Letter to the Editor

The active-site engineered “inactive hrsACE2” could be considered as a safer therapeutic agent in the treatment of COVID-19: An Opinion

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Dear Editor,

We read with great interest the inspiring paper by Monteil et al. (Monteil et al.). Their paper aimed to prove that human recombinant soluble ACE2 (hrsACE2) reduces SARS-CoV-2 recovery, *in vitro*, and also shows that SARS-CoV-2 can directly infect engineered human blood vessel/kidney organoids, which can be inhibited by this enzyme. The respectful authors expressed that hrsACE2 can block early entry of SARS-CoV-2 infections in various host cells, especially alveolar epithelial type II cells, as a viral reservoir and stated that they cannot make any predictions with respect to the effect of the recombinant protein on the later stages of COVID-19 and, also, honestly mentioned the study limitations. They also stated further (*in vitro*, pre-clinical and clinical) studies are needed to address the effect of hrsACE2 at later stages of SARS-CoV-2 infection. In the critical situation, these days, where hundreds of thousands of world population are diagnosed as COVID-19 confirmed cases and tens of thousands have lost their lives so far, and on the other hand, since we do not have the facilities to achieve our opinion in the laboratory; we share this opinion with you; dear editor, respectful authors of the above article and all my colleagues around the world.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) like SARS-CoV-1 interfere with the renin-angiotensin-aldosterone system (RAAS) through angiotensin-converting enzyme 2 (ACE2), an enzyme that physiologically counters RAAS activation but also functions as a receptor for both SARS viruses (Vaduganathan et al., 2020). RAAS includes angiotensinogen, angiotensin-converting enzyme (ACE) and also angiotensin converting enzyme 2 (ACE2), and angiotensin II (Ang II) type 1 and type 2 receptors; ACE produces the Ang II, while ACE2 reduces Ang II concentration; cleaves a single residue from Ang II to generate Ang 1-7. The RAAS is one of the determinant factors of acute respiratory distress syndrome (ARDS) which is frequently associated with multiple organ dysfunctions leading to high mortality, is an important clinical feature of COVID-19. Regarding a recently published

report in the Lancet (Huang et al., 2020), clinical characteristics in patients with COVID-19 pneumonia who developed ARDS are frequent and the mechanisms underlying ARDS are multi-factorial as well.

Only 20% of capillary endothelial cells in all other organs express ACE, while it is expressed in entire capillary network of the alveoli in human lung (Metzger et al., 2011), resulting in readily conversion of Ang I to Ang II in the lung pulmonary vessels. On the other hand, ACE2 is primarily produced in Clara cells and type II alveolar epithelial cells (Wiener et al., 2007). In COVID-19, due to the virus-mediated ACE2 internalization and epithelial injury as well, the ability of alveolar epithelial cells to produce ACE2 is severely impaired. Therefore, upon dominant ACE activities during ARDS (and/or ventilator-induced lung injury), patients encounter rapid vasoconstriction/low blood flow in the pulmonary circulation, followed by ventilation/perfusion mismatch (Vaduganathan et al., 2020). Moreover, since ACE2 is expressed in various tissues including the heart, kidney tubules, the luminal surface of the small intestine, and blood vessels (Monteil et al.) and references therein, SARS-CoV-2 could also infect these tissues, so that clinically, SARS-CoV-2 has been found in the urine and cardiovascular and renal dysfunctions have been reported for many patients with COVID-19.

As mentioned above, due to the virus-mediated ACE2 internalization and epithelial injury as well as dominant ACE activities during ARDS, increased plasma concentration of angiotensin II is anticipated. In a small study, Liu et al. revealed that patients with COVID-19 have significantly elevated levels of plasma angiotensin II compared to that of healthy individual, which were in turn correlated with total viral load and degree of lung injury (Liu et al., 2020). Moreover, it may be expected that (at least severe/critically ill) COVID-19 cases to have some degree of hypertension.

According to Zhou et al (Zhou et al., 2020), different comorbidities have been with nearly half of COVID-19 patients, with hypertension being the most common comorbidity (only 30%), followed by diabetes and coronary heart disease. In the other study with similar results, chronic hypertension was more frequent among deceased patients than recovered patients (48% vs. 24%) (Chen et al., 2020). They reported that more patients who died had arterial pressure of 140 mm Hg or higher, but median systolic blood pressure in deceased patients was 137.0 mm Hg, ~ 10 units greater than that of recovered patients (125.0 mm Hg). Normal blood pressure is considered to be between 90/60 mmHg and 120/80 mmHg. High blood pressure is considered to be 140/90 mmHg or higher. In this regard, blood pressure 120 to 139 mmHg is called pre-hypertension; blood pressure 140 to 160 mmHg is called Grade 1 hypertension and above 160 mmHg is called Grade 2 hypertension (Camm et al., 2009; RANA, 2015). So, hypertension appears not to be a COVID-19 clinical feature and it can be concluded most patients have normal blood pressure. Also, we must note that some cases may have hypertension as underlying diseases. A meta-analysis of the

comorbidities suggests that hypertension prevalent in approximately 17% of the patients, which almost equals the prevalence of hypertension in the entire adult population of China (~23%) (Yang et al., 2020). This work introduces hypertension comorbidity as an important risk factor for critical patients. In another study (Guan et al., 2020), with 1590 patients (the mean age was 48.9 years), severe cases accounted for 16.0% of the study population which was equal to prevalence of hypertension, as the most prevalent comorbidity (16.9%). They interestingly observed that, on admission, 10-15% of patients reported as having hypertension, and also found that younger patients (1191 patients with the mean age of 44.8 ± 15.2) had no morbidity and older cases (399 patients with mean age of 60.8 ± 13.4) had at least one morbidity (including hypertension). Regarding the above statements, and although significantly high levels of plasma angiotensin II for COVID-19 patients have been reported (Liu et al., 2020), it is logically concluded that **a)** hypertension cannot be considered as a consequence of COVID-19 and **b)** it is an absent clinical feature in mild COVID-19 cases.

As mentioned above, patients with COVID-19 have significantly elevated levels of plasma angiotensin II compared to that of healthy individual and membrane-bound ACE2 (in addition to protecting from lung injury, based on its catalytic domain) is the critical *in vivo* SARS-CoV spike glycoprotein receptor. In this regard, soluble but active ACE2 may be beneficial in COVID-19 patients (Vaduganathan et al., 2020); soluble ACE2 can effectively compete with the membrane enzyme (and limits virus binding/entrance into the host cells) and also decreases the plasma levels of Ang II. Several research groups (including (Monteil et al.)) believe that restoration of ACE2 through the administration of recombinant ACE2 reverse (though in part) this devastating lung-injury process. In a human study, using a recombinant ACE2 (GSK2586881), the intervention was well-tolerated in patients with ARDS, and rapidly modulated RAAS peptides (increased angiotensin II and increased angiotensins (1-7)/(1-9), but this work was not so powered to detect changes in acute physiology or clinical outcomes (Khan et al., 2017). Also, in another report, administration of rhACE2 for healthy individual was generally well tolerated and despite marked changes in angiotensin system peptide concentrations, blood pressure and heart rate were not significantly changed. In agreement with (Newby et al., 1997), the contribution of Ang (1-7)/(1-9) to blood pressure regulation is of minor importance in (not sodium-depleted) healthy persons and suggesting the presence of effective compensatory mechanisms in healthy volunteers (Haschke et al., 2013). It is noteworthy that the ACE2 activity depends on chloride ion, so that the catalytic efficiency of the enzyme increases in the presence of chloride ion (Guy et al., 2003). Recently, Zheng et al. observed significant differences, including abnormal biochemical indices (such as sodium concentration) between COVID and non-COVID patients and attributed lung tissue damage in COVID-19 patients to virus infection itself (Zheng et al., 2020). Since the mentioned compensatory mechanisms may not be adequately active in unstable COVID-19 patients with ARDS, they may experience

severe hemodynamic changes (and likely rapid change in blood pressure; hypotension) after ACE2 infusion. Moreover, as mentioned in (Khan et al., 2017), ACE2 intervention in such patients may expose them to all its adverse effects, but accompanied by a weak (or with no) clinical outcome in respiratory compensation. In agreement with the above statements, and as one of limitations of the study (Monteil et al.), authors stated that the RAAS system that represents a complex network of pathways and is influenced by external processes, has not been simulated in their model systems. Therefore, the effect of the hrsACE2 on the RAAS system appears to be ambiguous and unpredictable, especially in severe/unstable patients or complicated COVID cases with cardiovascular comorbidity.

According to WHO declaration, COVID-19 is a public health emergency of pandemic proportions (<https://www.who.int/>). Given the medical emergency of a growing contagion of COVID-19 and the thousands of lives at stake, expedient attempts to improve survival are needed (Solaimanzadeh, 2020). Since SARS-CoV-2 spike protein recognizes human ACE2 with even higher binding affinity than Spike from SARS-CoV regarding ACE2 expression in various organs (that may explain the multiorgan dysfunction observed in COVID patients), we recommend that researchers only focus on inhibiting the binding of spike glycoprotein of SARS-CoV-2 to the membrane-bound ACE2 on the host cells (and blocking the SARS-CoV-2 from entering target cells) using a catalytically inactive hrsACE2 and do not get caught up in the complexities of RAAS. Molecular structures as well as catalytic domain of ACE/ACE2 are well known. A strong similarity exists between the catalytic domains such that the active site structure is highly conserved in ACE2 (Guy et al., 2003). Using recombinant DNA technology, one (or more) of the residues that comprise the catalytic site of the enzyme, including the zinc coordination sphere (His³⁷⁴, His³⁷⁸, and Glu⁴⁰²), can be simply replaced with suitable (but inert) residues, with no gross structural change in the mutant/inactive enzyme (and with minimal neutralizing antibodies upon infusion). This can lead to the initiation of new drug development program of catalytically inactive soluble recombinant human ACE2 with no RAAS concerns.

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