The human-COVID-19 Tango: Connecting the Dots

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As the world grapples with a pandemic with various and expanding epicenters, a flurry of medical and scientific activity has gained speed and momentum in a race to halt COVID-19. A controversial topic has been the connection between COVID-19 and the Renin-Angiotensin system (RAS). COVID-19, like Sars before it, enters by way of the Angiotensin Converting Enzyme 2 (ACE2). ACE2 is ubiquitously expressed in many tissues in the body serving as the doorway by which the virus can enter and spread causing inflammatory havoc. Demographic evidence coming out of China and other locations make it clear that the elderly and those suffering cardiovascular complications such as hypertension etc are most at risk. The connection to RAS and the demographic nature of the data coming out has led many to advance hypothesis, recommendations and even therapies based on existing RAS inhibitors and other components of the renin-Angiotensin system. It is pertinent to review the literature in the context of our understanding of the renin-angiotensin system to allow better judgements to be made as well as lines of research initiated advancing a quick resolution to COVID-19. Covid-19 appears invincible as if dipped in the river Styx, but even Achilles had a vulnerable heel. Understanding the homeostatic balance that the coronavirus disrupts, we can discover the arrow in corona’s heel.

Key words: COVID-19, ACE, ACE2, Angiotensin II, Bradykinin, RAS inhibitors, ACE inhibitors, AT1 receptor blockers, Losartan, Bradykinin Antagonists, Ang II loading, Giapreza, Icatibant.

Introduction

As the scientific community focuses on the genome of COVID-19 overlooking that it takes two to tango. COVID-19, like Sars before it, enters by way of ACE2 (Angiotensin Converting Enzyme 2). ACE2 is a member of the of the Renin-Angiotensin system (RAS). Due to the urgency of the situation, it has becomes pertinent to connect the dots of the human-covid tango. This review will focus on RAS from the point of view of COVID-19. We apologize in advance if we did not include important papers on RAS in the context of the cardiovascular and other fields.

Survey Methodology

A literature search was conducted in the PubMed database that included the terms: “Angiotensin and COVID-19”. Other search terms that included ACE2 in particular yielded the same set of articles. The search was conducted on April 2, 2020. These key words were used because they yielded the appropriate topic under study. The articles where chosen in sequential order and reviewed for major ideas. Exclusion criteria were articles that mentioned ACE2 in passing as the COVID-19 receptor. We also excluded case reports and the ACE I/D polymorphism. A MOOSE flow diagram is presented in Figure 1. Since most articles were published recently and in open access journals, we were able to obtain full texts of the articles. Upon reviewing the articles, it became apparent that there were different schools of thought on the use of RAS inhibitors in the context of the Coronavirus outbreak. These different perspectives are
discussed in this review along with an accompanying discussion of the pertinent aspects of the renin-Angiotensin system with respect to COVID-19 for clarification.

**Background on the Renin-Angiotensin System as it pertains to COVID-19 for clarification purposes**

The Renin-Angiotensin system involves several human organs working in concert to ensure the function of this multi-faceted system. Components of RAS whether hormone peptides or receptors are widespread in the body and are found in all organs and organ systems (Fagerberg et al., 2014). RAS is a complex system of biochemical pathways involving various proteins, enzymes, peptides and receptors. RAS regulates homeostasis systemically (Azushima et al., 2020) as well as locally (De Mello & Frohlich, 2011). When discussing RAS we tend to think of vasorestriction (Benjamin, Collier, & Webb, 1988) and dilation of blood vessels (Vanhoutte et al., 1989) and assign negative and positive connotations to them respectively. In biological processes, however both play a role and it is the dysregulation of the balance between them that leads to a disease process. RAS is also associated with inflammation (Duprez, 2006), cell growth (Dai et al., 2019) and cell death (Wang et al., 2013) again forming negative connotations about necessary biological processes that lead to negative consequences because of a homeostatic imbalance. RAS has been known to play a major role in cardiovascular physiology (Unger, Azizi, & Belz, 2000), however in the past decade it has become a major player in cellular physiology dictating cell growth or death (apoptosis) by its two main receptors (Jacques et al., 2019; Cai et al., 2019; Namsolleck et al., 2014): the Angiotensin II receptor 1 (AT-1) and Angiotensin II receptor 2 (AT-2).

RAS is composed of two main pathways (axis) that are mutually antagonistic in maintaining homeostasis, despite the constant flow of mediators and perturbations to the local and systemic environments. The first axis represented by ACE/AngII/AT1 promotes inflammation, cell proliferation and vasoconstriction (Iwai & Horiuchi, 2009). The second pathway represented by ACE2/Ang1-7/MAS is an anti-growth pathway inhibiting inflammation and inducing vasodilation (Santos et al., 2013). Another important component of RAS that is often overlooked and in fact did not appear to be discussed (in COVID-19 context) in any of the articles that we have reviewed is the Bradykinin pathway (Marceau et al., 2020). Bradykinin is a pro-inflammatory proliferative peptide hormone inducing vasodilation, vascular permeability and edema (Kempe et al., 2020; Lopatko et al., 2019; Péterfi et al., 2019). Bradykinin is upregulated by ACE inhibitors (Bakhle, 2020) and may be playing a significant role that has gone unnoticed in the COVID-19 crisis. We will review all three components with regards to COVID-19, discussing the main ideas being published and conclude with an elucidation of future directions in this field.

**COVID-19 and the RAS connection**

![Figure 1. MOOSE flow diagram](image-url)
Unlike larger viruses (mimivirus-400nm) that require actin-dependent phagocytosis (Yaakov et al., 2019) COVID-19 (125 nm) enters the cell by clathrin mediated (CM) endocytosis (Wang et al., 2020). CM Endocytosis is a membrane process that needs a protein coat to induce curvature leading to the invagination of the membrane (Lherbette et al.,2019; Narasimhan et al.,2020). It is a complex process requiring many cellular mediators. COVID-19 targets ACE2 positive cells (Wan et al.,2020; Tai et al.,2020). ACE 2 is a membrane bound enzyme peptidase on the surface of cells found in the lung, arteries, heart, kidney and intestines (Fagerberg et al.,2014; Xu et al.,2020; Qi et al., 2020; Zou et al., 2020). ACE2 converts the peptide Angiotensin II to Ang 1-7 (Burrell et al.,2004). The catalytic domain of the enzyme faces the extracellular surface where it binds Angiotensin II and releases Ang 1-7. In the literature ACE2 has been called the COVID-19 receptor, since COVID-19, like Sars before it has been shown to bind specifically to ACE2 initiating the curvature needed for the endocytosis of the virus along with the endocytosis of ACE2. This process allows COVID-19 to enter the cell, depleting ACE2 from the cellular membrane as in Sars (Kuba et al 2005). Papers published in this category stressed the structural relationship between ACE2 and its viral binding protein as well as the homology in the viral proteins from various species (Liu et al.,2020; Chen et al.,2020; Tai et al.,2020; Qiu et al.,2020; Li, Qiao, & Zhang, 2020).

Chen et al. 2020, using sequence alignments, three-dimensional structural analyses, protein structure simulations and molecular docking were able to compare and contrast the interaction of human ACE2 with both Sars and COVID-19. Performing molecular simulations, they superimposed the predicted structure of the RBD protein of COVID-19 on the x-ray crystallographic structure of Sars with some structural variation. The inferred RBD (COVID-19) structure had a similar pattern of interaction with ACE2 as was seen with Sars and a more stable Gibbs free energy. In their discussion, they noted that the viral binding domain of ACE2 is different from the catalytic domain. They also hypothesized on the inhibition of ACE2 by ACE1 inhibitors and or other novel inhibitors. They speculated that the binding of ACE2 by specific catalytic domain inhibitors might change its conformation preventing it from binding the virus. To our knowledge, ACE1 inhibitors have not been shown to inhibit ACE2. This does not preclude their use in the context of the COVID-19 pandemic but more on this in the conclusion section of this review.

Other papers focused on the homologous relationship, the similarities and differences between COVID-19 and Sars (Liu et al.,2020; Li, Qiao, & Zhang, 2020; Tai et al.,2020). For example, Tai et al. showed the similarity between COVID-19 and Sars through the cross-reactivity of Sars-RBD specific antibodies with the RBD protein of COVID-19. The RBD protein of COVID-19 bound to human ACE2 expressing cells and did not bind to non-ACE2 expressing cells. The RBD protein from COVID-19 also bound tighter to ACE2 expressing cells than the RBD of Sars. Their results also showed that COVID-19 RBD bound to bat ACE2 expressing cells with the same intensity as it bound to the human ACE2 expressing cells alluding to the origin of COVID-19. The tighter binding they concluded explained the rapid transmission of COVID-19 in comparison to Sars. Using RBD protein from COVID-19 they were able to inhibit a pseudovirus construct from entering human expressing ACE2 cells alluding to a possible therapeutic mechanism.

ACE/AngII/AT1 Pathway (pro-inflammation) vs. ACE2/Ang(1-7)/MAS (anti-inflammation)

The Renin-Angiotensins system begins its venture in the kidney where the enzyme Renin is made (Bock et al., 1992). The liver, on the other hand, produces the other half of the system, the protein Angiotensinogen (Ohkubo et al.,1983). Both the kidney and liver release Renin (Toffelmire et al.,1989) and Angiotensinogen into the
bloodstream, where Renin cleaves the decapetide Angiotensin I from Angiotensinogen (Wu et al., 2011). Ang I travels by blood to the lungs (pertinent to COVID-19) where it is converted by another enzyme called the Angiotensin converting enzyme (ACE) to produce the octapeptide (active hormone) Angiotensin II (Corvol, Williams, & Soubrier, 1995). Depending on the availability of the Ang II receptors on human cells, Ang II induces inflammation through various biochemical and physiological mechanisms (Benigni, Cassis, & Remuzzi, 2010). This well studied ACE/Ang II axis is now complemented by the discovery of a second enzyme in RAS called ACE2 (Marian, 2013) which is also widely distributed in the body and is also found in the lungs (Fagerberg et al., 2014). ACE2 binds to Ang II converting it to the peptide Ang (1-7). This peptide in turn binds to its own receptor (MAS) inhibiting the inflammatory response (Varagic et al., 2014). In a twist of fate it is ACE2 that is the receptor for COVID-19 (Chen et al., 2020).

The ACE/AngII/AT1 (ACE) axis is a pro-inflammatory pathway characterized by cell proliferation and vasoconstriction. The driving force of this pathway is the production of Angiotensin II by the catalytic activity of the Angiotensin Converting Enzyme (ACE) upon the decapetide Angiotensin I. ACE is an enzyme peptidase located on the cell surface but is also found in a soluble form (Beldent et al., 1993). ACE is widely distributed in all human tissues including the lungs (Fagerberg et al., 2014). ACE also degrades the peptide hormone Bradykinin. Bradykinin is a blood vessel dilator and a powerful pro-inflammatory agent. The pro-inflammatory actions of the ACE pathway is initiated by the binding of Angiotensin II to the AT1 receptor. Many studies have revealed that Ang II facilitates the inflammatory cascade by regulating cells and factors involved in inflammation. Bernstein et al (2013) presents an excellent and comprehensive review on ACE. ACE is a key regulator of the various endothelial cell selectins (Larsson, Schwieler, & Wallén, 2000; Tayeh & Scicli, 1998) and promotes the extravasation of leukocytes to the site of tissue injury (Piqueras et al., 2000). As the inflammatory process ensues, the local Renin-Angiotensin system initiates vascular permeability via Ang II by inducing the expression of the vascular endothelial growth factor (Kang et al., 2006) as well as the expression of the endothelial adhesive molecules (Pueyo et al., 2002) and their ligands, the integrins (Kawano et al., 2000). Ang II also upregulates the production of cytokines (Sadoshima, 2000) and chemokines (Wolf et al., 1997) promoting the recruitment of neutrophils (Nabah et al., 2004) and lymphocytes (Crowley et al., 2010). This is by no means an exhaustive list of the role of RAS in the inflammatory process, but stresses the importance of using RAS inhibitors in cardiovascular and other pathologies, since RAS inhibitors modulate Ang II levels benefiting patients. In addition to benefiting patients by inhibiting ACE and the pro-inflammatory axis, RAS inhibitors increase the levels of ACE2 (Gilliam-Davis et al., 2011) inducing the vasodilatory and anti-inflammatory effect by the Ang 1-7 peptide (Simões et al., 2013). It is the role of ACE inhibitors and AT1 receptor blockers in upregulating ACE2 that gave pause to some in the medical community about the use of RAS inhibitors in the context of the COVID-19 pandemic (Fang et al., 2020; Esler & Esler, 2020). Since COVID-19 uses ACE2 as a recognition receptor by which to enter the cell, they argued its upregulation by RAS inhibitors could aid virus entry. Reports countering this argument allude to the rise of Ang II in COVID-19 patients, resulting from ACE2 depletion due to its endocytosis with the viral particle. They stress that the accumulation of Ang II, a pro-inflammatory hormone, would leave it unopposed to bind to the AT1 receptor promoting the inflammatory axis of RAS and RAS inhibitors would minimize the inflammatory effects of Ang II (Patel & Verma, 2020; Gurwitz, 2020; Sun et al., 2020;
There is evidence that Ang II levels are elevated in COVID-19 patients (Liu et al., 2020; Meng et al., 2020; Vaduganathan et al., 2020). In this context, it would be pertinent to understand the fate of ACE2, Ang II and Bradykinin in the context of COVID-19 and the progression of lung inflammation. The main enzyme producing Ang II and down regulating Bradykinin is ACE. By cleaving the decapeptide Ang I (Ang 1-10), ACE produces the active octapeptide hormone Ang II (Ang 1-8, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). ACE also cleaves the nanopeptide Bradykinin inactivating it.

Perhaps to be able to appreciate the connections between COVID-19 and RAS, it would be pertinent to understand the fate of ACE2, Ang II and Bradykinin in the context of COVID-19 and the progression of lung inflammation. The main enzyme producing Ang II and down regulating Bradykinin is ACE. By cleaving the decapeptide Ang I (Ang 1-10), ACE produces the active octapeptide hormone Ang II (Ang 1-8, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). ACE also cleaves the nanopeptide Bradykinin inactivating it. Historically, Ang II served as the holy grail of RAS. Ang II was at the center of much of the physiological activity of the
Renin-Angiotensin system. This however is changing due to the discovery of other active peptides and receptors adding to the complexity of RAS in systemic and local biological processes. Despite the added complexities, Ang II retains its prominent role in RAS as evidenced by the successful and widespread use of RAS inhibitors (ACEi and ARBs) targeting the production or function of Ang II. Besides having endocrine functions, regulating vasoconstriction, fluid homeostasis, cardiac output, Ang II affects local tissue by controlling many facets of cellular function such as cellular growth and death. In fact, the cellular role of Ang II is unconnected to its systemic role as a hormone. In this regard and relevant to our discussion in this review, Ang II was shown to mediate alveolar epithelial cell (AEC) death (apoptosis) via the AT1 receptor (Papp et al., 2002). Thus, Ang II is an endocrine as well as a paracrine and autocrine hormone effecting biological processes via the binding of receptors on the surface of cells. In summary, the concentration of Ang II systemically or locally depends on the concentration (gene expression, genetic control), and activity (enzyme kinetics) of various gene products. These include Renin, Angiotensinogen, ACE, and ACE2. Thus, the role of Ang II in the COVID-19 saga will depend on the concentration (Ang II concentration vs virus titer) of both actors, since both Ang II and COVID-19 bind to the same enzyme protein, ACE2. COVID-19 opportunistically binds to ACE2 gaining entry into the cell. In this case, both the virus and ACE2 are endocytosed. This depletes the levels of ACE2 on the cell surface, inhibiting the production of Ang 1-7, the anti-inflammatory peptide, and in the meantime raising the concentration of Ang II, the pro-inflammatory peptide. Indeed, it has been shown that COVID-19 patients have an elevated level of Ang II peptide (Liu et al., 2020). The rise in Ang II is understandable in the context of ACE2 depeletion after viral infection and does not give us a baseline for the patient before infection. It would be interesting to see whether increasing Ang II concentration can interfere or change the conformation of ACE2 to prevent it from binding to the receptor-binding domain of COVID-19. Is there any indirect evidence that can lead us to think along these lines encouraging a research effort? If we consider the demographics of the current pandemic, we see two important and recurring themes in most countries. Children are spared, incurring a milder infection, while adults and especially the elderly are the hardest hit (China CCDC, 2020; World Health Organization, 2020). Yet most of the publications we reviewed focused on the elderly population with cardiovascular complications and ignored the benign effect of the virus in children. This could be coincidental and explained away by children having less exposure to the virus. Yet when we apply the same logic vis a vie influenza, children are just as prone to infection and severe symptoms. In fact, children like the elderly are more susceptible to infection than other groups, which is not the case with COVID-19. Perhaps a more accurate and targeted explanation in the context of COVID-19 would be the Ang II/Bradykinin connection. COVID-19 enters via ACE2 and in children, Ang II, the natural substrate of ACE2, is elevated above adult levels (Broughton Pipkin, Smales, & O'Callaghan, 1981). We venture to guess that the lower infection rate and the absence of lethality in children is attenuated by the high Ang II levels (Figure 2). This could also be compounded by low Bradykinin levels, which would abort the ensuing inflammation and edema leading to a benign infection. In the elderly Ang II levels are depressed in comparison with the child population and they are further depressed by ACE inhibitors. ACE inhibitors are known not only to raise ACE2 levels (the viral port of entry) but also Bradykinin levels (inflammation and edema). The combination of higher ACE2/lower Ang II, which can lead to a much wider infection of the cell population and the higher Bradykinin levels, which can lead to a potent inflammatory/edemetic response results in a malignant infection (Figure
3). This demographic fact necessitates that we study the effect of increasing Ang II on the entry of virus in ACE2+ cells. It also necessitates that we consider Bradykinin inhibitors in the collection of drugs being investigated for COVID-19.

**Conclusion**

To appreciate the dynamics of COVID-19’s connection to RAS and its resultant infection and ensuing inflammatory response, we need to envision the two axes of the renin-Angiotensin system: the pro-inflammatory axis choreographed by ACE/Ang II via the AT1/AT2 receptors and Bradykinin, vs the ACE2/Ang 1-7 promoting the anti-inflammatory axis via the Mas receptor. By attacking the anti-inflammatory axis, COVID-19 down regulates ACE2, since the cells engulf the virus particle along with the ACE2 enzyme, leaving the pro-inflammatory axis unopposed resulting in severe lung inflammation. This has led to the proposition of opposing views on RAS inhibitors. In one view, Lei Fang and colleagues advocated withholding Angiotensin converting enzyme inhibitors (ACEIs) or Angiotensin receptor blockers (ARBs) due to the possibility that they can upregulate ACE2, COVID-19’s port of entry. Demographically the group with the highest risk of Infection complications was hypertensive patients, which tends to support Lei Fang et. al’s hypothesis. Christopher J Tignanelli et al. 2020, published an opposing view, that withholding RAS inhibitors is premature, stressing the protective nature of ACE2 and losartan infusions in preventing damage by induced lung injury in various animal studies. We believe both views have merit and are not necessarily in opposition when we consider the dynamic relationship between COVID-19 and the pro-inflammatory and anti-inflammatory axes of RAS. Perhaps the
solution lies in the timing. ACE and ACE2 are mutually antagonistic. RAS inhibitors are not identical in action nor targets. ACE inhibitors target ACE and Bradykinin and are ineffective against ACE2. ACE inhibitors decrease the production of the hormone Ang II inhibiting both axes by decreasing the binding of Ang II to AT1 and ACE2. The decrease in Ang II and increase in Bradykinin in hypertensive elderly patients using ACEI meds is in contrast to the high Ang II levels in children. This dichotomy in children (High Ang II, low Bradykinin, mild infection) and the elderly (low Ang II, high Bradykinin, lethal infection) can help us elucidate a working hypothesis when considering COVID-19 infection. ACE inhibitors reduce Ang II, while raising ACE2 and Bradykinin levels. Does COVID-19 bind tighter to ACE2 in the absence of Ang II? Does COVID-19 produce a malignant inflammatory response in the presence of Bradykinin? Could this be partly responsible for the virulence of the virus in the elderly? In this regard, we need to distinguish between patients on ACE inhibitors vs AT1 blockers. Hypertensive elderly patients on AT1 receptor blockers would have an inhibited pro-inflammatory pathway, while keeping Ang II levels high (as in children) occupying ACE2 and preventing COVID-19 binding. In the event of virus entry into the cells and the down regulation of ACE2, would leave Ang II unopposed to bind the AT1 receptor thereby driving the proinflammatory response in lung tissue. At this stage, the use of AT1 receptor blockers can indeed lessen the inflammatory response without inhibiting ACE and upregulating Bradykinin. This scenario would support the hypothesis of the use of AT1 blockers to minimize damage and inflammation to the lungs and other affected organs. We would venture to guess and propose the use of Bradykinin antagonists to prevent the pro-inflammatory response, vascular permeability and edema that could develop in the course of infection. Perhaps both hypotheses have merit when considering the timing of RAS inhibition prior to viral infection and during the course of viral infection. We propose the continuation of RAS inhibitor use due to its crucial medical benefit to the elderly until an infection has been confirmed. Once infection has occurred and progresses, we favor the use of AT1 receptor blockers, which would be beneficial in protecting lung tissue from inflammation and damage. We would also urge the medical and scientific community to test the possibility of using

Figure 4. Ang II loading coupled with AT1 receptor blockers and Bradykinin antagonists can minimize if not stop the continuing infection of tissue by budding viral particles. This configuration of of effectors mimics the state of RAS in children, where the higher Ang II levels serve as a guard preventing wide-spread and rapid infection of the cells. This attenuates the infection and does not trigger an inflammatory response leading to a benign infection.
Bradykinin inhibitors or in combination with AT1 receptor blockers to minimize the damaging inflammatory response and pulmonary edema associated with COVID-19. Finally, we propose a novel approach and that is the use of intravenous “Ang II loading” in these patients in combination with AT1 receptor blockers to prevent the further infection of tissues by the budding off viral particles (Figure 4). Ang II in this case would serve as a guardian of ACE2 in non-infected cells. A battle dictated by binding constants, conformational maneuvers and gibbs free energy. Ang II might turn out to be the arrow in Corona’s heel.

**Future Directions**

To elucidate future directions of research in this field, we propose a working model that can be tested and applied. COVID-19 produces a virulent infection in the elderly (hypertensive patients) and a benign infection in children. In children there appears to be high Ang II/low Bradykinin levels coupled with low ACE2. We propose that this combination of effectors leads to a benign COVID-19 infection. The exact mechanism remains obscure but we can predict that virus entry in the cells of children is attenuated due to the lower ACE2 and the higher basal Ang II levels prior to infection. Ang II, by occupying the catalytic site of ACE2, might change ACE2 conformation enough to interfere with viral binding. This can be tested in the laboratory by increasing the concentration of Ang II in the medium of ACE2+ cells and determine whether this inhibits viral entry. In the elderly population, ACE inhibitors lower Ang II, upregulate ACE2 and elevate Bradykinin. Under normal circumstances, this is an ideal health condition. If we consider COVID-19 infection, however, this “state” of RAS might predispose the elderly to a virulent infection. Since COVID-19 uses the ACE2 as a port of entry and ACE2 is elevated in this population accompanied by a depression in Ang II. The decrease in Ang II might give the virus an open target (ACE2) with the proper conformation for binding. A further complication in the elderly would be the elevated Bradykinin levels. Under normal circumstances, Bradykinin would promote vasodilation and heart health. During viral infection, however, Bradykinin becomes a potent inflammatory mediator promoting edema and vascular permeability. Inhibiting Bradykinin might mitigate the virulent inflammatory response in these patients. Perhaps a perfect therapy for COVID-19 patients, besides the combination of AT1 receptor blockers and Bradykinin antagonists, would be the counterintuitive use of “Ang II loading” in these patients. This would mimic the RAS “state” in children and perhaps abort the continuing infection of the budding viral particles.

We urge the scientific and medical communities to test this working model in the search for a solution for COVID-19.

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