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Mapping the effect of drugs on ACE2 as a novel target site for COVID-19 therapy

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Abstract. – Angiotensin converting enzyme 2 (ACE2) has potentially conflicting roles in health and disease. COVID-19 coronavirus binds to human cells via ACE2 receptor, which is expressed on almost all body organs. Boosting the ACE2 receptor levels on heart and lung cells may provide more cellular enter to virus thereby worsening the infection. Therefore, among the drug targets, ACE2 is suggested as a vital target of COVID-19 therapy. This hypothesis is based on the protective role of the drugs acting on ACE2. Therefore, this review discusses the impact and challenges of using ACE2 as a target in the current therapy of COVID-19.

Key Words:
COVID-19, Angiotensin converting enzyme 2 (ACE2), Azithromycin, Hydroxychloroquine, Zinc, Vitamin D.

Introduction

In late 2019, a novel coronavirus (CoV), named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused coronavirus disease-2019 (COVID-19), which first emerged in Wuhan, China. Later, SARS-CoV-2 spread around the globe, causing an unprecedented public health crisis[1]. The World Health Organization (WHO) declared it a pandemic in March 2020[2].

New mammalian CoVs are regularly recognized, such as severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in China in 2002, Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in Saudi Arabia in 2012 and the latest emerging human-infecting beta-CoV (SARS-CoV-2)[3,4]. Although COVID-19

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has a higher number of cases, the mortality rate is lower than in SARS-CoV and MERS-CoV[5]. Phylogenetic analysis suggests that the SARS-CoV-2 origin is bat-borne because of the 96% genetic similarity; however, pangolins may also be a potential intermediate that facilitates spill-over to human[6-8].

Angiotensin converting enzyme 2 (ACE2), which is a zinc metalloproteinase containing 805 amino acids with high sequence homology with ACE, is a potential host receptor for SARS-CoV-2 and is expressed by different cells[9-11]. SARS-CoV-2 binds to the host cell receptor ACE2, starting viral replication and pathogenesis. ACE2 levels expressed on the cells affect the virus ability to replicate. Thus, modulation of the expression and/or activity of ACE2 may have a role in COVID-19 management. In this review, we have discussed in detail the link between ACE2 and the drugs used in the management of COVID-19 in an attempt to discover a potential target for the used drugs.

Coronaviruses (CoVs)

SARS-CoV-2 is the 7th identified CoV, which belongs to the subgenus *sarbecovirus*, subfamily *Orthocoronavirinae* of the family *Coronaviridae*[12]. There are several identified CoVs that are pathogenic to humans such as 229E, NL63, OC43 and HKU1, and most of them result in mild disease[13]. However, few pathogenic CoVs including SARS-CoVs and MERS-CoVs are able to infect human and result in life-threatening infection[14]. Currently, SARS-CoV-2 moved the world toward a public health emergency. Bats are suggested to be the natural host of this virus because of the 96% genetic homology among SARS-CoV-2 and bat CoVs[6,7,12]. Interestingly, pangolins may also be a potential intermediate host due to the similarity the similarity between SARS-CoV-2 and pangolin CoVs (85.5-92.4%) genomes[8].

The SARS-CoV-2 particle is spherical with spikes on the surface, and it has a single-stranded, positive-sense RNA genome (from 29.8 kb to 29.9 kb) encoding large nonstructural polyproteins (ORF1a/b), which cleaves to 16 proteins, while the rest ORFs encode structural and accessory proteins[12,15,16]. The essential proteins for viral assembly and infection are envelope (E), membrane (M), nucleocapsid (N), and spike (S) (Figure 1)[17-19]. Trimeric S glycoprotein is

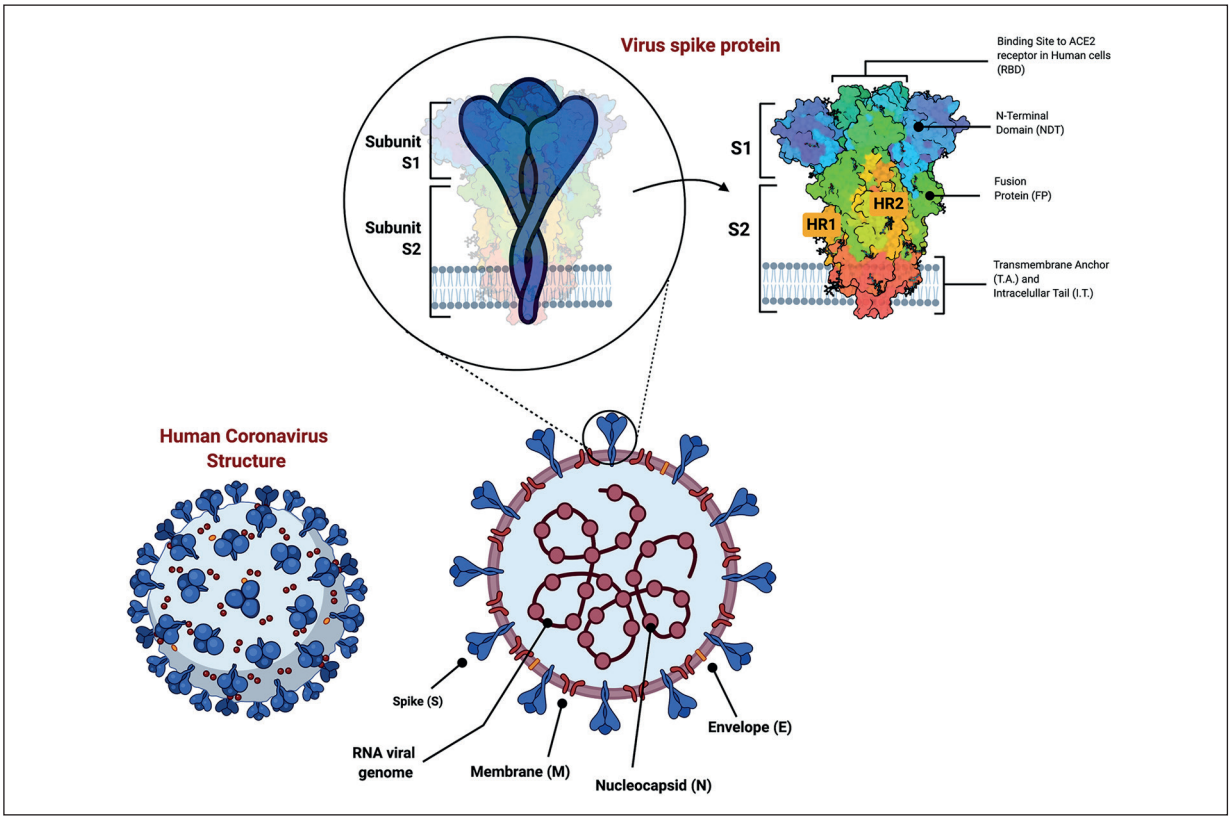

Figure 1. Representative illustration of human coronavirus structure showing the crystallographic structure of the virus spike proteins.

responsible for the attachment and binding to the host cells. It has two conserved domains S1, which contain the receptor-binding domains (RBDs) and S2 having heptad repeat 1 (HR1) and heptad repeat 2 (HR2) domains, which mediate the virus-cell membrane fusion (Figure 1)[20-22]. In the case of SARS-CoV-2, RBDs bind to the ACE2 receptor facilitating virus entry to the host cells[6,16].

Replication and Pathogenesis

Replication of SARS-CoV-2 starts with recognition and binding of the RBDs to host cell receptors (ACE2) (Figure 2)[6]. SARS-CoV-2 can easily spread in human population because of a higher binding affinity of viral spikes compared to SARS-CoV spikes (10 to 20-fold)[23]. After binding to its host receptor, S protein undergoes conformational changes to facilitate the fusion between the host cell membrane and the viral E protein and then viral entry (Figure 3)[24,25].

This fusion induces viral RNA release into the host cytoplasm to undergo translation, producing two polyproteins, pp1a and pp1ab that cleave into smaller proteins. Majority of the nonstructural proteins assemble to form the replicase-transcriptase complex (RTC) to subsequently synthesize a set of nested sub-genomic RNAs (sgRNAs), which encode different accessory and structural proteins (Figure 2)[17,26].

The formed genomic RNA, E and N proteins assemble, forming the virion particle. Finally, the virus particles are released after the virus-containing vesicles fuse with the host cell membrane[17,27].

Pathogenesis begins after recognition and binding of the virus to the host cell receptors. Once inside the cell, the virus rapidly replicates, inducing a strong immune response against SARS-CoV-2 and may cause the cytokine storm (hypercytokinemia), which is associated with uncontrolled production of proinflammatory cytokines[28]. The increased levels of cytokines and chemokines include interleukin (IL) 2, IL-7, GCSF, IL-8, GMCSF, IFNγ, MCP1, IP10, MIP1α, MIP1β and TNF-α[29,30]. Such virus-triggered cytokine storm can be the potential cause of acute respiratory distress syndrome (ARDS) and other organ damage and dysfunction[29]. The expression of ACE2 receptor on several host cell surfaces such as in the lungs, heart, liver and kidney explains the involvement of many organs and

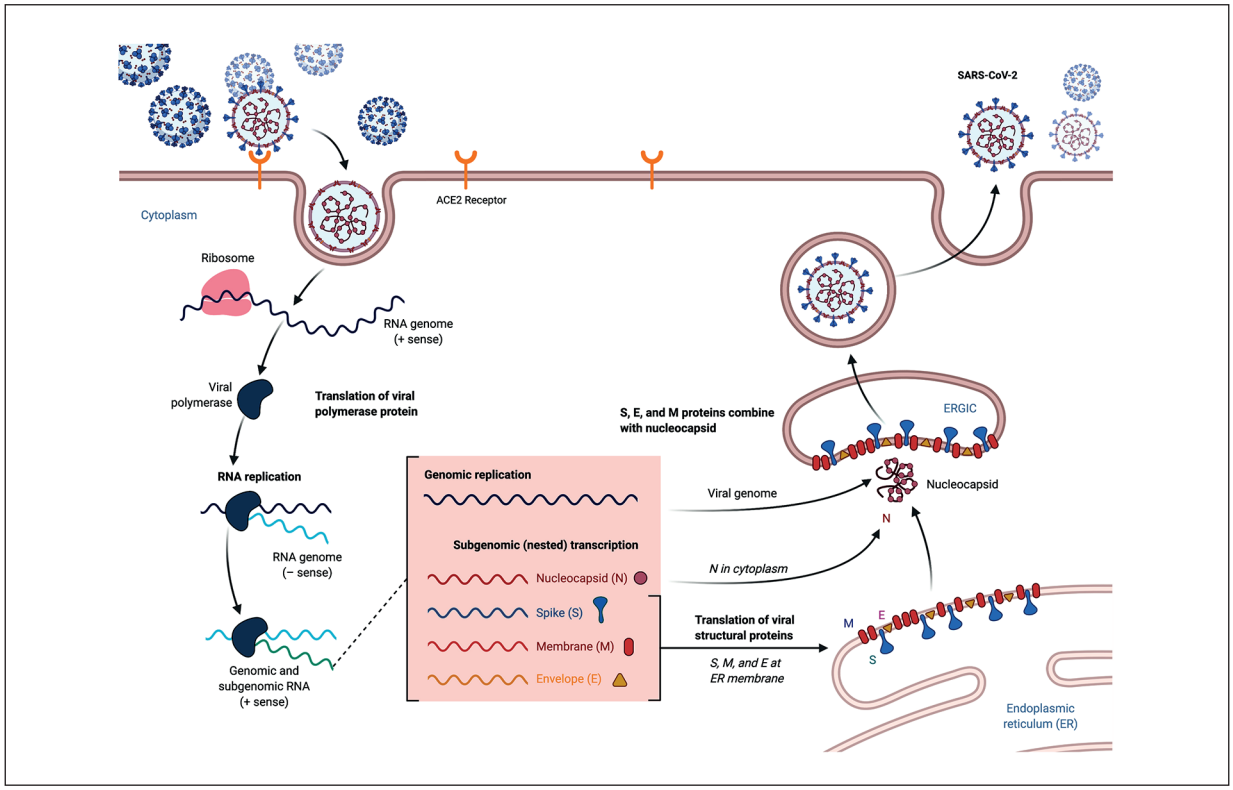

Figure 2. The replication cycle of SARS-CoV-2 in host cells.

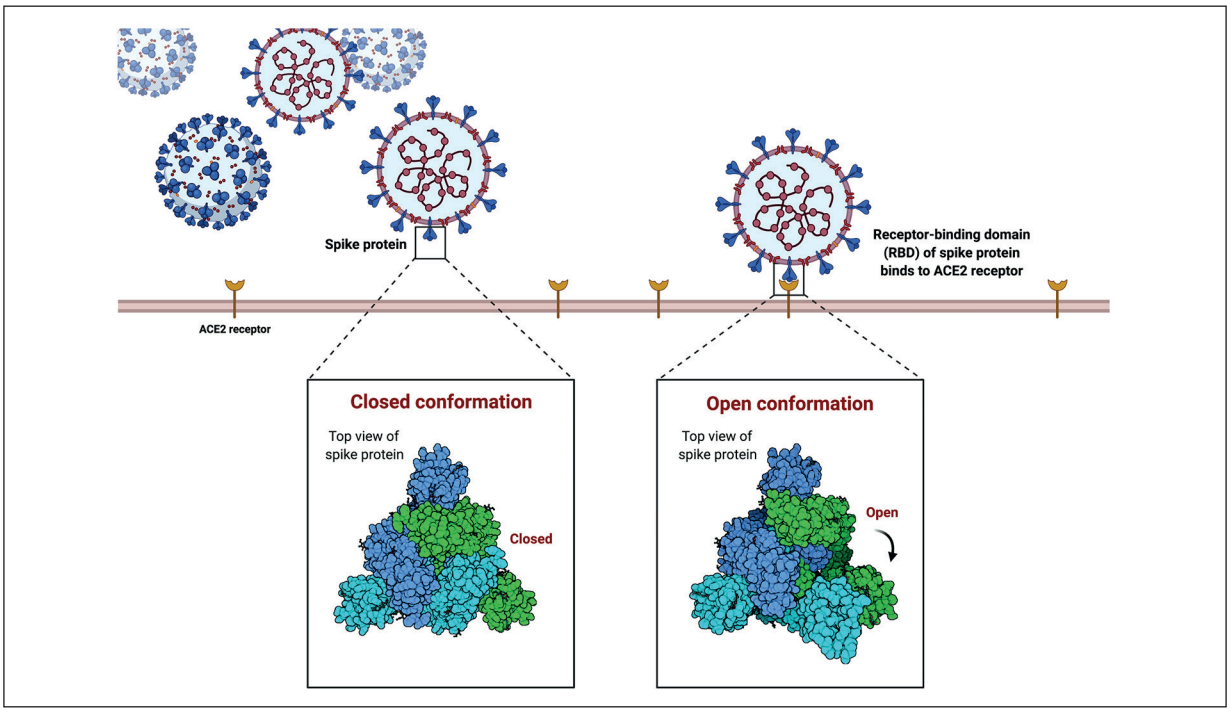

Figure 3. Conformational changes of SARS-CoV-2 spike protein.

death due to multiple organ dysfunction[6,29,31-33]. The dominant clinical symptoms of SARS-CoV-2 infected patients mainly appear as severe pneumonia and acute cardiac injury. Such rapidly developing pneumonia can easily be seen in chest X-rays. Coagulopathy is resulted by alveolar and interstitial inflammation[34,35].

Clinical Presentation

Patients with COVID-19 may be asymptomatic or suffer from multiorgan manifestations. Patients with mild disease may present with cough, fever, fatigue, headache, dyspnea and sore throat[36]. Gastrointestinal symptoms such as nausea, vomiting or diarrhea are observed in some patients. While severe COVID-19 cases show signs and symptoms of ARDS, which can precipitate septic shock and require intensive care. Computed tomography revealed a ground-glass opacity in the lungs and bilateral patchy shadowing[37].

Angiotensin Converting Enzyme 2 (ACE2)

Angiotensin Converting Enzyme 2, discovered in 2000, is a zinc metalloproteinase having 805 amino acids and with a significant genomic homology to ACE[9]. ACE2 is expressed by a variety of cells in the heart, lungs, arteries, kidney, and intestines and acts as a potential host receptor for SARS-CoV-2. It is predominantly expressed in vascular endothelial cells in the heart and kidneys[10]. Alveolar epithelial type II (AEII) cells represent around 83% of ACE2-expressing cells in the lung and have many genes that are closely linked to the viral life cycle and nucleic acid replication, so AEII cells are believed to act as a reservoir and facilitate the replication of SARS-COV-2 in the lung[11]. ACE2 functions as a carboxypeptidase rather than a dipeptidyl carboxypeptidase. Thus, ACE2 hydrolyzes Ang I to Ang (1–9) and Ang II to Ang (1–7). Consecutively, Ang (1–9) is changed to Ang (1–7). Thus, ACE2 facilitates the production of Ang (1–7). A characteristic feature of ACE2 is not to catalyze Ang I to Ang II conversion (Figure 4).

Another critical feature of ACE2 is that ACE inhibitors do not affect its enzymatic activity. ACE2 can inhibit the function of Ang II produced by ACE by 2 mechanisms; ACE2 can initiate another pathway for the degradation of Ang1 (Figure 4) and can enhance the production of Ang, which is a vasodilator peptide that can antagonize the harmful actions of Ang II (1–7). ACE2 generating Ang (1–7) can vary in different tissues. ACE2 is not specific for RAS since it also metabolizes apelins, kinins and neurotensin[38].

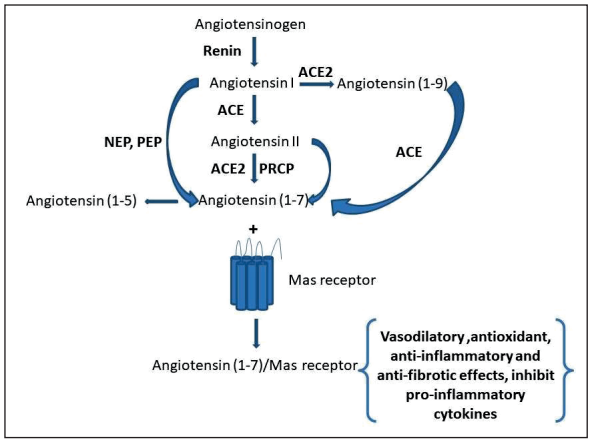

Figure 4. Metabolic pathway of renin angiotensin system (RAS). Renin converts angiotensinogen into angiotensin I and metabolized angiotensin-converting enzyme (ACE) into angiotensin II. Angiotensin I is processed by ACE2 into the inactive peptide angiotensin (1–9), which can be changed by ACE to Ang (1–7). through the neutral endopeptidase 24.11 (NEP) or prolylendopeptidase (PEP), angiotensin I is changed to Ang (1–7). Angiotensin (1–7) can be also produced from Angiotensin II under the effect of ACE2 or prolylcarboxypeptidase (PRCP). Ultimately, angiotensin (1–7) interacts with its receptor Mas to produce various effects.

Li et al[39] investigated the biological behavior of the virus and its relation to ACE2 levels, thereby dividing them into high and low levels. ACE2 expression was associated with various immune responses including B cell responses and different cytokines like IL-1, IL-6, IL-8 and IL-10 production. Interestingly, the higher ACE2 can prolong the virus replication cycle and promote the viral penetration into the host cell. Accordingly, drugs, which modulate the activity and/or expression of ACE2, may be valuable in the management of COVID-19.

Risk Factors for COVID-19 and ACE2

The COVID-19 infection shows the most prevalent comorbidities including hypertension, cardiovascular disease, diabetes, and respiratory system disease. One explanation may be the increased expression of ACE2 in those patients. ACE2 expression may also be a key factor that determines age- and sex- related susceptibility, disease severity and clinical outcome of COVID-19. Children and young adult, especially young women, show a relatively high expression of ACE2 so they are more susceptible to SARS-CoV-2 because SARS-CoV-2 may compete with angiotensin II for cell surface binding sites and cellular uptake[40]. However, they are relatively protected from COVID-19 associated complications than adults, probably due to higher titers of antibodies formed against CoVs enabling the maintenance of a less inflammatory state. Different childhood vaccinations resulting in a diverse T-cells repertoire in children and young adults may play a significant role in decreasing susceptibility to complications. Its expression decreases with age leading to higher fatality rates and unfavorable outcomes. This may be explained by the fact that adults having skewed memory T-cell repertoires that may establish a memory T cell response to cross-reactive epitopes with subsequent T cell mediated damage and disease progression[41].

Another risk factor that links ACE2 to severe COVID-19 infection may be race. ACE2 is more expressed in Asians than in Whites or African Americans[40].

Although the level of cardiac ACE2 is reduced in hypertension, diabetes and heart disease, but once CoV finds its way inside hypertensive, diabetic and heart patients, several biological functions, including regulation of blood pressure, blood glucose limitation of progressive renal diseases such as diabetic nephropathy can be disturbed. ACE2 limits the adverse vasoconstrictor, the oxidative stress and profibrotic effects of AngII[42].

Therapeutic Approaches of COVID-19 and ACE2

Azithromycin and ACE2

A virtual model for the binding point between the SARS-CoV-2 spike and the ACE2 receptors was established and azithromycin showed a high binding affinity and has a much better ability at directly targeting the binding interaction point between the SARS-CoV-2 spike and ACE2[21]. In addition to ACE2, CD147 also acts as a receptor of host cells for SARS-CoV-2 invasion. Anti-CD147 antibodies can prevent SARS-CoV-2 invasion of host cells. Preclinical and clinical evidence demonstrated that azithromycin could rapidly prevent COVID-19 infection by possibly decreasing the expression of metalloproteinases (molecules closely related to CD147). Moreover, azithromycin induces antiviral responses in epithelial cells by increasing the levels of interferon and interferon-stimulated proteins, decreasing viral replication and virus release[43]. *In-vitro* studies have demonstrated the capacity of azithromy-

cin to reduce the production of proinflammatory cytokines such as IL-8, IL-6, reduce oxidative stress and modulate T-helper functions, which can indicate immunomodulating and antiviral post-infection role of azithromycin in the fight against COVID-19[29]. Other researchers also suggested that the raised pH of the trans-Golgi network might alter glycosylation of ACE2 receptor and this glycosylation inhibits SARS-CoV-2 from binding to host cells[44].

Limitations of Use

Azithromycin is generally well tolerated, but it has some relatively common adverse effects (1-5% of patients) such as gastrointestinal upset, headache and dizziness. Transient increases in transaminases have also been reported in 1.5% of patients[45]. Serious adverse effects include QT prolongation and torsades de pointes, resulting in death particularly when used in combination with hydroxychloroquine (HCQ)[46,47]. Thus, QT assessment and monitoring are necessary when the two drugs are co-administered. Additionally, the US Food and Drug Administration issued a warning in 2012 to consider the risk of fatal heart rhythms as patients with a prolonged QT interval, with a history of torsades de pointes, arrhythmias or uncompensated heart failure.

Hydroxychloroquine and ACE2

The expression of ACE2 is not only essential before infection with COVID-19 but also during infection[48]. Numerous mechanisms of action of chloroquine (CQ) and hydroxychloroquine (HCQ) against COVID-19 have been suggested. COVID-19 was found to utilize the same cell surface receptor ACE2 as SARS-CoV. An *in-vitro* study of the mechanism of the anti-SARS-CoV effects of chloroquine showed a deficit in the glycosylation of a virus cell surface receptor ACE2 on Vero cells[49]. Zhuang et al[48] also reported that ACE2 is upregulated after infection by SARS-CoV and COVID-19, or by stimulation with IFN-β in human bronchial epithelial cells. Zhuang et al[48] added that IFN-α, IFN-β, IFN-γ, TNF-α, IL-1α, or IL-1β, using luciferase reporter assays, can stimulate the promoter region of ACE2 that contains several immune and cytokine-responsive transcription factors binding sites such as sites for c-Jun, STAT1, STAT3, STAT5, IRF1, and IRF8. COVID-19 may positively induce the cellular entry receptor, ACE2. Thus, the induction of ACE2 by inflammatory cytokines also implies that the "cytokine storm" caused by COVID-19 not only damages host tissues but may also accelerate the spread of the virus. Another study demonstrated that HCQ in combination with azithromycin acts synergistically to inhibit viral replication *in vitro,* which it is very effective in the early stages of COVID-19 before the onset of a cytokine storm[50].

In-vitro results on cell lines showed that HCQ is slightly more toxic to ACE2hi cells than CQ, both CQ and HCQ could bind to ACE2, and they also exhibit an equivalent suppression effect for the entrance of COVID-19 spike pseudo-type virus into ACE2hi cells. These findings provided a theoretical and experimental basis for the clinical treatment of using CQ and HCQ for COVID-19 and suggested that CQ and HCQ as ACE2 blockers to inhibit viropexis of COVID-19 spike pseudotype virus[25].

Limitations of Use

Although HCQ is preferable than CQ because it is associated with a lower risk of toxicity, there are potential risks associated with HCQ, including QT prolongation, myopathy, retinal toxicity, and rhabdomyolysis. Other adverse effects of HCQ including nausea, diarrhea, and abnormal liver function are reported. The use of HCQ and CQ was discouraged in an open label randomized trial that used high dose HCQ in mildly to moderately ill COVID-19 patients admitted to 16 government hospitals in China. It showed no difference regarding the time to negative conversion of COVID-19 by real-time reverse transcription polymerase chain reaction at 28 days between groups treated with HCQ *vs.* standard treatment using either an upper or lower respiratory sample. However, no patients died with few adverse side effects. Another observational controlled study showed radiographical findings of COVID-19 pneumonia requiring oxygen in French hospitals and there were no differences between HCQ *vs.* non HCQ-treated groups. Moreover, eight of 84 patients receiving HCQ had the drug stopped because of electrocardiogram changes[51]. Accordingly, the WHO discontinued the use of these antimalarial drugs (CQ and HCQ) in hospitalized patients because of its limitations and the nonsignificant reduction in mortality[27,52,53].

Nonsteroidal Anti-Inflammatory Drugs and ACE2

The worse outcomes of COVID-19 may be associated with NSAID use. Nonsteroidal anti-inflammatory drugs (NSAIDs) in particular,

ibuprofen, was found to upregulate the ACE2 receptors and increase susceptibility to the virus or worsen symptoms in existing diseases[11]. However, paracetamol has been used as an alternative to NSAIDs to decrease elevated body temperature. The discrepancy about the use of NSAIDs in COVID-19 may be due to the complex production of prostaglandins that can both promote and inhibit inflammatory mediators[54]. A recent publication stated that NSAIDs especially ibuprofen increase ACE2 expression, but the authors did not provide the reference that supports this statement[26]. However, the European Medicines Agency (EMA) concluded that there are currently no scientific evidences establishing a link between ibuprofen or NSAIDs and worsening of COVID-19[55].

Limitations of Use

Liver toxicity may occur at high doses of paracetamol. Reasonable evidence exists for a link between NSAIDs and both respiratory and cardiovascular adverse effects in several settings. Regular NSAID use should probably not be recommended as the first line option for managing the symptoms of COVID-19. Nephrotoxicity is a common side effect of NSAIDs, which is more likely to be exacerbated by fever and dehydration associated with COVID-19[56].

Zinc and ACE2

Generally, zinc (Zn) has a significant role in viral infection through modulation of viral entry, replication, viral protein translation and release of virus[57]. ACE2 receptor is considered as a potential entry receptor for COVID-19[10]. Deficiency of Zn reduces the activity of ACE in the testes of prepubertal rats by a change in the concentration of ACE protein. ACE activity in the testes of the Zn-deficient rats was reduced by 74% compared with that in the control group. This was accompanied by a 64% reduction in the amount of ACE protein in the testes. It was extensively reviewed that zinc at a physiological concentration reduced recombinant human ACE2 activity in rat lungs. Moreover, Zn was reported to increase ciliary beat frequency *in vitro*. Zinc is crucial for maintaining a healthy respiratory epithelium because Zn have antioxidant, antiinflammatory activity and play a role in the regulation of tight junction proteins ZO-1 and Claudin-1. Moreover, Zn treatment was shown to increase the production of interferon α (IFNα) from leukocytes in rhinovirus-infected cells. Additionally, CQ can act as an ionophore for Zn and enhances the uptake of Zn by the lysosomes as demonstrated in human ovarian carcinoma cell line A2780[58].

Zinc is considered as a potential supportive treatment in COVID19 therapy due to its immune modulatory effect and a direct antiviral effect[59]. Thus, the ameliorative effect of zinc on ACE2 activity or expression may be involved in its usefulness in COVID-19.

Limitations of Use

Zinc is considered safe even in pregnancy, provided the recommended daily maximum zinc intake (40 mg) is not exceeded[60].

Vitamin D and ACE2

Surprisingly, an experimental study by Cui et al[61] demonstrated that the neuroprotective efficacy of calcitriol (1,25-dihydroxyvitamin D3, an active form of vitamin D) in spontaneously hypertensive rats, is blood pressure-independent and added that calcitriol exerted pronounced impact on ACE2/Ang (1–7)/MasR axis with enhanced expression of ACE2, MasR and Ang (1–7)[61]. This might suggest an indirect or independent pathway of vitamin D to influence ACE2[62,63]. In diabetics patients, Vitamin D supplementation can protect the kidney by decreasing the expression of ACE2 in the kidney tubule cells and so hinder SARS-COV-2 entry into the cells[64].

Limitations of Use

Vitamin D is relatively safe, thus, prophylactic vitamin D supplementation and/or food–rich vitamin D might reasonably serve as a very convenient and incomparable/invaluable adjuvant therapy[65].

Remdesivir and ACE2

Remdesivir inhibits viral replication by premature termination of RNA transcription and has shown activity against beta-CoVs and *in vitro* activity against SARS-CoV-2[66]. There is a link between remdesivir and ACE2 by using theoretical calculations and computational methods based on the density functional theory[32]. This study indicated that remdesivir could be an effective barrier that inhibits the binding of S1 to the human cell.

Limitations of Use

Previous studies carried out on 500 individuals of healthy volunteers and Ebola virus infected patients showed that remdesivir have a favorable clinical safety profile[67].

Conclusions

SARSCoV2 similarly to SARSCoV requires ACE2 for entry into target cells. ACE2 is a common target for many therapeutic agents attempted for managing COVID-19. Therefore, modulation of ACE2 receptors may play a potential role in the therapeutic strategies of COVID19 treatment. Agents, which enhance the expression of ACE2, may worsen COVID-19; however, drugs which ameliorate the activity or expression of ACE2 may be useful in preventing or treating COVID-19.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

All authors contributed to Conceptualization, formal analysis, writing – original draft preparation, writing – review and editing. All authors have read and agreed to the publication of current version of the manuscript.

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