite Ang 1–5 (Chappell, Pirro, Sykes, & Ferrario, 1998). There is abundant capacity to synthesize Ang II in the lungs as well as AT_1 receptors to mediate its effects (Oakes, Fuchs, Gardner, Lazartigues, & Yue, 2018). In a mouse model of lung inflammation induced by bacterial lipopolysaccharide, ARBs reportedly reduced the pathological injury (Ye & Liu, 2020). Gurwitz (2020) noted that there is a large population of individuals who are taking ARBs for treatment of hypertension (Gurwitz, 2020). There are now several retrospective surveys registered with ClinicalTrials.gov (Table 1) to determine if the use of an ARB or ACE inhibitor is associated with SARS-CoV-2 infections and the degree of morbidity or death compared to individuals taking non-ARB antihypertensive medications that will readily inform us as to the utility of ARBs and ACE inhibitors to ameliorate this disease.

As of this writing, The American College of Cardiology, The American Heart Association and the Heart Failure Society of America all recommend that patients with hypertension continue to take ARBs and ACE inhibitors as directed https://www.acc.org/latest-in-cardiology/articles/2020/03/17/08/59/hfsa-acc-aha-statement-addresses-concerns-re-using-raas-antagonists-in-covid-19 as well as several other biomedical societies listed recently (Vaduganathan et al., 2020).

As of April 13, 2020, there were four additional trials listed with clinicaltrials.gov: one additional ARB therapy trial, one withdrawal of ACEinhibitor/ARB therapy, one retrospective study of ACE inhibitor/ARB therapy, and one study to observe RAS activity in COVID 19 patients.

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