

The Role of Soluble Recombinant ACE2 in SARS-COV-2 Patients

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Abstract

The aim of this thematic article is to go through the present literature about the role of soluble recombinant ACE2 during the last COVID-19 pandemic months. The reason is trying to understand its role and its possible clinical and therapeutic consequences in hospitalized SARS-CoV-2 patients. Further, more specific studies are needed to evaluate this approach and share light on potential research directions.

Keywords: ACE2; Soluble Recombinant ACE2; SARS-CoV-2; Pandemic; Health Care

Abbreviations

ACE: Angiotensin Converting Enzyme; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus (2); Ang: Angiotensin; RAS: Renin-Angiotensin System; AT1r: Angiotensin II Type 1 Receptor; AT2r: Angiotensin II Type 2 Receptor; BMJ: British Medical Journal; CFR: Case Fatality Rate; H1N1: Orthomyxovirus (Species Influenza A Virus of the Genus Influenza Virus A); ARB: Angiotensin Receptor Blocker (Hypertension Drug); ARDS: Acute Respiratory Distress Syndrome; LDH: Lactic Acid Dehydrogenase; CD8: Cluster of Differentiation 8; H5N1: Avian Influenza (or "Bird Flu"); PCR: Polymerase Chain Reaction; CVD: Cardiovascular Disease; CRP: C-Reactive Protein; PCT: Procalcitonin; BNP: Natriuretic Peptide Type B; BMI: Body Mass Index

Introduction

In this paper will be illustrated a summary of works that take into account the role of ACE inhibitors as well as the possibility of using them both in therapeutic and research studies. The intention of this monothematic dissertation is to shed light on other potential research directions and the various possibilities that ACE2 plays in several cellular functions.

Let us start with a work by Wu [1] who was the first to open the discussion about the role of ACE2. Starting from the now known observation that SARS-CoV-2 uses ACE2 to infect lung cells (which, as we know, has a local RAS); Wu states that in the lungs the activity of RAS, ACE and Ang II is intrinsically high, as well as but that of ACE2. ACE2 is an enzyme capable of synthesizing Ang-(1-7) and thus counterbalancing the effects of Ang II. Both viral replication and viral "spike protein" alone have shown to selectively reduce the expression of ACE2 but not ACE [2] in SARS-CoV infected mice.

In Wu's interesting work [1] published in *Virologica* this year, it is stated that SARS-CoV-2 viral infection causes clusters of severe respiratory diseases such as acute respiratory distress syndrome (ARDS) similar to that caused by SARS-CoV. Both SARS-CoV-2 and SARS-CoV use the same receptor, ACE2 (angiotensin-2 conversion enzyme), to infect cells. Activation of local RAS in the lungs can influence the pathogenesis of lung damage through multiple mechanisms, such as increased vascular permeability and alterations in alveolar epithelial cells. In addition, ACE2 is a counterpart of ACE and plays a key role in balancing the responses caused by ACE. (The Ang (1-7) counterbalances the effects of Ang II and is produced by the activity of ACE2).

ACE2 hydrolyzes:

- The Ang I generating Ang- (1-9)
- The Ang II generating Ang-(1-7), which binds to Mas (Mas is an endogenous receptor, coupled to phospholipase C, and is expressed in various organs such as heart, vessels, testicles and brain) to play its main role of antagonizing many of the effects mediated by Ang II.

The activity of RAS, ACE and Ang II are inherently high in the lungs, however ACE2 activity is also very high. This is to regulate the Ang II/Ang-(1-7) balance, as high levels of Ang II in the lung can lead to increases in vascular permeability and pulmonary oedema.

In SARS-CoV infection, viral spike protein replication occurs. They function as tiny picks that allow the coronavirus to unhinge the entrance doors of cells in the human respiratory system to penetrate and multiply. Blocking them means disarming the virus. In addition, SARS-CoV induces rapid downregulation of ACE2 from the cell surface and the release of active catalytic ACE2 ectodomains. Given that the spike protein of SARS-CoV-2 interacts with ACE2 like that of SARS-CoV, it is possible that the pathogenetic mechanism will be shared between the two viruses. Based on this assumption, compensation of ACE2 and balancing ACE/ACE2 functions may be a way to alleviate severe virus-induced lung lesions.

Several solutions could be adopted:

- Therapies to increase ACE2 expression could be developed in the future, through direct injection of recombinant protein ACE2, which has been shown to protect mice from severe acute lung damage and by obtaining therapeutic vectors expressing high levels of ACE2 directly into lung tissue to overcome its virus-induced deficiency.
- Some ACE-inhibitors such as Lisinopril can be used to balance the ACE / ACE2 function.
- Ang-(1-7) heptapeptide can be administered for therapeutic purposes to activate its Mas receptor and to counteract Ang II activities.
- Drugs that block Ang II receptors can also be tested. AT1r (but not AT2r) have been shown to promote the pathogenesis of the disease by inducing pulmonary oedema and compromising lung function. Therefore, an AT receptor blocker, such as losartan, could be tested to alleviate pulmonary damage from COVID-19.

In this study, we should cite the statements of Sommerstein (University Hospital Bern) [3] who, in a letter to the *BMJ*, states that RAS-I, in patients suffering from cardiovascular diseases and especially in patients suffering from diabetes (a disease in which RAS-I are particularly indicated drugs), could contribute to the development of a more severe form of COVID-19. As we know, SARS-CoV-2 uses ACE2 as a receptor to penetrate the target cell, the efficiency of ACE2 must be a determining factor of SARS-CoV transmissibility. However, the author himself does not feel like drawing conclusions, admitting, "we need urgent epidemiological and pre-clinical studies to clarify this report".

The 2019 outbreak of coronavirus disease (SARS-CoV-2) from Wuhan, China, is spreading worldwide and is a major international concern as it has the potential to become a pandemic (it is now pandemic-ed).

The largest clinical study in China, with 44,672 confirmed cases of COVID-19, shows an overall high mortality rate (CFR: case fatality rate) of 2.3%. Important comorbidity factors found are high blood pressure (CFR 6.0%), diabetes (CFR 7.3%), cardiovascular disease (CFR 10.5%) and age > 70 (CFR 10.2%). Similar co-morbidities were also observed for the SARS epidemic in 2003. It is not clear what the interaction of these risk factors is. This is surprising compared to the 2009 H1N1 pandemic influenza, for example, where immunosuppressed patients were primarily affected. Cardiopathic patients seem to be at higher risk in COVID-19 disease. A possible response could be the following: Patients with hypertension comorbidities, diabetes and cardiovascular disease may be indicated to use angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists.

The possible link between SARS-CoV2 and ACE-inhibitors

The question we pose is: is there a connection between the use of these drugs and the serious consequences of COVID-19?

While the epidemiological association has not yet been studied, several indicators underline the hypothesis of the link between ACE-inhibitors and SARS-CoV-2: it has been demonstrated that SARS-CoV-2 uses ACE2 for entry into target cells. The interaction between ACE2 and the SARS-S spike protein virus has been clarified and the efficiency of this interaction has been shown to be a determining factor in the transmissibility of SARS-CoV. On the other hand, animal experiments have shown that both Lisinopril (ACE-inhibitor) and losartan (angiotensin receptor blocker) can significantly increase mRNA expression of cardiac ACE2 (5 times and 3 times, respectively). In addition, losartan significantly increases cardiac ACE2 activity.

Is there a possible link between these observations? ACE2 receptor expression in the target cells of the virus is increased by the use of ACE inhibitor/angiotensin receptor blocker; therefore, is it possible to state that the patient may be at greater risk for a severe course?

This hypothesis is also supported by Fang and colleagues (March 2020) [4], who confirm that the overexpression of ACE2 (present among others in diabetics, especially if treated with glitazones and promoted by drugs such as ibuprofen but also by ACEI and ARB), is the key point for the penetration of the virus into the cell. But Fang poses another problem: it is well known that ACE2 has anti-inflammatory properties, so that it has been suggested as a potential new therapy in inflammatory lung diseases, cancer, diabetes and hypertension. So? What are the answers available? Important to note, the genetic polymorphisms of ACE for which the sensitivity of an individual could be derived from a combination of therapy and ACE2 polymorphism. Fang is therefore limited to suggesting greater control of patients affected by SARS-CoV-2 and in therapy with RAS-inhibitors. Human pathogenic coronaviruses (SARS-CoV and SARS-CoV-2) bind to their target cells through ACE2, which is expressed by the epithelial cells of the lung, intestine, kidney and blood vessels. The expression of ACE2 is substantially increased in patients with type 1 diabetes or type 2 diabetes, who are usually treated with ACE-I and ARB. Arterial hypertension is also treated with ACE-I and ARB with an over regulation of ACE2.

ACE2 can also be increased by glitazones and ibuprofen. These data suggest that the expression of ACE2 is increased in diabetes and treatment with ACE-I and ARB. Consequently, increased expression of ACE2 would facilitate COVID-19 infection. The authors therefore hypothesize that diabetes and the treatment of hypertension with ACE2 stimulant drugs increase the risk of developing severe and fatal SARS-CoV-2 pneumonia [4]. If this hypothesis is confirmed, it could lead to a conflict regarding treatment because we know that ACE2 reduces inflammation and has even been suggested as a potential new therapy for inflammatory lung disease, cancer, diabetes and hypertension. In this article [4] it is suggested that patients with heart disease, hypertension or diabetes, treated with ACE-I with increased ACE2 expression, are at greater risk for severe SARS-CoV-2 infection and should therefore be monitored; perhaps a substitution could be considered, for example with calcium channel blockers only for the patients with these pre-existing conditions.

In a Chinese article, Sun and colleagues [5] state the opposite leading us back to Wu's hypothesis [1], to the point that RAS inhibitors could be a good choice for the treatment of SARS-CoV-2 pneumonia, precisely because the virus "requires" all ACE2 by unbalancing the AngII/Ang(1-7) balance. SARS-CoV-2 infects patients by binding ACE2 and causing severe pneumonia and a high mortality rate in patients. There is currently no defined and effective treatment for COVID-19. ACE2 plays an important role in RAS and the imbalance between ACE/Ang II/AT1R and ACE2/Ang (1-7)/Mas in the RAS system leads to multi-systemic inflammation. The increase in ACE and therefore Ang II is an unfavourable prognostic factor for severe pneumonia. The confusion about this is remarkable: in a small Chinese study with only 12 cases, but thoroughly studied, Liu and colleagues [6] analysed a series of markers to predict the severity of the disease. Data collected from epidemiological, clinical, laboratory, radiological and potential biomarkers were analysed to predict the severity of SARS-CoV-2 infection. All 12 cases had developed pneumonia and half of the sample tested had developed ARDS. The most common laboratory data were hypoalbuminemia, lymphopenia and neutropenia, increased PCR and LDH and decreased CD8 counts. In addition, Ang II levels in plasma were significantly elevated and directly associated with lung damage. In this perspective, ARBs, especially losartan, already tested in SARS-CoV and H5N1 influenza could have a beneficial effect.

In a retrospective study carried out by Peng [7], on 112 COVID-19 patients with CVD admitted to Wuhan (from 20 January 2020 to 15 February 2020), 16 of whom were admitted to intensive care units, various markers were evaluated to explore the clinical features and prognosis of new patients with SARS-CoV-2 with associated cardiovascular disease. The patients were divided into:

- "Critical" group (ICU, n = 16)
- "General" group (n = 96).

Based on the severity of the disease and were monitored all the way to the clinical endpoint.

Indicators included haemachrome, C-reactive protein (CRP), arterial haema gas analysis, markers of myocardial damage, coagulation, liver and kidney function, electrolytes, procalcitonin (PCT), natriuretic peptide type B (BNP), lipid trim, pulmonary CT and pathogen detection. The results of this study reveal that within the "critical" group (in intensive care), compared to the "general" group, the lymphocyte count was extremely low, while CRP, PCT and BMI were significantly higher. The patients were further divided into:

- Group "non survivors" (17: 15.18%)
- "Survivors" group (95: 84.82%).

Among the "non survivors", 88.24% of patients had BMI > 25 kg/m², significantly higher than that of the "survivors" (18.95%, P < 0.001). Lactic acid was also significantly higher, while the oxygenation index was significantly lower. There was no significant difference in the percentage of ACEI/ARB drug use between the "critical" and the "general" group, nor between the "non survivors" and the "survivors" group (all P > 0.05).

The article therefore concludes that COVID-19 cardiovascular patients are associated with a higher risk of mortality. "Critical" patients are characterized by a lower lymphocyte count. In "critical" and "non surviving" patients, high BMI is more often observed. The use of ACEI/ARB does not affect the morbidity and mortality of COVID-19 with CVD but causes of death include fulminant inflammation, lactic acid accumulation and thrombotic events.

Soluble recombinant ACE2 and SARS-CoV2

A paper published by Batlle, *et al.* on Clinical Science (2020) [8], seems to agree on the RAS-I showing evidences of two functional forms of ACE2. The first form binds to the cell membrane and is the receptor of the spike protein of SARS CoV; but there is also a second

form of ACE2, soluble type, which does not bind to the membrane and which would compete with the other form of ACE2 for the link with the virus. Soluble recombinant ACE2 protein availability could have very interesting therapeutic perspectives.

But we add, do RAS-I increase the expression of this soluble form of ACE2?

ACE2 is a monooxypeptidase known for its effects on many molecules within the renin-angiotensin system and other substrates, such as apelin (a molecule already present in our body that has been shown to regulate blood sugar and increase sensitivity to insulin). This enzyme is present at a very low concentration in circulation but is widely expressed in organs such as the kidneys and gastrointestinal tract, with relatively low expression level in the lungs where ACE2 expression in type 2 pneumocytes has been reported.

Functionally, there are two forms of ACE2:

1. The ACE2 “full-length” contains a transmembrane domain capable of fixing its extracellular domain to the plasma membrane. The extracellular domain is a receptor for the spike protein of SARS-CoV, which has recently also been demonstrated as a receptor for SARS-CoV-2 as well. The new coronavirus is evolutionally related to Bat-SARS, which uses ACE2 bound to the membrane as a receptor in the same way.
2. The soluble form of ACE2 lacks membrane anchorage and circulates in small amounts in the blood.

The authors [8] assume that this soluble form may act as an interceptor of SARS-CoV and other coronaviruses by preventing the binding of the virus particle to ACE2 “full length”, which binds to the cell surface. In fact, *in vitro* studies have shown that replication of SARS-CoV has been blocked by the soluble form of ACE2 in the monkey kidney cell line. In addition, ACE2 fused with a portion of Fc immunoglobulin has recently been reported to neutralize SARS-CoV-2 *in vitro* and we know that SARS-CoV-2 binds ACE2 with higher affinity to SARS-CoV. In such a context, the availability of soluble recombinant human recombinant ACE2 protein could indeed be useful as a new biological therapy to combat or limit the progression of infection caused by coronavirus using ACE2 as a receptor. Soluble recombinant ACE2 protein has therapeutic potential for a wide range of indications and new, shorter variants of ACE2 are being tested in mice for the treatment of kidney disease.

Conclusion

Given these data in literature, we conclude that it is absolutely premature (and dangerous) to stop therapy with ACEI-ARB at the moment, but new and useful questions are opening up for a new line of research. It is therefore essential to investigate these aspects, by checking levels of soluble recombinant ACE2 in COVID-19 patients compared to the asymptomatic one (detectable in a group of uninfected cohabitants or the one who developed the disease in a mild form), in order to detect the quantitative differences of recombinant ACE2, respectively.

With this article, we wish to draw attention to this possible correlation between SARS-COV and ACE2, with the aim of developing more studies on the possible correlation between the amount of recombinant ACE2 and the etiological development of the disease, together with a study evaluating the quantitative incidence in relation to the development of the infection itself.

Conflict of Interest

The authors declare that no financial interest or any conflict of interest exists.

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