The Central Role of the Aldosterone in COVID-19 Provides a Treatment Target.

Richard Henry[1]

[1]Queen's University

July 13, 2020

Abstract

Striking features of the COVID-19 pandemic are the rapid spread of the SARS-CoV-2 virus and the relatively narrow profile of patients adversely affected. SARS-CoV-2 enters human cells by means of ACE2, destroying the protein in the process. ACE2 maintains renin-angiotensin-aldosterone system (RAAS) homeostasis and is upregulated in the setting of chronic RAAS disequilibrium seen in COVID-19-susceptible patients. The ACE2-depletion effect on angiotensin II (AngII) levels likely contributes to the spectrum of cytokine responses found in COVID-19 patients. However, as most of the inflammatory effects of AngII are amplified through aldosterone-activated mineralocorticoid receptors (MRs), blocking MRs may provide much-needed benefit. The MR-blockers, spironolactone and eplerenone, are relatively selective in exerting downstream dampening effects on RAAS stress and target the pathophysiological processes in COVID-19-susceptible patients where acute viral-induced ACE2 depletion could be responsible for a catastrophic aldosterone surge in severely ill patients.

Introduction

SARS-CoV-2 is a novel coronavirus similar to the SARS-CoV virus that caused the SARS epidemic in 2003, but with two critical differences. The infamous spike protein is vastly more adept at cell entry through ACE2, (1) destroying ACE2 in the process (2) and the replicating virus is able to subvert the innate immune response, silencing the incubation period until after viral shedding has occurred. (3) Both features hamper efforts to reduce spread by immediate isolation of symptomatic patients, as was done during the SARS epidemic. If SARS-CoV-2 does indeed inhibit the immune reactivity, what triggers the delayed immune response in some patients?

The variable impact of ACE2 depletion may explain the broad range of host responses to infection in which 20% of infected people become ill, 10% become severely ill and 1-5% die. Those most at risk for severe COVID-19 illness are the elderly, obese, males and certain race groups such as African Americans and those with predisposing conditions such as hypertension, diabetes, cardiovascular disease, cancer and chronic lung and kidney diseases. (4) The renin-angiotensin-aldosterone system (RAAS) appears to be the link between the at-risk population and the acute ACE2-deficiency state induced by COVID-19. (2) Could the severe inflammation seen in Covid-19 be simply viral-induced or does ACE2 depletion cause an aldosterone crisis in patients already affected by RAAS stress? Could reducing RAAS stress effectively prevent the cytokine storm that predicts severe illness or death with COVID-19?

The Aldosterone Crisis Hypothesis – "Covid Syndrome"

The RAAS helps maintain interstitial fluid homeostasis and tissue perfusion and has a central role in the cardiovascular injury through inflammatory mechanisms. Aldosterone is a powerful pro-inflammatory steroid hormone and aldosterone-mediated stress is directly implicated in obesity, hypertension, cardiovascular, renal and respiratory diseases, diabetes, cancer and inflammation; matching the profile of COVID-19-susceptible

patients. (5,6) RAAS-blocking pharmaceuticals have shown enough promise in COVID-19 to encourage further study.

This author hypothesizes that acute ACE2 depletion in COVID-19 induces acute-on-chronic RAAS stress mediated by excessive Ang II and aldosterone levels, which together activate the severe inflammatory and oxidative stress responses that contribute to the spectrum of organ dysfunctions, "Covid Syndrome", encountered by these patients. Blocking aldosterone production and activity, alone or in concert with other RAAS suppressing/counteractive treatments that correct the susceptible host derangement, may provide a logical, safe and immediately available treatment approach.

The RAAS

Angiotensinogen, produced primarily by the liver, is cleaved by the kidney protease, renin, to form angiotensin I (Ang I), a circulating pro-signaling decapeptide. RAAS-targeted cells express angiotensin-converting enzyme (ACE) that cleaves two amino acids off Ang I to form angiotensin II (Ang II), which then binds to Ang II receptors (ATR). ATR activation increases blood pressure and cardiac output both directly and indirectly through increased aldosterone production. ACE2 is also a membrane-bound protease expressed by RAAS-targeted cells, but it cleaves both Ang I and Ang II, removing only one amino acid from each to form Ang 1-9 and Ang 1-7 respectively. Both peptides bind to the MAS1 receptor in the same RAAS-targeted cells to exert a counter-regulatory balancing effect. In the setting of chronic RAAS stress, up-regulated ACE2 expression exerts an ameliorating homeostatic effect. However, increased ACE2 expression enables enhanced SARS-CoV-2 tropism with repeated viral cycling until ACE2 is depleted, disrupting RAAS homeostasis. (2)

Inexplicably overlooked in COVID-19, Ang II downstream signaling involves aldosterone and its receptor, the mineralocorticoid receptor (MR). Aldosterone is an *inflammatory* steroid, produced mainly in the adrenal glands and adipose cells, that was first recognized as a sodium-retaining hormone. (5) Ang II and low sodium/increased potassium extracellular concentrations stimulate aldosterone secretion interactively, such that Ang II-mediated aldosterone secretion is augmented in the setting of low sodium or high potassium. (6) Aldosterone synthesis is also increased by adrenocorticotrophic hormone while atrial and brain natriuretic factors and the synthetic steroid, dexamethasone, suppress aldosterone production.

The Mineralocorticoid Receptor (MR)

The actions of aldosterone on fluid homeostasis are well known and the first MR blocking drug, spironolactone, is still considered to be primarily a diuretic. The MR is more recently appreciated as a transcription factor of the family of steroid receptors that increases blood pressure and effects vascular repair using pathophysiological processes that cause cardiovascular and renal disease. MRs expressed in innate immune cells (macrophages and T-lymphocytes) and adipocytes exert paracrine effects on the cardiovascular system as well as contributing to widespread inflammatory disorders and insulin resistance. The MR exerts two strong genomic effects on MR-expressing RAAS cells: increasing expression of NADPH oxidase subunits and activating the inflammatory transcriptor, NF-kB. (6)

NADPH oxidase (Nox)

The NADPH oxidase (Nox) family is composed of seven Nox isoforms and the main catalytic function of Nox is the generation of superoxide from NADPH. Control of blood flow is mediated by NO produced mainly by endothelial nitric oxide synthase (eNOS). In order to reduce the vasodilating effects of NO, to effectively constrict blood flow from its 'normal' open state, the NO signal must be attenuated. This is achieved by Nox-derived superoxide released into the inter-cellular space where it reacts rapidly with NO to form peroxynitrite, converting the NO signal to one of vasoconstriction. Nox assembly is acutely regulated by Ang II while both Ang II and aldosterone affect Nox gene expression. (7) ATR blockers (ARB) suppress Ang II-stimulated production of superoxide by Nox, thereby increasing NO availability and lowering blood pressure. Statin drugs interfere with Nox assembly to exert acute cardio-protective effects. (8) In addition to cardiovascular cells possessing Nox, resident macrophages, neutrophils, T-cells and platelets also express

MR-activated Nox and contribute to vascular oxidative stress in disease. (9)

Simultaneous production of superoxide and NO can increase the production of peroxynitrite a million-fold, resulting in destruction of cellular components, dysfunction of critical cellular processes and disruption of signaling pathways that promote chronic hypertension and associated cardiovascular diseases. In chronic disease, peroxynitrite has further detrimental effects on redox balance by inducing additional superoxide production through mitochondrial electron transport chain disruption and by uncoupling eNOS. Peroxynitrite impairs free radical scavenging by decreasing the effectiveness of superoxide dismutase and glutathione anti-oxidant protection. Peroxynitrite damages DNA by oxidizing the nucleobase guanine and breaking the sugar-phosphate backbone to cause single-strand breaks. Guanine disruption results in mutagenesis and carcinogenesis while single-strand breaks cause the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) that repairs the DNA and promotes inflammation. When the DNA damage is severe, excessive PARP activation results in cell death through apoptosis or necrosis. (10)

Immune response – NF-kB activation

Innate and adaptive immunity are involved in chronic hypertension and vascular damage. The Ang II/aldosterone/peroxynitrite axis activates the key inflammatory gene transcriptor NF-kB to evoke genomic production of inflammatory cytokines such as Il-1, Il-6 and TNF-a, adhesion molecules (ICAM-1), chemokines, coagulation factors such as von Willebrand's Factor and IL-18 and plasminogen activator (t-PA) which promote clotting and collagens to promote fibrosis. Aldosterone and Ang II produce a concerted and interactive oxidative and inflammatory cascade by activating most of the cell types found in and around the cardiovascular system, including cardiomyocytes, vascular smooth muscle cells, mesangial cells and podocytes, fibroblasts and immune cells. Aldosterone induces macrophage and T-lymphocyte vascular infiltration through MRs expressed in both of these cell types. Aldosterone produces an M1 proinflammatory phenotype in macrophages through ROS-mediated activation of the NLRP3 inflammasome and can result in Macrophage Activation Syndrome. MR-activated T-lymphocytes infiltrate the cardiovascular system and secrete inflammatory mediators such as interferon-gamma (IFN-y), interleukins and TNF-a. There is a profound increase in $CD8^+$ IFN-y T cells, while regulatory T lymphocytes (Tregs), suppressors of the innate and adaptive immune response, are reduced, priming the system for an acute inflammatory response that is directly associated with COVID-19 severity. (9)

Settings of increased aldosterone activity – obesity, advanced age and disease

The MR is expressed in adipose tissue, driving stem cell differentiation and leptin secretion and contributes to Metabolic Syndrome in obesity. Leptin induces release of aldosterone in a positive feedback loop and may be a significant mediator of obesity-associated hyper-aldosteronism. MR-driven hypertrophic adipocytes, especially in visceral and perivascular fat, modulate vascular health and disease through release of proinflammatory cytokines, IL-1, Il-6, TNFa and monocyte-chemotactic-protein-1 (MCP-1) and pro-thrombotic factor plasminogen activator inhibitor type-1 (PAI-1). (11,12)

Aging is characterized by a transition from physiological aldosterone regulation to a pattern of renin-independent secretion. The adrenal gland shows increasing non-neoplastic foci of aldosterone-producing cells while cortisol-mediated MR activation increases. MR expression has also been shown to be increased in the vasculature with aging. Thus, the elderly may have increased risk of autonomous aldosterone production, cortisol-mediated MR activation, and increased MR expression, all contributing to increased aldosterone-mediated pathogenesis.

Evidence continues to accrue confirming chronic Ang II/aldosterone/MR hyper-activation in several cardiovascular disorders, including hypertension, coronary artery disease, ventricular hypertrophy, atrial fibrillation, congestive heart failure and related diseases such as chronic kidney disease, diabetes, stroke and Metabolic Syndrome. (13) In these disease settings the affected tissues are in a heightened state of oxidative and inflammatory stress with persistent interactive up-regulation promoting disease progression. The homeostatic response to chronic RAAS stress is up-regulation of ACE2 and atrial and brain natriuretic factors. The most common medical interventions include ACE inhibitors (ACEI), ATR blockers (ARB) and statins,

with MR blockers (MRB) reserved for end-stage disease. Abrupt discontinuation of these medications is associated with rapid increase in disease and death in patients after myocardial infarction or in cardiac failure. During acute events such as myocardial infarction and viral pneumonia, attenuating the RAAS stress response has been shown to be protective.

RAAS treatments in Covid-19

The role of ACE2 in COVID-19 and the protective role of ACE2 in different organs has recently been reviewed. Clinical trial and retrospective data pertaining to RAAS treatments during COVID-19 are beginning to surface. In one retrospective study looking at ARB use in COVID-19 patients, ongoing use of these medications compared to other hypertensive treatments, was associated with lower risk of all-cause mortality compared with ACEI/ARB non-users (adjusted HR 0.30; 95% CI, 0.12-0.70; P=0.01). (14) Another study also confirmed a lower risk of mortality (relative risk 0.65, 95% CI 0.45-0.94, P= 0.02). (15)

Statin drugs, which prevent ART-mediated activation of Nox and reduce oxidative stress, recently showed a lower crude 28-day mortality (IRR 0.78; 95% CI, 0.61-0.996; p=0.046) in COVID-19. (16) Equally, dexamethasone showed a reduced risk of 28-day mortality (age-adjusted ratio 0.83; 95% CI 0.743-0.92; P<0.00). (17)

Although ARBs are not being used as de-novo treatment during COVID-19, current recommendations and consensus is to continue established treatment. There is little discussion of either statin drugs or MR blockers in COVID-19. As with ARBs, discontinuing these RAAS-modifying medications should not be taken lightly in view of the acute RAAS stress experienced during this illness.

Confirming the "Covid Syndrome" hyperaldosterone hypothesis would require the measurement of renin/aldosterone levels and their correlation with serum cytokine levels and disease severity and outcomes. Cytokine levels, particularly IL-6, have been shown to predict outcome. Electrolyte and blood pressure levels are unlikely to directly correlate with aldosterone levels due to many confounding variables and influences. Elevated aldosterone levels with low/normal renin could result from ACE2-depletion/Ang II-mediated stimulation.

This hypothesis advocates for the use of a cocktail of medications that counter the acute loss of ACE2 during "Covid Syndrome" by blocking the downstream effects of acutely increased Ang II activity. These medications would include dexamethasone, ARBs and MRBs such as spironolactone and eplerenone. (18) Supportive treatment with a statin drug could also be included, but may not add much benefit in combination with the aforementioned drugs. (16) All of these medications are readily available worldwide and have established safety and efficacy records when used, including in combination, in patients with severe RAAS-mediated disease. (19,20) Patients who recover from severe illness often experience severe fibrosis of the lungs, heart and kidneys. Blocking RAAS until complete resolution of the cytokine storm is achieved may reduce these debilitating long-term effects.

Acknowledgements: No funding was sought or provided for preparation of the current work.

The author would like to thank Ngoc Nguyen for her assistance with preparation of the summary schematic (Figure 1).

Declaration of Interests: Dr. Henry currently has a patent application pending for the use of mineralocorticoid receptor (MR) blockers for the treatment of COVID 19.

References

1. Tan HW, Xu YM, Lau ATY. Angiotensin-converting enzyme 2: The old door for new severe acute respiratory syndrome coronavirus 2 infection. Rev Med Virol. 2020 Jun 30:e2122. doi: 10.1002/rmv.2122.

2. Sriram K, Insel PA. A Hypothesis for Pathobiology and Treatment of COVID-19: The Centrality of ACE1/ACE2 Imbalance. Br J Pharmacol. 2020 Apr 24. doi: 10.1111/bph.15082.

3. Zhang Y, Zhang J, Chen Y *et al.*The ORF8 Protein of SARS-CoV-2 Mediates Immune Evasion through Potently Downregulating MHC-I, BioRxiv, May 24, 2020 available at URL: https://doi.org/10.1101/2020.05.24.111823 (accessed July 3, 2020)

4. Guzik TJ , Mohiddin SA, Dimarco A *et al.* COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options, Cardiovasc Res. 2020 Apr 30:cvaa106. doi: 10.1093/cvr/cvaa106.

5. Gilbert KC, Brown NJ. Aldosterone and inflammation. Curr Opin Endocrinol Diabetes Obes. 2010:17(3):199-204.

6. Bene NC, Alcaide P, Wortis HH, Jaffe IZ. Mineralocorticoid receptors in immune cells: emerging role in cardiovascular disease. Steroids. 2014;91:38-45.

7. Golub AS, Pittman RN. Bang-bang model for regulation of local blood flow. 2013 Aug;20(6):455-83. doi: 10.1111/micc.12051.

8. Rodrigues-Diez PR, Tejera-Muñoz A, Marquez-Exposito L *et al.* Statins: Could an Old Friend Help the Fight Against COVID-19? Br J Pharmacol. 2020; 19;10.1111/bph.15166. doi: 10.1111/bph.15166.

9. Kasal DA, Barhoumi T, Li MW, Yamamoto N, *et al* . T regulatory lymphocytes prevent aldosterone-induced vascular injury. Hypertension. 2012;59(2):324-30.

10. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007;87(1):315-424.

11. Jia G, Aroor AR, Sowers JR. The role of mineralocorticoid receptor signaling in the cross-talk between adipose tissue and the vascular wall. Cardiovasc Res. 2017;113(9):1055-1063.

12. Vecchiola A, Lagos CF, Carvajal CA, Baudrand R, Fardella CE. Aldosterone Production and Signaling Dysregulation in Obesity. Curr Hypertens Rep. 2016;18(3):20.

13. Bothou C, Beuschlein F, Spyroglou A. Links between aldosterone excess and metabolic complications: A comprehensive review. Diabetes Metab. 2020;46(1):1-7.

14. Zhang P, Zhu L, Cai J *et al.* Association of Inpatient Use of Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers With Mortality Among Patients With Hypertension Hospitalized With COVID-19. Circ Res. 2020;126(12):1671-1681.

15. Gao C, Cai Y, Zhang K *et al.* Association of hypertension and antihypertensive treatment with COVID-19 mortality: a retrospective observational study. Eur Heart J. 2020;41:2058–2066.

16. Zhang XJ, Qin JJ, Cheng X, *et al.* In-Hospital Use of Statins Is Associated with a Reduced Risk of Mortality among Individuals with COVID-19. Cell Metab. 2020; 24:S1550-4131(20)30316-8. doi: 10.1016/j.cmet.2020.06.015. Online ahead of print.

17. Recovery Collaborative Group. Dexamethasone for COVID-19 – Preliminary Report. June 22, 2020. doi: https://doi.org/10.1101/2020.06.22.20137273.

18. Makani H. Efficacy and safety of dual blockade of the renin-angiotensin system: meta-analysis of randomised trials. BMJ. 2013;346: 360.

19. Yang J, MJ. Mineralocorticoid receptor antagonists — pharmacodynamics and pharmacokinetic differences Curr Opin Pharmacol. 2016;27:78-85.

20. Lainscak M, Pelliccia F, Rosano G, *et al.* Safety profile of mineralocorticoid receptor antagonists: Spironolactone and eplerenone. Int J Cardiol. 2015;200:25-29.