Inhaled modified angiotensin converting enzyme 2 (ACE2) as a decoy to mitigate SARS-CoV-2 infection

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ABSTRACT

COVID-19 is a new zoonotic disease caused by the SARS-CoV-2 virus. Since its emergence in Wuhan City, China, the virus has rapidly spread across the globe causing calamitous health, economic and societal consequences. It causes disproportionately severe disease in the elderly and those with co-morbidities, such as hypertension and diabetes. There is currently no proven treatment for COVID-19 and a safe and effective vaccine is at least a year away. The virus gains access to the respiratory epithelium through cell surface angiotensin converting enzyme 2 (ACE2). The receptor binding domain (RBD) of the virus is unlikely to mutate without loss of pathogenicity and thus represents an attractive target for antiviral treatment. Inhaled modified recombinant human ACE2, may bind SARS-CoV-2 and mitigate lung damage. This decoy strategy is unlikely to provoke an adverse immune response and may reduce morbidity and mortality in high-risk groups.

COVID-19 is an emerging zoonotic disease, caused by SARS-CoV-2, which appears to have been transmitted to humans in late 2019 in the Hubei province of China, probably from an intermediate host in a live animal market. The viral sequence bears close similarity to bat (Chiroptera) coronaviruses,[1] although the proximate animal host source for this spillover event remains unidentified.[2] SARS-CoV-2 belongs to the family of beta coronaviruses, which have previously caused pandemics including SARS-CoV in 2003 and the Middle Eastern respiratory syndrome (MERS-CoV) in 2012.[3]

Following the initial outbreak in Wuhan City, there has been rapid spread of the virus across the globe with catastrophic health, economic and societal consequences.[4] The virus spreads from human to human via respiratory droplets, aerosols, fomites and by other body contact including the hands. Countries around the world have been attempting to block transmission of the virus by physical distancing and restricting movement of individuals including extreme measures of quarantining entire regions and countries.[5] Occult transmission of the virus by presymptomatic and asymptomatic persons may challenge healthcare systems attempting to eliminate the virus.[6]

Morbidity and mortality

Current case fatality rates (CFR) vary widely between countries from approximately 0.1% to 11% with a more recent overall estimate closer to 0.99%.[7] There appears to be a steep age-related mortality gradient with rates approaching 20% in those over 80 years of age.[8] Younger patients have also been severely affected, including medical and nursing healthcare workers

(HCW) who were exposed to high concentrations of the virus before the use of personal protective equipment (PPE).[9]

Epicentres in Europe have experienced large numbers of cases and deaths, which have overwhelmed healthcare systems. At the time of writing, the US death toll is rapidly approaching 100,000 and modelling by various authorities predict up to two million deaths depending on the effectiveness of preventative measures.

Individuals with co-morbidities such as hypertension, obesity, ischaemic heart disease, chronic pulmonary disease and diabetes are at increased risk of severe outcomes. Current advice is that patients on ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs) should continue treatment for hypertension.[8] Patients at risk of severe morbidity and death may experience rapid spread of the virus through the respiratory tract leading to viral pneumonia, sepsis, acute respiratory distress syndrome (ARDS) and multi-organ failure. Many patients have died in spite of invasive ventilation and extracorporeal membrane oxygenation (ECMO).[8]

Receptor for SARS-CoV-2

Like SARS-CoV (2003), SARS-CoV-2 enters human cells through the angiotensin converting enzyme 2 (ACE2) expressed on the membranes of type 2 pneumocytes of the respiratory tract.[10] There are two subtypes of ACE in humans.[11] ACE1 catalyses angiotensin 1 to its more active form angiotensin 2. ACE2 has approximately 40% sequence similarity to ACE1. Its main function is to produce angiotensin 1–7 and 1–9, which are physiological antagonists to angiotensin 2.[12] ACE2 also hydrolyses apelin, a pleiotropic peptide ligand with multisystem effects.

Membrane-bound ACE2 is cleaved by a metalloproteinase, tumor necrosis factor alpha convertase (TACE, ADAM17)[13] to produce a soluble ectodomain that is shed into the extracellular space. This cleaved ACE2 appears to maintain its catalytic function. Its exact physiological role is uncertain but it may act as a negative regulator of blood pressure control.[14]

Strategies to combat the virus

Apart from the effective public health strategies to self-isolate and maintain social and physical distancing, a variety of antiviral methodologies have been considered to combat the virus. These include the recent trials of COVID-19 candidate vaccines in several countries.

Multiple clinical studies are evaluating the efficacy of antiviral drugs such as favipiravir, remdesivir and ritonivir as well as other drugs including hydroxychloroquine and azithromycin, which appear to be less effective.[15] The cell surface protease, TMPRSS2 plays a critical role in activating both SARS-CoV and SARS-CoV-2 viruses.[16] *In vitro* studies suggest drugs such as camostat mesilate, which inhibits TMPRSS2, are effective in preventing viral entry into respiratory epithelial cells.[17,18] Large clinical studies will determine the precise efficacy of these treatments. Currently there are limited supplies of some drugs.

Passive immunisation with convalescent neutralising sera has also been considered.[19] It is however concerning that some patients with high titres of anti-SARS-CoV-2 sera had high viral loads.[9] Although such antibodies neutralised virus *in vitro*, they seem to be less effective *in vivo*. There is also concern about antibody-dependent enhancement (ADE) of disease.[20] It is likely patients who recover from COVID-19 have qualitative differences in antibody responses analogous to hepatitis B.

Similarly, monoclonal antibodies to the receptor-binding domain (RBD) have been considered but have not yet been deployed in clinical trials.[21] Competitive inhibition with peptides has been used in animals to counter SARS-CoV, but given the differences in spike protein sequences, it remains to be determined if SARS-CoV-ACE2 interaction is identical to that of SARS-CoV-2-ACE2.[22,23] Such peptides, particularly if coupled to IgG Fc fragments, also risk provoking an adverse immune response.

Inhaled modified soluble recombinant human ACE2 to treat COVID-19

We believe the Achilles heel of SARS-CoV-2 is the RBD sequence of the spike glycoprotein, which is critical for viral entry. Viral evolution of SARS-CoV-2 RBD is unlikely to be tolerated without loss of pathogenicity. Our strategy is to produce modified recombinant soluble human ACE2 (shACE2) molecules, which are similar to those cleaved from the cell surfaces of the respiratory mucosa.[24] Two amino acid

substitutions will abolish the catalytic activity of ACE2 (R273A) and reduce N-glycosylation (N90D) to increase affinity for the RBD of unactivated SARS-CoV-2 to inhaled shACE2. If the structure of these inhaled modified shACE2 molecules is preserved, the virus will bind to these decoy receptors.[25]

Soluble human ACE2 has high affinity (14.7 nM) for the SARS-CoV spike (S) glycoprotein and has been demonstrated to block the SARS-CoV virus from infecting cells in culture.[26] This is comparable to the affinity of a single-chain variable region fragment neutralising antibody against SAR-CoV-S.[27] As the SARS-CoV-2 S spike glycoprotein shares 77% protein sequence similarity to SARS-CoV S glycoprotein, it is anticipated that modified shACE2 will bind SARS-CoV-2 with similar high affinity.

The shACE2 will be delivered to the lungs by the Respimat® inhaler to newly diagnosed infected patients, particularly those with co-morbidities and the elderly, who might not be offered ventilation.[28] The Respimat® is an ideal drug delivery device as it induces lower shear stresses, which is less likely to denature the protein. Furthermore, since it is a closed system, it does not pose an additional danger to HCWs or family members. Unlike nebulisers, the Respimat® does not generate hazardous aerosols and more of the drug will be deposited in the respiratory system. Dry powder inhalers are unsuitable as they generate high shear stresses which could denature the proteins.

Binding of SARS-CoV-2 to the modified shACE2 decoy could alter the trajectory of the infection, delaying or halting the destruction of the pulmonary epithelium and allowing appropriate protective immune responses to the virus. Soluble ACE2 has been shown to inhibit *in vitro* SARS-CoV-2 infection of human organoids, supporting our approach.[29,30] We shall check the binding affinity of modified shACE2 by ELISA and its *in vitro* efficacy by viral cytopathic inhibition studies both before and after passage through the Respimat® inhaler.

The proposed strategy includes administering several treatments over a few days until there is clinical evidence of improvement in terms of fever, cough, dyspnea, myalgia and lethargy. Early resolution of fever, improved gas exchange and reduction in inflammatory markers may be reliable signs of efficacy in randomised trials.

Another ACE2 product conjugated to an Fc domain will be created for systemic use.[25] A shACE2-Fc construct could however aggravate the cytokine storm in such patients, although it may be more effective in removing the virus rapidly and reducing damage to the respiratory epithelium.[19] It could be used in patients on ECMO to reduce the duration of pulmonary failure. Data from China shows patients who succumb to COVID-19 have persistent, unrelenting viral sepsis.[8] This product, with the appropriate ethics approvals could be considered in severely affected individuals at a future date. There are now clear prognostic markers of death in such patients including unrelenting viremia, persistent lymphopenia, raised d-dimers, etc.

Potential benefits

The use of modified decoy shACE2 is a novel and relatively low-risk approach to mitigate the effects of a lethal infection. A decoy strategy is a compromise between safety and efficacy for a new class of biopharmaceutical agents. While ACE2-Fc constructs might generate rapid antiviral responses, they may also aggravate ARDS. If the strategy is successful, it may reduce the morbidity and mortality of COVID-19. It will convert those with severe disease to milder forms. Apart from reducing mortality, it may ease pressure on intensive care units and reduce the need for ventilators.

It is also possible this treatment may enhance the efficacy of other antiviral drugs, which may have only modest efficacy against SARS-CoV-2. Similar to HCV or HIV, a combination of drugs may lead to rapid improvement of disease if administered early in the infection.

Such agents could be used prophylactically for family members of COVID-19 cases and HCWs, who are at high risk of infection and transmission to their families and other patients.[31] The molecules may also be useful as prophylaxis in care homes experiencing outbreaks of infection. In countries

with widespread community transmission, deployment of these products in new infection clusters may allow development of protective herd immunity with a lower risk of death in the elderly. In countries without herd immunity such as New Zealand, these biopharmaceuticals could play a role in reducing the reproductive number (R0) of the virus. By decreasing the viral burden in an infected person, these molecules might decrease the risk of transmission.

The best-case scenario is shortening the duration of the current pandemic with saving large numbers of lives with low risk of adverse effects. These molecules may bridge the gap until a safe and effective vaccine is identified. In the event SARS-CoV-2 becomes more virulent by increasing its affinity to ACE2, these biopharmaceuticals could become even more effective. This strategy may also mitigate future pandemics caused by novel coronaviruses utilising ACE2 for viral entry.

Efficacy

It is not known if this experimental strategy will be effective. It is uncertain if the inhaled modified shACE2 will bind the unactivated virus with the same high affinity as cell surface ACE2, following activation by the TMPRSS2 protease. It is possible larger doses of these biopharmaceuticals will be required but administration will be initially limited by the yields from *in vitro* production.

A similar product, APN401 (Apeiron Biologics AG, also known as GSK2586881) was well tolerated in high doses but ineffective when administered to ARDS patients intravenously.[32] Importantly, there was no evidence of disease enhancement. The key to efficacy in moderating the progression of COVID-19 may be early administration through the respiratory route, with a product, which blocks viral entry and replication.

Potential risks and adverse effects

Because of the sequence similarity of shACE2 to the physiologically cleaved wild-type ACE2, an immediate adverse immune response to the protein is unlikely. Even if the few amino acid differences prove immunogenic, treatment would have been discontinued before an adverse immune response develops. It is very unlikely shACE2 will provoke a long-term autoimmune disorder.[33–35]

It is uncertain if there will be an adverse immunological response to SARS-CoV-2-shACE2 complexes. These complexes are likely to be engulfed by macrophages, which are well equipped to eliminate the virus compared to pulmonary epithelial cells, which undergo cytopathic destruction. It is unlikely these soluble complexes will be internalised through the alternate endosomal pathway previously described for SARS-CoV leading to worsening damage to the respiratory mucosa.[16] Other SARS-CoV-2-shACE2 complexes will be removed by the mucociliary ladder and swallowed, likely resulting in hydrolytic destruction of the virus in the stomach.

The risks of this experimental treatment must be considered in the context of the known morbidity and mortality of this infection for which there is no effective treatment. Given the rapid reduction of new COVID-19 cases in New Zealand, large-scale randomised clinical trials of these biopharmaceuticals will be conducted internationally. Preclinical safety studies could be undertaken in New Zealand. If successful, these products will be made available to New Zealand patients and HCWs on a compassionate basis once relevant ethics and regulatory approvals have been received. The clinical availability of such biopharmaceuticals will depend on how quickly each jurisdiction assesses and approves such novel products in this global crisis.

Competing interests:
Dr Rolleston reports affiliation with South Pacific Sera Ltd outside the submitted work; and is the Chair of the Life Sciences Network. Dr Petousis-Harris reports grants from GSK outside the submitted work.

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