The role of angiotensin-converting enzyme 2 in the pathogenesis of COVID-19: the villain or the hero?

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Abstract

Angiotensin-converting enzyme 2 (ACE 2) is the entry receptor for the novel coronavirus SARS-CoV-2, the aetiological agent of COVID-19. At the same time, ACE 2 expression decreases during COVID-19. Two seemingly contradictory relationships between the expression of ACE 2 and COVID-19 have been reported. Increased level of expression of ACE 2 may be a risk factor for the development of COVID-19 infection, while reduced ACE 2 expression during COVID-19 leads to acute respiratory distress syndrome. This article provides a comprehensive overview of available scientific knowledge about the role of ACE 2 in the pathogenesis of COVID-19, which is available up to current day. Also, it discusses unknown factors that we will have to reveal in order to understand the whole role of ACE 2 in the pathogenesis of COVID-19.

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Introduction

COVID-19 (corona virus disease 2019) is a designation issued by the World Health Organization (WHO) on February 11, 2020 for the disease caused by SARS-CoV-2 coronavirus (severe acute respiratory syndrome-related coronavirus). The first cases appeared in December 2019 in the Chinese city of Wu-Chan, Hubei province, and the disease gradually spread to most countries all over the world.[1]

SARS-CoV-2 bears many similarities to the already known beta-coronavirus SARS-CoV.[2,3] SARS-CoV is the cause of SARS, which broke out in Guangdong Province, China in 2002.[4] In addition to their common phylogenetic origin in bats,[3(p2)] both viruses demonstrate an identical mechanism of entry into a host cell. Their entry receptor is the angiotensin-converting enzyme 2 (ACE 2) and the virion bond to ACE 2 is mediated by the spike protein of the virion envelope.[2,3] SARS-CoV and SARS-CoV-2 spike proteins shares

about 76% concordance in amino acid sequences and nearly identical 3D structure.[2] SARS-CoV and SARS-CoV-2 do not use aminopeptidase N or dipeptidyl peptidase 4 as an entry receptor, contrary to some other coronaviruses.[3]

Role of ACE 2 in homeostasis

ACE 2, a homologue of the much better known angiotensin-converting enzyme, was discovered in 2000.[5] This discovery, together with the newly discovered angiotensin (ANG) (1-7) and the*Mas* receptor, revealed another branch of the renin-angiotensin system (RAS)[6,7] (see Figure 1) *Schematic of the renin-angiontensin system* . Both ACE 2 and its ACE isoform are carboxypeptidases cleaving amino acids from the carboxyl end of the peptide. However, the difference in their enzymatic selectivity is crucial. While ACE cleaves 2 amino acids from ANG I decapeptide, producing an ANG II octapeptide, ACE 2 cleaves 1 amino acid from ANG I to form ANG (1-9). ACE converts ANG (1-9) to ANG (1-7). ANG II is analogously converted by ACE 2 to ANG (1-7).[6,7] ACE 2 activity is not inhibited by ACE inhibitors (ACE-I).[5]

ANG (1-7) is a ligand for the *Mas* receptor and seems to be the principal peptide of the ACE 2/ANG (1-7)/*Mas* branch of the RAS system. In general, the ACE 2/ANG (1-7)/*Mas* branch counteracts vasoconstriction, sodium retention, proliferation/hypertrophy, fibrosis, oxidative stress, and arrhythmogenesis caused by the ACE/ANG II/angiotensin receptor 1 (AT1 R) cascade.[6–8]

Role of ACE 2 in SARS/COVID-19

ACE 2 was identified as a functional receptor for SARS-CoV *in vitro* [9] and *in vivo* [10]. SARS-CoV replication was significantly lower in the group of ACE 2 knockout mice compared to the control group of wild-type mice.[10] Analogously, higher rate of SARS-CoV replication was demonstrated in the group of transgenic mice for human ACE 2 (with overexpressed ACE 2) compared to the control group of wild-type mice.[11] Thus, there is a positive correlation between the level of ACE 2 expression and the rate of SARS-CoV replication.

ACE 2 acts not only as the entry receptor for SARS-CoV, but its expression is also downregulated during SARS. It was proved *in vivo* , that reduction of ACE 2 expression is cause directly by interaction between SARS-CoV spike protein and ACE 2 protein.[10] Due to similarity between SARS-CoV and SARS-CoV-2[2,3]we may expect similar pathogenesis in COVID-19.

Reduction of ACE 2 activity leads to increased ANG II concentration and decreased ANG (1-7) concentration. The increased ANG II/ANG (1-7) ratio leads to the acute respiratory distress syndrome (ARDS).[12,13] Therefore, the reduction of ACE 2 activity with consequent RAS imbalance seems to be an essential factor contributing to the lethality of SARS/COVID-19.[10]

The above stated, seemingly contradictory roles of ACE 2 in the pathogenesis of COVID-19 point to the following consequences:

1. Increased ACE 2 expression is a risk factor for SARS-CoV-2 infection and the rate of COVID-19 development in the early phase of the disease. 2. Decreased ACE 2 activity (or more specifically increased ANG II / ANG (1-7) ratio) is a risk factor for severity/lethality of COVID-19.

"Increased ACE 2 expression is a risk factor for SARS-CoV-2 infection"

ACE 2 expression has been detected for example in the heart, kidneys, gastrointestinal tract (GIT), lungs, brain, testes, bladder, adipose tissue, vascular system.[5,8,14,15] Recently, ACE 2 expression was detected in cholangiocytes,[16] in the epithelial cells of the oral[17] and nasal mucosa.[18] Various modes of SARS-CoV-2 transmission are possible due to the ubiquitous expression of ACE 2. The possibility of infection through

the GIT is discussed in the initial transmission from an animal to humans.[19] Intrapopulation faecal-oral transmission is reported, especially in children.[20] Nonetheless, the droplet transmission appears to be the most significant way for the intrapopulation transmission.[21]

Respiratory tract

Why respiratory tract and ARDS?

Zhao et al.[22] analysed lung tissue of 8 human lung donors and detected concentrated expression of ACE 2 RNA in a small group of pulmonary cells. Most of them (83%) were type II alveolar cells (AT 2). Surprisingly, only 1.4 % of the AT 2 expressed ACE 2 RNA, and, compared to the AT 2 not expressing RNA ACE 2, this group expressed other genes facilitating viral reproduction and transmission. The SARS-CoV-2 spike protein contains a sequence that can be cleaved into the S1 and S2 domains. Subsequent cleavage facilitates fusion of the virion into the host cell.[23,24] This cleavage may be performed via a protease called furin [23] which is also used by other respiratory viruses to penetrate into a host cell (but it is not utilised e.g. by the original SARS-CoV),[23,25] or by transmembrane serine protease 2 (TMPRSS2).[24] Both proteases are significantly expressed in the respiratory tract.[25] So, it seems to be crucial that in addition to the ACE 2 expression itself, SARS-CoV-2 requires the expression of further genes and activation of metabolic pathways for its replication. This explains why lungs are the most vulnerable organ.

Smoking

Several studies documenting increased ACE 2 expression in smokers have been published in relation to the current COVID-19 pandemics. Immunohistochemistry analyses revealed higher expression of ACE 2 proteins in smokers as compared to non-smokers and increased ACE 2 expression in smokers with chronic obstruction pulmonary disease (COPD) as compared to healthy smokers.[26,27] Similarly to Zhao et al.,[22] ACE 2 was expressed in AT 2,[26] in alveolar macrophages[26] and the small airway epithelium.[26,27]

Also studies[28–30] based on transcriptome analyses confirm the increased expression of RNA ACE 2 in lungs of smokers when compared to non-smokers. Increased expression of ACE 2 RNA correlated with the expression of genes included in the process of replication of SARS-CoV-2.[28,29]Furthermore, there is a positive correlation between expression of ACE 2 RNA and the length and intensity of exposition to cigarette smoke in mice.[28] Higher ACE 2 expression in smokers seems to be part of a complex change of metabolic processes facilitating the replication of SARS-CoV-2. Considering the fact that smoking is a general risk factor for many respiratory diseases[31], including the Middle East Respiratory Syndrome (MERS),[32] smoking seems to be a clear risk factor for incidence of COVID-19 infection.

It is therefore surprising that we do not have evidence significantly showing epidemiological association between smoking and increased incidence of SARS[33] or COVID-19. Contrary to that, Miyara et al.,[34] for instance, compared the ratio of smokers among 482 French patients with symptomatic COVID-19 with the ratio of smokers in the general population and found out that smoking seems to be a protective factor for development of symptomatic COVID-19. Pharmacologically, they explained their results by nicotine-induced reduction of ACE 2 expression.[35] This hypothesis is therefore contrary to studies where smoking increased ACE 2 protein expression in pulmonary tissue in humans.[26,27]However, the level of ACE 2 expression in pulmonary tissue of patients using nicotine patches compared to smokers would be of interest.

We have evidence of undetected patients infected with SARS-CoV-2.[36–38]It is therefore possible that current data does not correspond to the prevalence of SARS-CoV-2 infection among smokers compared to non-smokers in the general population, but only to the ratio of smokers and non-smokers in severe cases. But, studies analysing smoking as a risk factor worsening the course of COVID-19 provide contradictory findings. There are studies showing smoking as a risk factor for COVID-19 severity,[39,40]while other studies disprove the association between smoking and COVID-19 severity.[41,42] So, it will be extremely interesting to wait for the results of larger epidemiological studies on smoking, e.g. from Iceland colleagues who are testing a large part of the population for the presence of SARS-CoV-2.[43,44]

Other factors increasing the expression of ACE 2

The relationship between other factors and possible increase in ACE 2 expression in the respiratory tract is more ambiguous. Several transcriptome analyses with non-coherent conclusions are available. Cai[30] did not detect a difference in ACE 2 RNA expression between women and men, individuals younger and older than 60 years of age, or various ethnic groups, which contradicts the study of Chen et al..[45] Chen et al. found increased expression of ACE 2 RNA in women, East Asian population and suggests negative correlation between ACE 2 expression and age. Furthermore, he found decreased expression of ACE 2 RNA in diabetic patients, contradicting Pinto et al.,[29] who found increased expression of ACE 2 RNA in patients with diabetes mellitus, hypertension and COPD. In addition to the ambiguity, the possible discrepancy between RNA expression and protein levels of ACE 2[46] is yet another limiting factor of these analyses.

Benefit of reduced pulmonary ACE 2 expression?

With increased expression of ACE 2 being a risk factor for SARS-CoV-2 infection, a theoretical possibility to reduce the risk of SARS-CoV-2 infection appears to be ACE 2 blockage in the pulmonary tissue. Inhibitor of ACE 2 was discovered *in silico* and it is supposed to prevent the interaction between ACE 2 and SARS-CoV,[47]but the consequences of the inhibition of the generally protective ACE 2 have not been evaluated *in vivo* . Chloroquine and hydroxychloroquine are used in the clinical practice wherein the inhibition of the synthesis of sialic acids and the subsequent decrease in ACE 2 glycosylation have been described as one of their mechanisms of action in the treatment of COVID-19. Reduced ACE 2 glycosylation decreases the affinity between SARS-CoV-2 and ACE 2.[48]

Tissues outside the respiratory tract

Despite high ACE 2 expression, tissues outside the respiratory tissue seem less significant for the pathogenesis of COVID-19. This may be caused by the absence of further enzymes amplifying the replication cycle of the SARS-CoV-2. However, extrapulmonary tissue cannot be completely excluded from the pathogenesis of COVID-19. It is clearly important for the initial contact between SARS-CoV-2 and the host with the subsequent transfer of the virus into the pulmonary tissue. Furthermore, there is evidence of the role of SARS-CoV-2 in the pathogenesis outside the respiratory tract. The presence of SARS-CoV-2 and the subsequent tissue damage are suggested in GIT,[20,49] central nervous system (CNS),[50] kidneys[51] and liver.[16] Decreased expression of ACE 2 on the surface of platelets may contribute to the procoagulant state.[52] Similarly, SARS affected extrapulmonary tissue, e.g. in a study analysing cardiac tissue of patients who succumbed to SARS, virus was detected in 35% of patients, with correlating downregulation of ACE 2 expression in cardiac tissue.[53]

Pharmacological increase of ACE 2 expression

We hold evidence that some medication can increase the expression of ACE 2. Regrettably, there are no studies focusing on the pharmacological modulation of ACE 2 expression specifically in the pulmonary tissue. The influence of ACE-I and AT1 R blockers is a matter of intense discussion.[54–59] But, mineralcorticoid receptor antagonists, statins, thiazolidinediones,[59] ibuprofen, as representatives of non-steroidal anti-inflammatory drugs,[60] are also associated with increased ACE 2 expression.

Recently published studies analysing the available evidence linking the effect of ACE-I and AT1 R blockers to increased ACE 2 expression did not demonstrate their clear and general effect on the increase of ACE 2 expression in humans.[55,59] Furthermore, the different effect of ACE-I and AT1 R blockers on ANG II concentration is also discussed. While ACE-I, beta blockers or direct renin inhibitors decrease the plasma concentration of ANG II, AT1 R increases the plasma concentration of ANG II.[58] Some authors[57] state that the increased concentration of ANG II as a substrate for ACE 2 may lead to an increase in ACE 2 expression. On the other hand, ANG II has been shown to downregulate ACE 2 mRNA and protein expression levels in

hypertensive conditions.[61] Therefore, some authors[58] warn of a possible upregulation in ACE 2 expression induced by beta blockers or direct renin inhibitors.

The extent of the possible increase in ACE 2 expression is also unknown. There are data pointing to a fold increase in ACE 2 expression compared to control groups,[59] as well as studies where pharmacotherapy significantly increased the expression of ACE 2, but by far did not reach the level of the control groups.[60,62] It is clear that any suggestions to changes in pharmacotherapy[56] must be better supported by experimental data. All the more so, as the data published so far have not shown any negative association between drugs affecting the RAS system and COVID-19.[63–68]

"Decreased ACE 2 activity is a risk factor for severity/lethality of COVID-19"

The patient's immune response seems to be the most important factor influencing the course of COVID-19. SARS-CoV uses several strategies to escape or suppress non-specific immune response. Comparison of patients who succumbed to SARS with survivors showed that the development of an adaptive immune response was a crucial factor. Specifically, the synthesis of antibodies against the spike protein of SARS-CoV.[69] However, more detailed immunopathological analysis is beyond the scope of this paper.

Decreased activity of ACE 2 in high-risk comorbidities

Risk factors correlating positively with severity of COVID-19 have been described as higher age, male sex, hypertension, diabetes and cardiovascular diseases.[70,71] Associations between some of these factors and the change of ACE 2 expression/activity may be found in the literature. Patients with heart failure have been reported to have upregulated expression of both ACE 2 mRNA and protein in cardiac tissue.[72,73] However, there is also a study which failed to find differences in the expression of ACE 2 protein in the cardiac tissue between heart failure patients and healthy individuals.[5] In animal models, ACE 2 deficiency is named as a risk factor worsening cardiac pathogenesis.[6,73] In patients with hypertension, decreased vascular expression of ACE 2 may be expected, but ACE 2 activity in the CNS also plays an important role in the pathogenesis of hypertension.[6,73] Also diabetes mellitus is associated with the reduction of ACE 2 activity [15,73]. There are studies reporting decreased expression of renal ACE 2 protein in patients with diabetic nephropathy compared to healthy control.[74] On the other hand, Lely et al.[75] failed to find difference in the renal expression of ACE 2 protein in healthy individuals and patients with renal damage, including diabetic nephropathy. A study in rats showed correlation between higher age and decreased ACE 2 protein expression. This decrease was more pronounced in males[76]. However, there are studies which do not show any age-related changes of ACE 2 activity in rats[77] and people[78] with ARDS. Non-coherent transcriptome analyses stated in the section 2.1.3 do not provide a clear conclusion either.

Therefore, there are data supporting the theory that some risk factors of COVID-19 severity correlate with lower baseline expression/activity of ACE 2. And several authors are in favour of this theory.[52,79,80] On the other hand, the data not fully compliant with this theory calls for caution. It is also important to mention that several comorbidities, e.g. diabetes, older age, and cardiovascular diseases, are negative prognostic factors in infectious diseases with a different pathogenesis than that of COVID-19 as well.[81]

Benefit of increasing ACE 2 activity

In addition to the patient's adaptive immune response, the survival of COVID-19 requires maintained ACE 2 activity to avoid the development of fatal ARDS, as stated above. During ARDS caused by SARS-Cov-2, ACE 2 activity in AT 2 is likely to be substantially reduced, and it is therefore appropriate to restore it. The logical solution seems to be the infusion of recombinant ACE 2 protein. In COVID-19, serum ACE 2 protein can bind circulating SARS-CoV-2 and suppress virus replication, in addition to reducing the unfavourable

ANG II/ANG (1-7) ratio. [19,82,83] From this point of view an inhalation form of an ACE 2 analogue would be interesting because of possible reduction of the viral load in the airways. The safety of recombinant ACE 2 has been demonstrated in pilot clinical trials in humans.[8,19] It can therefore be expected for results of valid clinical trials in patients with COVID-19 to be shown in the near future.

Protective effect in ARDS may be expected from the administration of ANG (1-7) as a product of ACE 2.[84,85] A study wherein high doses of calcitriol alleviated reduction of ACE 2 mRNA and protein expression during acute lung injury in mice seems to also be of interest.[86] To provide a complete picture, calcitriol itself has not significantly increased natural expression of ACE 2 mRNA and protein in control group.

Some authors[87,88] hypothesise a beneficial effect of AT1 R blockers in the treatment of COVID-19 patients. They speculate that AT1 R antagonism decreases harmful effects of ANG II. Furthermore, increase of circulating ANG II as an ACE 2 substrate will lead to quicker conversion to ANG (1-7). Consequently, Gurwitz assumes upregulation of ACE 2 expression.[88] There is evidence supporting this hypothesis in ARDS conditions[85] and very limited population-based data associating treatment with AT1 R blockers as a protective factor of COVID-19 severity.[63,64] But clear and general effect of AT1 R blockers on the increase in ACE 2 expression in humans is not confirmed,[55,59] as noted in section 2.2.1.

The currently available evidence supports the attempts to maintain ACE 2 activity in patients with severe COVID-19 as a life-saving strategy. It also seems rational to avoid increasing ACE 2 activity by its expression in the cells potentiating the replication cycle of SARS-CoV-2, e.g. AT 2. Further, a speculation on the use of direct ACE 2 activators: there have been several studies supporting the use of 1-[[2-(dimethylamino) ethyl] amino]-4-(hydroxymethyl)-7-[[(4-methylphenyl) sulfonyl] oxy] -9H-xanthone-9 (XNT) and diminazene aceturate (DIZE) in tissue and animal models, where ACE 2 activation increased ANG (1-7) and simultaneously decreased ANG II. [7,89] Unfortunately, their toxicity is not known. Therefore, their introduction into the clinical practice during the current COVID-19 pandemics is not plausible, but they indicate a possible direction of development.

Conclusion:

- In addition to the expression of ACE 2, the pathogenesis of COVID-19 requires the presence of further enzymes important for the replication cycle of SARS-CoV-2 – this happens e.g. in AT 2.
- The only proven factor increasing ACE 2 expression in AT 2 is smoking and COPD in smokers.
- However, smoking has not been proven as a risk factor for COVID-19 infection in epidemiological studies.
- There are no studies focusing on pharmacological upregulation of ACE 2 expression specifically in the pulmonary tissue. Only limited data showing that certain drugs may increase ACE 2 expression in extrapulmonary tissue are available, but the association with the risk of SARS-CoV-2 infection is not known.
- It is necessary to consider the extent of the ACE 2 increase. It is possible that some patients profit from pharmacologically-induced upregulation of ACE 2 expression followed by cardiovascular benefits, though the overall extent of ACE 2 expression may be lower than in the healthy population.
- There are cautious confirmations of reduced ACE 2 expression in comorbidities as diabetes, hypertension, and higher age. However, the theory that decreased baseline activity of ACE 2 contributes to more severe course of COVID-19 has not been confirmed yet.
- Increasing ACE 2 activity during COVID-19 seems to be a life-saving strategy.

Competing of interests:

The author have no conflicts of interest to declare.

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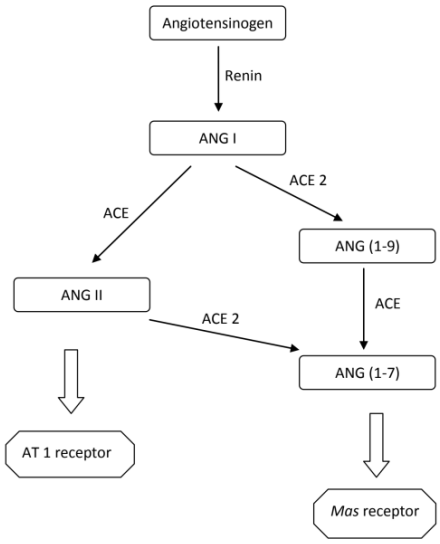

Figure 1. *Schematic of the renin-angiontensin system.* ANG I - angiotensin I; ACE - angiotensin-converting enzym; ANG II - angiotensin II; AT 1 receptor - receptor for angiotensin II type 1; ACE 2 - angiotensin-converting enzym 2; ANG (1-9) - angiotensin (1-9); ANG (1-7) - angiotensin (1-7)