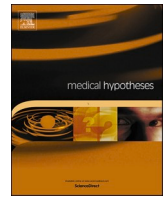




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A tale of two diseases: Sarcoidosis, COVID-19 and new therapeutic options with dual RAS inhibition and tetanus-diphtheria vaccine

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ABSTRACT

Sars Cov-2, the pathogen which belongs to the beta coronavirus family that is responsible for COVID-19, uses Angiotensin Converting Enzyme 2 (ACE2) as a receptor, which is responsible for controlling the actions of renin-angiotensin system (RAS). Sars Cov-2 - ACE2 binding leads to a RAS mediated immune response, which targets especially lungs to form ARDS, which in turn, is the most important cause of mortality in COVID-19. CD8⁺ T cell response dominates over CD4⁺ T cell response and natural killer cell dysfunction also leads to CD4⁺ cell dysfunction in COVID-19; this immune dysregulation leads to inappropriate (ARDS) and inadequate (low or quickly waning antibodies) responses to the disease and unfortunately, prepares the patients for re-infections. The peripheral anergy seen in chronic sarcoidosis has much resemblance to COVID-19; CD8⁺ T cell accumulation is also responsible for inadequate reaction to tuberculin and antigenic stimulus. This article, based on the similarity of COVID-19 and sarcoidosis, discusses a combination of the therapeutic strategy of the tetanus-diphtheria vaccine and dual RAS inhibition, alongside with hydroxychloroquine and antiviral agents, as a solution to overcome the problems described above.

Introduction

On December 31st, 2019, an unknown disease, later called COVID-19 and caused by a yet unrecognized microorganism, was reported to have been appeared in a Huanan seafood market in Wuhan city, China [1]. It was eventually recognized as a new coronavirus variant that was designated Sars Cov-2. The COVID-2019 epidemic had become a pandemic by the World Health Organization on March 3rd 2020 and since the onset of the disease, many articles have been published, describing the etiology, pathogenesis, clinics and the treatment approaches to the patients. An international effort on creating a feasible treatment and producing vaccines has been on-going ever since.

Sars Cov-2 belongs to coronavirus family that had similarities to earlier Sars Cov and Mers Cov [2,3]. The infection starts with fever and non-productive cough and proceeds to dyspnea, respiratory failure and adult respiratory distress syndrome (ARDS), related to bilateral interstitial pneumonia [4]. Guan and colleagues have described the clinical and laboratory properties of 1,099 patients who contracted COVID-2019 in Wuhan and found that fever (87.9%) and cough (67.7%) was the most common presenting symptoms of the disease [4]. The median incubation period was 3 days, and the median age was 47 years. Leukopenia, lymphopenia and thrombocytopenia were the most common laboratory

findings of these patients. During the hospitalization, pneumonia, ARDS and shock were the most common complications of the disease (79.1%, 3.37% and 1% respectively). On admission, 81% of patients have been reported to be mild, 14% of the patients severe and 5% of them critical and treated in intensive care unit [5]. Overall fatality rate has been reported at 2.3%, rising to 8% in patients aged between 70 and 79 years and up to 14.8% in patients older than 80 years. The overall fatality rate was 49% in critical patients [5]. In contrast, in Italy, 24.9% of patients have been reported to be severe and 5% of them critical [6] although 16% of all hospital admissions [7] have been warranted to apply to intensive care unit. This clearly highlights the risk of overcapacity of the intensive care units and all health systems. Several drugs have been tested for the treatment of the disease. Remdesevir [8] lopinavir-ritonavir [9] hydroxychloroquine (HCQ) [10 11] teicoplanin [12] azitromycin [13] favipravir [14] and some combinations of these were all tested with some success, but so far death tolls have risen in all parts of the world. In addition, there have already been some reports and studies announcing that antibody response against COVID-19 might not be adequate to protect from re-infections; hence the danger of being infected by Sars Cov-2 repeatedly can be a real threat [15-17]. Here, the author assumes that none of the drugs or vaccines by themselves are capable of bringing an abrupt end to the search for a cure and an

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adequate antibody response; rather, a combination strategy (dual renin-angiotensin system (RAS) inhibition and tetanus-diphtheria vaccine along with antiviral and HCQ) is required to overcome COVID-19, as a result of the complex nature of the disease.

Hypotheses

Sars Cov-2 is a unique virus that seems to project the host's immune system onto itself; therefore the mortality and morbidity come from the effects of immunity, not primarily from the direct effects of the virus [18]. A change in the direction of the immune response to the virus itself, away from the host appears to be required, and immune modulation seems to be the key pathway to achieve a conclusive result. Nevertheless, corticosteroids, chloroquine, HCQ, anakinra, tocilizumab and Jak inhibitors have been tested, especially in patients with Macrophage Activation Syndrome (MAS) which is associated with cytokine storm and mortality [19]. The author hypothesizes that dual RAS inhibition with Ramipril and Losartan, and tetanus-diphtheria vaccine administration at the beginning of the disease, may alter the immune response, avert MAS and ARDS pathway, produce a sustained, durable antibody response and prevent re-infections until the end of the pandemic.

Relation between COVID-19 and RAS

Coronavirus and RAS activation

In 2002, a novel beta coronavirus infection that had later named Sars Cov had emerged in Guangdong, China. It had infected 8,000 patients and caused 774 deaths in 37 countries [20]. Later on, in 2012, Mers Cov, also a beta coronavirus originating in Saudi Arabia, caused 2,494 confirmed cases and 858 fatalities [3]. These corona viruses had a common binding site called Angiotensin Converting Enzyme 2 (ACE2), which has been linked to ARDS [21,22]. RAS, as an important regulator of blood pressure, has direct effect on bone marrow and has unique capabilities affecting the immune response [23-25]. Renin cleaves angiotensinogen to generate angiotensin (Ang) I. Ang I is inactive: it requires to be cleaved by ACE in the lungs to be active and then Ang I activates Ang II. Ang II is a key regulator of the RAS and exerts biological functions through the specific receptors called Ang II receptor type 1 (AT1R) and Ang II receptor type 2 (Fig. 1) [22,23,26]. Ang II, which acts a vasoconstrictor in the smooth muscles of peripheral vessels through ATR1 binding and RAS pathway inhibition, is a major treatment option of hypertension [23].

For many years, ACE has been known to be the only pathway regulating the functions of RAS. In 2000, two independent study groups isolated a new homolog of ACE, named ACE2. This homolog has unique properties: it was found to cleave one nucleotide from Ang I and one nucleotide from Ang II, to form Angiotensin 1-9 and Angiotensin 1-7 respectively (Fig. 2) [27,28]. Angiotensin 1-7 has been found to exert counter-regulatory effects of ACE on lungs, kidney, heart, testes and colon [22]. After the SARS outbreak in 2003, it was demonstrated that ACE2 as a receptor in the lungs, was the main binding and disease activation site of Sars Cov and this binding was a key factor: the inability of the virus to bind the receptor effectively prevents the formation of ARDS [29,30]. It was demonstrated that, after binding of the virus to receptor, ACE2 concentrations in the lungs dropped significantly, and loss of counter-regulatory actions led to the activation of RAS [17]. ACE2 knockout mice had developed lethal ARDS, whereas blocking ACE, ATR1 receptor or treating with ACE2 recombinant protein, had significantly reduced or improved acute lung injury in the Sars Cov infection [22,30,31]. When ACE2 functions ceased to exist, excessive RAS activation occurred in the mouse and human body, which caused severe inflammation, acute lung injury and ARDS. Through these mechanisms it was assumed that Angiotensin Converting Enzyme Inhibitors (ACEi) or Angiotensin Receptor Blockers (ARBs) could have a positive impact on prognosis of the disease [32].

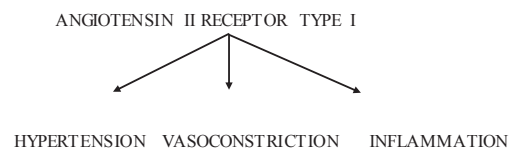
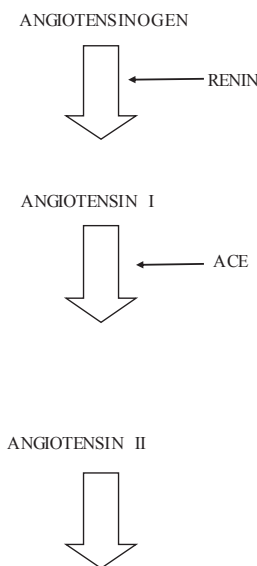


Fig. 1. Schematic explanation of renin-angiotensin system.

Gene Polymorphism, ARDS and COVID-19

The human ACE gene is located on chromosome 17q23 and contains a restriction fragment length polymorphism within the coding sequence of intron 16, defined by the presence (insertion, I) or absence (deletion, D) of a 287-bp repeat, that led to determine the function of ACE [22,33]. It has been shown that ACE activity was significantly increased in patients with D/D and D/I phenotype. Patients with ARDS had carried the D phenotype significantly compared to those who had not had ARDS in intensive care units; mortality rates of these 'D phenotype' patients were also significantly higher [22,33-35]. It was shown that ACE2 receptor binding was essential for the entry of Sars Cov-2 to the lungs, so RAS must be the responsible pathway, as in Sars Cov, for acute inflammation, lung injury and ARDS (Fig. 3) [36]. In the light of these findings, ACE inhibition and/or AT1R blockade, may have a therapeutic value in COVID-19 [37]. In fact, recently, Gomez and colleagues found that in COVID-19 patients, ACE D/D and D/I polymorphism significantly associated with worse disease outcome; but the significance of the latter polymorphism seemed to be associated with hypertensive status [38].

RAS inhibition and immune modulation

How RAS and ACE2 related pathways could lead to these responses is a matter of debate and many studies have been done to clarify whether RAS also has immune-modulatory effects. Zhao and colleagues have found that ACE affected the presentation of MHC class II antigens by producing an increased CD4⁺ T cell and antibody response, and ACE inhibition with Lisinopril downregulated the activation of antigen presenting cells. The authors concluded that manipulation of ACE expression by antigen-presenting cells might be a novel strategy to alter the immune response [24]. Winfield and colleagues evaluated 1,952 obese patients and found that patients taking ACEi or ARBs, had improved T cell function and leukocyte maturation [39]. Schmeisser and colleagues had found that Losartan and Ramiprilat, an ARB and an ACEi, inhibited

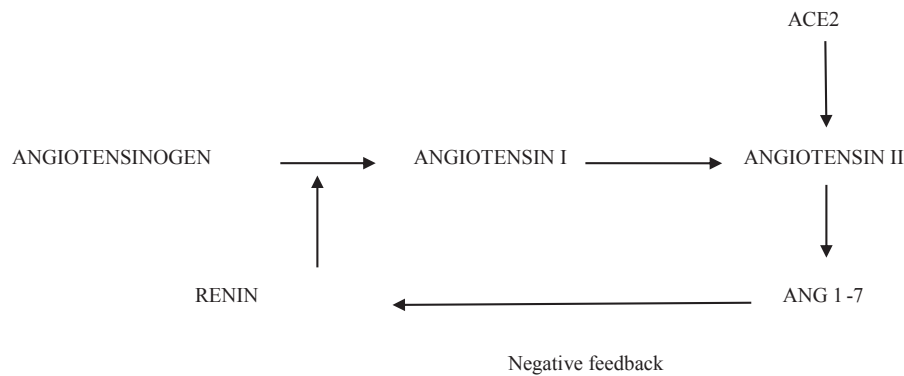


Fig. 2. Down-regulation of renin by Angiotensin 1–7. Angiotensin Converting Enzyme 2 cleaves one nucleotide from Angiotensin II to form Angiotensin 1–7.

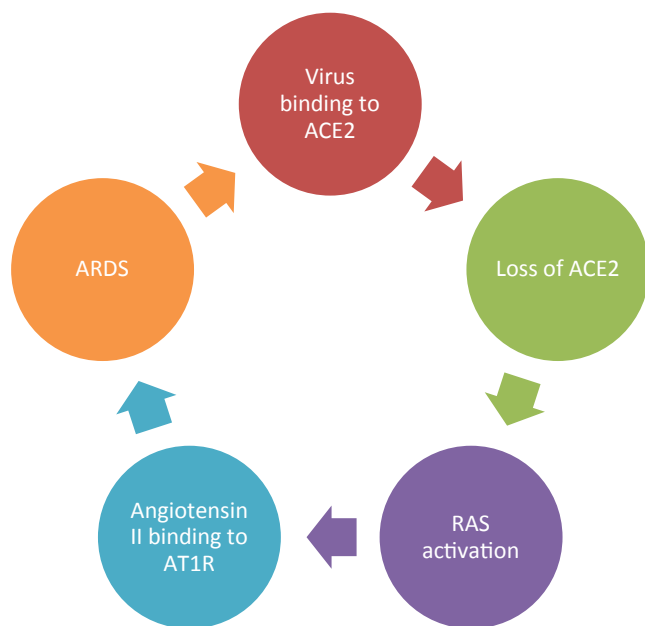


Fig. 3. Renin-angiotensin system activation due to SARS COV-2 and its relation to Acute Respiratory Distress Syndrome. AT1R: Angiotensin II receptor Type 1.

Ang II-induced up-regulation of IL-8 and MCP-1 protein in vitro, due to reduced AT1R expression and down regulation of NF- κ B activity [40]. In Sars Cov and Mers Cov, down regulation of CD4⁺ T cell count has led to a severe interstitial pneumonia and a delay in the clearance of Sars Cov in the lungs; up regulation of IL-8 exacerbates the situation [41]. ACE and ARB inhibition has been shown to resolve both of them in cell culture studies [40]. Local pulmonary RAS is also directly responsible for pulmonary homeostasis. ACE and ACE2 are found on both pulmonary endothelium and epithelium [42,43]. There are two types of alveolar epithelial cells: alveolar type 1 cells are responsible for gas exchange and alveolar type 2 cells secrete surfactant [43]. In the alveolar epithelium, minor ACE2 expression is found (1–7% of AT2 cells) [44,45], but a very recent study showed that pulmonary epithelial stem cells also expressed ACE2 and could be infected with Sars Cov-2 [43]. This study explained the widespread distribution of lung disease despite minor ACE2 expression in lungs, and ACE inhibition may also affect the pathogenesis of COVID-19 via local RAS inhibition. In fact, local pulmonary RAS was responsible for apoptosis of pulmonary alveolar epithelial cells, Angiotensin II played a pivotal role in Fas and TNF- α related apoptosis and RAS inhibition seemed to prevent this process in previous studies [46,47].

Dual RAS inhibition

Sars Cov-2, like the former Sars Cov, exhibits its deleterious effects on lung injury by binding to ACE2 as a receptor in the lung. All human coronaviruses have the capability to bind ACE2 and bat-coronaviruses do not have any, but it is thought that before infecting to humans, these bat-coronaviruses also evolved to gain ACE2 binding capabilities [48]. After binding, the ACE2 activity is blocked and counter-regulatory mechanism is lost so that RAS activation and RAS related ARDS occurs because of unchecked Ang II influx to the lungs; RAS inhibition was shown to increase mRNA activity or direct concentration of ACE2 in the studies [49–51]. Therefore, it can be said that in COVID-19, deaths occur not from the direct pathogenic effects of the virus, but rather from the unbalanced immune response to Sars Cov-2. It has been debated that RAS inhibition must be harmful, as the virus used ACE2 as its receptor to enter the pulmonary system [52]. Blocking RAS should hence introduce more virus entry in the lungs, heart or other organs which ACE2 expression incurred, but after a more careful evaluation, the author of this paper proposes that the immune modulatory and protective/treating effects of RAS inhibition from ARDS, can be an effective weapon for fighting with COVID-19 and a two steps approach is needed to suppress RAS activity, based on some evidence. First, ACEi / ARB's have an immune-modulator activity, such as increasing the CD4⁺ T cell count, improving the imbalance of CD4/CD8 ratio and improving its functions so as to prevent severe interstitial pneumonia, down-regulating IL-8, which plays an important role in the pathogenesis of COVID-19, and enhance antibody production [18,40,41]. Also, RAS inhibition was shown to increase mRNA activity or direct concentration of ACE2 in the studies [49,50,53], so as ACE2 inhibits RAS via Ang 1–7, the increase in ACE2 concentration and/or mRNA should add an additional weapon to suppress RAS activity and modulate immunity to treat or prevent pneumonia and ARDS. A detailed review recently evaluated the particular possibility of using ARBs for COVID-19 [51]. The authors speculated that the increase in Ang II would lead to attachment to ACE2 and this binding could make conformational changes on ACE2, so that the binding of Sars Cov-2 might be prevented. In fact, recently, two meta-analyses have clearly shown that RAS inhibition had significantly decreased severe disease and death [54,55] and Flacco and colleagues found that the use of ACEi/ARBs did not relate to COVID-19 associated mortality [56]. Therefore, a two-step approach should be useful for effectively suppressing RAS in a limited time.

COVID-19, sarcoidosis and Tetanus-Diphtheria vaccine

Sarcoidosis is a chronic multi-system inflammatory disease that is presented with chronic granulomas and mononuclear cell inflammation and destruction to affected organs [57]. The lung is the most involved organ in sarcoidosis, though every system in the human body can be affected [58]. Its etiology is currently unknown and at about two thirds

of the patients achieved a durable remission, whereas one third of them presented with a chronic disease that eventually led to fibrosis and severe organ dysfunction. Sarcoidosis is a true immune paradox, a very busy immune system with peripheral anergy associated with reduced delayed type hypersensitivity reaction to tuberculin and antigenic stimulus [58,59]. CD4⁺ T lymphocytes are responsible for regulating B lymphocytes which mediate antibody response and CD8⁺ T lymphocytes, which are associated with responses associated with eliminating microorganisms and Natural Killers (NKs), are the major factor managing the CD4⁺ T cells [60]. In sarcoidosis, this anergy was explained by the accumulation of CD8⁺ T lymphocytes in peripheral blood of the patients [61]. In addition to that, a disequilibrium between effector and regulator T lymphocytes was seen in chronic sarcoidosis [59]. NKs also play an important role in the pathology of the disease; reduced number of NKs was associated with chronicity and impaired antibody response in patients with chronic sarcoidosis as CD4⁺ T cell responses are also directly affected. [60]. The same pathways are also active in COVID-19: reduced number and impaired maturation of NKs were found in severe COVID-19 patients [18,62]. Increased interleukin-6 (IL-6) activity and checkpoints on NKs seemed to be related to these abnormalities on NKs [63,64]. Also, the number of both CD4⁺ and CD8⁺ T lymphocytes decreased in correlation with the disease severity in COVID-19 and when IL-6 activity was blocked Tocilizumab, lymphocyte counts increased [65]. CD8⁺ T lymphocyte responses, like sarcoidosis, seemed to be much more activated than CD4⁺ positive ones [66] regardless of disease severity, so we can say that a peripheral anergy can be expected in COVID-19 as in sarcoidosis. This can be an explanation of why antibodies towards Sars Cov-2 seemed to wane over a few months, or did not appear at all in some patients. Without a proper antibody response, patients are at risk of contracting the virus repeatedly, which could lead to catastrophic results. Aside from dual RAS inhibition, the Tetanus-diphtheria vaccine may be an additional weapon that helps to regulate the immune dysfunction and can lead to better and long-lived antibody responses. Haghghat and colleagues have shown that co-administration of hepatitis B and tetanus-diphtheria vaccines significantly increased the titers of the antibodies against hepatitis B, compared to hepatitis B vaccine administration alone [67]. The augmented response seemed to be related to increased activity of CD4⁺ T lymphocytes which mediate increased antibody activation. The undersigned, as a patient of chronic sarcoidosis, successfully applied this approach after an unsuccessful attempt to vaccinate for hepatitis A, a second vaccination trial with tetanus-diphtheria vaccine produced a high titer antibody response against hepatitis A. Ietto G. published this hypothesis for using tetanus-bordetella and diphtheria trivalent vaccine for treatment of COVID-19 and also highlights the importance of CD4⁺ T cell activation, and suggested that vaccine administration must be applied at the beginning of the disease so as modulate immune system and avert ARDS [68]. The author of the present paper suggests that, based on published data and his personal examination, with dual RAS inhibition: vaccine administration not only can help to avert ARDS, but also modulate the immune system to produce a durable antibody response to Sars Cov-2, as the immune dysregulation and pathology of impaired antibody response are similar in chronic sarcoidosis and COVID-19. In summary, the immunopathology of COVID-19 is complex and there must be an active approach for the treatment of the disease not to allow the immune influx to lungs and other systems. An antiviral is required to combat this virus, but any antiviral alone cannot protect the patients who are driven to ARDS by their immune system, so immune modulation is clearly needed to overcome this problem. The author suggests using Tetanus-diphtheria vaccine, dual RAS inhibition and hydroxychloroquine at the beginning of the infection, along with an antiviral. HCQ acts as an immune modulator and has been shown to exert an antiviral effect during pre- and post-infection conditions, by interfering with the glycosylation of ACE2 and blocking virus fusion with the host cell. In this case, we can speculate that ACEi or ARBs along with HCQ can be used to block the formation of ARDS and to treat it without much risking of more virus

entry into the lung or heart, as HCQ blocks the binding of the virus to ACE2 [69]. There had been some randomized trials announcing the ineffectiveness of the HCQ in COVID-19 [70-72] but more recently, two meta-analysis have shown that HCQ had been effective both in active disease and prophylaxis [73,74]. Dual RAS inhibition should start by 50 mg of Losartan and 2.5 mg of Ramipril and a "step by step increase" approach should be used to avoid intolerance. First, the dose of Losartan should be increased to a maximum of 100 mg, then the Ramipril dose should be increased to a maximum of 10 mg if the patient tolerates it; otherwise, the drugs dose should be adjusted to the maximum tolerable dose. Patients who took ACEi or ARBs should use whichever class he/she have not used. In hypertensive patients already using ACEi/ARBs, chronic RAS inhibition would lead to cause an increase in renin levels by negative feedback, to such a level that the inhibitor function of the drugs is overwhelmed and more Ang II would be formed by an increase in plasma renin activity, to continue the cycle [23]. In addition, it has been shown that patients with D phenotype of ACE gene polymorphism had more Ang II formation and were more susceptible to ARDS and death [22,33,38]. Therefore, with time, chronic ACE inhibition/AT1R blockade could be insufficient to block the RAS activity. The author proposes that dual inhibition of RAS by both an ACEi and ARBs, can overcome both an increase in gene polymorphism-related ACE activity and negative feedback to increase plasma renin, even in patients already using a RAS inhibitor (Fig. 4). The time to prevent/treat the pneumonia of the patient with Sars Cov-2 is short, at approximately 7–14 days, so the belief is also that there will not be much time for the formation of an increase in negative feedback-related plasma renin activity, and the RAS inhibition should be rapid with the two drugs. The treatment should be started as soon as the patient is diagnosed with COVID-19, along with an antiviral agent which is shown to reduce the Sars Cov-2 load and HCQ which is shown to interfere the binding of virus to ACE2. The patients who will most benefit from the treatment will be those who are in the initial stage of pneumonia/ARDS and do not use any of these RAS inhibitors; nevertheless, some treatment activity can be expected, even for patients with severe ARDS, as shown in rat studies [22,31]. By adding dual RAS blocker, we would be treating COVID-19 with three different pathways: the antiviral reduces the viral load, HCQ blocks the entry of Sars Cov2 via ACE2 blockade and modulate immunity, and the ACEi plus ARBs plus tetanus-diphtheria vaccine inhibits and modulates the RAS dependent immune response.

Several problems may complicate this hypothetical protocol. First, the tetanus-diphtheria vaccine may stimulate fever, which is also a common symptom in COVID-19. Second, it is well known that taking a dual RAS blocker may cause excessive hypotension, which may dangerously increase blood potassium levels and creatinine levels to such an extent that urgent hemodialysis would need to be performed on the patient [75,76]. In addition, such problems can already be in place in patients with

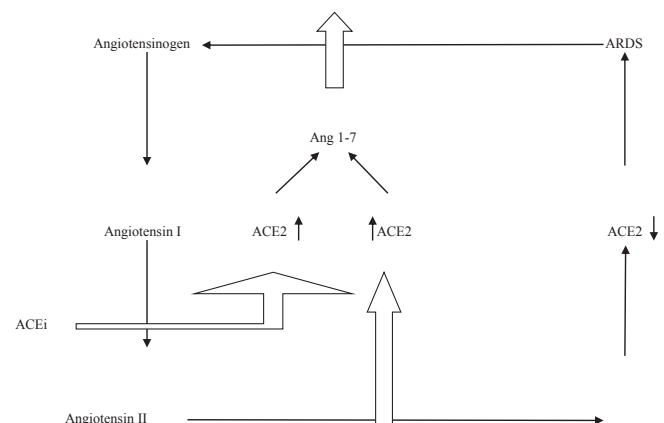


Fig. 4. Effects of dual renin-angiotensin inhibition.

severe COVID-19 disease. Dual RAS inhibition has been fallen out of sight after several large trials showed complications and worsening of renal function or no additional benefit on the long run [75,77]. We are discussing, for only a short period of time, the use of the dual RAS blockade starting with low doses and then increased if patient tolerates, in order that it does not complicate the treatment. In fact, in On-Target study, when the patients used Telmisartan plus Ramipril for 18 days in run-in period, of 29,919 patients enrolled for the study, 492 patient (1.7%) had symptomatic hypotension, 223 patients (0.8%) had hyperkalemia and 64 patients (0.2%) had a worsened renal function [75]. Given the few number of patients experiencing complications, in a short limit of time such as in our hypothetic protocol, it can be wise to consider using a dual RAS inhibition and tetanus-diphtheria vaccine in COVID-19, with close monitoring for fever, symptomatic hypotension, elevated potassium levels and worsening renal function with a new hope to save patients from being "struck down" with their own immune response. Urgent prospective studies are clearly needed to evaluate the possible benefits of this proposal.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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