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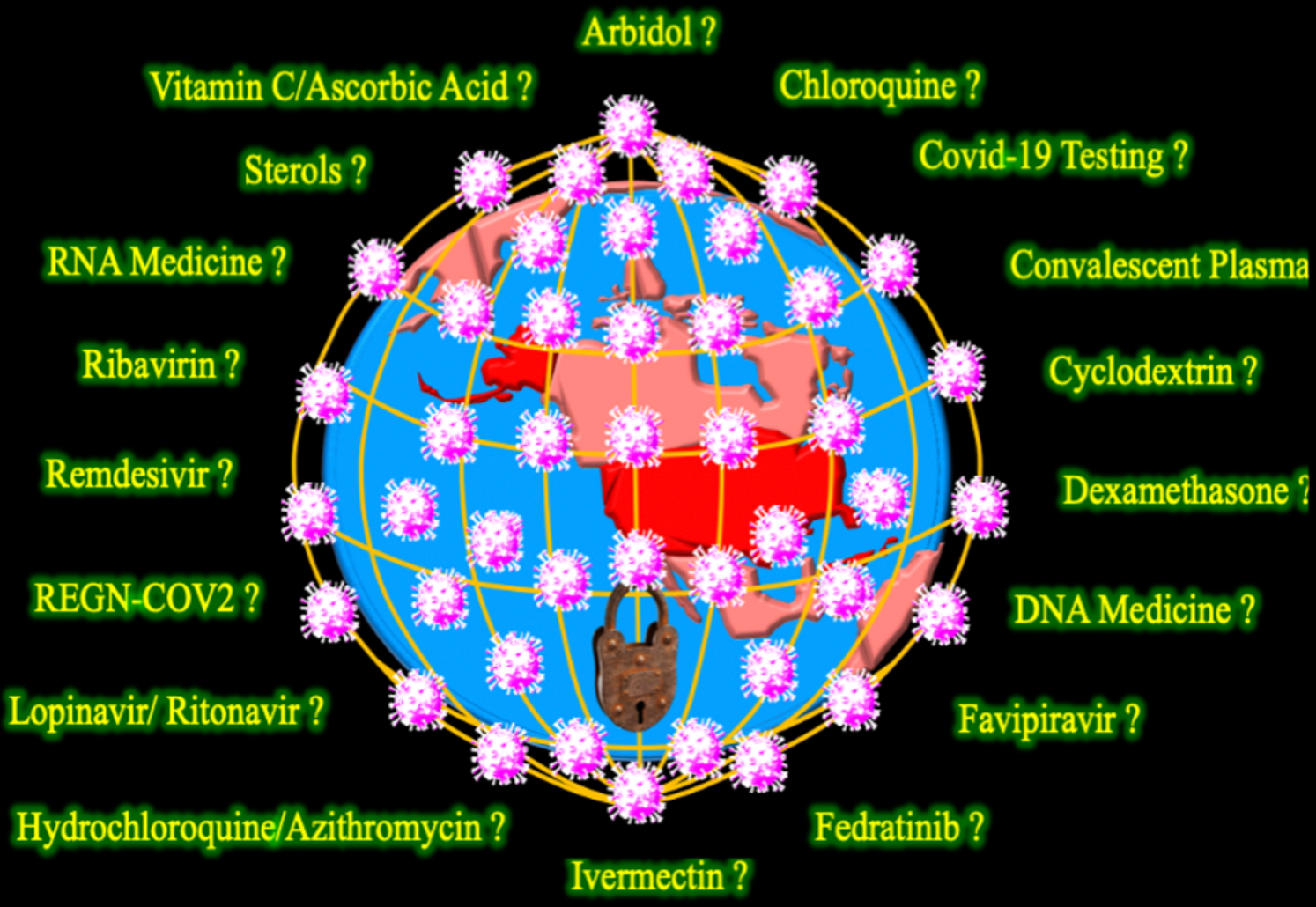

Fear of coronavirus locks down the world and resets activities on the earth

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Review Article

Implications of COVID-19 in metabolic syndrome and therapeutic strategy for prevention and cure of the disease

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From what started as pneumonia, whose origin was unknown, in China at the end of 2019 to now well established coronavirus disease-2019 (COVID-19), havoc has been created all over the world with unprecedented impacts socially, economically and medically. At the end of January 2020 World Health organisation (WHO) had declared a public health emergency in China and in mid March the disease had been declared a pandemic. This novel pathogen of microscopic size, now named the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has shaken the most developed western countries that once lauded their health care set up, forcing lockdowns in majority of countries to curb the spread of this highly contagious virus. In the present review we highlight the impact of SARS-CoV-2 on the cardiovascular system with systemic injury and a dysfunctional RAS. We have also tried to take a stand on the continuation of the use of the much debated chloroquine and its hydroxyl analogue, hydroxychloroquine and also on the use of RAS inhibitors as a treatment measure for those infected by SARS-CoV-2. This review also suggests various pathways for development of therapeutics for the purpose of treatment of coronavirus infections because there are currently no known medications for this family of viruses. In the end we have tried to derive future perspective in vaccine development as a measure to provide herd immunity and subsequent elimination of the virus.

Keywords: SARS-CoV-2, metabolic syndrome, renin angiotensin system, therapeutics, vaccine

Introduction

An outbreak occurred in China in a city called Wuhan at the end of 2019 with cases of severe pneumonia being reported. This outbreak is now named as Coronavirus Disease-2019 or COVID-19, as is commonly called, and is continuously on the rise worldwide. COVID-19 is caused by a novel member of the family of coronaviruses, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).[1] SARS-CoV-2 is responsible for causing even more infections, mortality and economic disruptions than SARS-CoV of 2002–2003[2] and Middle East Respiratory Syndrome Corona virus (MERS-CoV) of 2012. Coronaviruses have been haunting mankind since a very long time with the very first infection seen in the 1960s in humans. These are respiratory viruses that extensively exist in nature with its natural hosts being humans, dogs, cats, pigs, mice and bats.[3] Coronaviruses are reported as large, single–stranded, enveloped positive-sense RNA viruses[4-7] belonging to coronaviridae, subfamily coronavirinae and the nidovirales order.[5,8,9] The subfamily, coronavirinae, is further divided into four genera; alpha- (α), beta- (β), gamma- (λ) and delta- (δ) coronaviruses.[5,9] SARS-CoV-2 comes under the β-coronavirus genus[10] which is further categorised into A, B, C, and D lineages. SARS-CoV-2 and SARS-CoV both come under lineage B of β-coronaviruses.[11] Coronaviruses (CoV) represent a diverse virus family having the capability of causing disease in human and animal respiratory illnesses.[4] Since the 21st century two β-CoVs have caused epidemics of pneumonia in humans that include the SARS-CoV that was responsible for an epidemic with a mortality rate of 10% and the MERS-CoV that had started out in the Middle Eastern countries and caused an outbreak in humans with a mortality rate of 35%. The most recent and the third type of β-CoV is the SARS-CoV-2 that is by far the most contagious of all known coronaviruses with 9,124,002 cases worldwide as on 23rd June 2020 and 472,068 deaths. The fatality rate so far is low with about 6% that may change as the pandemic progresses. These three viruses cause more severe infections of the lower respiratory tract causing pneumonia and lung injuries.[9] They cause zoonotic infections in humans and are found to be transmitted from animals with

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vast reservoirs found in bats. Four other coronaviruses of zoonotic origin are the α-CoVs, HCoV-229E, HCoV-NL63, and the β-CoVs HCoV-OC43, HCoV-HKU1 that account for about 30% infections of the upper respiratory tract that are mild and complications and mortality are only reported in the elderly and immune-compromised individuals as well as young children.[5,9] The origin of SARS-CoV-2 is yet uncertain but bats are thought to be their original sources. Among human CoVs, SARS-CoV is thought to be its closest relative with 79% genetic similarity. It is however most similar to RaTG13, a Coronavirus in bats, with 98% genetic similarity.[2,10] Population genetic studies that were done on 103 genomes of SARS-CoV-2 have shown the evolution of these viruses into two types; the L and S type. The prevalence of L type was more (with a frequency of about 70%) than the S type (with frequency of about 30%). The ancestral version, however, was found to be the S type whereas the L type is found to transmit more quickly, was more prevalent and aggressive during the initial period of the outbreak in Wuhan as compared to the S type. After early January 2020 the L type frequency had decreased and that of S type, which was evolutionarily less aggressive and spread less quickly, must have increased due to weaker selective pressure.[3] The COVID-19 diagnosis was primarily done by utilising real-time reverse transcriptase-polymerase chain reaction (RT-PCR) using respiratory samples.[7,12-14]

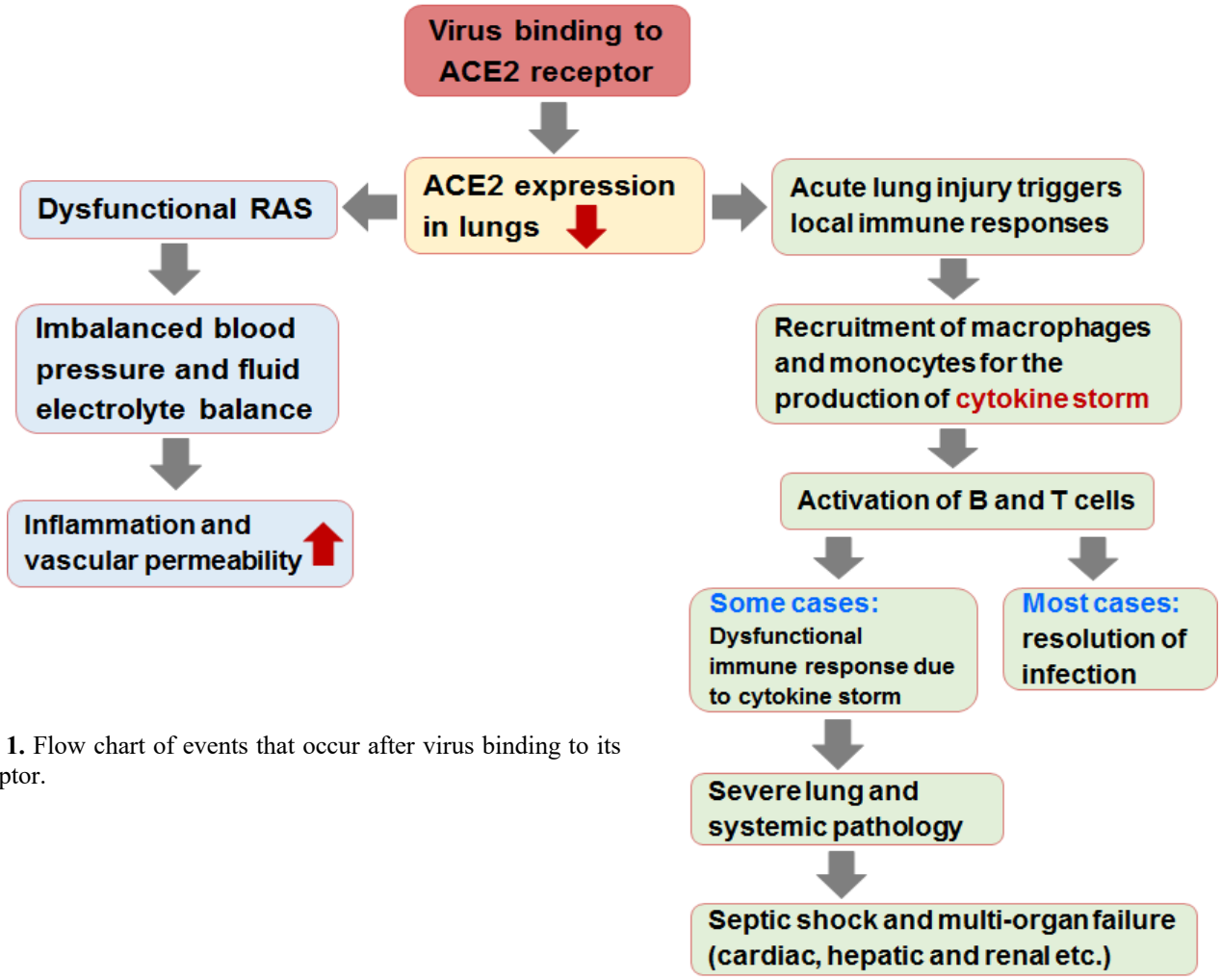

Fig. 1. Flow chart of events that occur after virus binding to its receptor.

Viral entry

The primary contraction of SARS-CoV-2 is caused by coming into contact with large respiratory droplets and infected fomites. It is also transmitted by self-contamination of the eyes, mouth or nose which is why it is advised to not touch the face unnecessarily.[15-17] The most significant feature of coronaviruses is the presence of a club-shaped spike protein on their surface which appears like a 'corona' hence the name, coronavirus.[3] All coronaviruses encode this surface glycoprotein, spike (S), and uses its homotrimer for recognition of host cell receptor, fusion of cell membranes of both the organisms, and subsequent entry of viral components into the host cell.[1,4,5,11] CoVs use

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variety of host cell receptors for binding and infection of these cells. Some of the receptors known to be recognised by CoVs are the Dipeptidyl peptidase 4 (DPP4) recognised by the MERS-CoV and the Angiotensin converting enzyme 2 (ACE2) receptor recognised by SARS-CoV. The recently emerged SARS-CoV-2 is also known to use the same receptor as SARS-CoV to gain entry into cells, a carboxypeptidase, ACE2.[1,4,9,11,18-21] The spike glycoprotein of the virus is essential for receptor recognition, binding, infection and causing COVID-19 associated pneumonia as well as myocardial injury and various cardiovascular pathologies and multiple organ failure as observed in certain cases.[4,18]

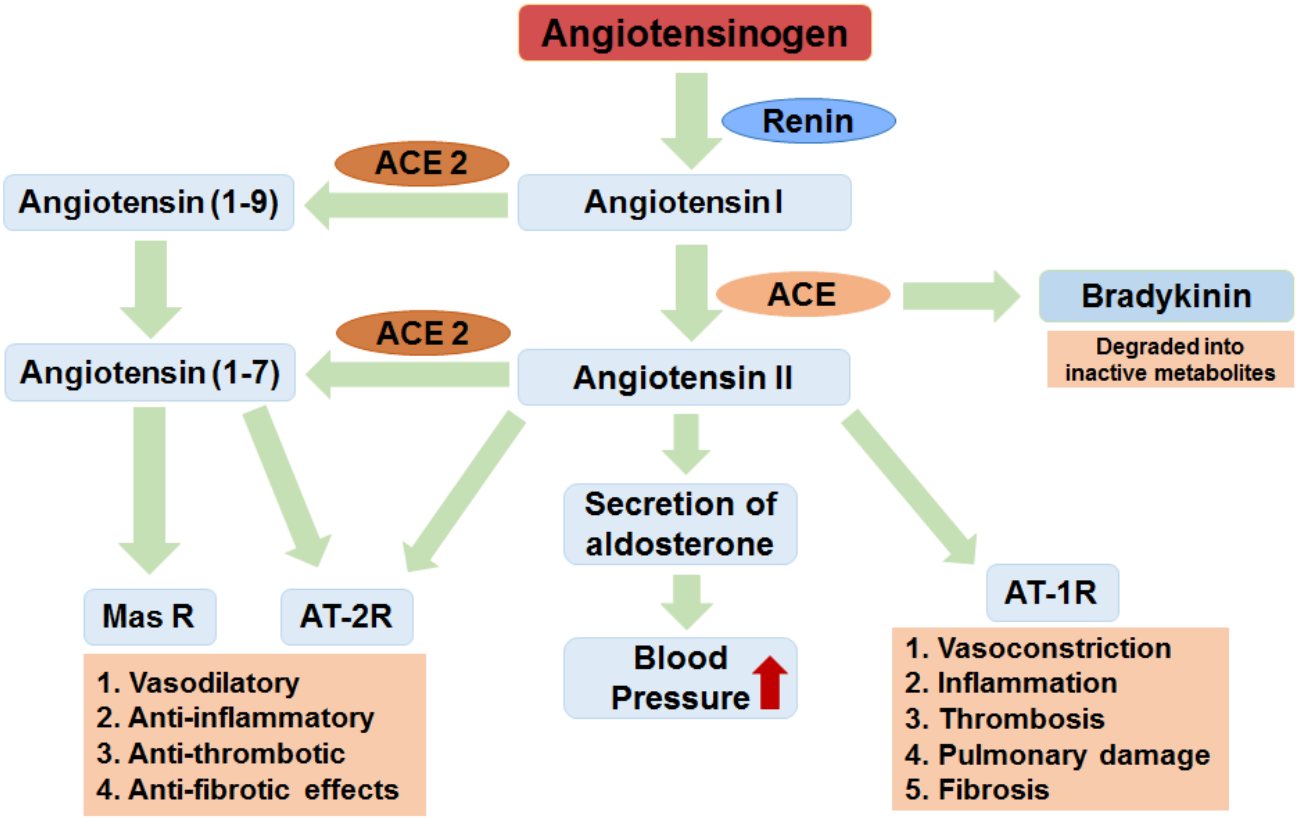

Fig. 2. Representation of the Renin-Angiotensin-Aldosterone system.

The spike or 'S' glycoprotein

The spike, S, protein of the virus is known to undergo a number of conformational changes after its binding to the host receptor.[4] Several studies using an Electron microscope have shown that the S protein is a clover-shaped trimeric structure with three heads of the S1 subunit and a stalk of trimers of S2 subunit.[9] The tip of each S1 subunit localises a domain called the receptor binding domain (RBD) whereas the S2 subunit has a machinery for membrane fusion consisting of a fusion peptide and two heptad repeats (HR1 and HR2)[4,10] The RBD binds to ACE2 on its outer surface marking the first step of viral entry process.[4,9,11,22] A transmembrane protease serine 2 (TMPRSS2) present alongside ACE2 carries out the second and most important step in the viral entry process which is the cleavage of clover shaped structure into an S1 and an S2 subunits. The latter is internalised by endocytosis that is initiated by binding of S protein to the receptor, where the endosomal proteases out its second cleavage.[10] This second cleavage occurs at the S2' site and exposes a fusion peptide which subsequently gets incorporated into the host's membrane.[4,5,10,11,22] The HR1 and HR2 regions then fold to come together to form a helical transmembrane structure of six helices thereby causing fusion of the viral and host membranes causing subsequent release of the viral contents, replication of viral genome, and infection of nearby cells.[4,22] Evidence also suggests that the protease not only activates the viral S protein after cleavage but also acts on the receptor to activate it.[5,11] A study carried out by Letko *et al* shows that on addition of a protease during the process of infection of SARS-CoV the entry into those cells that had low ACE2 expression was facilitated. Through these findings they also propose that SARS-CoV-2 is as efficient as SARS-CoV in the

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use of ACE2 that may explain the transmissibility of this virus between humans.[11] The process of host cell infection by SARS-CoV-2 can therefore be divided into 5 steps; receptor binding, internalisation/endocytosis, protease cleavage, membrane fusion, and entry and replication.

Clinical presentation of COVID-19 and associated co-morbidities

COVID-19 is seen to progress through different stages of infection. First stage is the viral infection, second is the cytotoxic effects of the virus, and third is the heightened inflammatory response. The first stage constitutes mild symptoms with severe symptoms seen in the second and third stages of infection. Mild symptoms include fever, and dry cough (common symptoms), breathing difficulty, headache/dizziness, muscle or joint pain, nausea and coughing with traces of blood,[10] sore throat, nasal congestion, diarrhoea[7] (less common symptoms). Lately, loss of taste and smell is also considered a symptom of COVID-19 in asymptomatic patients. Severe symptoms include pneumonia, multiorgan failure, cardiovascular damage, acute respiratory distress syndrome (ARDS), hyper-inflammatory response, septic shock.[7,10,15,18] Among all the fatal cases of COVID-19, 70% deaths are caused as a result of ARDS which directly leads to failure of the respiratory tract and 28% of deaths are caused as a result of large amounts of cytokine release leading to a cytokine storm and a subsequent septic shock.[10]

Severity of infection is caused not only as a result of viral infection but also as a result of host's response to that infection. Various clinical studies have reported that some patients present more severe symptoms due to the presence of co-morbid cardiovascular disease (CVD). Among these, the proportion of coronary artery disease, hypertension and cerebrovascular disease was higher than that of any other co-morbidity suggesting that people with CVD might be more sensitive to coronavirus infection.[3] The presence of CVD is proven to worsen the severity of COVID-19 and increase the risk of death.[3,22,23] CVDs such as coronary heart disease, hypertension and cerebrovascular disease are all related to metabolic diseases and it has been reported that these metabolic diseases cause down regulation of key players in host's innate immune response important against pathogenesis thereby affecting the function of the immune system (both humoral and innate immune responses). Coronary heart disease is associated with chronic low-grade inflammatory disease and its presence is known to be a cause of inflammation. Hypertension, on the other hand, is known to cause oxidative stress, activation of the immune system by secretion of

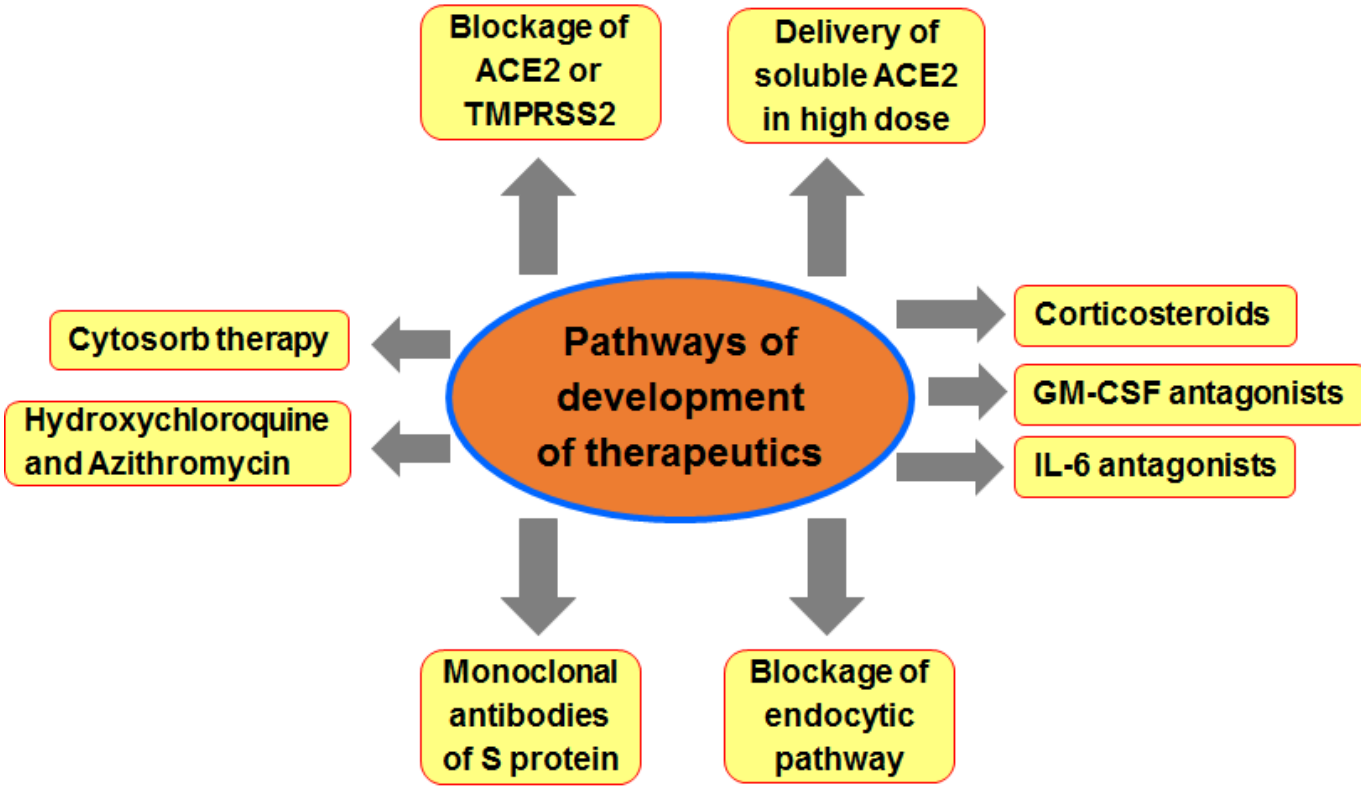

Fig. 3. Schematic representation of the pathways for therapeutics development

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cytokines such as Tumor Necrosis Factor α (TNF-α), and Interleukins (IL-6, IL-1). This may explain the susceptibility of patients with underlying CVDs to infection.[3] National Health Commission of China (NHC) reported 11.8%, among those who succumbed to COVID-19 with no cardiovascular disease history, had heart damage NHC also released the mortality data of those with SARS-CoV-2 infection which reported 17% patients with coronary heart disease, and 35% with hypertension.[18] A study reported that patients who were suffering from severe symptoms of COVID-19, frequency of heart disease, hypertension, and arrhythmia in them was 25%, 58%, and 44% respectively. Data released by the NHC reported that 35% patients with coronavirus infection were those who had a history of hypertension and 17% were those who had a history of coronary heart disease.[18] China Centre for disease Control (CDC) carried out a survey on those with a diagnosis of COVID-19 and showed that among these 13% had hypertension, 4% had a history of underlying cardiovascular disease, and 5% had diabetes mellitus. In the same survey done on patients who had succumbed to COVID-19 the results showed that 40% had hypertension, 22% had pre-existing cardiovascular disease, and 20% had diabetes mellitus.[15-17] Li *et al*, in their study, tried to establish a role of cardiovascular disease in the prognosis of COVID-19. The study indicated that COVID-19 patients having underlying CVD had more serious clinical symptoms including lung injury, release of enzymes associated with tissue injury, and surge of cytokines (cytokine storm) compared to the non-CVD patients.[23] Many studies have reported multiple organ failure in those with SARS-CoV-2 infection; a study by Li et al showed that the apart from lungs the next most common organ to be damaged is the heart.[3] In a study of 191 patients, pre-existing CVDs were not among the independent factors responsible for death among COVID-19 patients. Therefore observations where cardiovascular co-morbidities are prevalent among those who died of this infection may be because of the high prevalence of these conditions in older age groups.[15-17] This uncertainty should be cleared by future studies.

ACE2

ACE2 is a membrane bound carboxypeptidase that has also recently been recognised as an important SARS-CoV-2 receptor. It has a critical role in immune and cardiovascular systems, in the development of metabolic syndrome (diabetes and hypertension) and is involved in protecting the lung.[1,,18,24] ACE2 is extensively expressed in the lungs (on type II alveolar epithelial cells), heart, kidney, liver, and gastrointestinal tract.[7,18,19] This widespread localisation of ACE2 may explain the multiple organ failure scenarios prevalent in certain patients of COVID-19 (Zheng *et al*, 2020). ACE2 has a major role in the Renin-Angiotensin-Aldosterone System (RAAS).[3,23] It is present both as membrane bound and soluble form where its main function is the formation of angiotensin 1-7 by the degradation of angiotensin II.[3,22,23] In addition to this ACE2 also can catalyse the conversion of angiotensin II to angiotensin 1-9 that is further cleaved to angiotensin 1-7 by ACE, a homolog of ACE2.[3] Angiotensin 1-7 further activates downstream signalling through binding to the Mas receptors thereby exerting the desired anti-inflammatory and anti-proliferative effects. Angiotensin 1-9 can directly bind to the angiotensin II type 2 receptors (AT2R) resulting in effects that are anti-inflammatory and anti-angiotensin II type 1 receptor (AT1R).[3] Angiotensin II binds to AT1R to exert vasoconstriction, inflammation and thrombosis. ACE2 therefore regulates the angiotensin II levels in the body to subsequently trigger counter-regulatory protective effects in the lungs and the cardiovascular system.[22] Leading evidence suggest the protective role of ACE2 in cardiovascular disease and its loss or down-regulation may result in harmful effects resulting in lung and cardiac injury.[3] ACE2 gene is present on the X-chromosome with large polymorphisms associated with it.[25] Its presence on the X-chromosome might be a cause of increased susceptibility to COVID-19 in men as reported by some studies from China and Italy. A study on 201 COVID-19 patients showed that 63.7% of them were men.[22] ACE2 polymorphisms are also associated with the occurrence of hypertension in Han Chinese men and in women of different race and ethnicities. In Asian populations there seems to be a correlation of ACE2 with incidence of diabetes, coronary artery disease, septal wall thickness, and cerebral stroke.[25] There are speculations however about the fact that ACE2 polymorphism may put an individual at the forefront of COVID-19 infection and severity. Ethnically and demographically the prevalence of these polymorphisms are different which might provide us with a reason as to why some countries may have lower cases of this disease; for example in Africa the incidences of COVID-19 are very low.[25] Chinese and Italian

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studies have shown that frequently observed co-morbidities among those with COVID-19 were diabetes, CVD, and hypertension with other factors including old age and male sex. One such study shows the percentage of men among those infected by SARS-CoV-2 was 63.7% with mean age of 51 years. The frequency of hypertension, diabetes, and cardiovascular disease was 19.4%, 10.9%, and 4% respectively. Another study from Italy reports that 82% of those infected were males with mean age of 63 years. Proportion of those with diabetes was 17%, with CVD was 21%, and with hypertension was 49%.[22]

ACE2, COVID-19 and the Renin Angiotensin System (RAS)

As established from the previous section that patients with cardiovascular disease present more severe symptoms of COVID-19 and have poor prognosis, this is due to the fact that ACE2 expression in these patients is increased.[18] Also patients with COVID-19 without previous cardiovascular co-morbidities show increased cardiovascular damage as recognised by increased incidence of myocardial injury. ACE2 and cytokine storm, triggered by an uncontrolled immune response, are considered to be the mechanisms involved in myocardial injury.[18] SARS-CoV-2 infection initiates with the virus binding to ACE2 receptor that leads to a reduction in the expression of ACE2 in lungs which in turn triggers acute lung injury as well as a dysfunctional RAS. A localised immune response is initiated at the site of injury which is responsible for the recruitment of immune cells; monocytes and macrophages. These immune cells secrete cytokines which function by activating T-cells (both T_H (helper) cells and T_C (cytotoxic) cells) and B-cells. T-cells upon activation either release cytokines to resolve infection or directly kill the infected cells, B-cells, on the other hand, secrete neutralising antibodies to clear infection. Triggering of B- and T-cells, therefore, is sufficient to resolve infection in most of the cases but in certain rare cases a cytokine storm ensues due to a dysfunctional immune response. The uncontrolled and continuous secretion of cytokines results in severe lung and systemic pathology leading to septic shock and multi-organ failure (especially the heart, liver, and kidneys)[10] as demonstrated in Fig. 1.

ACE2 is also involved in RAS regulation and its down-regulation as a result of viral binding to it resulting dysfunction of RAS as demonstrated (Fig. 2). In this system, the first step is the conversion of Angiotensinogen to Angiotensin I carried out by the enzyme, Renin. Further cleavage of Ang I by Angiotensin Converting Enzyme (ACE) results in the product, Ang II. ACE2, a homolog of ACE, converts Ang I to Ang 1-9 and Ang II to Ang 1-7. Ang II binds to Angiotensin II type 1 receptor (AT1R) and triggers adverse effects including vasoconstriction, inflammation, thrombosis, pulmonary damage, and fibrosis via the ACE→Angiotensin II→AT1 receptor axis. Ang 1-7 counter-balances the effects of Ang II by triggering vasodilatory effects and decreasing inflammation via the ACE2→Angiotensin1-7→Mas receptor axis.[19,22] The entry of SARS-CoV-2 into cells causes loss of function of ACE2 receptors due to its decreased expression. Effect of this down-regulation is an increase in Ang II levels which binds to AT1 receptors thereby increasing inflammation and coagulation in the lungs. Various clinical studies have established that patients infected by SARS-CoV-2 possess conditions including old age, hypertension, diabetes, and cardiovascular disease that are all associated with ACE2 deficiency. Therefore it maybe suggested that ACE2 deficiency caused by the viral infection maybe particularly harmful to those patients who already have existing ACE2 deficiency due to the conditions mentioned above. Reduction in ACE2 expression may also result in an imbalance between adverse effects caused by Ang II binding to AT1 receptors and the protective effects caused by Ang 1-7 binding to Mas receptors. This imbalance would result in uncontrolled Ang II activity that would trigger inflammation and thrombosis in lungs due to lack of Ang 1-7.[3,22]

Hypertension, COVID-19 and the use of RAS inhibitors

One of the major underlying conditions that is shown to increase a person's susceptibility to COVID-19 is hypertension. Hypertension is a condition where the body fails to regulate the blood pressure levels as a result of which there is significant increase in blood pressure levels from the normal 120/80 mmHg. Blood pressure is regulated in the body by various mechanisms; the most significant among these is the Renin-Angiotensin System (RAS). Associations between RAS, ACE2 and COVID-19 have been highlighted previously. Treatment of hypertension is done by the administration of ACE inhibitors (ACEI) or Angiotensin Receptor Blockers (ARBs) known as the RAS inhibitors. ACE2 levels are known to

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increase by the administration of RAS inhibitors,[3] which trigger its protective effects thereby regulating elevated blood pressure levels. The upregulation of ACE2 in hypertension and by RAS inhibition theoretically increases the susceptibility to CoV infection. In ACE2 deficient mice models with severe lung injury restitution of ACE2 levels mitigates lung injury. These two scenarios have led to a debate on the administration of ACEI or ARBs in patients who are at risk of COVID-19.[15,19] There is an uncertainty regarding the discontinuation of ACEI or ARBs in these patients with one school of thought being that ACEI/ARBs may increase susceptibility to the virus while the other school of thought being that their use may have a protective effect.[19-21] We agree with the second school of thought. Increase in the levels of Ang II results in lung injury by the activation of adverse effects via the AT1 receptors producing inflammation and fibrosis. ACEI decrease the levels of Ang II and ARBs block the AT1 receptors thereby increasing Ang 1-7 levels and activating Mas receptors. They counter the role of Ang II by decreasing inflammation and fibrosis thereby decreasing lung damage. Therefore we believe and suggest the continuation of these drugs for treating patients having CVDs and who are at risk of coronavirus infection since discontinuation of established therapies like these would lead to more complications.

Pathways for therapeutics development

In a pandemic like this one it is important not only to treat the patients with infection but also to effectively contain the spread of the virus which is done by the use of vaccines. Treatment requires the development of new therapeutics as well as testing the existing ones as there is currently no available drug or prevention strategy for any human coronavirus.[1,9] Another important strategy in this direction is to determine the targets for drug development. Existing knowledge of SARS-CoV can be very helpful in determining these targets since there is a significant similarity between SARS-CoV and the novel SARS-CoV-2, about 79% genetic similarity.[10] Since the RBDs of both these viruses are highly conserved, various anti-RBD antibodies should be tested for their efficacy[9] making the 'S' protein as the suitable target for therapeutic generation. A recent study from April 2020 reports the efficacy of a recombinant fusion protein in neutralising a virus that was pseudotyped with SARS-CoV-2 spike. The recombinant protein was generated by joining the IgG1 Fc region to the extracellular domain of ACE2. This fusion protein bound to the RBD of SARS-CoV-2 with high affinity and also exhibited cross-reactivity against the coronavirus.[1] One other important pathway of therapeutic development is to block the host receptor (ACE2 in this case) or the cellular serine protease TMPRSS 2. As an alternative strategy high concentrations of soluble ACE2 can be delivered to reduce viral entry into host cells. Yet another interesting adjunct therapy is cytosorb. Cytosorb works by absorbing a broad spectrum of cytokines, death associated molecular patterns (DAMPs), and pathogen associated molecular patterns (PAMPs) thereby ameliorating the immunopathology by reducing the circulating levels of these substances. Antibodies from convalescent serum samples are also been administered with successful clinical results.[10] Apart from the above mentioned strategies that target the 'S' protein, Serine protease TMPRSS2 and the ACE2 receptor, there are various other targets for therapies and one of them is targeting the endocytic pathway of viral internalisation. Among known drugs that inhibit this pathway chloroquine and hydroxychloroquine are widely used (Fig. 3). It is necessary to determine the pathway utilised by SARS-CoV-2 by which internalisation of the virus takes place in the host cell; clathrin dependent or caveolin-1 mediated endocytosis. This step can act as an important therapeutic target for future drug development. The propagation of coronaviruses in the host cells is regulated by various transcriptional and post-translational mechanisms.[6] After infecting host cells, coronaviruses assemble their multi-subunit RNA-synthesis machinery. This machinery is an arrangement of non-structural proteins (nsp) formed when the open reading frames, ORF1a and ORF1ab, are cleaved. Viral polyproteins among the non-structural proteins, nsp12, nsp7, and nsp8 are the minimum requirements for nucleotide polymorphism. An RNA-dependent RNA polymerase, nsp12 has some amount of activity alone but its polymerase activity is tremendously stimulated by the presence of co-factors, nsp7 and nsp8 forming an nsp12-nsp7-nsp8 complex. This complex seems to be the minimum requirement to carry out processes of transcription as well as replication although additional subunits of viral nsp may also be necessary.[26]

Evidence on the use of chloroquine

Chloroquine and hydroxychloroquine are anti-malarial drugs but are known to exert an anti-viral response as well by interfering with the endosome-

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mediated pathway of viral internalisation, by inhibiting replication of virus and also by decreasing the secretion of pro-inflammatory cytokines thereby decreasing inflammation and its associated deteriorating effects.[7,15] A non-randomized, small study from France showed that administration of chloroquine and azithromycin had faster than expected viral clearance. Another randomized study from Shanghai, China of 62 patients showed that the treatment group, which was administered with hydroxychloroquine (200 mg twice daily for between days 1-5), had fever and cough for shorter duration than normal.[15-17] Human immunodeficiency virus (HIV), Nipah, Ebola, Hepatitis C virus (HCV), Mouse Hepatitis, Vesicular stomatitis, Influenza and now SARS Coronavirus are some of the viruses against which the in vitro anti-viral activity of chloroquine has been demonstrated. However in human clinical trials these in vitro, anti-viral effects were replicated only for HIV and HCV. The in vitro, anti-viral efficacy of chloroquine against SARS-CoV-2 was very promising results. A French study recently reported randomized human trial results of chloroquine administration to COVID-19 patients. Out of 36 patients in the study, hydroxychloroquine was administered to 20 patients and out of these 6 were given azithromycin in addition to hydroxychloroquine, remaining 16 patients served as controls. 6 days after treatment proportion of those who tested negative for Coronavirus was 100% in case of hydroxychloroquine and azithromycin combination treatment, 57% in case of hydroxychloroquine treatment alone, and 12% in case of the control group.[7] This study in particular is very promising however it is a small study and more such studies are required to establish their efficacy. Based on the clinical data available at present it is difficult to advocate the continuation of the use of chloroquine and its derivative. The director of Council of Scientific and Industrial Research (CSIR) has however advised the WHO to resume trials of hydroxychloroquine.

Vaccine strategy

The strategy for treating viral infection is highlighted in the previous sections. Treatment alone is not effective in a pandemic of this scale and therefore containment of the virus is extremely important. This is where the importance of a vaccine comes into play. We know from the history of Immunology how a vaccine is important against viral infections; small pox and polio serve as the best examples of viral diseases that have been completely wiped out from the human population. Vaccines are therefore a key in reducing coronaviral disease burden.[27] Coronaviruses are present in the zoonotic pool with a vast reservoir found in bats. Therefore there is always a risk of future animal–human transmission resulting in a pandemic. Designing a vaccine for coronaviruses hence becomes important for outbreak preparedness.[27] The goal of a vaccine is to provide the individual with specific immunity against the pathogen that is long-lived and illicit a memory response when future encounter with the real pathogen happens. There are currently five broad classes of vaccines in use; avirulent live/attenuated, inactivated/heat-killed, subunit vaccines, viral vectors carrying recombinant genes (live, recombinant), and DNA vaccines. In order to exert a strong immunogenic, protective effect against a pathogen it is important to activate both the humoral and cell-mediated branches of immunity and for this the most successful are the live vaccines both live/attenuated and viral vectors.[28] However, live/attenuated vaccines have a limitation because of the reversion into virulent type[25] as any recombination event may cause the reversion of attenuated strain into a virulent strain. Coronaviruses are known to replicate their subgenomic RNAs through a transcription mechanism that is discontinuous. This is performed by a conserved sequence network of nucleotides that are present near the 5'end of the genome and also at 5' end of each downstream open reading frame known as the Transcription Regulatory Sequences (TRSs). A guide sequence, 6 to 8 nucleotide core sequence, within this TRS helps in base pairing. A study by Graham *et al* on SARS-CoV showed the possibility of rewiring this guide sequence to produce an infectious virus with the ability to excite an immunogenic response but not the ability to cause SARS infection. The study also proved that it is highly unlikely that the rewired strain would revert back to virulent virus because the product formed after recombination of wild type virus and rewired virus did not turn out to be viable.[25] Since S is the main component of the virus that interacts with the host cell to initiate infection it is the main focus of vaccine design.[5] Tremendous research is underway to achieve this mammoth task of vaccine development against coronaviruses and many in the scientific community believe that it will be done in record time. Development of a successful vaccine otherwise requires anywhere between 7-10 years. Even after vaccine development its safety, availability and cost

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effectiveness will be some great challenges for the governments and concerned health organisations.

Conclusion

SARS-CoV-2, a virus of the family coronaviridae, is highly transmittable and causes infection of human cells by utilising their cell surface receptor, ACE2. ACE2 is present on the X-chromosome with various polymorphisms. ACE2 polymorphisms leave some individuals at an increased susceptibility to COVID-19. Presence of co-morbidities, including metabolic syndrome, also leads to a higher susceptibility to SARS-CoV-2 infection and are associated with worsening of symptoms and poor prognosis. Chloroquine is not as promising as their in vitro results and their use in the treatment of COVID-19 remains uncertain and require further randomized clinical trials to end this debate. ACE2 regulates RAS by acting on Ang II and decreasing its levels in the body. ACE2 is down-regulated in case of viral infection as well as in case of CVD. ACE2 levels, on the other hand, increase during hypertension and also on administration of RAS inhibitors that include ACE inhibitors and ARBs. Due to low expression levels of ACE2, Ang II levels goes up and as a result of this AT1 receptors gets activated. Downstream effects of AT1R activation would be increased inflammation, increased blood pressure, and pulmonary injury. Therefore, we suggest that administration of these RAS inhibitors should be kept continuous in patients of COVID-19. This review stresses the importance of vaccines and a strategy for their development in the future.

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Conflict of interest

None

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