Journal of Medical Research and Health Sciences
Received 08 June | Revised 26 June 2023 | Accepted 23 July 2023 | Published Online 10 Sept 2023

DOI: https://doi.org/10.52845/JMRHS/2023-6-9-4

JMRHS 6 (9), 2742–2753 (192023)

ISSN (O) 2589-9031 | (P) 2589-9023

Original Article **Open Access Journal**

Advances in Angiotensin Converting Enzyme-2 and Renin Angiotensin System Against COVID 19: A Pharmacotherapy and Physicochemical Review

Suhad Hafidh [1], Hayder Abdul-Amir Makki Al-Hindy [*1], Abbas Jaafar Al-Anbari [2], Hussein Ali Abdulabbas [3], Auday Majeed [4]

[1]College of Pharmacy, University of Babylon, Babylon, Iraq.

[2]College of Medicine, Al-Nahrain University, Iraq.

[3]College of Nursing, University of Babylon, Hilla, Iraq.

[4]Radiologist, Tehran, Iran.

Corresponding Author: Hayder Abdul-Amir Makki Al-Hindy

Abstract:
The renin-angiotensin-aldosterone system (RAAS) is a key regulator of cardiovascular function through components like angiotensin II (Ang II) and angiotensin 1–7 (Ang 1–7). Angiotensin-converting enzyme (ACE) plays a pivotal role, existing as ACE1 and ACE2 isoforms. The COVID-19 pandemic, resulting from coronavirus, exploits ACE2 for cell entry, leading to a global outbreak. The virus's impact on ACE2 and the RAAS system influences disease severity. This review concentrates on the interplay between ACE2, RAAS, and SARS-CoV-2, investigating viral entry, binding patterns, and effects on RAAS balance. The virus binds to ACE2's receptor-binding domain (RBD) and enters cells via endocytosis, involving TMPRSS2 protease. SARS-CoV-2's higher ACE2 affinity contributes to its infectivity. ACE2 expression varies in health and disease, impacting COVID-19 outcomes. The RAAS has two opposing arms—classical and counter-regulating. ACE2 bridges these arms, converting Ang II to Ang 1–7 with vasodilatory and protective effects. The pandemic introduces a "third arm," the RAAS-SARS-CoV-2-axis, impacting ACE2 expressions and RAAS balance. Recombinant ACE2 (hrsACE2) shows promise in inhibiting viral replication and reducing viral load. ACE2-loaded extracellular vesicles (EVs) extend ACE2's effectiveness, inhibiting virus infectivity. Immunological factors such as cytokines, interferons, and cell count influence COVID-19 severity. Understanding ACE2's role and its interactions with RAAS and SARS-CoV-2 is vital for potential therapeutic strategies and disease management.

Keywords: ACE2, SARS-CoV-2, RAAS, COVID-19, viral entry, therapeutic strategies

Introduction

The renin-angiotensin-aldosterone system (RAAS), is one of the most important systems governing cardiovascular function through various components, notably angiotensin II (Ang II) and angiotensin 1–7 (Ang 1–7) Angiotensin-converting enzyme (ACE), which exists in two physiologic isoforms, is a key enzyme in this system. The first form is ACE1 expressed extremely in the pulmonary vessels to synthesize Ang II and has potent vasoconstrictor activity. As well, it augments sympathetic tone and has mitogenic and pro-inflammatory effects over the epithelial or endothelial cellular layer. ACE2 is the second isoform forms a polypeptide named; Ang 1–7 that is opposite to Ang II and has vasodilating, anti-inflammatory, besides its cardioprotective properties (1).

The COVID-19 pandemic arose in Dec. 2019 in China, spreading rapidly and prompting WHO's official designation of the virus as novel coronavirus-2019 on January 12, 2020 (2-6). This highly transmissible virus caused a global outbreak of pneumonia, affecting over 190 countries, including severely impacted nations like the USA, India, and Brazil. By October 6, 2020, the infection had surpassed 34 million cases and resulted in a death toll exceeding one million (7). The virus's entry into alveolar cells involves binding with angiotensin-converting enzyme 2 (ACE2), triggering infection propagation (8).

Clinical symptoms of COVID-19 vary, fluctuating from asymptomatic to severe signs (8). Severe cases often involve respiratory and organ failure, notably refractory hypoxemia (9). While some patients recover, the disease has a more adverse prognosis in older persons and those with underlying health disorders. Despite significant progress, the exact virus-body interaction and severe conditions remain unclear.

The ACE2's role in susceptibility and immunity to SARS-CoV-2 is complex (10). Inhibition of proinflammatory cytokines IFNγ and TNF appears to reduce immunopathology, with disease severity tied to ACE2 (11). Understanding the disease's underlying pathobiology is vital, as ACE2 could serve as a therapeutic target. ACE inhibitors might benefit severe cases (12, 13)

The pharmacological and physiochemical characteristics of the COVID-19 virus and its complex interactions with ACE2 and RAAS will be the main topics of this review.

Structure, location, and role of ACE2 as the entry point of Coronavirus

The ACE enzyme exists in two states: incorporated into the soluble (sACE2) or cell membrane (mACE2) (14). Both small intestinal epithelial cells and lung alveolar epithelial cells have highly expressed ACE2 on their cell surface. Such finding explains why SARS-CoV-2 has a significant impact on the respiratory and gastrointestinal systems (15). Certain cells, the skin, and the nasal epithelia all express ACE2. SARS-CoV-2 is one coronavirus that employs mACE2 as an entrance route into the cells. In actuality, SARS-CoV-2 enters cells mostly through mACE2. In more detail, the mACE2 on the surface of human cells is bound by the spike protein of the virus (at the enzymatic domain site). The enzyme-virus complex is endocytosed as a result of this reaction, and then it is translocated into intracellular endosomes (16). The priming of the spike protein necessary for the virus entry course is carried out by the host serine protease TMPRSS2. In this line, scientists are presently looking at TMPRSS2 suppression as a method for preventing viral entry into body cells. Other investigators also showed that the appropriate viral entry is significantly hampered by disruption of S-protein glycosylation (17).

Binding pattern of SARS-CoV-2 spike protein and ACE2

Cryo-electron microscopy was used in 2020 to determine the atomic-level binding pattern of the SARS-CoV-2 spike protein and ACE2 (Figure 1). The interaction of ACE2 and SARS-CoV-2 spike protein monomer is mediated mostly by the alpa1 helix (mostly residues 21- 43), a few dispersed residues in alpha 2, β 3, and β 6 cell surface adherence. Since ACE2 is a key step of SARS-CoV-2 endocytosis, blocking the "protein-protein interaction (PPI)" of ACE2's "receptor-binding domain (RBD)" is a crucial strategy for clinical SARS-CoV-2 prevention. This preventive approach is especially important because coronaviral alterations that result in immune

evasion are predicted to decrease the virus's affinity to the cell surface ACE2 (18).

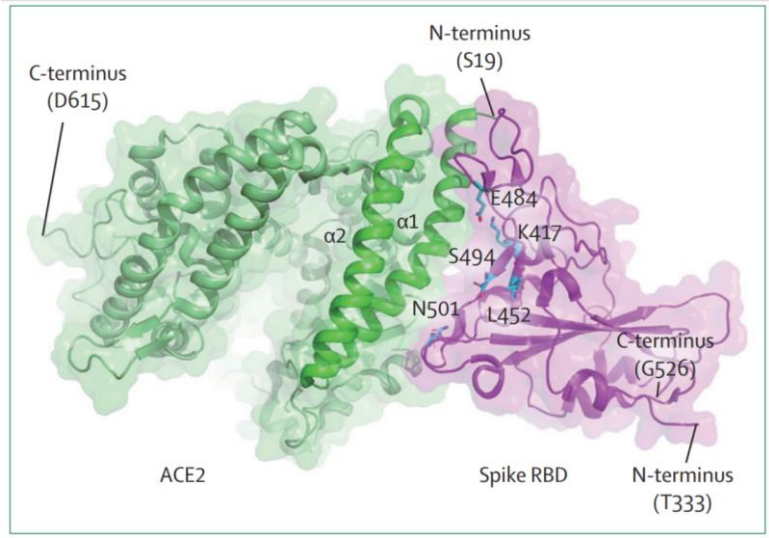

Figure 1: Assembly of the ACE2 complex (greenish) and coronavirus spike receptor binding domain (RBD) in purple (18)

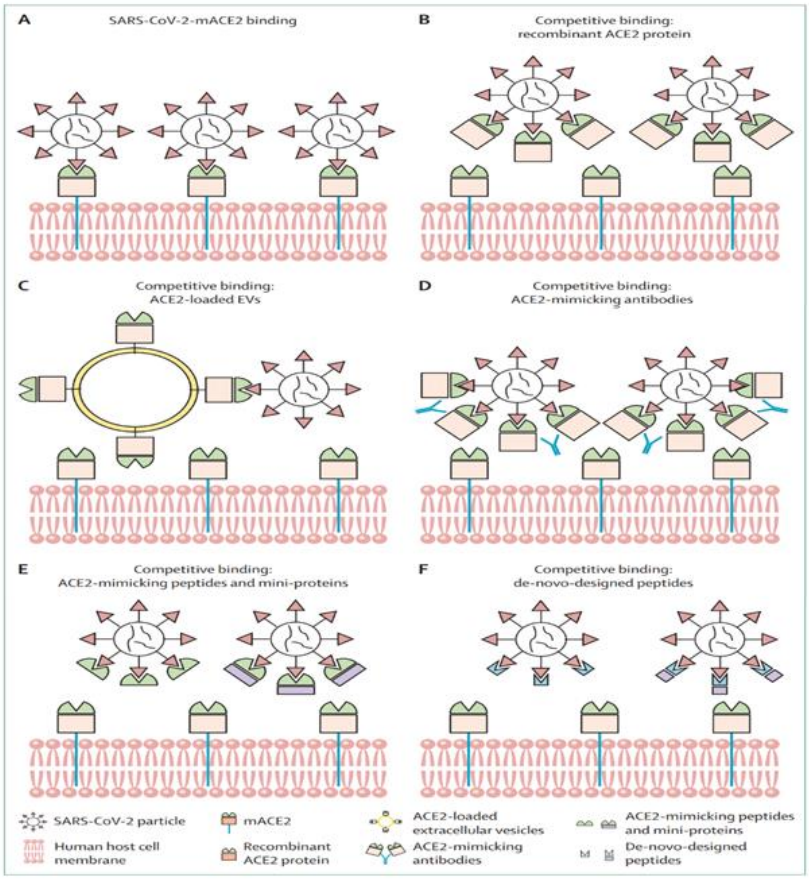

Figure 2: Representations of the binding patterns of SARS-CoV-2 with the native ACE2 and its derivatives.

ACE2 = angiotensin-converting enzyme 2, **mACE2 =** membrane-bound ACE2 (18).

SARS-CoV2 penetrates human cells via the ACE2 receptor to induce COVID-19 illness

ACE2 was initially identified 20 years ago (19). ACE2 is extensively present in human organs, like the small intestine, kidney, lungs, heart, nasal

canals, and oral mucosa (20). ACE2 is mostly expressed in macrophages and type II alveolar cells in the lungs, with only expressed less in the epithelial lining of the trachea and bronchi (21), (Figure 2).

The coronavirus that triggered the SARS epidemic in China in 2002 - 2003, SARS-CoV-2, is the same virus as COVID-19. Coronavirus accesses target cells via its cell ACE2 surface receptor. As a result, it seems that these coronaviruses all use the ACE2 receptor as a mechanism of entrance. When SARS-CoV-2's viral spike glycoprotein (S protein) links to the membrane-bound ACE2, it makes it easier for the virus to connect to its target endothelium and/or epithelial cells (22). According to Yan et al. (15), the trimeric SARS-CoV-2 spike protein interacts with ACE2 via binding to the peptidase domain of the latter.

Transmembrane protease serine 2 (TMPRSS2) then cleaves the ACE2 C-terminal section, allowing the SARS-CoV-2 viral particles to merge with the host cell membrane, leading to viral entrance and reproduction in target cells.

Although the S-protein of the SARS-CoV and the SARS-CoV-2 share about 80% of the same amino acids, there are some significant changes. Contrary to SARS-CoV, SARS-CoV-2 appears to not use the human dipeptidyl peptidase-4 (DPP4) as a receptor and has an affinity of 10 to 20-fold higher for the ACE2 protein, which likely explains its increased infectivity and led to the current COVID-19 outbreak (23). (Figure 3)

In various additional clinical disorders, such as diabetes, hypertension, and respiratory disorders, ACE2 levels are altered (24). Depending on the date of the measurements, ACE2 activity and/or expression in the body of various fluids or tissues may be caused by the primary pathology, medication, or compensatory pathways (25). There are also documented differences in ACE2 expression and ACE2 genomic polymorphisms that affect variations in enzymatic activity (26). Since ACE2 is often expressed at lower levels in women and children, it is tempting to link these to the better results in these cases.

On the other hand, subjects who are older or who have co-morbid conditions like diabetes or hypertension also have changed ACE2 levels and are linked to worse COVID-19 results. Therefore, more research is needed to completely understand the effects of changes in ACE2 hereditary variants besides levels of gene expression on COVID-19 disease.

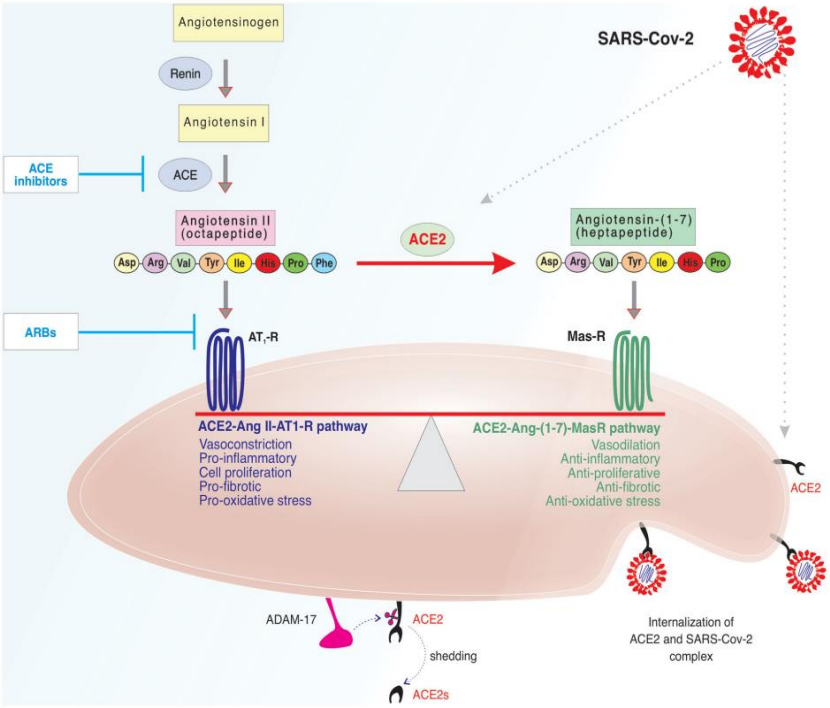

Figure 3. Coronavirus can enter cells easily using the ACE2 enzyme, which functions as a surface receptor in the RAAS.

The two opposing pathways that make up the RAAS system decide whether physiological or pathological consequences take place. Angiotensinogen is converted into Ang I through renin in one pathway, and subsequently, Ang II is produced via the ACE. Ang II can function in a variety of ways through the Mas receptor (MasR) or the AT1 receptor (AT1R), depending on how ACE2 converts it into Ang-1-7. While the ACE2-Ang-(1-7)-MasR route has the opposite consequences, such as vasodilation and anti-inflammatory actions, the ACE-Ang II-AT1 pathway results in vasoconstriction, inflammation, and other detrimental signals. ACE2 serves as the entry point for the virus in addition to its function in the RAAS. The virus and ACE2 may interact to lower ACE2 levels on cell membranes. By being broken down by ADAM-17, ACE2 can also be released as soluble ACE2 (ACE2s), maintaining its functionality and possibly interfering with virus binding. The levels of ACE2 in various tissues and fluids are raised by medications like ACE inhibitors and blockers, which are used to treat hypertension and cardiac conditions. The synthesis, shedding, and breakdown mechanisms that take place in cells and bodily fluids are what keep ACE2 levels in check (27).

The two counter-regulating arms of RAAS

The physiopathologic effects of the RAAS are determined by the relative balance of its two pathways or "arms", which are antagonistic in their functions (Figure 3).

Physiological arm

Blood pressure and electrolyte balance are significantly regulated by the traditional RAAS 'arm' (28). Angiotensin II (Ang II) is a vital part of the traditional RAAS. Angiotensinogen is transformed into angiotensin I by renin from the kidneys. Angiotensin I is then converted into Ang II by the kidneys' and lungs' released angiotensin-converting enzyme (ACE). The latter causes vasoconstriction, the cardiovascular response, the production of aldosterone and anti-diuretic hormones, and the subsequent increase in blood pressure and volume through potassium, sodium, and water absorption.

Pathophysiological arm

When the ACE-Ang II-AT1 receptor is active, cell proliferation, pro-oxidative stress, pro-inflammatory, and pro-fibrotic signals are generated (29-31). RAAS inhibitors, like ARBs, ACEIs, and MCRIs, can be used to avoid the hyperactivity of this RAAS arm, which has been related to several diseases, including hypertension, cardiovascular disease, issues from diabetes, and respiratory illnesses. Inhibitors of the RAAS are frequently used to treat hypertension, heart failure, and cardiac myopathies, and to halt the course of cardiac and renal disorders (31).

The primary link between the two RAAS counter-regulating arms is ACE2

The usual arm of the RAAS and its antagonistic arm appear to be connected by ACE2. Finally, Ang II is transformed by ACE2 into Ang-1-7. When this arm of RAAS predominates, anti-inflammatory, anti-proliferative, vasodilation, oxidative stress, and anti-fibrotic signals are in opposition to one another (28).

Prior experiments demonstrated blood pressure-lowering effects of Ang-(1-7) opposite to those of Ang II meanwhile, these actions most possibly involved a novel A (1-7) receptor rather than AT1 or AT2 receptors (32). Others have since demonstrated how Ang-(1-7) affects the cardiovascular and pulmonary systems with anti-inflammatory, anti-oxidative, and anti-fibrotic effects. In several diseases, such as problems brought on by diabetes, the respiratory, cardiovascular, and ACE2/Ang (1-7) regulatory arm of the RAAS, activation is protecting (33). Consequently, ACE2 plays an essential role in preserving a steadiness between the dual pathways of RAAS.

The third arm: RAAS-SARS-CoV-2-axis

Given that coronaviral infections need ACE2-receptor-mediated penetrance and the potential for ACE down regulation after access of the virus into infected host cells, it stands to reason that SARS-CoV-2 may also regulate the levels of ACE2 that are readily accessible. The general balance of the RAAS pathways is consequently likely to be affected by coronavirus interactions with ACE2, and accordingly, this interface might be assumed as a new RAAS "third arm" in SARS-CoV-2 cases or what can be called "RAAS-SARS-CoV-2-axis". Acute pulmonary injury is characterized by

decreased ACE2 levels in the lung parenchyma, whereas hypertension and diabetes mellitus are well-known co-morbidities correlated with poor clinical outcomes in coronavirus and are also may be linked with changed ACE2 bioactivity. The specific metabolic alterations caused by SARS-CoV-2 in the different RAAS's components are still unclear, hence these investigations would be ascertaining the precise pathophysiological effect of the RAAS-SARS-CoV-2-axis (34).

Recombinant ACE2 protein

Due to its ability to prevent SARS-CoV-2 infection, recombinant ACE2 protein (hrsACE2) has attracted interest. The binding affinity of ACE2 derivatives can be further improved by efficacy-enhancing changes (Figure 2), pointing to their potential as therapeutic approaches. Human membrane-bound ACE2 (805 amino acids) is broken down by the protease TMPRSS2 into naturally occurring soluble ACE2 (sACE2) proteins, which may hinder viral binding by serving as a deceptive tool (35). Despite the modest levels of sACE2 in the blood, different amounts have had diverse effects on SARS-CoV-2 infection in cell lines (36). Notably, clinical investigations have shown that exogenous sACE2, such as hrsACE2 (residues 1-740 or APN01), is safe and has antiviral efficacy (37).

Clinical-grade hrsACE2 effectively inhibits SARS-CoV-2 replication in cell culture, reducing viral load by 1000-to-5000-fold. The efficacy extends to variants like alpha, beta, delta, and omicron. Ongoing clinical trials assess hrsACE2's safety and effectiveness in COVID-19 patients (NCT04335136), revealing reduced viral load and improved mechanical ventilator-free days (38, 39).

HrsACE2's mechanisms benefit patients by neutralizing SARS-CoV-2 and downregulating the RAAS to prevent organ injury and inflammation (40). HrsACE2 demonstrates promise against coronavirus and other ACE2-targeting viruses, presenting a non-destructive neutralization approach. Ongoing clinical trials will further elucidate hrsACE2's efficacy and potential as a therapeutic agent (41).

ACE2-loaded extracellular vesicles

Since ACE2 has strong antiviral action against SARS-CoV-2, researchers are looking into ways to increase its efficacy. With its elimination half-life of roughly 10 hours, one drawback is its short efficacy lifetime (37). Extracellular vesicles (EVs) have been researched as ACE2 protective carriers to remedy this (42). EVs are tiny, spherical cell secretions with the ability to encapsulate bioactive compounds and extend their bloodstream presence (43). To sustain therapeutic levels in clinical trials using human recombinant soluble ACE2 (hrsACE2), intravenous dosages were given twice a day (41). In contrast, because of their prolonged blood circulation half-life, EVs may be able to provide a longer duration of activity.

Since the lungs are the primary target of SARS-CoV-2, EV-based treatments have shown promise in animal models for reducing lung inflammation, and fibrosis, and encouraging lung tissue repair (44). Plasma from COVID-19 patients had elevated amounts of endogenous circulating EVs that included ACE2 (evACE2), and these EVs were successful in preventing cell death brought on by SARS-CoV-2 infection (45). Additionally, when compared to soluble ACE2, EVs loaded with ACE2 showed a much stronger inhibitory effect on virus infectivity due to their strong binding affinity for spike proteins (46).

An interesting finding was that EVs embedded with modified ACE2 (EVs-ACE2) showed strong neutralization capabilities both in vitro and in vivo. When it came to preventing virus entry into mouse nasal epithelial cells, these modified EVs-ACE2 performed noticeably better than untreated controls. To improve ACE2's interaction with cell membranes and release into EVs, researchers also looked at palmitoylating it at particular residues. In vitro and human ACE2 transgenic mice, engineered EVs-ACE2 with these changes demonstrated increased neutralization power against pseudo-typed and real coronaviruses (47).

ACE2-mimicking antibodies

The spike protein of SARS-CoV-2 is a target for neutralization antibodies that can block cell entry. In convalescent serum samples, neutralization antibodies targeting the RBD of the S protein for 90% of the entire response. The RBD-targeting neutralization antibodies are crucial in counteracting the virus. However, key mutations in the virus make neutralization challenging. P2C-1F11 and S2K146 are class 1 neutralization antibodies that mimic ACE2 and effectively

neutralize different SARS-CoV-2 variants. These ACE2-like antibodies show promise as potential vaccine candidates due to their ability to compete with ACE2 for RBD binding and their efficacy against variant strains (48).

The effectiveness of class 1 neutralization antibodies, specifically P2C-1F11 and S2K146, against SARS-CoV-2 and its variants of concern (VOCs) was the focus of concern. P2C-1F11 exhibits high clash volumes with ACE2, indicating a neutralization mechanism through ACE2 mimicry. It successfully neutralizes the alpha, beta, and gamma variants through ACE2 mimicry, unlike many other neutralization antibodies. S2K146, another ACE2-mimicking antibody, retains its efficacy against the Omicron variant. Both antibodies demonstrate superior viral neutralization potency and broad-spectrum properties due to their competitive binding to the receptor-binding domain (RBD) against ACE2, making them promising candidates for potential vaccines.

Immunological factors affecting body response to SARS-CoV-2

The immune system is the most efficient line of defense for enhancing the body's innate capacity to fight against resistant illnesses and viruses because there are no effective therapies for COVID-19 (49).

Numerous immunological factors have been extensively researched in COVID-19 infections, including interleukins (50-52), interferons (53, 54), tumor necrosis factors (52, 54), myeloperoxidase (55, 56), and a wide range of additional immunoregulatory factors (49, 54, 57, 58). ICU patients had higher levels of growth factors like hepatocyte growth factor (HGF), cytokines like IL-8, and monocyte chemoattractant protein-3 (MCP-3), while the levels of some TNF-family like “TNF-related activation-induced cytokine (TRANCE)” and stem cell factor were lower. Along with this, common severe COVID-19 traits include increased MCP-1, G-CSF, and IP-10 concentrations, decreased CD4+, CD8+, Treg, B, and NK cell numbers and activity, increased plasmablast and neutrophil numbers, downregulated type I interferon signaling, and decreased antigen presentation (58, 59).

Future steps and directions

A few recent trials to make suitable anti-COVID-19 agents were also promising including optimized synthesis of anti-COVID-19 drugs of Favipiravir and sabizabulin aided by retrosynthesis software (60). Systemic corticosteroids (dexamethasone), IL-6 receptor antagonists (tocilizumab), and Janus kinase inhibitors (baricitinib) decrease mortality in those with severe COVID-19 besides have additional advantages, like shortening hospital stays and minimizing time spent on a ventilator. It further mentions that non-severe COVID-19 has also been proven to be responsive to the antivirals nirmatrelvir/ritonavir (Paxlovid), molnupiravir (Lagevrio), and remdesivir (Veklury) (61). As well, as evaluation of the practice of prescribing and monitoring antivirals among hospitalized patients (62).

Moving forward in the study of coronavirus, highlighting the importance of understanding its cell entry pathway for combating the virus. While vaccines have shown initial effectiveness against COVID-19, their long-term potency might decline due to the rapid mutation rate of the virus.

There's also concern about equitable vaccine distribution worldwide. Existing COVID-19 drugs could have uncertain outcomes. This review suggests that COVID-19 is not just an infectious disease, but a disruption of homeostasis caused by the virus exploiting cellular pathways. Preventing virus cell entry is seen as crucial to stopping infectivity and the emergence of new variants. The study recommends rebalancing disrupted signaling pathways using repurposed safe drugs as a key strategy for patient survival, regardless of the virus's presence or mutation status.

Conclusion

The severity and susceptibility of SARS-CoV-2 are greatly influenced by the complex interaction between the RAAS and ACE2. Disease outcomes are impacted by the RAAS system's delicate balance between its two opposing arms, which ACE2 controls. Recombinant ACE2 protein and ACE2-imitated antibodies are two cutting-edge treatment modalities in addition to some recent agents that have the potential to block viral impacts. The continuous pandemic can be

combated and practical solutions can be created by comprehending this complicated relationship.

References

1. Alabsi S, Dhole, Atharva, Hozayen, Sameh, Chapman, Scott A. Angiotensin-Converting Enzyme 2 Expression and Severity of SARS-CoV-2 Infection. Microorganisms. 2023;11:612. PubMed PMID: doi:10.3390/ microorganisms11030612.
2. Al-Hindy Hayder AM A-MA, Mousa MJ. Association of dental caries in the era of COVID-19 with the number of occluded coronary vessels: A non-traditional risk factor in patients with acute coronary syndrome. Journal of Emergency Medicine, Trauma & Acute Care. 2023;3:1-7. doi: 10.5339/ jemtac .2023.midc.8.
3. Amal Talib Al Sa'ady ZAA, Ali Fadhil Obaid, Hayder Abdul-Amir Makki Alhindy, Amir S. Al-Mumin Prevalence of adverse effects from COVID-19 vaccine among Iraqi adults: A retrospective cross-sectional study. Journal of Emergency Medicine, Trauma Acute Care. 2022;3:1-9.
4. Hajir Karim Abdul-Husseein KAA-A, Mustafa Karim Abdul-Husseein, Hayder Abdul-Amir Makki Al-Hindy. The Liver Function Abnormalities in COVID-19 Patients and Their Association with Age and Sex: A Cross-Sectional Study. Archives of Razi Institute. 2023;77:453-8. doi: 10.22092/ARI. 2022.359755.2474. PubMed Central PMCID: PMCPMC10258244.
5. Hayder Abdul-Amir Maki Al-Hindy JM, HO Hashim. BCG Vaccine in preventing COVID-19 epidemic had to be reviewed: correlation does not imply causation. Australian Journal of Basic and Applied Sciences. 2021;14:58-63.
6. Mohialdeen S, Hummadi Fayadh NA, Khaleel Al-Anbari AJ, Maddah H. Al-Alosi B. Acute arterial thrombosis in patients admitted with COVID-19 infection: Clinical experience. 2022;2022. doi: https://doi.org/10.5339/jemtac.2022.ismc.5.
7. Lu L, Liu X, Jin R, Guan R, Lin R, Qu Z. Potential Roles of the Renin-Angiotensin System in the Pathogenesis and Treatment of COVID-19. BioMed Research International. 2020;2020:7520746. doi: 10.1155/2020/7520746.
8. Wu Y-C, Chen C-S, Chan Y-J. Overview of The 2019 Novel Coronavirus (2019-nCoV): The Pathogen of Severe Specific Contagious Pneumonia (SSCP). Journal of the Chinese Medical Association. 2020;83:1. doi: 10.1097/ JCMA.0000000000000270.
9. Adhikari SP, Meng S, Wu YJ, Mao YP, Ye RX, Wang QZ, et al. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. Infect Dis Poverty. 2020;9:29. doi: 10.1186/s40249-020-00646-x. PubMed PMID: 32183901; PubMed Central PMCID: PMCPMC7079521.
10. Yin J, Li C, Ye C, Ruan Z, Liang Y, Li Y, et al. Advances in the development of therapeutic strategies against COVID-19 and perspectives in the drug design for emerging SARS-CoV-2 variants. Computational and Structural Biotechnology Journal. 2022; 20: 824-37. doi: https://doi.org/10.1016/j.csbj. 2022.01.026.
11. Gawish R, Starkl P, Pimenov L, Hladik A, Lakovits K, Oberndorfer F, et al. ACE2 is the critical in vivo receptor for SARS-CoV-2 in a novel COVID-19 mouse model with TNF- and IFNγ-driven immunopathology. eLife. 2022;11:e74623. doi: 10.7554/eLife.74623.
12. Nemati H, Ramezani M, Najafi F, Sayad B, Nazeri M, Sadeghi M. Evaluation of Angiotensin-converting Enzyme 2 (ACE2) in COVID-19: A Systematic Review on All Types of Studies for Epidemiologic, Diagnostic, and Therapeutic Purposes: Angiotensin-converting enzyme 2 (ACE2) in COVID-19. Open Access Macedonian Journal of Medical Sciences. 2020;8:84-91. doi: 10.3889/oamjms.2020.4763.
13. Dambha-Miller H, Albasri A, Hodgson S, Wilcox C, Islam N, Khan S, et al. Drug treatments affecting ACE2 in COVID-19 infection: a systematic review protocol. BJGP Open. 2020;4. doi: 10.3399/ bjgpopen 20X101115. PubMed PMID: 32665234; PubMed Central PMCID: PMCPMC7465570.
14. Scialo F, Daniele A, Amato F, Pastore L, Matera MG, Cazzola M, et al. ACE2: The Major Cell Entry Receptor for SARS-CoV-2.

Lung. 2020;198:867-77. doi: 10.1007/s00408-020-00408-4. PubMed PMID: 33170317; PubMed Central PMCID: PMCPMC7653219.
15. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020;367:1444-8. doi: 10.1126/science. abb2762. PubMed PMID: 32132184; PubMed Central PMCID: PMC PMC7164635.
16. Mahmoud Gheblawi KW, Anissa Viveiros, Quynh Nguyen, Jiu-Chang Zhong, Anthony J. Turner et al. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System. Circulation Research. 2020;126:1456-74. doi: doi:10.11 61/CIRCRESAHA.120.317015.
17. Safarzadeh Kozani DS, Baharifar, N., Sheikhi, S., Khalaf Shamsabadi, F., Chamaie Nejad, F. et al. . ACE2 in SARS-CoV-2-Mediated COVID-19: A Brief Review. Trends in Medical Sciences. 2022;2:e130682. doi: 10. 5812/tms-130682.
18. Zhang H, Lv P, Jiang J, Liu Y, Yan R, Shu S, et al. Advances in developing ACE2 derivatives against SARS-CoV-2. The Lancet Microbe. 2023;4:e369-e78. doi: 10.1016/S266 6-5247(23)00011-3.
19. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res. 2000;87:E1-9. doi: 10.1161/01.res.87.5.e1. PubMed PMID: 10969042.
20. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. 2020;12:1-5.
21. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203:631-7. doi: 10.1002/path.1570. PubMed PMID: 15141377; PubMed Central PMCID: PMCPMC7167720.
22. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417-8. doi: 10.1016/s0140-6736(20)30937-5. PubMed PMID: 32325026; PubMed Central PMCID: PMCPMC7172722.
23. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020;367:1260-3. doi: 10.1126/science.Abb 2507. PubMed PMID: 32075877; PubMed Central PMCID: PMC PMC7164637.
24. South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. Am J Physiol Heart Circ Physiol. 2020; 318:H1084-h90. doi: 10.1152/ ajpheart. 00217.2020. PubMed PMID: 32228252; PubMed Central PMCID: PMC PMC7191628.
25. Santos RAS, Oudit GY, Verano-Braga T, Canta G, Steckelings UM, Bader M. The renin-angiotensin system: going beyond the classical paradigms. Am J Physiol Heart Circ Physiol. 2019;316:H958-h70. doi: 10.1152/ ajpheart.00723.2018. PubMed PMID: 30707 614; PubMed Central PMCID: PMC PMC7191626.
26. Devaux CA, Rolain JM, Raoult D. ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. J Microbiol Immunol Infect. 2020;53:425-35. doi: 10.1016/j.jmii.2020.04.015. PubMed PMID: 32414646; PubMed Central PMCID: PMCPMC7201239.
27. Akhtar S, Benter IF, Danjuma MI, Doi SAR, Hasan SS, Habib AM. Pharmacotherapy in COVID-19 patients: a review of ACE2-raising drugs and their clinical safety. J Drug Target. 2020;28:683-99. doi: 10.1080/1061186x.2020 .1797754. PubMed PMID: 32700580.
28. Ferrario CM, Ahmad S, Joyner J, Varagic J. Advances in the renin angiotensin system focus on angiotensin-converting enzyme 2 and angiotensin-(1-7). Adv Pharmacol. 2010;59 :197-233. doi: 10.1016/s1054-3589 (10)5900 7-0. PubMed PMID: 20933203; PubMed Central PMCID: PMCPMC5863743.
29. Al-Saad R, Shaker, AK, Dleikh, FS, Al-Hindy, Hayder AA Is there any association between highly sensitive C-reactive protein and dental-status in ischemic heart diseases Comparative-study Biochem Cell Arch. 2020;20:6069-75.

30. Asseel K. Shaker RA-S, Raad Jasim, Hayder Abdul-Amir Makki Al-Hindy. Biochemical Significance of Cystatin-C and High Sensitive CRP in Patients with Acute Coronary Syndrome; any Clinical Correlation with Diagnosis and Ejection Fraction. Systematic Reviews in Pharmacy. 2020;11:301-8.
31. Arendse LB, Danser AHJ, Poglitsch M, Touyz RM, Burnett JC, Jr., Llorens-Cortes C, et al. Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure. Pharmacol Rev. 2019;71:539-70. doi: 10.1124/pr.118.017129. PubMed PMID: 31537750; PubMed Central PMCID: PMC PMC6782023.
32. Association AH. Clinical Implications. Hypertension. 2016;68:1321-. doi: doi:10.1161/HYPERTENSIONAHA.116.08492.
33. Almutlaq M, Alamro AA, Alroqi F, Barhoumi T. Classical and Counter-Regulatory Renin-Angiotensin System: Potential Key Roles in COVID-19 Pathophysiology. CJC Open. 2021;3:1060-74. doi: 10.1016/j.cjco.2021.04.004. PubMed PMID: 33875979; PubMed Central PMCID: PMC PMC8046706.
34. Babajani F, Kakavand A, Mohammadi H, Sharifi A, Zakeri S, Asadi S, et al. COVID-19 and renin angiotensin aldosterone system: Pathogenesis and therapy. Health Sci Rep. 2021;4:e440. doi: 10.1002/hsr2.440. PubMed PMID: 34869917; PubMed Central PMCID: PMCPMC8596942.
35. Wang J, Zhao H, An Y. ACE2 Shedding and the Role in COVID-19. Front Cell Infect Microbiol. 2021;11:789180. doi: 10.3389/fcimb.2021.789180. PubMed PMID: 35096642; PubMed Central PMCID: PMC PMC8795668.
36. Yeung ML, Teng JLL, Jia L, Zhang C, Huang C, Cai JP, et al. Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system. Cell. 2021;184:2212-28.e12. doi: 10.1016/j.cell.2021.02.053. PubMed PMID: 33713620; PubMed Central PMCID: PMCPMC7923941.
37. Haschke M, Schuster M, Poglitsch M, Loibner H, Salzberg M, Bruggisser M, et al. Pharmacokinetics and Pharmacodynamics of Recombinant Human Angiotensin-Converting Enzyme 2 in Healthy Human Subjects. Clinical Pharmacokinetics. 2013;52:783-92. doi: 10.1007/s40262-013-0072-7.
38. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Critical Care. 2017;21:234. doi: 10.1186/s13054-017-1823-x.
39. Zhang H, Lv P, Jiang J, Liu Y, Yan R, Shu S, et al. Advances in developing ACE2 derivatives against SARS-CoV-2. Lancet Microbe. 2023;4:e369-e78. doi: 10.1016/s2666-5247(23)00011-3. PubMed PMID: 36934742; PubMed Central PMCID: PMCPMC10019897.
40. Zanganeh S, Goodarzi N, Doroudian M, Movahed E. Potential COVID-19 therapeutic approaches targeting angiotensin-converting enzyme 2; An updated review. Rev Med Virol. 2022;32:e2321. doi: 10.1002/rmv.2321. Pub Med PMID: 34958163.
41. Zoufaly A, Poglitsch M, Aberle JH, Hoepler W, Seitz T, Traugott M, et al. Human recombinant soluble ACE2 in severe COVID-19. The Lancet Respiratory Medicine. 2020;8:1154-8. doi: 10.1016/S2213-2600(20)30418-5.
42. Shoemaker RH, Panettieri RA, Jr., Libutti SK, Hochster HS, Watts NR, Wingfield PT, et al. Development of an aerosol intervention for COVID-19 disease: Tolerability of soluble ACE2 (APN01) administered via nebulizer. PLoS One. 2022;17:e0271066. doi: 10.1371/journal.pone.0271066. PubMed PMID: 35816490; PubMed Central PMCID: PMCPMC9273060 Apeiron Biologics A.G. Apeiron supplied the APN01 for study. Josef M. Pettinger was a founder of Apeiron, is a current shareholder and inventor of APN01. David L. McCormick is a Section Editor for PLOS One. Other authors declare no competing interests.
43. Kim OY, Lee J, Gho YS. Extracellular vesicle mimetics: Novel alternatives to extracellular vesicle-based theranostics, drug delivery, and vaccines. Semin Cell Dev Biol. 2017;67:74-82. doi: 10.1016/j.semcdb.2016.12.001. Pub Med PMID: 27916566.
44. Khalaj K, Figueira RL, Antounians L, Lauriti G, Zani A. Systematic review of extracellular vesicle-based treatments for lung injury: Are

EVs a potential therapy for COVID-19? J Extracell Vesicles. 2020;9:1795365. doi: 10.1080/20013078.2020.1795365. PubMed PMID: 32944185; PubMed Central PMCID: PMCPMC7481829.

45. El-Shennawy L, Hoffmann, Andrew, Dashzeveg, Nurmaa, Mehl, Paul, Yu, Zihao, Tokars, Valerie et al. Circulating ACE2-expressing Exosomes Block SARS-CoV-2 Infection as an Innate Antiviral Mechanism2020.
46. Zhang Q, Jeppesen DK, Higginbotham JN, Franklin JL, Crowe JE, Jr., Coffey RJ. Angiotensin-converting Enzyme 2-containing Small Extracellular Vesicles and Exomeres Bind the Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein. Gastroenter ology. 2021;160:958-61.e3. doi: 10.1053/j. gastro.2020.09.042. PubMed PMID:330222 77; PubMed Central PMCID: PMCP MC78 32655.
47. Xie F, Su P, Pan T, Zhou X, Li H, Huang H, et al. Engineering Extracellular Vesicles Enriched with Palmitoylated ACE2 as COVID-19 Therapy. Adv Mater. 2021; 33:e2103471. doi: 10.1002/adma.20210 3471. PubMed PMID: 34665481; PubMed Central PMCID: PMC PMC8646473.
48. Piccoli L, Park YJ, Tortorici MA, Czudnochowski N, Walls AC, Beltramello M, et al. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell. 2020;183:1024-42.e21. doi: 10.1016/j.cell.20 20.09.037. PubMed PMID: 32991844; PubMed Central PMCID: PMCPM C7494283.
49. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021;371. doi: 10.1126/science.abf4063. PubMed PMID: 33408181; PubMed Central PMCID: PMCPM C7919858.
50. Amjed H. Abbas MAR, Hayder Abdul-Amir Al-Hindy, Mazin J. Mousa, Hadeel Abd Ameir Al-Shalah. The Role of Serum IL-1β in Combination with Fractional Exhaled Nitric Oxide in the Diagnosis of Adult Bronchial Asthma. NeuroQuantology. 2021;19:13-9.
51. Hayder Abdul-Amir Makki Al-Hindy AJHA-A, Mazin J. Mousa, Safa Jihad Hameed, Suhad Hafidh Obeed. The Utility of Serum IL-1β and CRP Together with Fractional Exhaled Nitric Oxide in the Diagnosis of Asthma in Adults. NeuroQuantology. 2021;19:119-24.
52. Al-Shimmery AHS A-AM, Chabuck ZAG, Al-Mammori RTO, Mokif TA, Mahdi ZA-A, et al. Assessment of tumor necrosis factor-α, interleukin-17, and vitamin D3 levels on a group of gastrointestinal tumor patients in Babylon Provence, Iraq. Med J Babylon. 2023;20:362-7.
53. AL-Shimmery AHS, Mahdi, Zena Abdul-Ameer, Al-Hindy, Hayder Abdul-Amir Makki, Al-Mammori, Raheem Tuama Obayes, Mokif, Thanaa Abdulmahdi, Al-Dahmoshi, Hussein O M, Al-Khafaji, Noor S K Immunological Study of IFN-γ, ICAM-4, and Vitamin D3 Markers among Gastrointestinal Tumor Patients in Babylon Province, Iraq. The Asian Pacific Journal of Cancer Prevention. 2023;24 :253-7.
54. Hekmatnia Y, Rahmani F, Feili Z, Ebrahimzadeh F. A review of the effect of COVID-19 on immune responses of the body. J Family Med Prim Care. 2022;11:1624-32. doi: 10.4103/jfmpc.jfmpc_677_21. PubMed PMID: 35800568; PubMed Central PMCID: PMCPMC9254812.
55. Ahmed Khalid Aldhalmi AJHA-A, Hayder Abdul-Amir Makki Al-Hindy Association of Tumor Necrosis Factor-α and Myeloperoxidase enzyme with Severe Asthma: A comparative study. Reports of Biochemistry and Molecular Biology. 2022 ;11:238-45.
56. Shaymaa Obaid Abdullah GMR, Hayder Abdul-Amir Makki Al-Hindy, Mazin Jaafar Mousa, Amir Al-Mumin, Safa Jihad, Suhad Hafidh, Yaqeen Kadhum Serum Myeloperoxidase as A Biomarker of Asthma Severity Among Adults: A Case Control Study. Reports of Biochemistry Molecular Biology. 2022;11:182-9.
57. Teo A, Chan LLY, Cheung C, Chia PY, Ong SWX, Fong SW, et al. Myeloperoxidase inhibition may protect against endothelial glycocalyx shedding induced by COVID-19 plasma. Commun Med (Lond). 2023;3:62. doi: 10.1038/s43856-023-00293-x. PubMed PMID

: 37147421; PubMed Central PMCID: PMCP MC10160718.

58. Kilic G, Bulut O, Jaeger M, Ter Horst R, Koeken V, Moorlag S, et al. The Immunological Factors Predisposing to Severe Covid-19 Are Already Present in Healthy Elderly and Men. Front Immunol. 2021;12: 720090. doi: 10.3389/fimmu.2021. 720090. PubMed PMID: 34434199; PubMed Central PMCID: PMCPM C8380832.
59. Li D, Sempowski GD, Saunders KO, Acharya P, Haynes BF. SARS-CoV-2 Neutralizing Antibodies for COVID-19 Prevention and Treatment. Annu Rev Med. 2022;73:1-16. doi: 10.1146/annurev-med-042420-113838. Pub Med PMID: 34428080.
60. Qi W, Zhai D, Song D, Liu C, Yang J, Sun L, et al. Optimized synthesis of anti-COVID-19 drugs aided by retrosynthesis software. RSC Med Chem. 2023;14:1254-9. doi: 10.1039/ d2md00444e. PubMed PMID: 3748 4565; Pub Med Central PMCID: PMCP MC1 0357945.
61. Looi M-K. What are the latest covid drugs and treatments? 2023;381:p872. doi: 10.1136/bmj. p872 %J BMJ.
62. Qayssar Joudah Fadheel RTN, Hayder Abdul-Amir Al-Hindy. Evaluation Of Practice of Prescribing and Monitoring Anticoagulants Among Hospitalized Iraqi Patients. HIV Nursing. 2022;22:71-6.

Cite this: Hafidh , S., Al-Hindy, H. A.-A. M., Al-Anbari , A. J., Abdulabbas , H. A., & Majeed, A. (2023). Advances in Angiotensin Converting Enzyme-2 and Renin Angiotensin System Against COVID 19: A Pharmacotherapy and Physicochemical Review. Journal of Medical Research and Health Sciences, 6(9), 2742–2753. https://doi. org/10.52845/JMRHS/2023-6-9-4