Review article

ACE2 as Drug Target of COVID-19 Virus Treatment, Simplified Updated Review

Gomaa Mostafa-Hedeab*[1,2]

Abstract

Background: Since its first appearance in December of 2019, regular updates around the world demonstrates that the number of new Corona Virus 2019 (COVID-19) cases are increasing rapidly, indicating that not only does COVID-19 exhibit a rapid spread pattern, but human intervention is necessary for its resolution. Up until today (27-5-2020) and according to the World Health Organization (WHO), the number of confirmed COVID-19 cases has surpassed 4.5 million with more than 307, 500 deaths. Almost all countries have been affected by COVID-19, and resultingly, various drug trials have been conducted, however, a targeted treatment remains to be made accessible to the public. Recently, Angiotensin-Converting Enzyme-2 (ACE2) has gained some attention for its discovery as a potential attachment target of COVID-19.

Methods: We reviewed the most recent evidence regarding ACE2 distribution and action, the binding mechanism of COVID-19 and its correlation to cellular injury, ACE2 polymorphisms and its association to fatal COVID-19 and susceptibility and, finally, current ACE2-based pharmacotherapies against COVID-19.

Results: Blocking the ACE2 receptor-binding domain (RBD) using a specific ligand can prevent COVID-19 from binding, and consequently cellular entry and injury. Comparatively, soluble ACE2, which has a higher affinity to COVID-19, can neutralize COVID-19 without affecting the homeostatic function of naturally occurring ACE2. Lastly, ACE2 mutations and their possible effect on the binding activity of COVID-19 may enable researchers to identify high-risk groups before they become exposed to COVID-19.

Conclusions: ACE2 represents a promising target to attenuate or prevent COVID-19 associated cellular injury.

Keywords: ACE, ACE2, COVID-19.

ACE and ACE2

Since the end of 2019, the rapid spread of the novel Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first appeared in Wuhan, China, led to widespread severe respiratory infection and pneumonia, and a mortality rate of 1%-2.5% (1). ACE2 belongs to the ACE family, and ACE proteins, in particular, are zinc metallopeptidases. Moreover, the ACE2 gene is located on position Xp22 on chromosome X and contains 18 exons (2).

ACE2 is primarily expressed in arterial and venous endothelial cells, arterial smooth muscle (3), and oral mucosa (4). Jackman et al. (5) showed that in human pulmonary endothelial cells, angiotensin (Ang) (1–9) was significantly more active than Ang- (1–7), which enhanced the effect of ACE-resistant bradykinin analog on the beta receptor type-2 (B2) adrenergic receptor. In addition, Ang-(1–9) was found to augment arachidonic acid and nitric oxide (NO) release by kinin (5).

Though ACE2 is approximately 40% similar to the somatic form of ACE (2), they differ in biological function and substrate specificity (6).

ACE is located on chromosome 17 (7) and mediates the conversion from Ang I (inactive decapeptide) to Ang II (octapeptide active form) (8). Here is a list of the following actions that may occur as a consequence of this conversion:
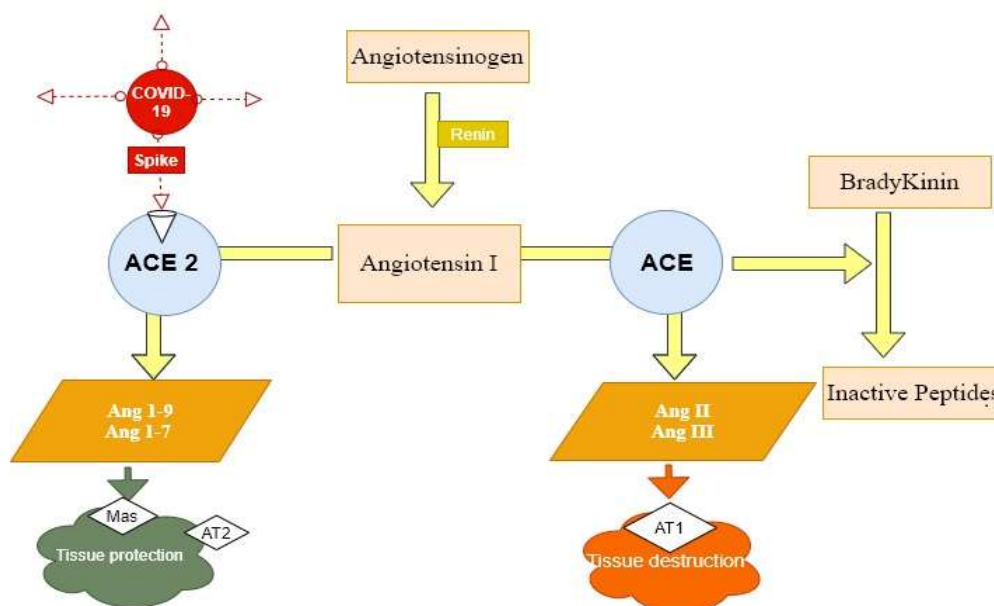
- Ang II is potent vasoconstrictor (8)

1: Pharmacology department, Medical College, Jouf University, KSA.
2: Pharmacology department, Faculty of Medicine, Beni-Suef University, Egypt.
*Corresponding author: Gomaa Mostafa-Hedeab; Tel: +96 6534627393; E-mail: Gomaa_hedeab@yahoo.com.

Fig. 1. Diagrammatic demonstration showed the main differences between ACE and ACE2 and its effect on COVID-19. COVID-19 bind specifically to ACE2 on cell membrane od different tissues, followed by ACE2 downregulation and COVID-19 cellular entry and consequent cytokine storm and lung tissue injury. ACE2 downregulation results in decrease Ang 1-9 and Ang 1-7 decreasing their tissue protection and leaving ACE destructive power unopposed that result in increasing the rate and extend of lung tissue injury.

- Preserves and prevents the degradation of bradykinin, a vasodilator peptide (9).
- Triggers the release of aldosterone which enhances sodium and water reabsorption by the kidneys (10).
- Enhances the action of anti-diabetic drugs such as dipeptidyl peptidase-4 (DPP4) (11). ACE2 does not degrade in the presence of bradykinin and it is not inhibited by classic ACE inhibitors (ACEIs) (12).
- Stimulates cell proliferation through the activation of various cytokines (13).

Bradykinin, a vasodilator that acts through the B2 receptor, is produced from its precursor kininogen by kallikrein, and subsequently degraded by ACE (14).

ACE2 is expressed in the membrane of tissues such as the heart, kidneys, lungs, intestine, and testis (15). ACE2 is also shown to be downregulated in the context of cyclic vomiting syndrome (CVS) related diseases (16).

ACE produces Ang-(1-7) via Ang Ⅱ hydrolysis and Ang-(1-9) via Ang I hydrolysis. Ang-(1–7) is a vasodepressor peptide that antagonizes the vasopressor action of Ang Ⅱ (17). Furthermore, Ang-(1–7) is converted into inactive Ang-(1–5) by ACE (6) or by neutral endopeptidases (12).

ACE2 activity may, therefore, counterbalance the effects of ACE by preventing the accumulation of Ang Ⅱ in tissues where both ACE2 and ACE are expressed (18).

Moreover, Ang-(1–7) binds to the mitochondrial assembly (Mas) receptor, a G-protein-coupled receptor (GPCR), resulting in prostaglandin vasodilation and antiproliferative actions (19). The Mas receptor can hetero-oligomerize with the angiotensin Ⅱ receptor type 1 (AT1) and act as a physiological antagonist of Ang Ⅱ (20). Ang-(1–7) induces endothelial NO production via protein kinase B (Akt)-dependent pathways (21). Ang-(1–9) may be converted to Ang-(1–7) by ACE (12) (Fig. 1).

Interestingly, pancreatic ACE2 plays a crucial role in glycaemic control (22). Downregulation of renal ACE2 was shown to be linked to the decline in renal disease and diabetic nephropathy (23). ACE2 transcription is controlled by various DNA binding proteins including Sirtuin 1 (SIRT1) (24).

The human ACE2 was discovered during human heart failure and in lymphoma complementary deoxyribonucleic acid (cDNA) libraries (12). Previously, ACE2 was considered to have no role in immune cell function, however, Zhao et al. recently discovered ACE2 micro ribonucleic acid (mRNA) in CD 14+ and CD16+ human monocytes (25).

In a bleomycin-induced pulmonary fibrosis animal model, the intraperitoneal injection of recombinant human ACE2 treatment decreased lung inflammation and fibrosis and had improved lung function (26). In a

rat model for ischemic heart disease, the subcutaneous infusion of diminazene aceturate (DIZE) significantly increased cardiac ACE2 mRNA expression and ACE2 protein catalytic activity, reduced ACE mRNA expression, and improved cardiac remodeling (27).

Further, the rate of ACE2 expression increased in diabetic and hypertensive patients receiving ACEI or angiotensin II type-I receptor blockers (ARBs) (28) (29).

2. Link between ACE2 Receptors and COVID-19

COVID-19 is a positive single-stranded RNA virus (+ssRNA) that causes severe respiratory syndrome in humans (30). COVID-19 binds to their cellular target via ACE2 (28), and its receptor recognition and fusion of cellular membrane are being mediated by SARS-CoV spike glycoprotein (SP) (31). SARS-CoV-2 SP binds to the RBD of ACE2 (32).

The similarity between SARS-CoV2 and SARS-COV1 (33), and the similarity between SARS-CoV2 SP and the RBD of ACE2, indicates a strong binding affinity to human ACE2 in both cases (34).

Furthermore, SARS-CoV2 has a stronger affinity to ACE2 compared to SARS-CoV, a factor that likely caused increased human to human transmission of SARS-CoV2 (28).

The binding of the +ssRNA virus to the RBD of ACE2 determines viral entry into the cell and the rate of cell injury (35), which is directly proportional to ACE2 expression (36).

3. COVID-19 binding to ACE2 and Tissue injury

ACE2 receptors are found in various extrapulmonary tissues including the heart, kidneys, endothelium, and intestine (15) (37). When COVID-19 binds to the ACE2 receptor, a cytokine storm and the inflammatory process is initiated, and the lung was found to be the most affected site. This begs the question, why?

The outcome of the lung was explained by Zhao et al. who demonstrated that alveolar epithelial type II cells (AECII) may express 83% of ACE2 in normal lung tissue donors (38). Moreover, using gene ontology analysis, they found that AEC2 expressing cells carried several viral process-related genes

including viral replication and assembly (38), which could explain the presence of viral replication in the lung tissue and initiation of lung injury.

In addition, also ACE2 is expressed in the gastrointestinal tract (GIT), specifically the luminal surface of epithelial tissue, and acts as co-receptor for amino acid and nutrient uptake (39). This finding suggests that we should consider the feco-oral transmission route and COVID-19 related-GIT symptoms. Indeed, the widespread distribution of ACE2 across more than one organ may explain the observed multiorgan dysfunction in COVID-19 patients (40) (41).

4. ACE2 gene polymorphisms

ACE2 gene polymorphisms are linked to several diseases, particularly Asian populations, which may play a role in ACE2 expression and increased risk of COVID-19 infection (42). The variability in ACE2 mRNA expression compared to race is not well understood, and there seems to be conflicting evidence in the literature. Firstly, Zhao et al. showed that the lungs of Asian males expressed higherACE2 mRNA compared to their female counterparts. Zhao et al. also found that Asian populations expressed higher ACE2 mRNA compared to Caucasian or African-American populations (38), whereas, Gai et al. showed that there was no significant difference in ACE2 mRNA expression between Asian and Caucasian populations (43). To clarify these conflicting results, the exact genetic basis of ACE2 expression in different populations warrants future investigation (44).

4.1 ACE2 Gene polymorphisms and diseases

As previously mentioned, ACE2 is a vasodilator agent that opposes the action of Ang II. The disruption of ACE2 results in a relative increase in Ang II levels and a decrease in cardiac performance (45).

Previous studies correlate the association of diminished cardiac ACE2 with diabetic heart disease, hypertension (HT) (46) (47) and dyslipidemia (48).

The association between ACE2 gene polymorphisms among different species and certain diseases are summarized in Table 1. Most polymorphisms are associated with a specific type of HT.

Table 1. Association between different ACE2 SNPs and diseases in different populations.

Population	Association	Reference
Chinese population	rs2074192 was associated with Essential Hypertension (EH) rs4240157, rs4646155, and rs4830542 were associated with EH- and hypertension-related AF and left atrial	(49)
Chinese population	rs2106809 determine the efficacy of ACEI in hypertensive patients	(50)
Chinese population	No association with either hypertension or orthostatic hypertension	(51)
Chinese population	A1075G associated with hypertension	(52)
Canadian Nicotine Dependence in Teens Canadian	rs2074192, rs233575, and rs2158083 variants associated with pathological variations of blood pressure	(53)
Nicotine Dependence Canadian of European descent	rs2074192 and rs233575 associated with both Systolic Blood Pressure (SBP) and diastolic Blood Pressure (DBP) and rs2158083 was associated with SBP only	(53)
French Canadian Males Nicotine Dependence	rs233575 and rs2158083 were associated with DBP	(53)
Canadian females of European Descent Nicotine Dependence	rs2074192, rs233575, and rs2158083 associated with a change in SBP	(53)
Chinese	rs2106809 affect benazepril efficacy.	(54)
Indian	rs21068809 association HT	(55)
Brazilian patients	ACE2 G8790A polymorphisms reveal susceptibility to HT	(56)
Uygurs	rs2048683 is associated with cardiovascular risk in Uygurs with type 2 diabetes mellitus.	(57)
Chinese	rs714205 is associated with retinopathy in type 2 diabetes mellitus among Chinese individuals	(58)
Chinese	rs2074192 and rs2106809 are associated with Left Ventricular Hypertrophy in hypertensive patients.	(59)

4.2 ACE2 receptor polymorphism and coronaviruses infection

In a murine model, Wysocki et al. found that the expression level of ACE2 may be affected by ACE2 mutations (60). In a separate study, increased cardiovascular tissue damage in mice with a deleted ACE2 gene was found to be
concomitant to increased Ang II levels (61). In Iran, the presence of SARS-CoV-2-induced inflammation was explained by the attenuation of Ang II conversion to Ang (1-7) via ACE2 (62).

Cell type II transmembrane protein dipeptidyl peptidase 4 (DPP4/CD26) is required for COVID-19 to bind to the host cell through the SP (63). Within endosomes, the SP activates cellular transmembrane serine protease 2 (TMPRSS2) and cysteine protease cathepsin L. Then, the kallikrein-related peptidase 5

(KLK5) cleaves dipeptidyl-peptidase-4 (DPP4) and releases it into circulation in its soluble form (64).

Among the 14 mutations reported within the DPP4 gene, four polymorphisms (K267E, K267N, A291P, and D346-348) were reported to decrease the binding and penetration power of the Middle East respiratory syndrome (MERS-CoV) into target cells, leading to decreased viral replication (65).

Among the 32 identified variants of ACE2 by Cao and colleagues, seven mutant points were considered to affect SARS-CoV-2 infections. Thus, some individuals may be more resistant to SARS-CoV-2 infections than others (44), however, these findings require further confirmation.

A recent publication by Asselta et al. stated that

TMPRSS2 variants, but not ACE2 gene polymorphisms, determines the severity and progression of COVID-19 (66).

Calcagnile et al. studied the potential interaction between ACE2 single nucleotide polymorphisms and COVID-19 S-glycoproteins. They found that the S19P (rs73635825) mutation, which is more common in African populations, may genetically protect against COVID-19. In addition, they found that K26R SNPs (rs75548401), which is more prevalent in individuals with a European background, may be predisposed to severe SARS-CoV-2 infection (67), however, a clinical association may be required to prove this conclusion.

5. Recombinant ACE2

The binding of COVID-19 to the ACE2 receptor binding site which is located on the cell membrane is followed by the downregulation of ACE2 and COVID-19 entry, and, therefore, the initiation and exacerbation of lung injury [10].

This raises the question of whether it is possible to neutralize COVID-19 to prevent cell entry by administrating soluble ACE2.

Yu et al. state that soluble ACE2 could theoretically result in COVID-19 neutralization and decrease the renin-angiotensin system (RAS)-induced lung injury during the acidic phase or ventilation (68).

Moreover, one clinical trial showed that recombinant human ACE2 (rhACE2; APN01, GSK2586881) is safe and reduced ang II proteolytic target peptide and interleukin-6 (IL-6) substantially when given to healthy volunteers suffering from acute respiratory distress syndrome (ARDs) (69) (70). Based on these conclusions, soluble ACE2 could be a possible therapeutic target in COVID-19 patients.

6. COVID-19 interfaces with ACE2

The COVID-19 RBD is similar to SARS-CoV-RBD (34), however, several sequence variations and conformational deviations can be found in their respective interfaces with ACE2.

When Yan et al. compared the interaction interface of SARS-CoV-2-RBD and SARS-CoV-RBD with ACE2, it was suggested that some variations strengthened the interaction between SARS-CoV-2-RBD and ACE2, while others likely weakened the interaction (71). He demonstrated that a change in amino acids, such as Valine 404 (Val404) to Lysine

317 (Lys317) resulted in a tighter association due to the formation of a salt bridge in ACE2 between Lys317 and Aspartic acid 30 (Asp30). Furthermore, the change from Leucine 472 (Leu472) to Phenylalanine 486 (Phe486) strengthened the van der Waals forces with Methionine (Met82) (71).

Comparatively, the replacement of Arginine 426 (Arg426) with Asparagine 439 (Asn439) in ACE2 appeared to weaken the interaction due to the loss of an important salt bridge with Asp32 (71).

He concluded that the development of a ligand or antibody could be carried out based on protein structure. Taken together, synthesizing an agent with an enhanced affinity to either ACE2 or COVID-19 could attenuate virulence and infection (71).

7. ACE2-based pharmacotherapy for COVID-19

COVID-19 could be attacked or at least partially attenuated by one of the following strategies:

- Blocking the COVID-19 ACE2 receptor binding site either by specific ligand or antibody.
- The soluble form of ACE2 will neutralize COVID-19 by binding to its surface, and, thus, attenuate its binding to ACE2 on the cell surface and decreasing collateral tissue damage, specifically in the lungs.
- Using a structural based design strategy that considers the interface between SARS-CoV-2 and SARS-CoV with ACE2 (71) will allow us to synthesize a novel neutralizing agent (soluble ACE2) that strongly adheres to COVID-19 compared to human ACE2
- Developing a COVID-19 spike vaccine that is based on its protein structure will prevent it from binding to the ACE2 receptor binding site.

8. Conclusion

A suitable target that can help attenuate or prevent cellular injury associated with COVID-19 infection is ACE2 since it is the known binding site for COVID-19. By blocking the RBD of ACE2 with a specific ligand, we can prevent COVID-19 binding and, consequently, cellular entry and injury. Overcoming possible constraints, and achieving a soluble ACE2 molecule with a higher affinity to COVID-19 could neutralize COVID-19 without affecting the beneficial effects of naturally occurring ACE2. Lastly, ACE2 mutations and their associated effect on the binding activity of COVID-19 could enable us to identify groups at higher risk of developing COVID-19.

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