Mechanisms of COVID-19 Entry into the Cell: Potential Therapeutic Approaches Based on Virus Entry Inhibition in COVID-19 Patients with Underlying Diseases

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ABSTRACT

The Coronavirus disease 2019 (COVID-19) virus spread from Wuhan, China, in 2019 and is spreading rapidly around the world. COVID-19 victims are almost associated with cardiovascular disease, high blood pressure, diabetes, and other underlying diseases. Concerning the high prevalence of these disorders, widespread mortality threatens global society, and its fatality rate may increase with increasing COVID-19 prevalence in countries with older populations. Therefore, evaluating patients' clinical status with severe COVID-19 infection and their medical history can help manage treatment. Currently, one of the considered treatments is angiotensin-converting enzyme 2 (ACE2) inhibition. This study investigated virus entry mechanisms through membrane receptors, their role in the pathogenesis of COVID-19 and underlying diseases, and treatment methods based on the viral entrance inhibition. According to existing studies, inhibition of ACE2 can increase oxidative stress, inflammation, fibrosis and ultimately exacerbate underlying diseases such as cardiovascular disease, kidney disease, diabetes, and hypertension in individuals with COVID-19. The ACE2 inhibition is not suitable for patients with COVID-19 with underlying diseases, but it seems that the recombinant ACE2 solution is more appropriate for inhibiting the virus in these patients if hypotension would be monitored.

Keywords: Angiotensin-converting enzyme 2; COVID-19; SARS-COV-2; Therapeutics

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INTRODUCTION

In early December 2019, an ambiguous acute respiratory patient's clinical symptoms from an unknown origin in Wuhan, China, were observed and reported. Initially, it consumes as simple flu, but after a short time, this disease, which is identified as a viral infection, has spread to other cities in China and worldwide at once. Moreover, the number of people infected with the virus is over 100 million so far. In 2020, the World Health Organization (WHO) called this pathogenic virus Coronavirus disease 2019 (COVID-19). Many aged individuals with more severe illnesses have been reported to suffer from underlying disorders, including cardiovascular, liver, and kidney diseases. Unfortunately, these patients expired due to the aggravation of their primary diseases.

In an exploration monitoring of an observational database from 169 hospitals in Asia, Europe, and North America, of the 8910 patients with Covid-19, 515 died in the hospital (5.8%). Factors founded to be independently associated with an increased risk of in-hospital death were at age greater than 65 years (mortality of 10.0%, vs. 4.9% among those ≤65 years of age), coronary artery disease (10.2%, vs. 5.2% among those without disease), heart failure (15.3%, vs. 5.6% among those without heart failure), cardiac arrhythmia (11.5%, vs. 5.6% among those without arrhythmia), chronic obstructive pulmonary disease (14.2%, vs. 5.6% among those without disease).

A broader investigation of 72,314 COVID-19 cases in China reported an increase in mortality among people with diabetes. The overall case-fatality rate was elevated among those with preexisting comorbid conditions 10.5% for cardiovascular disease, 7.3% for diabetes, 6.3% for chronic respiratory disease, 6.0% for hypertension, and 5.6% for cancer.

It is clear that patients with underlying conditions, such as high blood pressure, cardiovascular disease, and diabetes, are more likely to receive intensive ICU care after a coronavirus infection. Therefore, it is necessary to accurately assess the characteristics of individuals with COVID-19 and provide appropriate treatment. In this reading, we investigated virus entry mechanisms through membrane receptors [angiotensin-converting enzyme 2 (ACE2), cluster of differentiation 147 (CD147), and glucose-regulated protein 78 (GRP78) receptor], their role in the pathogenesis of COVID-19, and underlying diseases, and treatment methods based on the viral entrance inhibition.

Ethical Statement

The Ethics Committee of Babol University of Medical Sciences approved this study (IR.MUBABOL.REC.1399.215).

Cell Entry Mechanisms of COVID-19

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome and cellular behavior are similar to both SARS-CoV and bat coronavirus. Recent studies have shown that coronaviruses use numerous mechanisms to enter host cells after binding to the receptor.

Virus Transmission through GRP78

The GRP78 or binding immunoglobulin protein is the unfolded protein pathway's main chaperone protein. In normal circumstances, GRP78 is found in the endoplasmic reticulum (ER) lumen and activate three critical enzymes responsible for cell differentiation or cell death. Initiating cell stress results from GRP78 overexpression, enhancing the GRP78 chance to alter its location from ER to cell membrane. By this translocation, GRP78 becomes more susceptible to recognizing the virus through its substrate-binding domain and leading to its entry into the cell. In a recent study, the spike protein of COVID-19 was modeled by solved structures in the protein data bank, and after model validation, molecular docking was proceeded to assess its binding affinity against GRP78. Results suggested that GRP78 binds to COVID-19 same as the Middle East respiratory syndrome coronavirus (MERS-CoV) case, and researchers tried to predict the binding site using the similarity between Pep42 and the COVID-19 Spike protein. Eventually, four spike regions were suggested to be the binding site to GRP78 based on structural and sequence similarity. The results were promising and propose identifying the COVID-19 spike by the cell-surface GRP78 upon cell stress. Potential inhibitors include small molecules and peptides that can interfere with the interaction of SARS-CoV-2 and its target cells by preventing the GRP78 cell receptor from being detected by the viral spike protein. Therefore, it inhibited the interaction of SARS-CoV-2 and its target cells by preventing the detection of GRP78 cell receptors by viral spike proteins. In silica screening of existing databases of
active peptides, over 130 natural products, synthetic molecules, specific peptides, and monoclonal antibodies that target GRP78.\textsuperscript{12} One of these inhibitors, AR12, is a derivative of celecoxib that inhibits the ATPase activity of several chaperone proteins, particularly GRP78. AR12 suppressed the production of infectious virions via autophagosome formation, which was also associated with the degradation of GRP78.\textsuperscript{13}

**Virus Transmission through CD147**

Based on previous studies, the interaction between CD147 and the protein spike of the viruses facilitates virus entry and invasion on host cells.\textsuperscript{14} Besides, CD147 mediates viral entry into host cells through an interaction between the virus and one of the CD147 ligands called Cyclophilin, another essential factor in the SARS-CoV-2 entry mechanism.\textsuperscript{15,16} Cyclophilin A (CypA) is a cellular protein involved in viral invasion or proliferation through interaction with cellular receptors such as CD147.\textsuperscript{17,18} Interestingly, in a given tissue, the cells that express these molecules are the main determinants of susceptibility to SARS-CoV-2 infection.\textsuperscript{15,16} CypA might be secreted from cells in response to inflammatory stimuli such as infection and oxidative stress. The CypA activity is mediated through the CD147 cell receptor's activation and has a potential role in CD147-related diseases.\textsuperscript{19}

**CD147 Expression in Different Cells and Their Role in Diseases**

Many types of cells, like hematopoietic, endothelial, epithelial, neuronal, lymphoid, and myeloid cells, blood leukocytes, keratinocytes, and platelets, express CD147.\textsuperscript{20,21} This receptor is involved in numerous diseases include ischemic myocardial injury, lung-injury models, atherosclerosis, systemic lupus erythematosus, graft versus host disease, rheumatoid arthritis, multiple sclerosis, and pathogenic invasion.\textsuperscript{19}

**CD147 and Cardiac Remodeling**

CD147 is an essential receptor during the inflammation process and may include various disorders, including cardiovascular diseases like atherosclerosis and myocardial infarction. CypA is one of the pivotal associates of CD147, which is involved in activating platelets. CypA-activated platelets can bind monocytes and cause monocyte platelet aggregation. The study results confirm that the CypA and its receptor, CD147, make a leading figure in cardiac remodeling, containing the proliferation of cardiomyocytes, hypertrophy, interstitial fibrosis, and apoptosis and post-MI reconstruction.\textsuperscript{23}

**CD147 and Cytokine Secretion**

CD147 can act as an essential mediator of inflammatory and immune responses.\textsuperscript{24} Inflammatory stimuli secretion of a potent mediator called CypA can produce various types of inflammatory cytokines by binding to its receptor, CD147. Cyclophilins are thought to be involved in diverse signaling pathways, including adaptive immunity.\textsuperscript{24}

**CD147 and Lung Interstitial Fibrosis**

Multiple studies have announced that CD147 expression is developed in epithelial cells and alveolar macrophages during fibrosis. A mice model examination of the intermediate-stage pulmonary fibrosis attested that the prescription of CD147 antibodies improves fibrosis's clinical symptoms. However, this healing's exact cellular mechanism is still unknown, and cytokines regulation might be the leading cause of this improvement.\textsuperscript{22}

**CD147 and Kidney Diseases**

Based on various studies discussed above, CD147 is involved in pathophysiological processes by inducing cytokines produced by leukocytes. Stimulation of leukocytes in cytokines’ secretion through activating CD147 might initiate systemic inflammation, followed by dysfunction of some organisms, including kidney damage.\textsuperscript{23} A 2018 study reported that developed CypA and CD147 in the bloodstream are associated with the rapid progression of kidney disease in type 2 diabetic patients.\textsuperscript{26,27}

**Virus Transmission through ACE2**

Previous studies have noted that SARS-CoV binds to the ACE2 for entering the cell via the spike glycoprotein of the viral surface (S protein). Transmembrane serine protease 2 (TMPRSS2) is involved in the SARS-CoV-2 entry into host cells by processing the spike (S) protein.\textsuperscript{28} Based on studies, alveolar type II (AECII) epithelial cells comprise about 83% of the cells expressing ACE2. SARS-CoV viral infection enhances AngII expression and exacerbates lung damages.\textsuperscript{29} Besides the lungs, ACE2 is also available in intestinal epithelial
cells through the lumen's surface, acting up as a receptor for food nutrients absorption, particularly amino acids. The gut's ACE2 carries out a substantial impression in inflammation, and researchers claim that besides several other factors, ACE2 might lead to diarrhea in patients with coronavirus infection. Besides pulmonary injury and acute respiratory failure, the entered virus infects provider ACE2 organs, including the heart, kidneys, and gastrointestinal tract. After a long-term infection, the virus permeates other organs like the kidney via the bloodstream, leading to the accumulation of infection and the destruction of the host's cells. For instance, ACE2 is expressed on the renal tubular cell; it results in kidney infection and severe damage. Differences may influence the association between cardiovascular disease and mortality from SARS-CoV-2 infection in the expression or function of the ACE2. Studies also have claimed that the liver, bile ducts, and pancreas are affected by high coronavirus infection due to the increased ACE2 expression.

ACE2/Angiotensin (Ang) 1-7/ Mas receptor (MasR) Axis in the Management of Disease

The ACE/AngII/AT1R axis and the ACE2/Ang1-7/MasR axis are two axes of the renin-angiotensin system (RAS), maintaining hemostasis in humans. ACE2 is an ACE homolog. Despite the similarities between ACE and ACE2, the performance of these two enzymes is quite different. ACE2 prevents the classic RAS system's activation and protects the body against damages such as high blood pressure, inflammation, diabetes, cardiovascular disease, and fibrosis.

ACE2/Mas Receptor and Hypertension and Diabetes Management

Numerous studies show that low ACE2 expression and activity are associated with hypertension (HT), indicating that ACE2 can inhibit HT progression. Therefore, ACE2 has been suggested as a therapeutic target for the treatment of HT. Also, noticeable documents indicate the possible role of the ACE2/Ang 1-7/Mas axis in controlling insulin sensitivity. Impaired glucose tolerance and diabetes were observed in ACE2 Knock out (ACE2 

ACE2/Mas Receptor in the Pathophysiology Management of Coronary Heart Disease

Experimental models illustrated that myocardium ACE2 loss accompanied by an increased amount of tissue AngII causes severe cardiac complications, including cardiac fibrosis, dysfunction, and increased ACE2 activity levels in circulation. Based on the previous investigation, although circulating ACE2 activity was low in healthy individuals, it was increased in CVD patients, predicting detrimental events including coronary artery disease, heart failure, and arterial fibrillation in these patients. Ramchand et al claimed that in patients with Aortic Stenosis, the plasma ACE2 activity's elevation is relevant to myocardial structural abnormalities. They also reported that plasma ACE2 activity was related to decreased myocardial ACE2 expression in myocardial fibrosis.

ACE2/Mas Receptor and Fibrosis Management

There is plenty of evidence indicating the ACE2 protective effects against pulmonary dysfunctions such as acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), asthma, pulmonary fibrosis, and pulmonary hypertension. It has been mentioned in the literature that a significant decrease in ACE2 and Ang1-7 serum levels has been observed in pulmonary arterial hypertension (PAH) patients, and their expression was also reduced. Shao et al reported that in the lungs of mice with idiopathic pulmonary fibrosis (IPF) induced by bleomycin, the expression of the ACE2 gene and MAS1 gene and protein expression of ACE2 was considerably reduced. Their data indicate that Ang1-7 may be a contributor agent in IPF development as an antifibrotic factor.

ACE2/Mas Receptor and Renal Disease Management

Numerous studies have suggested that the deregulation of ACE2/ACE may lead to kidney injury. In experimental acute kidney injury (AKI), levels of ACE2 and Ang1-7 were noticeably decreased. Moreover, the downregulation of ACE2 resulted in tubular injury, macrophage infiltration and interstitial fibrosis, development of glomerular mesangial expansion, and accelerated glomerulosclerosis progression. Anguiano et al reported that in Chronic kidney disease (CKD) patients, circulating ACE2 activity was decreased.

ACE2/Mas Receptor and Oxidative Stress and Inflammation Management

ACE2/Ang1-7 signaling in preventing oxidative stress was reported previously. Strong evidence demonstrated that loss of ACE2 in mice leads to
nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and reactive oxygen species (ROS) production in aortas on a large scale and the aortic expression of inflammatory cytokines and chemokines monocyte chemoattractant protein 1 (MCP-1), interleukin-6 (IL-6), and IL-1β induce by AngII. In general, it seems that Ang1-7 administration or ACE2 overexpression concludes in reduced ROS formation and oxidative stress destruction in animal model studies. Ang1-7 infusion has also resulted in a rise in total superoxide dismutate (SOD) activity. Additionally, Ang1-7-induced neuroprotection due to its anti-inflammatory action in ischemia and mediated injuries was discussed. Through significant suppression of NF-κB activation, Ang1-7 treatment interrupts expression of pro-inflammatory genes including IL-1β, TNF-α, and COX-2 induced by NF-κB activation after cerebral stroke.51-53

Therapeutic Methods and Options in COVID-19

The person-to-person transition of COVID-19 has led to trying a variety of therapies to improve viral infection (Figure 1). There are no specific vaccines or antiviral drugs recommended for COVID-19.54,55

Prescription of Nonspecific Antiviral Drugs

The only existing option is to prescribe a wide range of antiviral drugs such as remdesivir (Veklury; Gilead Sciences company) and chloroquine (Aralen; Sanoficompamy) that could reduce COVID-19 infection.56,57 Hydroxychloroquine or chloroquine decreases the activity of dendritic cells and the inflammatory response by reducing TLR signaling. Therefore, hydroxychloroquine inhibits the entry and endocytosis of the SARS-CoV-2 virus.58,60 The role of remdesivir, ribavirin (Copegus; F.HOFFMANN-LA ROCHE company), and favipiravir (Favicovid; Optimuscompany) in controlling viral infection is to inhibit viral RNA dependent RNA polymer.56,61,82

Another report meant that HIV-protease inhibitors like lopinavir/ritonavir (Kaletra; Abbottcompany) benefits patients with virus infection by suppressing the 3–chymotrypsin-like protease.54,63,64 In addition to prescribing antiviral drugs and antibiotics, oxygen therapy is a therapeutic intervention in patients with viral infections, or ARDS.6,55,65 Recent studies have shown that oxidative stress affects pulmonary inflammation development, so antioxidant therapy and hydrogen therapy are some of the treatment strategies to reduce oxidative stress.60,66 Moreover, Russo et al have described the prevalence of pre-admission antithrombotic therapies in patients with COVID-19 and investigated the potential association between antithrombotic therapy and ARDS. They showed that antithrombotic therapy before admission did not affect the clinical presentation of COVID-19 in ARDS and in-hospital mortality.52 On the other hand, Anti-cytokines and chemokines such as Tocilizumab, Sarilumab, Siltuximab (Actemra, Roche company) block the interaction between the cytokine and its receptor, avoiding the amplification of inflammation associated with lung injury that leads to respiratory distress. As a support agent, Azithromycin (Sandoz company) has been used as adjunctive therapy to provide antibacterial coverage. It exerts potential immunomodulatory and anti-inflammatory effects in the treatment of some viral respiratory tract. The results showed that treatment with methylprednisolone (Medrol, Pfizer company) may be beneficial for patients who develop ARDS to reduce death risk.62

Treatment Roles of Ras Blockers in High-risk Patients and its Effect on ACE2 in the COVID-19

New hypotheses have emerged in treating patients with coronavirus, which has led to various studies and theories about inhibiting RAS and angiotensin receptors in patients with coronavirus.6 Patients with cerebrovascular disease, diabetes, hypertension, proteinuria, chronic kidney disease, and coronary heart disease are usually treated with RAS blockers, including angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). In diabetic and hypertensive patients treated with ACE inhibitors and ARBs, individuals took drugs such as thiazolidinediones and ibuprofen, leading to enhanced expression of the ACE2.68 Various efficacies of ACE inhibitors or ARB's drugs on the activity and concentration of the ACE2 have been reported. Studies have shown that ACE inhibitors or ARBs in rats increase the ACE2 level, losartan increases the ACE2 activity, and captopril increases this enzyme's expression. Generally, RAS inhibitors increase ACE2/Ang1-7 pathway. Few comparisons have examined the effect of RAS inhibitors on patients. A study of heart patients receiving RAS-inhibiting drugs illustrated that plasma ACE2 activity was not higher than untreated circumstances. Another study in Japan found that individuals with high blood pressure who
Figure 1. Investigate the relationship between angiotensin-converting enzyme 2 (ACE2) receptor inhibitor and other pathways of the virus entrance. The virus can enter the cell through three cellular surface receptors of ACE2 and glucose-regulated protein 78 (GRP78), cyclophilin A (CYP)/CD147. The ACE2 receptor inhibition leads to a decrease in its production (angiotensin-1-7) and therefore cancels the function of the mas receptor; and on the other hand, by increasing its substrate, angiotensin 2, it leads to increased inflammation, reactive oxygen species (ROS), fibrosis, and tissue damage. Increased ROS results from Angiotensin 2 function may increase GRP78 and CYP release and thus activate CYP/CD147 and increase the virus's entry into the cell and leads to tissue damage. Cyclosporine A (CsA) affects various viruses' replication by binding to Cyclophilin, and cytotoxic concentrations of CsA strongly inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV) proliferation. (Designed by our team)
took long-acting inhibitors (ARB-Olmesartan) displayed higher urinary levels of the ACE2 than other patients. Recent trials have proved that ACE2 inhibitors and ARBs alter the expression of ACE2 and its effect on the heart and kidneys. Since ACEIs/ARBs, besides their particular inhibiting role, can re-regulate the expression of ACE2, they may be able to protect the lungs, heart, and kidneys against damage to the RAS system. ACEIs/ARBs have two opposing mechanisms. The increased ACE2 level augments the coronavirus infection risk due to this inhibitor's prescript while reducing lung damage and improving it because of the ACE reduction and increased Ang1–7. A trial by Meng et al showed that treatment with these inhibitors might reduce the severity of the disease, positively affect the immune system, and indirectly prevent the virus from multiplying.

In patients with coronavirus, an increase and accumulation of AngII develop inflammatory cytokines. This inflammatory cytokine enhancement initiates severe damage to patients with coronavirus. Recent experiments show that ACEI/ARB remedy reduces inflammatory cytokines and reduces the risk of pneumonia and heart disease in patients. Therefore, the prescription of ACEI/ARB may be efficacious in COVID-19 patients.

Based on another study, ACE inhibitors or ARBs reduced the mortality in 112 individuals with heart disease who also caught the coronavirus. It is confirmed now that patients with coronavirus who had formerly been taking RAS inhibitors exhibit reduced drug effects.

Due to incomplete information about RAS inhibitors and their vague mechanism, these advantages have not been confirmed yet. For more clarity, RAS inhibitors should be examined on patients with COVID-19. The American and European Heart Association recommends continued use of RAS inhibitors for heart failure, hypertension, and cardiac ischemia.

Potential Approaches Based on the Virus Entry Inhibition

To the best of our knowledge, the virus may enter the cell through membrane receptors, auxiliary proteins, or unique mechanisms. There are various possible upcoming therapeutic approaches.

CD147 Block and Reducing the Virus Entry

As explained in various studies, since CD147 is involved in the onset and progression of various diseases, it has recently emerged as a promising target for treating various diseases. Since the CD147-SP pathway facilitates the SARS-CoV-2 invasion of host cells, this pathway can be considered a new target for antiviral drugs to treat COVID-19. CypA is a notable one among the molecules that bind to CD147 extracellularly and transmit their signals. N protein of SARS-CoV binds to CypA, which interacted with CD147. The N protein of SARS-CoV has been shown to induce apoptosis. CypA facilitates the proliferation of viruses such as coronaviruses by interacting with virus proteins (D8). Cyclosporine A (CsA), known as an immunosuppressive medicine, affects various viruses' replication by binding to Cyclophilin. Cell culture studies have indicated that cytotoxic concentrations of CsA strongly inhibit the proliferation of severe acute respiratory syndrome coronavirus (SARS-CoV). Examining the antiviral effect of mepolizumab on cultured Vero E6 cells showed that this drug significantly reduced the SARS-CoV-2 invasion by inhibiting CD147. The antiviral tests were performed to investigate the possible function of CypA in the SARS-CoV-2 invasion for host cells. Huh-7 cells were cultured, and CypA on Huh-7 cells was blocked with Alisporivir. Alisporivir is a non-immunosuppressive CsA derivative that inhibits replicating four different coronaviruses, including MERS- and SARS-coronavirus. Non-immunosuppressive CsA derivative alisporivir (ALV) and NIM811 inhibit replication of NL63 (a member of the coronavirus family) in cell culture. Utilizing an anti-CD147 monoclonal antibody in a mouse model reduced the lungs' acute inflammation and asthma by more than 50%.

Considering these results are indicated, blocking host cells' CD147 has an inhibitory trace on SARS-CoV-2, suggesting a substantial role of CD147 in facilitating SARS-CoV-2 invasion for host cells. These consequences provide a basis to explore the potential of Cyp inhibitors and broad-spectrum inhibitors of coronavirus family subunits, including SARS-CoV-2 replication.

Inhibition of Cellular Protease Activity

SARS-CoV-2 spread depends on ACE2 protein and TMPRSS2 activity. The spike (S) protein merges with transmembrane serine protease TMPRSS, interacts with the ACE2 receptor, and enters target cells. So, TMPRSS2 inhibitor might constitute the leading drug and treatment target. The serine protease inhibitor

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is Camostat Mesylate, which blocks TMPRSS2 activity and is considered a therapeutic option for SARS-CoV-2-infected patients.\textsuperscript{85-87} Besides, in the absence of TMPRSS2 cellular categories, SARS-CoV utilizes cysteine proteases cathepsin B and L (CatB/L) to break down protein S. So, both protease blockages are effective in inhibiting the virus entry into cells. CatB/L can be used to prime the spike protein similar to that of TMPRSS2.\textsuperscript{59,88,89} Nafamostat mesylate has been shown to inhibit MERS-CoV S protein-mediated viral membrane fusion with TMPRSS2 expressing lung Calu-3 host cells by inhibiting TMPRSS2 protease activity.\textsuperscript{62}

**ACE2 Blockers and Spike-protein-based Antibodies**

Many pharmaceutical development programs have focused on the interactions of ACE2-SARS-CoV-2 spike and observed that clinical-grade human ACE2 molecule could specifically suppress SARS-CoV-2 infections. Meanwhile, some studies have found that human recombinant soluble ACE2 (hrsACE2) inhibits SARS-CoV-2 binding to the target tissues. Human recombinant ACE2s such as rhACE2, APN01, GSK2586881 are harmless compounds that do not have adverse hemodynamic effects in healthy individuals and patients with ARDS.\textsuperscript{90} Considering ACE2 as the recipient of SARS-CoV-2, the protein-based vaccine spike virus's development could be a treatment option.\textsuperscript{4} In this case, SARS-CoV triggers a neutralizing antibody response in patients with coronavirus that inhibits viral (S) protein. Therefore, SARS laboratory vaccines, including recombinant (S) protein and inactive virus, provide neutral antibody responses.\textsuperscript{91} Recent studies have determined the receptor-binding domain (RBD) in SARS-CoV-2 S protein, which found that RBD protein binds to ACE2 receptors and can inhibit virus binding. Therefore, the binding of SARS-CoV-2 RBD to ACE2-supplying cells results in viral infection inhibition in host cells. Furthermore, SARS-CoV-2 RBD-based vaccines may be necessary for preventing SARS-CoV-2 infection.\textsuperscript{92}

Umifenovir (also known as Arbidol) is an antiviral substance that targets S / ACE2 protein and inhibits the viral envelope's membrane fusion.\textsuperscript{93} Another treatment based on the interaction of ACE2 and SARS-CoV-2 is blocking the ACE2 receptor through antibodies or small molecules.\textsuperscript{4,66} Recent reports have shown that ACE2 fusion protein (ACE2-Ig) may associate with both SARS-CoV and 2019-nCoV neutralization.\textsuperscript{92,94}

**The Effect of ACE2 Inhibition on other Viral Transmission Pathways**

CypA and its corresponding receptor, CD147, present in different cell types, play an important role in adjusting active oxygen species (ROS). It has been reported that the administration of AngII has significantly increased CypA and CD147 compared to the control group.\textsuperscript{95} Various studies have indicated that long-term AngII injection elevates blood pressure, leading to ATR activation, myocardial hypertrophy, and fibrosis in mice.\textsuperscript{96} Studies proved that AngII upregulates CypA by ROS formation; on the other hand, AngII is involved in ROS production through ATR. Take et al also confirmed that AngII-induced ROS production through NADPH oxidase2 in endothelial cells.\textsuperscript{97} It was identified that AngII promoted CypA expression and secretion and ROS production in H9C2 cells. Perrucci et al showed a direct relationship between CypA expression and ROS production.\textsuperscript{98} Therefore, inhibition of ACE2 can increase oxidative stress by increasing the amount of AngII, resulting in increased CypA and CD147 signals.

**Delivering Soluble ACE2**

Some papers have proclaimed that in mice, the binding of SARS-CoV-2 viral spike protein (S) downregulates the ACE2 receptor. Therefore, the excessive form of ACE2 solution may be competitively bound to SARS-CoV-2, which causes neutralize the SARS-CoV-2, increases cellular ACE2 activity, reduces the viral spread, and protect the lung from infection.\textsuperscript{99,100} Consequently, recombinant soluble ACE2 plays a more active role in blocking the SARS-CoV S1 protein with its cellular receptor.\textsuperscript{60}

**DISCUSSION**

Many COVID-19 patients with severe clinical manifestations have underlying diseases like cardiovascular, renal, diabetes, and hypertension. Therefore mortality in these patients is often happened due to exacerbation of their underlying disease. Evaluating patients' clinical status with severe COVID-19 infection and their medical history can effectively manage treatment. No specific vaccine or antiviral drug has been developed for COVID-19 yet. In this review,
mechanisms of the virus entrance through membrane receptors and auxiliary proteins were studied, their role in the pathogenesis of COVID-19, underlying diseases, and therapeutic methods based on inhibition of virus entry are also discussed. One of the considered treatments is ACE2 inhibition; this inhibition increases oxidative stress and enhances GRP78 release through switching to the ACE/AntII/ATR axis, and CypA production in the host cell facilitates virus entry through GRP78 and CD147 (Figure 2). It also leads to inflammation, fibrosis, cytokine secretion, and oxidative stress due to increased ACE and CD147 action, ultimately exacerbating underlying diseases such as cardiovascular disease, kidney disease, diabetes, and hypertension in people with COVID-19. Thus, it can be concluded that treatments based on ACE2 inhibition (antibody and RBD) are not suitable for patients with COVID-19 with underlying diseases, but prescribing ARB and ACE blockers may reduce the complications in these patients. In conclusion, it seems that the method of prescribed recombinant solution ACE2 is more appropriate for controlling the virus in these patients if hypotension does not occur.

CONFLICT OF INTEREST

The authors have stated that no conflict of interest occurs.

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