Keywords: ACE inhibitor, SARS-CoV-2, COVID19, Hypertension

Binding of SARS-CoV-2 and Angiotensin Converting Enzyme 2: Clinical Implications.

Eleanor Murray, Maciej Tomaszewski, Tomasz J. Guzik

The emergence and consequent impact of COVID-19 has led to clinicians and academics looking for evidence, with clinical controversies to parallel socio-economical and political ones.

A key clinical controversy, reaching media attention raised by COVID-19, has been whether the use of Angiotensin converting enzyme (ACE) inhibitors and Angiotensin receptor blockers (ARBs) might be detrimental in COVID-19. This is the most widely prescribed classes of drugs with a strong evidence base in hypertension and cardiovascular risk reduction. Individuals have proposed stopping such drugs, due to concern over upregulation of ACE2 receptor acting as SARS-CoV-2 entry point; others paradoxically suggesting prescribing this class of drugs to counteract a dysregulated angiotensin-aldosterone system. We therefore explore the links between the renin-angiotensin-aldosterone system (RAAS) and ACE2 receptor specifically; expanding on the observation that hypertension is prevalent among those diagnosed with COVID-19.

SARS-Cov2 virus: The pathogen responsible for COVID-19, the SARS-Cov2 virus achieves cell entry through an S (spike) high affinity protein binding of the catalytic domain of the ACE2 receptor [1]; pneumocytes are particularly vulnerable.

ACE2: The RAAS is well characterised in hypertension, heart failure and beyond. Angiotensin Converting Enzyme I (ACE) converts Angiotensin I to Angiotensin II and it occurs, predominantly in the lungs. Angiotensin II effects are dependent on receptor binding: AT1 receptor which is the target of ARBs is responsible for numerous pathological effects of angiotensin II ranging from increased oxidative stress, through vasococnstriction. In principle, AT receptor 2 regulates opposing effects. Angiotensin converting enzyme 2 (ACE2) is an enzyme attached to cell membranes in the lungs, endothelium, heart as well as kidneys. Its main pharmacological effect is to lower blood pressure by catalysing the cleavage of angiotensin I to Angiotensin 1-9 and Angiotensin II to Angiotensin 1-7 (vasodilatory, anti-inflammatory activity). ACE2 has additional affinity for other vasoactive substrates, including apelin-13, and bradykinin. Soluble ACE2 has also been described, cleaved from the cell surface but with a preserved catalytic-activity. A sex difference exists, with men displaying higher ACE2 levels [2,3]. Circulating levels are low in good health, but rise in heart failure, atrial fibrillation, and kidney disease:

- In 79 patients with obstructive coronary artery disease (59% on ACE inhibitor/ARB), 10 year MACE rate was higher in those with higher plasma ACE2 activity at baseline (p=0.035); ACE2 remained an independent predictor in multivariable analysis (HR 2.4, 95% CI 1.2-4.7) [2].
- Hospitalisation due to heart failure in the same cohort was associated with higher ACE2, HR of 1.0, 95% CI 1.4-11.5, p=0.009) [2].
- Post-operative ACE2 levels following orthopaedic surgery in 187 patients were significantly greater in patients with a subsequent in-hospital cardiac event (25.3 vs 39.5 pmol/ml/min, p= 0.012), though this did not remain significant in multivariate analysis [4].
- In 103 participants, increased ACE2 activity was associated with hypertension, impaired left ventricular systolic function and older age; atrial fibrillation (p= 0.04) and vascular disease (p<0.01) were independent predictors of plasma ACE2 activity [3]. ACE inhibitor/ARB use was reported: 28% of controls, 36% of paroxysmal AF and 55% of persistent AF participants.

Differing rates of ACE inhibitor/ARB use between groups is important considering the aforementioned effect on membrane-bound ACE2, and largely unknown effect on soluble ACE2.

Is ACE2 upregulated by ACE inhibitor/ARB use, and does this facilitate COVID-19 cell entry and increase severity of infection?: The upregulation of ACE2 concept arises from animal studies; rats demonstrating reduced plasma Ang II, increased ACE2 mRNA, and higher plasma Ang 1-7 in response to lisinopril or enalapril, and the latter two effects also found with losartan [5]. Though conflicting evidence can also be found, with Ramipril failing to increase ACE2 despite similar duration of ACE inhibition [6]. Mechanistically, Ang II induces lysosomal internalization of ACE2, thus reduced tissue expression; losartan prevents this through interaction and stabilisation of ACE2 with AT1 receptors [7]. This leads to the converse argument, that ARBs could reduce SARS-CoV-2 cell entry by reducing availability of binding sites and reducing internalisation of ACE2. However, a virus only needs one receptor to infect a cell, and the effect of ARB on Ang II breakdown to Ang 1-7 was not studied.

There has not been any good evidence of ACE2 upregulation associated with these drugs in humans [2,3], and despite 61% sequence similarity between ACE1 and ACE2, ACE inhibitors do not effect ACE2 receptors. It is notable that animal work is predominantly based on tissue expression, and human work on soluble/circulating ACE2, when the relationship between these remains unclear. Furthermore, mice and rats are not intermediate hosts of SARS-CoV, raising questions about generalisability of data to humans.

Loss of regulation of Ang II: Considering if there is evidence of RAAS dysfunction in COVID-19, hypokalaemia has been a reported complication not clearly explained by GI loss, and correlating with disease severity, though the study omitted to report medication use [8]. Renal potassium losses (as seen in hyperaldosteronism), may be a consequence of elevated Ang II due to SARS-CoV S protein binding to and reducing ACE2 expression, thus removing the homeostatic mechanism limiting Ang II [1]. ACE2 knockout mice support the protective effect of regulating Ang II through ACE2 metabolism to Ang 1-7, as in a viral influenza model, pathology and survival were inferior in knockout animals [9]. Use of losartan to block AT1 receptor improved lung injury in this mouse model [9].

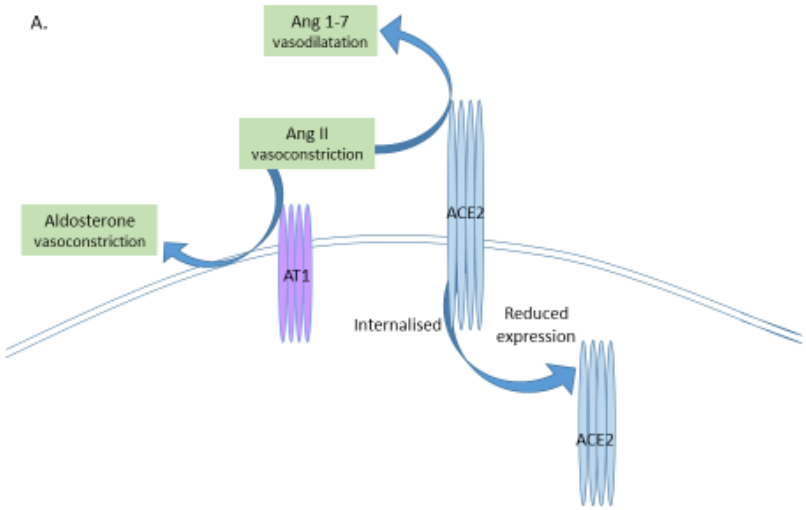
Clinical Outcomes with ACE inhibitors/ARBs: A cohort of 539 viral pneumonia patients found ACE inhibitor use was associated with an increased risk of death or need for intubation (OR 3, 95% CI 1.3-7.0), but continued use during the admission was possibly beneficial (OR 0.25; 95% CI 0.1-0.6)[10]. However, demographic and clinical features broken down by ACE inhibitor use/continuation were omitted, despite association of ACE inhibitor use with cardiovascular disease; and discontinuation of the drug more likely to occur in deteriorating patients.

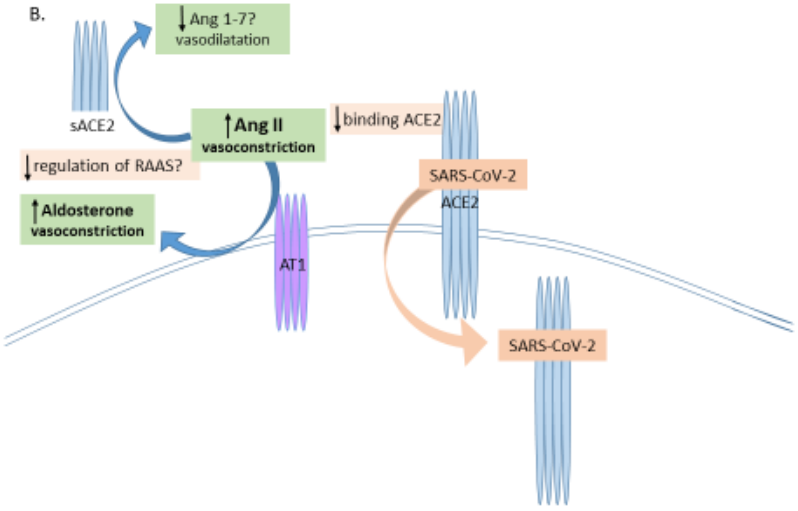
Long term outcomes: This remains largely unknown. Following SARS, lipid metabolism may remain altered even at 12 years follow up in comparison to both healthy controls (age and BMI matched) and those with bacterial pneumonia; though authors hypothesise that this may relate to use of methylprednisolone rather than the virus itself [15]. Compared to healthy controls, higher rates of cardiovascular and glucose metabolism abnormalities were reported, as was further hospitalisation.

Conclusion

In relation to the above evidence, American College of Physicians, Canadian Cardiovascular Society, European Society of Cardiology Council on Hypertension, European Society of Hypertension, Hypertension Canada, International Society of Hypertension and The Renal Association (UK) have all supported continued ACE inhibitor/ARB use, unless there is an alternative clinical reason to suspend

them in the face of COVID-19. The morbidity and mortality risk of stopping such drugs is significant, particularly given the myocardial damage that may occur in COVID-19.





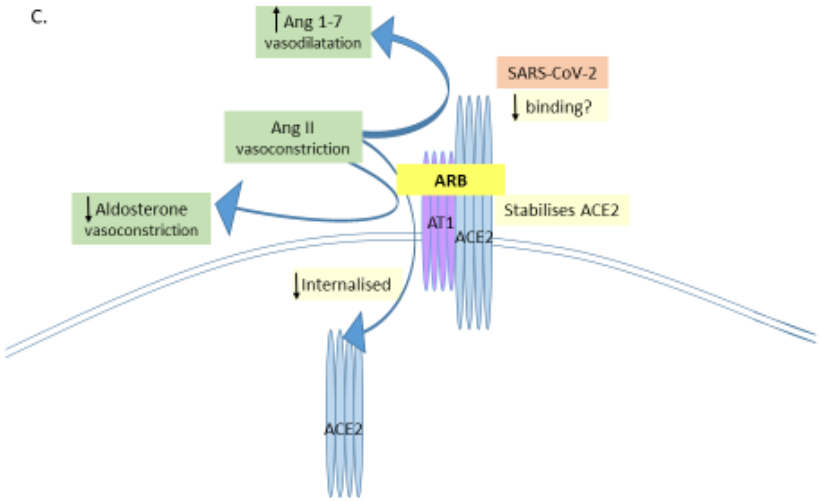


Figure 1. A. ACE2 contributes to regulation of Ang II, catalysing it to Ang 1-7. **B**. SARS-CoV-2 competes with Ang II for ACE2 for cell entry; may impair regulation of RAAS. **C**. ARB stabilises ACE2 with AT1 and may reduce availability for SARS-CoV-2 entry whilst reducing aldosterone excess.

Biography

Dr Eleanor Charlotte Murray is current Clinical Research Fellow at the University of Glasgow, funded by the European Research Council. Her clinical training is in General Medicine and Nephrology. Current research involves studying the immune system and vascular function in hypertension.

References

1. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. 2005;11(8):875-879. doi:10.1038/nm1267

2. Ramchand J, Patel SK, Srivastava PM, Farouque O, Burrell LM. Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. *PLoS One*. 2018;13(6). doi:10.1371/journal.pone.0198144

3. Walters TE, Kalman JM, Patel SK, Mearns M, Velkoska E, Burrell LM. Angiotensin converting enzyme 2 activity and human atrial fibrillation: Increased plasma angiotensin converting enzyme 2 activity is associated with atrial fibrillation and more advanced left atrial structural remodelling. *Europace*. 2017;19(8):1280-1287. doi:10.1093/europace/euw246

4. Chong CP, Lim WK, Velkoska E, et al. N-terminal pro-brain natriuretic peptide and angiotensin-converting enzyme-2 levels and their association with postoperative cardiac complications after emergency orthopedic surgery. *Am J Cardiol*. 2012;109(9):1365-1373.

doi:10.1016/j.amjcard.2011.12.032

5. Ferrario CM, Jessup J, Chappell MC, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111(20):2605-2610. doi:10.1161/CIRCULATIONAHA.104.510461

6. Burrell LM, Risvanis J, Kubota E, et al. Myocardial infarction increases ACE2 expression in rat and humans. doi:10.1093/eurheartj/ehi043

7. Deshotels MR, Xia H, Sriramula S, Lazartigues E, Filipeanu CM. Angiotensin II mediates angiotensin converting enzyme type 2 internalization and degradation through an angiotensin II type I receptor-dependent mechanism. *Hypertens (Dallas, Tex 1979)*. 2014;64(6):1368-1375. doi:10.1161/HYPERTENSIONAHA.114.03743

8. Chen D, Li X, Song Q, Hu C, Su F, Dai J. Hypokalemia and Clinical Implications in Patients with Coronavirus Disease 2019 (COVID-19). *medRxiv*. 2020;2019:2020.02.27.20028530. doi:10.1101/2020.02.27.20028530

9. Yang P, Gu H, Zhao Z, et al. Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci Rep*. 2014;4:7027. doi:10.1038/srep07027

10. Henry C, Zaizafoun M, Stock E, Ghamande S, Arroliga AC, White HD. Impact of angiotensin-converting enzyme inhibitors and statins on viral pneumonia. *Baylor Univ Med Cent Proc*. 2018;31(4):419-423. doi:10.1080/08998280.2018.1499293

11. Guan W, Ni Z, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. February 2020:NEJMoa2002032. doi:10.1056/NEJMoa2002032

12. Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. March 2020. doi:10.1001/jamainternmed.2020.0994

13. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA - J Am Med Assoc*. 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585

14. Zhou F, Yu T, Du R, et al. Articles Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan , China : a retrospective cohort study. *Lancet*. 2020;6736(20):1-9. doi:10.1016/S0140-6736(20)30566-3

15. Wu Q, Zhou L, Sun X, et al. Altered Lipid Metabolism in Recovered SARS Patients Twelve Years after Infection. *Sci Rep*. 2017;7(1):1-12. doi:10.1038/s41598-017-09536-z