



The ACE2 as a “rescue protein” or “suspect enzyme” in COVID-19: possible application of the “engineered inactive hrsACE2” as a safer therapeutic agent in the treatment of SARS-CoV-2 infection

Reza Khodarahmi¹ · Babak Sayad² · Mahsa Sobhani¹

Received: 25 May 2020 / Accepted: 25 August 2020 / Published online: 7 September 2020
© Iranian Chemical Society 2020

Abstract

COVID-19 is a devastating global pandemic around the world. While the majority of infected cases appear mild, in some cases, individuals present respiratory complications with possible serious lung damage. There are no specific treatments for COVID-19 as yet. Many repurposed antiviral drugs have had disappointing outcomes. Angiotensin-converting enzyme 2 (ACE2), an enzyme that physiologically counters renin–angiotensin–aldosterone system activation, functions as a receptor for both SARS viruses. The current study discusses on vague role of ACE2 under physiologic/pathologic conditions. The catalytically inactive hrsACE2 has been also proposed as an efficient treatment of SARS-CoV-2 infection.

Keywords SARS-CoV-2 infection · ACE2 · COVID-19 · RAAS

COVID-19 is a devastating global pandemic around the world. While the majority of infected cases appear mild, in some cases, individuals present respiratory complications with possible serious lung damage. There are no specific treatments for COVID-19 as yet, though a number are under evaluation, including experimental antivirals [1] and therapeutic proteins. We encountered/read the inspiring paper by Monteil et al. [2]. 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 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 that further (in vitro, pre-clinical and clinical) studies are needed to address the effect of hrsACE2 at later stages of SARS-CoV-2 infection. While the COVID-19 outbreak continues to spread all around the world, the absence of a clinically proven antiviral therapy (or a treatment specifically targeting the critical SARS-CoV-2 receptor ACE2 on a molecular level) has meant an empty arsenal for health care providers struggling to treat severe cases of COVID-19 [<https://www.sciencedaily.com/releases/2020/04/200402144526.htm>]. 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, we share our opinion/concern, combined with the literature review, with the scientific community all 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 [3]. ACE2, a type I transmembrane metalloprotease with the catalytic domain on the extracellular surface, is constitutively expressed and released from the apical surface of airway epithelial cells into airway

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13738-020-02049-z>) contains supplementary material, which is available to authorized users.

✉ Reza Khodarahmi
rkhodarahmi@mbr.ac.ir

¹ Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

² Infectious Diseases Research Center, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

surface liquid. Catalytic domain of ACE2, anchored at the apical surface of the epithelial cells, can be cleaved and released into alveolar space/blood by “a disintegrin and metalloproteinase domain-containing protein 17” (ADAM17) [4, 5].

Angiotensin-converting enzyme (ACE, EC 3.4.15.1) belongs to the gluzincin family of metalloproteases and is a peptidyl dipeptidase with broad substrate specificity. The predominant physiological function of ACE in RAAS is in cardiovascular homeostasis through cleavage of the C-terminal dipeptide from angiotensin I to produce the potent vasoconstrictor, angiotensin II. ACE1 also inactivates the vasodilator, bradykinin, by the sequential cleavage of two C-terminal dipeptides. Two isoforms of ACE are found; somatic ACE, with two homologous domains each, and testicular ACE (tACE), which corresponds to the C domain of somatic ACE. ACE2 was identified by screening an EST database [6] and also by 5' sequencing of a human heart failure ventricular cDNA library. Its transcripts are found mainly in the heart, kidney, and testis, whereas ACE mRNA expression is more widespread. Except for a HEXXH zinc-binding motif, other amino acid residues found to be critical for ACE activity are conserved in ACE2. It has only 42%

sequence identity with ACE and even 61% sequence similarity in a region surrounding the active site. The substrate specificities of ACE2 and ACE are different so that ACE2 functions exclusively as a carboxypeptidase and is able to cleave a number of physiologically relevant peptides including both angiotensin I and angiotensin II as well as (des-Arg9) bradykinin but unable to cleave bradykinin (Fig. 1). However, the classical ACE inhibitors captopril, lisinopril and enalaprilat do not inhibit ACE2 [7].

ACE2 expression was previously found to correlate with susceptibility to SARS-CoV infection in vitro [5, 10]. This enzyme (with homology to ACE) has been also identified in the heart, blood vessels, kidney and brain, as well as the retina, liver and gastrointestinal tract [11]. SARS-CoV-2 cell entry also depends on TMPRSS2 (a cellular serine protease) and is blocked by a clinically proven protease inhibitor [12].

Lower susceptibility of children to COVID-19 might be attributable to the difference in expression of ACE2 between children and adults. However, no evidence exists to show that ACE2 expression varies with age [13]. Schouten et al. have analyzed the age-dependent differences in pulmonary host responses in ARDS, and found that there is no difference in activity of ACE2 in bronchoalveolar lavage

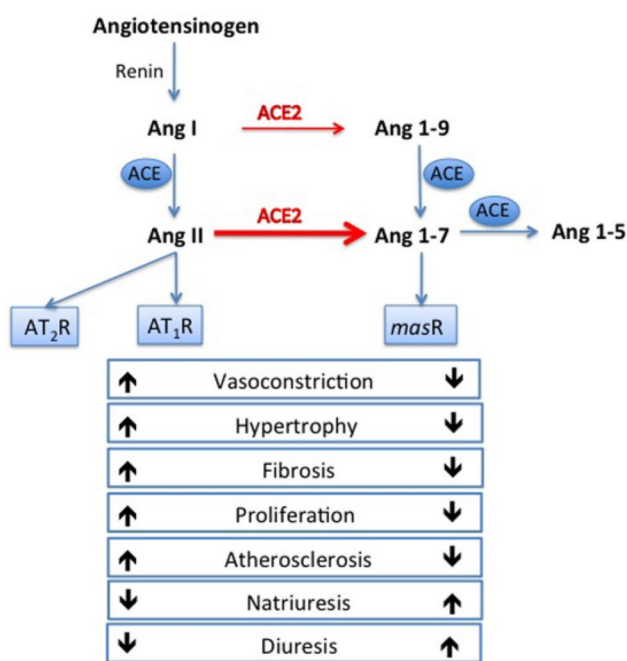
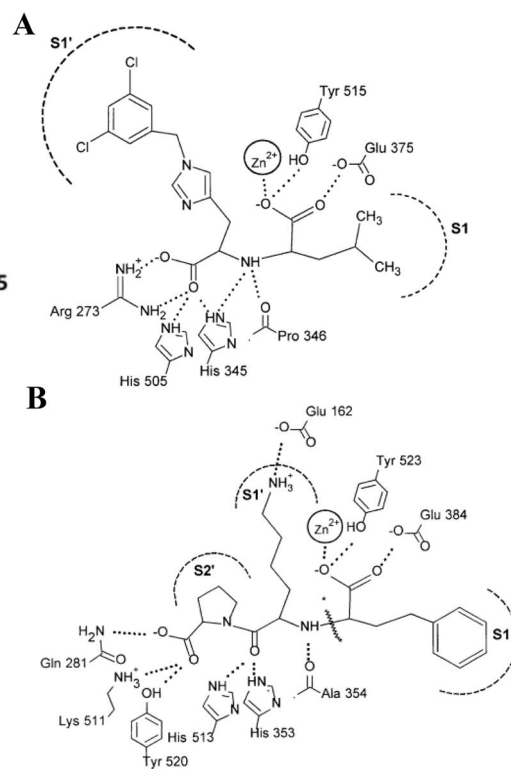


Fig. 1 (Left) Opposing arms of the renin-angiotensin system. Angiotensin-converting enzyme (ACE) converts angiotensin I to Ang II, which exerts its deleterious effects via the angiotensin type 1 receptor (AT1R). Ang II can also act on the angiotensin type 2 receptor (AT2R) which can counteract AT1R-mediated effects. In the oppos-



ing arm, ACE2 degrades Ang II to Ang 1–7, which is thought to exert beneficial effect via the *mas* receptor. (Right) Schematic view of the active site of ACE2 (a) with MLN-4760 and ACE (b) with lisinopril at the active sites. The different binding sub-sites are labeled. Adapted from [8, 9]

fluid from neonates, children, adults and older adults with ARDS [14]. Although Chu et al. found that the expression of ACE2 was relatively higher in cells with higher pseudotype SARS-CoV-2 entry in agreement with “association of higher initial viral load and worse prognosis in SARS” [15], however, no direct evidence exists to indicate a connection between ACE2 expression and the susceptibility and severity of SARS-CoV-2 infection. Preliminary data suggest that a high viral load of the SARS coronavirus is associated with adverse outcomes in ICU, but the relation of viral load to survival is unclear, so mortality data (for patients with SARS) should be interpreted in light of age, comorbidity and viral load [5]. Other studies suggest that serum ACE2 (Xp22) activity is sex-dependent; with higher levels in males compared with females [16].

RAAS includes angiotensinogen, ACE and also 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 determinant factors of acute respiratory distress syndrome (ARDS) which is frequently associated with multiple organ dysfunctions leading to high mortality, is important clinical feature of COVID-19. Regarding a recently published report in the Lancet [17], 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 [18], 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 [19]. 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 [3]. Moreover, since ACE2 is expressed in various tissues including the heart, kidney tubules, the luminal surface of the small intestine and blood vessels [2] 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 [20]. 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. [21], 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%) [22]. 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 [23, 24]. So, hypertension appears not to be a COVID-19 clinical feature, and it can be concluded that 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%) [25]. This work introduces hypertension comorbidity as an important risk factor for critical patients. In another study [26], 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 [20], 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. In patients with SARS-CoV-2 infection, underlying CVD can aggravate the pneumonia and increase the severity of symptoms. Among the people who died from COVID-19 reported by the National Health Commission of China (NHC), only 11.8% of patients without underlying CVD had substantial heart damage [27]. Similarly, according to the Pneumonitis Diagnosis and Treatment Program for New Coronavirus Infection (Trial Version 4, http://www.gov.cn/zhengce/zhengceku/2020-01/28/content_5472673.htm), adult/elderly people with hypertension/coronary heart disease comorbidities

are more likely to be infected with SARS-CoV-2 and patients with CVD are more likely to develop severe symptoms and account for a large proportion of deaths from COVID-19.

On the other hand, membrane-bound ACE2, expressed in cardiomyocytes, cardiac fibroblasts and coronary endothelial cells, as regulators for heart function [5], shows the ability to prevent angiotensin II-induced inflammation, myocardial hypertrophy, diastolic dysfunction and myocardial fibrosis [4]. ACE2 gene is upregulated in idiopathic and ischemic cardiomyopathy [28] and higher serum ACE2 activity is correlated with increasing severity of heart failure [29]. Ang (1–7), as ACE2 product, also exhibits antiproliferative, antiapoptotic and mild vasodilating abilities and presents various cardiovascular protective effects, including anti-heart failure, antithrombosis, anti-myocardial hypertrophy, anti-fibrosis, antiarrhythmia, anti-atherogenesis and attenuating vascular dysfunction related to metabolic syndrome [5, 30, 31]. ACE2 activity, either as a cause or an effect of CVD, may be also influenced by intrinsic factors such as genetic variation and single nucleotide polymorphism (SNP) [32] within and around the gene encoding ACE2. It is noteworthy that whether the increase in cardiac ACE2 represents an important adaptive mechanism to retard the progression of adverse cardiac remodeling (and the exact role of cleaved ACE2) are not clear yet [16], though some reports have confirmed that over-expression of ACE2 can prevent or even reverse the heart failure phenotype, whereas loss of ACE2 can accelerate the progression of heart failure and claimed that shedding of the membrane-bound ACE2 may be responsible for the increased circulating ACE2 activity in patients with heart failure [5].

The myocardial dysfunction can be indirectly caused by reduced oxygen supply, severe lung failure, and the cytokine storm after the SARS-CoV-2 infection. However, there is also the possibility that it might be attributable to the decreased activity of ACE2 in the heart, just like SARS [5]. However, direct evidence demonstrating that SARS-CoV-2 infects the heart and decreases the ACE2 expression is currently lacking.

ACE2 as rescue agent?

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, the soluble but active ACE2 may be beneficial in COVID-19 patients [3]; 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 [2]) 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 has not been so powered to detect changes in acute physiology or clinical outcomes [33]. 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 [34], 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 [35]. 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 [7]. 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 [36]. 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 [33], 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 [2], 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.

As stated, recombinant human ACE2 (rhACE2, APN01) was previously developed for the treatment of acute lung injury (ALI), acute respiratory distress syndrome (ARDS) and pulmonary arterial hypertension (PAH). After licensing in February 2010, several clinical trials from 2014 to 2017 to treat ALI/ARDS and PAH patients were conducted, and the drug candidate, administered intravenously as an infusion, has been shown to be safe and well tolerated in a total of 89 healthy volunteers and patients with PAH and ALI/ARDS in previously completed Phase I and Phase II clinical trials [https://www.apeiron-biologics.com/wp-content/uploads/2020/04/20200402_APEIRON_Phase-2-EU_trial_APN01_ENG.pdf]. Khan et al. [33] showed that recombinant catalytic ectodomain of human ACE2 was well tolerated in patients with ARDS and modulated RAAS peptides rapidly, although the study had not been powered

to detect changes in acute physiology or clinical outcomes. These days, regulatory approvals obtained for the treatment of 200 severely infected COVID-19 patients in Austria, Germany and Denmark, and the first patients are expected to be dosed shortly [https://www.apeiron-biologics.com/wp-content/uploads/2020/04/20200402_APEIRON_Phase-2-EU-trial_APN01_ENG.pdf]. Although, the same drug has already been evaluated against acute lung injury, acute respiratory distress syndrome and pulmonary arterial hypertension in clinical phase I and II studies, in our opinion, there is a serious ambiguity as to whether it is reasonable to enter the phase II/III clinical trials on SARS-CoV-2 infected patients using the active sACE2? 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. It is noteworthy that at the time of preparing the revised form of the current paper, we realized that contents of the above-mentioned link (by APEIRON) is no longer available (*‘the page you are looking for does not exist’*), but since we had downloaded the content at that time (Early March, 2020), the previously downloaded PDF file can be found as S.I.

In this regard, in a joint statement from the ACC, American Heart Association and Heart Failure Society of America, posted online on March 17, 2020 [<https://www.acc.org/lates-t-in-cardiology/articles/2020/03/17/08/59/hfsa-acc-aha-state-ment-addresses-concerns-re-using-raas-antagonists-in-covid-19>], we can see: “ACE2, ACE, angiotensin II and other renin–angiotensin–aldosterone system (RAAS) system interactions are quite complex, and at times, paradoxical. Furthermore, tissue expression of ACE2 differ in heart, kidneys and lungs of healthy patients, cardiovascular disease patients and coronavirus-infected patients, and its role in the setting of COVID-19 infection in patients with cardiovascular disease is unclear. In the event patients with cardiovascular disease are diagnosed with COVID-19, individualized treatment decisions should be made according to each patient’s hemodynamic status and clinical presentation. Therefore, be advised not to **add** or **remove** any RAAS-related treatments, beyond actions based on standard clinical practice.” In this regard, although, some hypothetical papers on ACE/ACE2 inhibition have been published [37], till now, no registered clinical trials against COVID-19 has been registered/run, using ACE/ACE2 inhibitors (or using inhibitors of the respective receptors).

ACE2 as suspect?

In a different article, Zamai raised an opposing opinion and described the rational for inhibition of ACE2 pathways as specific targets in patients with critical, advanced and untreatable stages of SARS-CoV-2 infection [38]. Due to

downregulation of cell-surface ACE2 expression [39], the ACE2-related pathway may be downmodulated during SARS-CoV-2 infection. However, due to constitutive expression (and release into airway surface liquid) of ACE2 on the apical cell surface of human airway epithelia [11], its surface downmodulation (upon SARS-CoV-2 infection) has been shown to be due to activation of extracellular matrix metalloproteinase; ADAM17 (A disintegrin and metalloproteinase 17), also known as TNF α converting enzyme (TACE, as “TACE:ADAM17”), leading to “ACE2 shedding.” Concomitantly “TNF α shedding/production” also occurs; tumor necrosis factor α (TNF α), a type II transmembrane homotrimer protein, exists as a membrane-bound form (mTNF α , 26 kDa) and can be processed into 17 kDa soluble TNF α (sTNF α) through the action of TACE: ADAM17 [40]; the soluble TNF α , with potent endocrine function, circulates throughout the body [41–43]. Interestingly, pro-inflammatory cytokines (such as IL-1 β , TNF α), with increased levels during nCoV-induced cytokine storm, trigger surface ACE2 downmodulation and the free enzyme shedding [11].

Moreover, elevated free sACE2 (which retain its binding ability for viral S-protein) reduces the viral entry into alveolar epithelial cells, so it appears that engagement of membrane-bound ACE2 by SARS-CoV binding induces an initial “protective ACE2 shedding feedback,” limiting viral entry at early stages of infection. On the other hand, various researchers found an intriguing positive correlation between ACE2 shedding (or soluble ACE2 activity) and viral infection, disease severity/complications [41, 44, 45], suggesting this possibility that subsequent events, downstream of ACE2 shedding/soluble ACE2 activity (directly/indirectly), exacerbate viral infection (and favor disease complications).

SARS-CoV spike protein binding to ACE2 does not interfere with its catalytic activity [46], thus ACE2 enzymatic activity is retained by spike protein-ACE2 complex and virus-bound sACE2 as well [11, 41, 47]. In 2009, Epelman et al. found association of catalytically active sACE2 with myocardial pathology [48]. Yu et al. also reported cardiovascular complications, including hypotension, as common clinical features in SARS-CoV patients [49]. As already mentioned, ACE2 processes several substrates; Ang I, Ang II and also bradykinin (the major functional vasodilator) metabolites. Ang (1–7) peptide, as one of the products of this terminal carboxypeptidase, induces vasodilative (hypotension), antiproliferative and apoptotic effects through Mas receptor [50–52]. In agreement with the latter observation, dramatic increase in ACE2 expression in the SARS-CoV-infected bronchial cells has been shown by bioinformatics analyses [53].

Elevated plasma sACE2 activity, Ang (1–7) or sACE2 upregulation have been associated with pathological conditions including GI tract inflammation, inflammatory bowel disease, cirrhosis, lung injury/fibrosis and greater severity

of myocardial dysfunction/infarction [48, 54, 55]. Moreover, inhibition of fibrosis/hypertrophy by ACE2 inhibitor in animal model myocardium confirms the mentioned adverse effect of “ACE2 activity [56].

Upregulated ACE2 (mRNA and protein) expression in human hepatocytes [57] and pulmonary artery smooth muscle cells [58] under hypoxic conditions, possible ACE2 pathway upregulation induced by hypercapnia/hypoxia (an observed condition in SARS patients) [59], ang II-induced lung microvascular permeability and subsequently favored “sACE2 and Ang (1–7)” diffusion in neighboring lung tissues [38], activation of bax/caspase-dependent apoptotic pathway in lung fibroblasts after Ang-(1–7) treatment [60], promoted morphological lung alterations as well as inflammatory cytokines (including TNF- α , and IL-6) upon in vivo administration of Ang-(1–7) alone in rats [60], Ang-(1–7)-promoted eosinophil apoptosis in lung bronchoalveolar lavage (washing) fluid [52] (SARS-CoV-2 infection is associated with Lymphopenia and eosinopenia [38]), inhibited allergic airway inflammation and reduced asthma symptoms upon ACE2 pathway activation [61], upregulated IL-10/IL-6 cytokines by a nonpeptide angiotensin-(1–7) receptor agonist [62] and in the most severe SARS cases [63], increased circulating ACE2 activity in subjects with “hypertension or diabetes or dyslipidemia” as well as confirmation/identification of “advanced age” as predictor of enhanced ACE2 activity [64], increased circulating ACE2 activity in subjects under treatment with angiotensin II receptor blockers (ARBs) but not ACE inhibitors, oral antidiabetic agents and insulin (or smokers, although not significantly), suggesting that ARBs should be avoided to reduce ACE2-mediated viral entry [64]; all these evidences, together, are reminiscent of a strong positive correlation between ACE2/Ang (1–7) axis activation (circulating sACE2 activity) and SARS-CoV-2 infection and the disease progression.

Since these data imply that the SARS coronavirus sustains ACE2 pathway activation and also the clinical picture as a whole (eosinopenia, hypotension and elevated pro-inflammatory cytokine profile, as downstream events stemming from an excessive ACE2 pathway activation/upregulation), is consistent with a ACE2 gain of function (rather than loss of function), inhibition of ACE2/Ang (1–7)/Mas receptor axis has been recently suggested [38] (Fig. 1).

As already mentioned, plasma ACE2 activity is elevated in patients with cardiovascular risk factors or cardiovascular disease [9]. Patients with heart failure, possessing increased cardiac/plasma ACE2 activity (which may be an adaptive mechanism and reflect renal, cardiac or blood vessel ACE2 activity) [16] have possibly experienced ACE2 shedding and are probably more susceptible to severe forms of SARS-CoV-2 infection and the disease progression. Since ACE2 (which is widely expressed in the cardiovascular system) is involved in heart function and the development of

hypertension and diabetes mellitus, SARS-CoV-2 might cause chronic damage to cardiovascular system, so attention should be given to cardiovascular protection during treatment for COVID-19.

Our suggestion: how to use the therapeutic potential of free ACE2?

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 [65]. 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 multi-organ dysfunction observed in COVID patients and considering contradictory reports on the role of ACE2 in the pathogenesis of SARS-CoV-2 and in inducing lung injury), 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. In other word, considering the highly expression of ACE2, SARS-CoV-2 mainly invades alveolar epithelial cells, resulting in respiratory symptoms. ACE2 is also over-expressed in heart failure, arterial hypertension and diabetes mellitus. The disease symptoms are more severe in patients with CVD, which might be associated with increased secretion of ACE2 in these patients, compared with healthy individuals. Given that ACE2 is a functional (or catalytically active) receptor for SARS-CoV-2, the safety and potential effects of “functional hrsACE2” therapy in patients with COVID-19 should be carefully considered.

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 [7]. Using ($H^{374}H^{378}/N^{374}N^{378}$) mutant of rhsACE2, Lei et al. showed that both SARS-CoV and SARS-CoV-2 viruses were potently neutralized, exactly with inhibition potency similar to the wild-type ACE2 [66]. Moore et al. also confirmed the above observation and found that catalytically inactive sACE2 can potently inhibit SARS-CoV infection [44]. It is also noteworthy that pharmacokinetic studies have revealed that half-life of rACE2, in human/mice, is only hours, and this pharmaceutical protein experiences a fast clearance rate [35, 67]. For production of catalytically inactive ACE2, 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.

Acknowledgement The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant No. 97468).

References

1. B. Sayad, M. Sobhani, R. Khodarahmi, Arch. Med. Res. (2020). <https://www.sciencedirect.com/science/article/pii/S0188440920305518>
2. V. Monteil, H. Kwon, P. Prado, A. Hagelkrüys, R.A. Wimmer, M. Stahl, A. Leopoldi, E. Garreta, C.H. del Pozo, F. Prosper, Cell **181**, 905–913 (2020)
3. M. Vaduganathan, O. Vardeny, T. Michel, J.J. McMurray, M.A. Pfeffer, S.D. Solomon, N. Engl. J. Med. **382**, 1653–1659 (2020)
4. R.A.S. Santos, W.O. Sampaio, A.C. Alzamora, D. Motta-Santos, N. Alenina, M. Bader, M.J. Campagnole-Santos, Physiol. Rev. **98**, 505 (2018)
5. J. Guo, Z. Huang, L. Lin, J. Lv, J. Am. Heart Assoc. **9**, e016219 (2020)
6. S.R. Tipnis, N.M. Hooper, R. Hyde, E. Karran, G. Christie, A.J. Turner, J. Biol. Chem. **275**, 33238 (2000)
7. J.L. Guy, R.M. Jackson, K.R. Acharya, E.D. Sturrock, N.M. Hooper, N.M. Turner, Biochemistry **42**, 13185 (2003)
8. J.L. Guy, R.M. Jackson, H.A. Jensen, N.M. Hooper, A.J. Turner, FEBS J. **272**, 3512 (2005)
9. S.K. Patel, E. Velkoska, M. Freeman, B. Wai, T.F. Lancefield, L.M. Burrell, Front. Physiol. **5**, 227 (2014)
10. H. Hofmann, M. Geier, A. Marzi, M. Krumbiegel, M. Peipp, G.H. Fey, T. Gramberg, S. Pöhlmann, Biochem. Biophys. Res. Commun. **319**, 1216 (2004)
11. H.P. Jia, D.C. Look, P. Tan, L. Shi, M. Hickey, L. Gakhar, M.C. Chappell, C. Wohlford-Lenane, P.B. McCray Jr., Am. J. Physiol. Lung Cell. Mol. Physiol. **297**, L84 (2009)
12. M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.-H. Wu, A. Nitsche, Cell **181**, 271–280 (2020)
13. Q. Li, X. Guan, P. Wu, X. Wang, L. Zhou, Y. Tong, R. Ren, K.S. Leung, E.H. Lau, J.Y. Wong, N. Engl. J. Med. **382**, 1199–1207 (2020)
14. L.R. Schouten, A.H. van Kaam, F. Kohse, F. Veltkamp, L.D. Bos, F.M. de Beer, R.T. van Hooijdonk, J. Horn, M. Straat, E. Witteveen, Ann. Intensive Care **9**, 55 (2019)
15. C.-M. Chu, L.L. Poon, V.C. Cheng, K.-S. Chan, I.F. Hung, M.M. Wong, K.-H. Chan, W.-S. Leung, B.S. Tang, V.L. Chan, CMAJ **171**, 1349 (2004)
16. A. Soro-Paavonen, D. Gordin, C. Forsblom, M. Rosengard-Barlund, J. Waden, L. Thorn, N. Sandholm, M.C. Thomas, P.-H. Groop, FS Group, J. Hypertens. **30**, 375 (2012)
17. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Lancet **395**, 497 (2020)
18. R. Metzger, F.E. Franke, R.M. Bohle, F. Alhenc-Gelas, S.M. Danilov, Microvasc. Res. **81**, 206 (2011)
19. R.S. Wiener, Y.X. Cao, A. Hinds, M.I. Ramirez, M.C. Williams, J. Cell. Biochem. **101**, 1278 (2007)
20. Y. Liu, Y. Yang, C. Zhang, F. Huang, F. Wang, J. Yuan, Z. Wang, J. Li, J. Li, C. Feng, Sci. China Life Sci. **63**, 364 (2020)
21. F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, Lancet **395**, 1054–1062 (2020)
22. T. Chen, D. Wu, H. Chen, W. Yan, D. Yang, G. Chen, K. Ma, D. Xu, H. Yu, H. Wang, BMJ **368**, m1091 (2020)
23. D. RANA, Scientific Publisher, New Delhi, India (2015). <https://www.researchgate.net>
24. A.J. Camm, T.F. Lüscher, P.W. Serruys, *The ESC Textbook of Cardiovascular Medicine* (Oxford University Press, Oxford, 2009)
25. J. Yang, Y. Zheng, X. Gou, K. Pu, Z. Chen, Q. Guo, R. Ji, H. Wang, Y. Wang, Y. Zhou, Int. J. Infect. Dis. **94**, 91–95 (2020)
26. W.-J. Guan, W.-H. Liang, Y. Zhao, H.-R. Liang, Z.-S. Chen, Y.-M. Li, X.-Q. Liu, R.-C. Chen, C.-L. Tang, T. Wang, Eur. Respir. J. **55**, 2000547 (2020)
27. Y.-Y. Zheng, Y.-T. Ma, J.-Y. Zhang, X. Xie, Nat. Rev. Cardiol. **17**, 259 (2020)
28. A.B. Goulter, M.J. Goddard, J.C. Allen, K.L. Clark, BMC Med. **2**, 19 (2004)
29. S. Epelman, W.W. Tang, S.Y. Chen, F. Van Lente, G.S. Francis, S. Sen, J. Am. Coll. Cardiol. **52**, 750 (2008)
30. L. te Riet, J.H. van Esch, A.J. Roks, A.H. van den Meiracker, A.J. Danser, Circ. Res. **116**, 960 (2015)
31. V.B. Patel, J.-C. Zhong, M.B. Grant, G.Y. Oudit, Circ. Res. **118**, 1313 (2016)
32. L.M. Burrell, S.B. Harrap, E. Velkoska, S.K. Patel, Clin. Sci. **124**, 65 (2013)
33. A. Khan, C. Benthin, B. Zeno, T.E. Albertson, J. Boyd, J.D. Christie, R. Hall, G. Poirier, J.J. Ronco, M. Tidswell, Crit. Care **21**, 234 (2017)
34. D.E. Newby, S. Masumori, N.R. Johnston, N.A. Boon, D.J. Webb, Cardiovasc. Res. **36**, 268 (1997)
35. M. Haschke, M. Schuster, M. Poglitsch, H. Loibner, M. Salzberg, M. Bruggisser, J. Penninger, S. Krähenbühl, Clin. Pharm. **52**, 783 (2013)
36. Y. Zheng, Z. Huang, G. Ying, X. Zhang, W. Ye, Z. Hu, C. Hu, H. Wei, Y. Zeng, Y. Chi, *medRxiv* (2020)
37. L. Zamai, Preprints (2020). <https://doi.org/10.20944/preprints202003.0338.v3>
38. L. Zamai, Preprints (2020). <https://doi.org/10.20944/preprints202003.0338.v2>
39. K. Kuba, Y. Imai, S. Rao, H. Gao, F. Guo, B. Guan, Y. Huan, P. Yang, Y. Zhang, W. Deng, Nat. Med. **11**, 875 (2005)
40. S. Yang, J. Wang, D.D. Brand, S.G. Zheng, Front. Immunol. **9**, 784 (2018)
41. S. Haga, N. Yamamoto, C. Nakai-Murakami, Y. Osawa, K. Tokunaga, T. Sata, N. Yamamoto, T. Sasazuki, Y. Ishizaka, Proc. Natl. Acad. Sci. **105**, 7809 (2008)
42. I. Glowacka, S. Bertram, P. Herzog, S. Pfefferle, I. Steffen, M.O. Muench, G. Simmons, H. Hofmann, T. Kuri, F. Weber, J. Virol. **84**, 1198 (2010)
43. J. Scheller, A. Chalaris, C. Garbers, S. Rose-John, Trends Immunol. **32**, 380 (2011)
44. M.J. Moore, T. Dorfman, W. Li, S.K. Wong, Y. Li, J.H. Kuhn, J. Coderre, N. Vasilieva, Z. Han, T.C. Greenough, J. Virol. **78**, 10628 (2004)
45. S. Haga, N. Nagata, T. Okamura, N. Yamamoto, T. Sata, N. Yamamoto, T. Sasazuki, Y. Ishizaka, Antivir. Res. **85**, 551 (2010)
46. F. Li, W. Li, M. Farzan, S.C. Harrison, Science **309**, 1864 (2005)
47. W. Li, C. Zhang, J. Sui, J.H. Kuhn, M.J. Moore, S. Luo, S.K. Wong, I.C. Huang, K. Xu, N. Vasilieva, EMBO J. **24**, 1634 (2005)
48. S. Epelman, K. Shrestha, R.W. Troughton, G.S. Francis, S. Sen, A.L. Klein, W.W. Tang, J. Card. Fail. **15**, 565 (2008)
49. C. Yu, R.S. Wong, E. Wu, S. Kong, J. Wong, G.W. Yip, Y. Soo, M. Chiu, Y. Chan, D. Hui, Postgrad. Med. J. **82**, 140 (2006)
50. H. Jia, Shock **46**, 239 (2016)

51. P. Xu, S. Sriramula, E. Lazartigues, *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **300**, R804 (2011)
52. G.S. Magalhaes, L.C. Barroso, A.C. Reis, M.G. Rodrigues-Machado, J.F. Gregório, D. Motta-Santos, A.C. Oliveira, D.A. Perez, L.S. Barcelos, M.M. Teixeira, *Front. Immunol.* **9**, 58 (2018)
53. X. He, L. Zhang, Q. Ran, A. Xiong, J. Wang, D. Wu, F. Chen, and G. Li, *medRxiv* (2020) 2020.02.03.20020206
54. J.T. Ortiz-Perez, M. Riera, X. Bosch, T.M. De Caralt, R.J. Perea, J. Pascual, M.J. Soler, *PLoS ONE* **8**, e61695 (2013)
55. M. Garg, L.M. Burrell, E. Velkoska, K. Griggs, P.W. Angus, P.R. Gibson, J.S. Lubel, *J. Renin-Angiotensin-Aldosterone Syst.* **16**, 559 (2015)
56. M.-A. Kim, D. Yang, K. Kida, N. Molotkova, S.J. Yeo, N. Varki, M. Iwata, N.D. Dalton, K.L. Peterson, W.-E. Siems, *J. Card. Failure* **16**, 777 (2010)
57. G. Paizis, C. Tikellis, M.E. Cooper, J.M. Schembri, R.A. Lew, A.I. Smith, T. Shaw, F.J. Warner, A. Zuilli, L.M. Burrell, *Gut* **54**, 1790 (2005)
58. R. Zhang, Y. Wu, M. Zhao, C. Liu, L. Zhou, S. Shen, S. Liao, K. Yang, Q. Li, H. Wan, *Am. J. Physiol.-Lung Cell. Mol. Physiol.* **297**, L631 (2009)
59. X. Liao, L. Wang, C. Yang, J. He, X. Wang, R. Guo, A. Lan, X. Dong, Z. Yang, H. Wang, *Mol. Med. Rep.* **4**, 1145 (2011)
60. Y. Meng, C.-H. Yu, W. Li, T. Li, W. Luo, S. Huang, P.-S. Wu, S.-X. Cai, X. Li, *Am. J. Respir. Cell Mol. Biol.* **50**, 723 (2014)
61. A.Z. El-Hashim, W.M. Renno, R. Raghupathy, H.T. Abduo, S. Akhtar, I.F. Benter, *Br. J. Pharmacol.* **166**, 1964 (2012)
62. M. Rodrigues-Machado, G. Magalhães, J. Cardoso, L. Kangussu, A. Murari, M. Caliari, M. Oliveira, D. Cara, M. Noviello, F. Marques, *Br. J. Pharmacol.* **170**, 835 (2013)
63. Z. Leng, R. Zhu, W. Hou, Y. Feng, Y. Yang, Q. Han, G. Shan, F. Meng, D. Du, S. Wang, *Aging Dis.* **11**, 216 (2020)
64. L. Anguiano, M. Riera, J. Pascual, J.M. Valdivielso, C. Barrios, A. Betriu, S. Mojal, E. Fernández, M.J. Soler, *IFTN Study, Nephrol. Dial. Transpl.* **30**, 1176 (2015)
65. I. Solaimanzadeh, *Cureus* **12**, e7343 (2020)
66. C. Lei, W. Fu, K. Qian, T. Li, S. Zhang, M. Ding, and S. Hu, *BioRxiv* (2020)
67. J. Wysocki, M. Ye, E. Rodriguez, F.R. González-Pacheco, C. Barrios, K. Evora, M. Schuster, H. Loibner, K.B. Brosnihan, C.M. Ferrario, *Hypertension* **55**, 90 (2010)