SARS-CoV-2 and the ACE2 Receptor

Introduction

Coronaviruses (*Coronaviridae*) are a group of related spherical, non-segmented RNA viruses that usually infect mammals and birds (Lau et al 2005). In humans, these viruses cause respiratory-tract infections that can range from mild (common cold caused by rhinovirus) to lethal such as 2002's SARS and 2012's MERS (Yin et al 2017). In December 2019, a new infectious respiratory disease emerged in Wuhan, Hubei province, China(Shi et al 2020). Shortly thereafter, a large cluster of cases suddenly appeared in the aforementioned location, where the cause was linked to a seafood market. Subsequently, human-to-human transmission occurred (Chan et al 2019) and the disease, now termed coronavirus disease 19 (COVID-19) rapidly spread within China and other countries. Specifically, coronaviruses may gain entrance into host cells through a process called membrane fusion, where the membranes of the virus and host cell fuse together allowing the cell to inject its own RNA (DTR 2020). Furthermore, in order for this process to work, the protein on the membrane of the virus, called the spike glycoprotein, must bind to a receptor on the host cell. In SARS-COV-2, the receptor was discovered to be the Angio-Tensin Converting Enzyme II (ACE2) (Lan et al 2020). As of June 20, 2020, a total of 8.75 million laboratory-confirmed cases are reported worldwide, including 463 000 deaths in 184 countries outside China. A novel coronavirus, SARS-coronavirus 2 (SARS-CoV-2), which was found to be closely related to SARS-CoV, was detected in patients and is believed to be the etiologic agent of the new lung disease COVID-19 (Zhu et al). This essentially means that since SARS-COV-2 is similar to SARS-COV structurally, treatment known to work against the latter may also work against the former.

Structure of SARS-CoV-2 and its Spike Glycoprotein

A SARS-CoV-2 virion is approximately 50-200 nm in diameter, making it much smaller than the smallest bacteria. This virus has 4 major structural proteins: S, E, M, and N proteins all of which are required to create a functionally complete viral particle(Bartlam et al 2007). Only the Spike Glycoproteins, which are proteins abundant with carbohydrate chains that aid in viral infection, will be discussed extensively since the specificity of these glycoproteins are directly related to receptor activation on a host cell (Walls et al 2020).

The spike (S) protein is a major surface glycoprotein that the virion uses to bind to a host cell's receptor; the precise shape for SARS-CoV-2's spike protein (SARS-COV-2-S) was recently mapped in early 2020 (Saplagoklu 2020). More specifically, this protein performs two primary tasks that aids the virus in infecting a host cell:

1. It mediates the attachment between the virus and host cell surface receptors physically
2. It facilitates the spreading of its viral RNA into a host cell by assisting in the fusion of the viral and host cell membranes (RayBiotech 2019).

Because this protein is directly responsible for the virion's entry into a host cell, further investigation regarding treatment and vaccine developments could potentially entail preventing recognition of this protein by the host cell receptor. Accordingly, more research is required here because the receptor ACE2 plays a critical role in blood pressure and fluid control within the body, and inhibiting the expression of such a protein could instigate severe side effects.

SARS-COV and SARS-COV-2 Spike Protein Comparison

In May 1, 2020, Jaimes et al (Jaimes et al 2020) found that spike (S) protein amino sequences in SARS-COV and SARS-COV-2 were overall 76% similar. The ectodomain of all CoV spike proteins share the same organization in two domains: a N-terminal domain named S1 that is responsible for receptor binding and a C-terminal named S2 responsible for fusion (Sinobiological 2018). Specifically, the domains of the S protein on SARS-COV-2 compared to SARS-CoV revealed that the S1 receptor-binding domain was less conserved (64% similarity) than the S2 fusion domain (90% similarity) in SARS-CoV-2. The relatively high degree of sequence similarity for the S1 is consistent with the assumption that SARS-CoV-2, like SARS-CoV, may be able to use ACE2 as its host cell receptor. However, since the S1 similarity between SARS-COV-2 and SARS-COV is still not as high compared to the S2 similarity; this may indicate possible differences with the receptor and binding affinities.

This finding is significant because it shows that the spike proteins on SARS-COV-2 and SARS-COV share similar amino acid sequences which means that they will most likely be triggered by similar receptors on host cells. As previously mentioned, spike proteins act as ligands and are only activated by complementary enzymes on host cells. In a greater context, similar spike proteins that are activated by similar receptors will most likely lead to the finding that viruses bearing these spike proteins will infect similar cell lines in an organism.

SARS-COV-2 and SARS-COV Infect Similar Cell Lines

A study done by Hoffman et al (Hoffman et al 2020) confirmed that SARS-COV and SARS-COV-2 infect similar cell lines. Briefly, replication-defective vesicular stomatitis virus (VSV) particles are a harmless version of a virion capable of infecting hosts but does not replicate or trigger biological reactions. These VSV particles were pseudotyped to express SARS-COV-S and SARS-COV-2-S; as such, this will allow a side-by-side comparison. Pseudotype means that VSV particles do not naturally express SARS-COV-S or SARS-COV-2-S proteins but are synthetically transfected to do so. Afterwards, both types of spike proteins as well as the naturally occurring VSV-G spike protein were released to infect multiple cell lines to compare infection effectiveness. The human cell lines, 293T & Caco-2, and animal cell lines ,Vero & MDCKII, were completely susceptible to entry driven by both SARS-COV-S and SARS-COV-COV-2-S and at similar levels of effectiveness. In conclusion, SARS-COV-2-S infects an identical spectrum of cell lines as SARS-COV-S and at similar rates of infectivity, suggesting similarities in choice of entry receptors and thus mechanisms of entry.

Significantly, therapeutics known to work against SARS-COV may also work against SARS-COV-2, but only if these drugs target either S spike proteins or receptors themselves.

ACE2: Common Receptor for SARS-COV and SARS-COV-2:

In a separate study conducted by Hoffman et al, human (HEK293) cell culture studies have demonstrated that human angiotensin converting enzyme (hACE2), an enzyme that controls blood pressure and regulates the amount of fluids in the body, is an essential receptor directly responsible for SARS-COV entry into the host cell. In order to explain why SARS-COV-S and SARS-COV-2-S target the same cell lines, it is important to determine whether SARS-COV-2-S contains amino acid sequences required for interaction with ACE2. Consequently, sequence alignment analysis revealed that SARS-CoV-2 clusters with other coronaviruses originating from bats which use ACE2 for host cell entry. Analysis of the receptor binding motif (RBM), a portion of the ligand that makes contact with ACE2, revealed that most amino acid sequences essential for ACE2 binding by SARS-COV-S were also found in SARS-COV-2-S. Even more, amino acid residues of coronaviruses that do not utilize ACE2 for entry were missing from S proteins of SARS-COV-2 . In conclusion, these various experiments indicate that SARS-COV-2-S, like SARS-COV-S, uses ACE2 for cellular entry.

ACE2 Expression in Humans

A key question is why the lung appears to be one of the most vulnerable organs (The Hindu 2020). One possible explanation is that the large surface area of the alveoli in the lungs makes respiratory tissue highly susceptible to SARS-CoV-2 and SARS-CoV, but there is also a biological factor which is ACE2 expression levels. Logically, SARS-COV-2-S is activated by ACE2, meaning that areas expressing ACE2 in high levels could be at higher risk of being infected.

For instance, using normal lung tissue from eight adult donors, Zhao et al (Zhao et al 2020) found that over 83% of all human ACE2-expressing cells were alveolar epithelial type II cells (AECII) suggesting that these cells can easily serve as a target for viral infection. In addition to this, Zhao et al also used gene ontology enrichment analysis (a form of analysis that maps gene attributes across all species) and learned that these AECII have high levels of genes related to viral processes, including regulatory genes for viral life cycle, assembly, and genome replication. This suggests that the ACE2-expressing AECII facilitates viral replication and proliferation from within the lung. Other than the 83% expressed in lungs, ACE2-receptor expression is also found in many other types of tissues such as the heart, kidney, endothelium, and intestine. More importantly, 12% of ACE2 is also expressed in the intestinal epithelial cells which is responsible for nutrient uptake and amino acid resorption from food. Therefore, this might prove that the intestine is also a major entry site for SARS-CoV-2 and corroborate the suspicion that the infection had been initiated by eating food from a Wuhan market.

Similarly, another study performed by Li et al (Harmer 2002) found that, in 72 samples of adult human tissue, expression of ACE2 was on average 10 000 times higher in the digestive tract, (ileum, jejunum, duodenum) and the respiratory tract (lung, alveolar epithelium) than other organs such as the spleen, umbilical cord, and the brain. Interestingly, testicular ACE2 expression was on par with organs known to have high risk such as the lung, and originated from the testes only; this may suggest that men are more susceptible than women in contracting this virus.

ACE2 Expression levels do not correlate with disease severity

The gender discrepancy was also noted in a study conducted by Jin et al (Jin et al 2020). Here, Jin and company found that men's cases tended to be more lethal than women's (up to 2.4 times more lethal) and that older age had a greater impact on health in men than women, independent of ACE2. Moreover, the study by Li et al did not notice any statistical discrepancies between ages–-ACE2 was expressed in similar levels between those aged under 49 and those over 49. Although it is known that older populations report more severe cases of SARS-COV-2, according to public data sets, higher ACE2 expression levels do not increase disease severity–higher ACE2 expression levels only correlate with a higher probability of being infected.

Conclusion

In summary, the disease COVID-19 caused by the virus SARS-COV-2 was responsible for many deaths and lethal injuries . Each SARS-COV-2 virion contains a variety of critical proteins necessary to make a fully-functioning viral particle. However, the spike glycoprotein must be thoroughly researched as it will be a target of therapeutics. This protein is further divided into the N terminal, which is responsible for physical connection with ACE2, and C terminals which allows the fusing of membranes. Additionally, the glycoproteins of SARS-COV-2 were compared to the glycoprotein of SARS-COV and were found to be overall 76% similar. Notably, this may indicate that both these viruses are activated by the same receptor, since receptors must be perfectly complementary in shape to the spike glycoprotein it triggers. Further analysis showed that, indeed, both SARS-COV and SARS-COV-2 use ACE2, an enzyme that plays a critical role in blood pressure and fluid regulation in the body, as the direct receptor. Importantly, may lead scientists to possibly inhibit or diminish the expression of this enzyme to lower the risk of infectivity . Analysis of ACE2 expression within the body indicates that respiratory, digestive, and testicular organs express ACE2 in high levels making them more susceptible to SARS-COV-2 infection. Moreover, these studies as well as public data sets agree in the fact that men’s COV cases are on average 2.4 times more severe than that of women, possibly explained by the fact men express high levels of testicular ACE2. However, even though older population’s account for the majority of casualties due to COVID-19, there is no discernable difference in ACE2 expression between older and younger populations. This therefore indicates that ACE2 may not play a role in the severity and lethality of the disease, but only the likelihood of being infected. This notion can also be linked to the gender discrepancy -- men reporting

more lethal cases of COVID-19 may not be due to high testicular ACE2 expression, but some other unknown reason. Naturally, there are many other factors at play when analysing COVID-19 infectivity and lethality other than receptors (such as genetic variations, past or ongoing illnesses) that may provide scientists a better understanding of preventing SARS-COV-2 infection.

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A Review of SARS-COV-2 Receptors and Inhibitors To Prevent Viral Infection and Proliferation

Introduction:

Coronaviruses (Coronaviridae) are a group of related spherical, non-segmented (viral genome is all on one string of non-fragmented RNA) RNA viruses that usually infect mammals and birds (Lau et al 2005). In humans, these viruses cause respiratory-tract infections that can range from mild (common cold caused by rhinovirus) to lethal such as 2002's SARS-COV (SARS outbreak) and 2012's MERS-COV (Yin et al 2017). In December 2019, a new infectious respiratory disease emerged in Wuhan, Hubei province, China (Shi et al 2020). As of June 20, 2020, a total of 8.75 million laboratory-confirmed cases are reported worldwide, including 463 000 deaths in 184 countries outside China.

To infect a cell, SARS-COV and 2019's SARS-COV-2 (COVID-19) first bind onto a cell's membrane receptor for viral attachment, enter endosomes , and then fuse membranes, allowing the viral particle to inject its RNA, thereby hijacking the cell (Shang et al 2020). Moreover, the spike glycoproteins on SARS-COV and SARS-COV-2 are highly similar in terms of their structure, therefore discussion of an aspect of one virus will implicitly include the other. SARS-CoV-2 spike glycoproteins contains a receptor-binding domain (RBD) that specifically recognizes angiotensin-converting enzyme 2 (ACE2), serine protease TMPRSS2, and lysosomal proteases cathepsins Cathepsin L and B as receptors. In order to prevent SARS-COV-2 from latching onto a cell in the first place, it will be necessary to identify all currently known receptors, which will be outlined in this review. Since receptors allow the virus to infect the cell, eliminating interactions between the viral spike glycoprotein and host cell receptor is worthy of research and subsequently become a treatment option. For instance, all receptors are proteins synthesized by their cell's genotypic expression for that particular protein; decreasing genotypic expression for a protein will decrease the amount of that protein to be synthesized, leading to less receptors for SARS-COV-2 to utilize. The three receptors that will be discussed in this review are ACE2, TMPRSS2 and Cathepsin L/B, followed by research on inhibition for its corresponding receptor as a method of treatment.

ACE and ACE2 Work In Tandem To Maintain Blood Pressure

As an enzyme belonging in the renin angiotensin aldosterone system (RAAS) which plays a crucial role in regulating blood pressure, the Angiotensin Converting Enzyme ii (ACE2) acts as an antagonist to ACE. ACE converts the hormone angiotensin I into the vasoconstricting hormone angiotensin II. ACE2 in turn cleaves the carboxyl-terminal on the amino acid phenylalanine from angiotensin II and hydrolyses it into the vasodilating hormone angiotensin 1-7. In simpler terms, ACE activity elicits vasoconstriction (the narrowing of the blood vessels elevating blood pressure) and ACE2 activity elicits vasodilation (the widening of blood vessels lowering blood pressure) to maintain homeostasis. Because of ACE2's role in the RAAS system,

the effects of SARS-COV-2 on ACE2 may affect the organism's overall blood pressure, thus increasing the risk of the organism to develop comorbidities in addition to COVID-19.

ACE2 Receptor and Inhibitors

However, even though ACE2's primary function is to control blood pressure, it was also discovered to catalyze the Spike glycoprotein (SARS-COV-2-S) on the SARS-COV and SARS-COV-2 viruses, meaning that the receptor binding domain on the spike glycoprotein is identical in shape with angiotensin II, complimentary to ACE2. Today, many studies have proven that ACE2 is the primary receptor for SARS COV 2 and SARS-COV infection (Hoffman et al; Lan et al), which is a significant milestone as it provides researchers a place to begin research for a treatment.

The Angiotensin II receptor (AT1R) blocking pharmaceutical drugs losartan and olmesartan, which are drugs commonly administered for reducing blood pressure in hypertensive patients, were shown to increase cardiac and respiratory ACE2 expression up to three-fold (Ishiyama 2004). As a result, this particular study suggests that an increased ACE2 expression due to AT1R blockers may potentially increase the probability of being infected by SARS-COV-2. However, several different studies on SARS-CoV, which are relevant for SARS-CoV-2, suggest that the benefits of higher ACE2 expression–from the use of AT1R blockers– outweigh the consequences inferred by Ishiyama.

More specifically, AT1R blockers reverse ACE2 downregulation caused by SARS-COV-2 infection and protects the body from severe lung injury. First, it has been demonstrated that the binding of the SARS-COV-2 spike glycoprotein to ACE2 (the infection) leads to ACE2 downregulation (the process by which a cell decreases the quantity of a cellular component, such as RNA or protein, in response to an external stimulus) (Imai et al 2005). This results in excessive production of angiotensin (vasoconstrictor) by ACE, while less ACE2 – from its downregulation due to the infection – is capable of converting it back to the vasodilator angiotensin 1–7. According to Kuba et al, reduced ACE2 expression contributes to lung injury, as angiotensin-stimulated AT1R results in increased pulmonary vascular permeability, thereby allowing increased lung pathology. Therefore, higher ACE2 expression caused by SARS-CoV-2 patients administered with AT1R blockers, contrary to inferences made by Ishiyama, may protect them against acute lung injury (Kuba et al 2005).

Chloroquine: A new ACE2 Inhibitor

Previously, the AT1R blockers losartan and olmesartan were commonly administered drugs meant to lower blood pressure, however, these drugs are not dedicated towards SARS-COV-2 infection prevention. Consequently, we will be discussing drugs that do inhibit ACE2 expression for the sole purpose of reducing probability of infection by SARS-COV-2, or to reduce the spread of infectivity. A study performed by Vincent et al (Vincent et al 2005) on the monkey cell line Vero e6 discovered a potent compound called chloroquine that inhibits not only ACE2 but

also cathepsins, a co-receptor enzyme for SARS-COV-2[1]. Specifically, in addition to the well-known functions of chloroquine such as elevations of endosomal (membrane-bound organelles responsible for endo and exocytosis) pH, this drug interferes with spike protein activation by ACE2. This negatively influences the virus-receptor relationship and hinders infection probability, resulting in the inhibition of infection and spread of SARS CoV at clinically admissible concentrations. According to the experiment, pretreatment with 0.1, 1, and 10 μM chloroquine reduced infectivity by 28%, 53%, and 100%, respectively. Hence, this data demonstrates that exposing Vero E6 cells to chloroquine prior to SARS-COV-2 rendered these cells “immune” to viral infection.

Vincent et al further extended their research to see if a postinfection chloroquine treatment would be just as effective in preventing the spread of SARS-CoV infection within neighbouring cells of the same sample. Interestingly, as little as 0.1–1 μM of chloroquine reduced the infection by 50%, and up to 90–94% inhibition was observed at higher concentrations of the drug. At concentrations of chloroquine greater than 1 μM, only a small number of individual cells were initially infected, and the spread of the infection to adjacent cells was nearly eliminated altogether. This experiment clearly shows that addition of chloroquine can effectively reduce the establishment of infection and spread of SARS-CoV if the drug is added immediately following virus absorption. Unfortunately, for a drug to become a clinically/FDA-approved drug, there must be extensive research to ensure the benefits outweigh the risks, which has not been sufficiently researched. Furthermore, there has not been research on chloroquine with human-culture cells.

Isolating Spike Glycoprotein and blocking ACE2

Instead of utilizing synthetic drugs, Tai et al proposes the following: Why not use the virus’ own spike protein by itself to bind to ACE2 to reduce the chances of being infected by the actual virus? In this study performed on human embryonic kidney cells (HEK293t), Tai et al (tai et al, 2020) identified the receptor-binding domain (RBD) in SARS-CoV-2 Spike glycoprotein and found that the RBD protein bound strongly to ACE2 receptors. Moreover, SARS-CoV-2 RBD exhibited significantly higher binding affinity to ACE2 receptors than SARS-CoV RBD. In other words, SARS-COV-2 RBD binds more easily and strongly to ACE2 compared to SARS-COV, meaning that SARS-COV-2 RBD is a more suitable drug to prevent viral infection.

They found that SARS-COV-2 RBD could block the binding and, hence, attachment of SARS-CoV-2 RBD and SARS-CoV RBD to ACE2-expressing cells, thus inhibiting their infection to host cells. Expectedly, SARS-CoV-2 RBD protein inhibited SARS-CoV-2 pseudovirus entry into ACE2-expressing 293T cells with 50% inhibition concentration (IC50) as low as 1.35 μg/ml. This is very low compared to other viruses such as IC50 of 5.47 μg/ml for SARS-CoV pseudovirus into ACE2-expressing 293T, and 11.63 μg/ml for hCOV-229E. Lower IC50 values means that the inhibitor requires lower concentration to inhibit the virus by 50% and indicates

[1] See Section: Cathepsin L/B Receptor and Inhibitors)

that it is more potent and effective. In sum, this study entails using the actual harmless Receptor Binding Motif and having it bind to the receptor, instead of the actual virus.

However, while ACE2 inhibitors, or Angiotensin II Receptor Blockers (ARB's) are already clinically-approved pharmaceuticals proven to treat hypertension and other vascular illnesses, there is not enough research on these drugs to prove that they work in preventing SARS-COV-2 infection, spread, or reduce tissue injury.

TMPRSS2 Receptor

Transmembrane protease, serine 2 (TMPRSS2) is an enzyme encoded by the TMPRSS2 gene. Furthermore, it is a plasma membrane anchored protein that is abundantly expressed in the respiratory and digestive tract (The Human Protein Atlas). The precise function of this protein still remains largely unknown, although it was discovered to aid SARS-COV-2 infection by priming Spike glycoproteins on SARS-COV-2 virions (Glowacka 2011). This essentially means that this enzyme cleaves the S1 and S2 domains on the spike glycoprotein which facilitates infection via plasma membrane fusion (Shabir 2020). There is not enough research dedicated to TMPRSS2 inhibition and its relationship to SARS-COV-2 to discuss; any research done on it will be discussed in the following section: **Cathepsin L/B Receptor and Inhibitors**

Cathepsin L/B Receptor and Inhibitors

Cathepsin B and L are members of the cathepsin family which are a group of proteases that degrade proteins. Most cathepsins, including B and L, work in low pH environments such as in the lysosomes and endosomes. In terms of cathepsins, it was proven that SARS COV 2 uses cathepsin L and B to prime S proteins and facilitate entry into host cells (Huang 2005; Gnirss et al 2011).

K11777:

Zhou et al discovered the compound K11777, a protease inhibitor that is capable of effectively inhibiting the activity of cysteine cathepsins (Cat B/L) in humans (Choy 2013). As previously mentioned, Cat B/L are enzymes that break down proteins and are part of the Cysteine protease family. Since CatB/L is a co-receptor for SARS-CoV-2, inhibiting this co-receptor will also be necessary for complete prevention of SARS-CoV-2 infection. In this study, K11777 blocked all the major enveloped viruses known to require cathepsins for entry, including a variety of coronaviruses and filoviruses. K11777 inhibited SARS-CoV entry with a remarkably low IC50 (Concentration required to block the virus by 50%) of 0.68 nM, with no cytotoxic effects observed. The fact that the IC50 is low for SARS-CoV inhibition indicates that K11777 is efficient in preventing SARS-CoV infection, and thus could be a suitable treatment.

Simultaneous treatment of Camostat and EST:

Kawase et al (Kawase 2012) conducted an experiment using HeLa cells that attempted to fully inhibit SARS-COV infection by using cysteine and serine inhibitors (CAT L/B and TMPRSS2 inhibitors respectively) simultaneously, since both types of receptors are interchangeable in SARS-COV Spike protein activation. Furthermore, this study established that this virus is able to use both CAT L/B and TMPRSS2 but not fully. More specifically, Camostat (a known TMPRSS2 inhibitor) inhibition of SARS-S entry never exceeded 65%, even in the presence of a high concentration of the drug (100 μM). This indicates that, despite full inhibition of TMPRSS2, 35% of the viruses utilized endosomal cathepsins CAT L/B for cell entry. Conversely, inhibiting only cathepsins CAT L/B using EST, a well-researched CAT L/B inhibitor, in the same cell lines only inhibited viral entry by 20%.

Afterwards, Kawase et al decided to treat the cells with both TMPRSS2 serine inhibitors and CAT L/B inhibitors simultaneously in order to maximize inhibition. Within the same HeLa cells, simultaneous treatment with camostat and EST drastically blocked infection, with an inhibition rate of more than 95%. Even more, this group extended their research into Calu-3 cells, an immortalized human airway epithelial cell line, and found that these inhibition rates were conserved, and that viral replication was suppressed (>6,000-fold in cells cultured in the presence of both 10 μM camostat and 10 μM EST). Taken together, inhibiting all of SARS-COV's receptors simultaneously is the clear administrative method when utilizing protease inhibitors.

A study conducted by Hoffman et al, similar to the one done by Kawase et al, also tested the serine protease (TMPRSS2) inhibitor camostat by itself, and then coupled with other drugs to use simultaneously. In the african monkey Vero cell line, camostat mesylate only inhibited SARS-COV-2 entry by 50% at high concentrations; gradually decreasing the concentration also drastically lowered SARS-COV-2 inhibition rate. Hoffman et al used the cysteine protein (CAT L/B) inhibitor E-64d, which on its own, inhibited SARS-COV-2 infection by around 65% in the same vero cells. Expectedly, referring to the results of Kawase et al's study, an inhibition rate of approximately 98% was achieved when administering camostate and e-64d were used simultaneously.

Conclusion:

In summary, the spike glycoprotein which resides on the surface of the virus, physically attaches the host cell receptor. For SARS-COV and SARS-COV-2, these receptors are the ACE2, TMPRSS2, and CAT L and/or B. Of all three, ACE2 is the most well-known enzyme that is a part of the Renin-Angiotensin aldosterone system (RAAS), a system responsible for blood pressure regulation. When SARS-COV infects a cell, the cell's expression for ACE2 is downregulated. Furthermore, this downregulation of ACE2 is threatening for the cell because it worsens lung injury and promotes viral proliferation. Angio-tensin Receptor Blockers (ARB's) are commonly prescribed for hypertension patients by elevating ACE2 expression; this in turn also helps patients infected with SARS-COV-2 since the downregulation of ACE2 induced by infection is offset by upregulation of ACE2 induced by ARB's. Chloroquine is a recently discovered ACE2 inhibitor that not only inhibits ACE2 but also cathepsins, possibly blocking two receptors with one drug. Another interesting method of blocking ACE2 interaction with SARS-COV-2 is by using its own spike glycoprotein to bind to the receptor instead of the virus. Nevertheless, more elaborate and extensive research must be conducted to corroborate evidence outlined in this review. Besides ACE2, TMPRSS2 is a serine protease and Cathepsins L and B (CAT L/B) fulfills a similar role by aiding in viral infection. Simultaneous treatment entailing inhibiting both TMPRSS2 and CAT L/B prevented SARS-COV-2 infection by 95%, but at much lower percentages when administered separately. Therefore, should inhibiting receptors become a critical part of clinically-approved drugs, it will most likely necessitate inhibiting two or all receptors simultaneously. Additionally, two separate studies discovered similar comorbidities associated with COVID-19; of all patients examined, 30% suffered from hypertension, 27% from diabetes, and 7% from any form of cardiovascular disease (Zhou et al 2020; Wu et al 2019). However, COVID-19 may not be responsible for the onset of the aforementioned illnesses because those illnesses are prevalent among older populations regardless of COVID-19. As of today, there is still no definitive relationship between SARS-COV-2 infection and blood pressure. Nevertheless, inhibiting the ACE2 receptor could constitute a measure to prevent SARS COV 2 infection and spread because ACE2 is the enzyme that allows S-driven membrane fusion.

For future research, it is encouraged to:

1. Discover any new and definitive receptors;
2. Discover the risks of inhibiting receptors within the physiology of the organism of interest;
3. Weigh the risks of side-effects and the benefits of preventing SARS-COV-2;
4. Learn the time-period of effectiveness for each receptor inhibitor;
5. Devise a logical method of consuming these drugs, for it is not realistic for the entire population to constantly consume pills.
6. Conduct more research on inhibitors on human tissue to corroborate findings outlined in this review

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