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EDITORIAL

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Covid-19: From structure to therapeutic targeting in studying approved drugs and local DNA vaccination

The coronavirus disease 2019 (COVID-19) pandemic presents an unprecedented threat to global public health. The spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections on a global scale has affected more than 30.6 million people suffering the COVID-19, resulting in more than 955 000 deaths globally as of the 20 September 2020. The current lack of specific and effective therapies for the COVID-19, and the continuous spread of coronavirus SARS-CoV-2 across many parts of the world, represents one of the major challenges in controlling the disease severity and consequences, posing a huge threat to the global health. In this article, we highlight several previously approved drugs for potential effect on combating SARS-CoV-2 coronavirus infection, and modulating pulmonary inflammation and immune response.

Despite unprecedented efforts to contain the virus spread and prevent infection, SARS-CoV-2 pneumonitis can still rapidly strike to incapacitate the lung causing severe acute respiratory distress syndrome (ARDS), resulting in severe disease aftermath and sometimes death.[1] Similar to the SARS virus and dissimilar to the seasonal influenza virus, SARS-CoV-2 causes the high rates of mortality in COVID-19 patients without evident immunodeficiency, and sequelae of survivors, instigating panic in the general population.[2] Therefore, as currently highest challenges in medical cares, identifying existing treatments that can be quickly and effectively repurposed to reduce morbidity and mortality, in addition to creating immune vaccine, has led to an explosion of enormous interest in combating COVID-19.

To repurpose drugs for a faster, as well as far more economical, effect than starting development from scratch on COVID-19, recent studies by Xiao-Jing Zhang and colleagues demonstrated in a retrospective cohort study that statin use has a beneficial effect on COVID-19.[3,4] The authors have found a significantly reduced risk of mortality in COVID-19 patients having statin therapy, with the risk for 28-day all-cause mortality being 5.2% in the matched statin group and 9.4% in the matched non-statin group ($P = .001$)[3] While the mechanism of statin action on COVID-19 requires investigation, it has been noted that statins potentially withhold the host's immune system and attenuate a 'cytokine storm', which likely imposes the greatest risk of death.[4] Previous studies showed that statins suppress TLR4/MyD88/NF-kB signalling and modulate the NLRP3 inflammasome.[5] In addition, statins modulate pro-inflammatory cytokine release such as interleukin-6 and interleukin-8,[6-9] which are considered to be the potential drivers of the COVID-19 'cytokine storm' or cytokine release syndrome to cause ARDS.[4,10]

Recently, Ronald M. Evans and Scott M. Lippman[1] suggest that vitamin D deficiency or the failure to activate the vitamin D receptor (VDR) can aggravate ARDS by triggering a wounding response in the pulmonary stellate cells. The FDA-approved drug paricalcitol, an synthetic analogue of vitamin D, suppresses pro-inflammatory and pro-fibrotic events initiated by murine hepatic and pancreatic damages by binding to VDR.[1] As a member of the nuclear hormone receptor transcription factor family, VDR operates as an important anti-inflammatory anti-fibrotic checkpoint in hepatic stellate cells (HSCs) that expands about 100-fold in response to tissue injury with excessive cytokine and matrix-component release.[1] Genetic disruption of VDR, or vitamin D deficiency, triggers severe inflammation and fibrosis, by transcriptionally activating TGF-β signalling via unleashing genomic competition with Smad3 occupancy on pro-fibrotic and pro-inflammatory (eg, IL-6) genes; however, calcipotriol functions as a vitamin D3 agonist by binding to VDR with high affinity to attenuate the pro-inflammatory and pro-fibrotic pathogeneses.[1] Additional to the anti-inflammatory effect in hepatic injury, calcipotriol also suppresses pancreatitis and pancreatic cancer progression in mice.[1] Moreover, intravenous paricalcitol alleviates the acute lung injury induced by lipopolysaccharide (LPS) and preserves the pulmonary alveolar barrier function, attenuating neutrophil recruitment.[1] Thus, with a probable VDR anti-inflammatory program in COVID-19 patients, repurposing paricalcitol infusion therapy to restrain the COVID-19 cytokine storm could prove to bring a beneficial effect to patients in higher COVID-19 mortality rates and lower vitamin D levels.[1]

In this issue of CEPP, 1791–1797, Hend Ashour and colleagues[11] suggest that the clinically used drug nicorandil (N-[2-hydroxyethyl]-nicotinamide nitrate) for treating angina by increasing nitric oxide availability and opening ATP-sensitive potassium (KATP) channels may worth considering for oxidative stress-induced pulmonary cell death and dysfunction in COVID-19. Nicorandil has been shown to attenuate the acute injuries induced by LPS in human pulmonary artery endothelial cells and suppress monocyte-endothelial adhesion, which is a key step in inflammatory infiltration.[11] Pre-treatment with nicorandil (100 μg/kg·hour) has also been showen to prevent non-ventilated lung collapse and protect re-expansion in one-lung ventilation in rabbit model.[11] In addition, nicorandil effectively protects rat lung from ischaemic injury, with the drug decreasing the extent of pulmonary microvascular permeability in 60 minutes of reperfusion in association with reduced filtration coefficient and the wet-to-dry lung weight ratio.[11] Furthermore, nicorandil has been

shown to improve the levels of cyclophosphamide-induced lung fibrosis, silica-induced lung inflammation and fibrosis, and bleomycin-induced lung fibrosis in different animal models.[11] Notably, about one-third of the Middle East Respiratory Syndrome-related coronavirus (MERS-CoV) and the old SARS-CoV cases were associated with the radiological findings of lung fibrosis, and SARS-CoV-2 infection has a high tendency for pulmonary parenchymal and interstitial fibrosis.[11] Moreover, a bronchodilator effect of nicorandil has been recorded for 6 mg/h intravenous infusion in preventing thiamylal-fentanyl-induced bronchoconstriction in humans, consistent with the airway smooth muscle relaxation through NO and the KATP opening activity. Further mechanism of nicorandil action in oxidation/inflammation suppression appears to involve a reduction of the inflammatory signalling molecules such as NF-κB and TNF-α in the lung, and restoration of the oxidant/antioxidant balance through down regulating iNOS and upregulating GSH, SOD, and Nrf-2 in murine models of lung injury.[11] By contrast, nicorandil increases the levels of phosphatidylinositol-3-kinase (PI3K), hypoxia-inducible factor (HIF-1α), and SOD in the injured lungs. Most importantly, nicorandil's potential of anti-fibrotic effect has been studied in clinical patients of acute myocardial infarction subjected to coronary angioplasty, coagulation disorders with platelet dysfunction with unstable angina, and nephroprotective effects of renal disease (see Ashour et al[11]).

In the page 1874–1878[12] in this issue of CEPP, Dean Tatlow and associates discuss an alternative way to fight the SARS-CoV-2 viral infection by facilitating antigen presentation and mobilization of T- and B-lymphocytes of the immune system. It was thought to be possible that DNA vaccination can be achieved from an inhaled DNA plasmid construct containing a portion of the coronavirus spike protein. Translation of a particular antigenic protein portion may take a shortcut to process antigen in the endoplasmic reticulum and facilitate antigenic epitope presentation on the cell surface through MHC class I mechanism. Given that viral antigenic protein processing is subjected to complex regulation,[13] purposely designed potential antigenic peptides coded by inhaled DNA plasmids may provide a source of antigenic epitope for immunization at the right place of the lungs. In addition to producing potentially secreted spike protein fragments to competitively interfere with viral binding to the angiotensin-converting enzyme 2 (ACE2) receptor, a local administration of the SARS-CoV-2 vaccines may also limit potential systemic side effect while potentially igniting both humoral and cytotoxic immunity (for details, see Tatlow et al[12]).

With the ideas of shared molecular targets across different conditions of diseases, we note a potentially fundamental interaction among the most abundant polyphenol compound — quercetin, human ACE2, SARS-CoV-2 3CL protease, and SARS-CoV-2 spike glycoprotein. Since SARS-CoV-2 binds ACE2 to enter pulmonary epithelial cells, inhibition of the viral spike glycoprotein binding to ACE2 represents a vital strategy to prevent the infection. As a drug with free radical scavenging and antioxidative activity, quercetin may also potentially serves as a promising candidate in inhibiting SARS-CoV-2 virus from entering pulmonary epithelial cells. Recent studies have shown quercetin anti-inflammatory effect on the upper respiratory tract infection in three human clinical trials with low risk of bias, and oral quercetin has been shown to have a beneficial effect on the incidence and duration of respiratory tract infections in certain populations.[14] In addition, quercetin is an important ingredient of Qingfei Paidu decoction which shows a beneficial effect and has been recommended for the treatment of COVID-19 by the National Health Commission of China.[15] Moreover, quercetin is one of the five ingredients in the Gene-Eden-VIR/Novirin formula with efficacy in conquering SARS-CoV-2 infection.[16] Furthermore, consistent with a role of vitamin D deficiency in ARDS,[1] quercetin and vitamin D have been identified as putative COVID-19 mitigation agents by manifesting their effects on gene expression on ACE2 and FURIN.[17]

By supercomputer SUMMIT drug-docking screen and Gene Set Enrichment Analyses (GSEA), quercetin is identified as one of the top-scoring candidate therapeutics for COVID-19.[18] When performing molecular docking analysis, however, we found that quercetin binds to not only human ACE2, but also two SARS-CoV-2 components, SARS-CoV-2 3C-like (3CL) protease and the spike glycoprotein, with high affinities (Figure 1). These findings indicate the potentially multiple targeting sites of quercetin on pathogen virulence of SARS-CoV-2 at multiple steps including virus entry, virus replication, and protein assembly.

Together, these studies based on molecular targeting with potential uses of previously approved drugs offer potentially important insightful strategies for therapeutic consideration of the drugs for clinical trials in order to accelerate the development of effective drugs against SARS-CoV-2 coronavirus infection. These strategies of COVID-19 intervention include the mechanisms of modulating pulmonary inflammation and immune response, facilitating antigen processing and presentation to boost vaccine development, and targeting the interface between the virus SARS-CoV-2 protein structures, the viral host receptor, and drug molecule.

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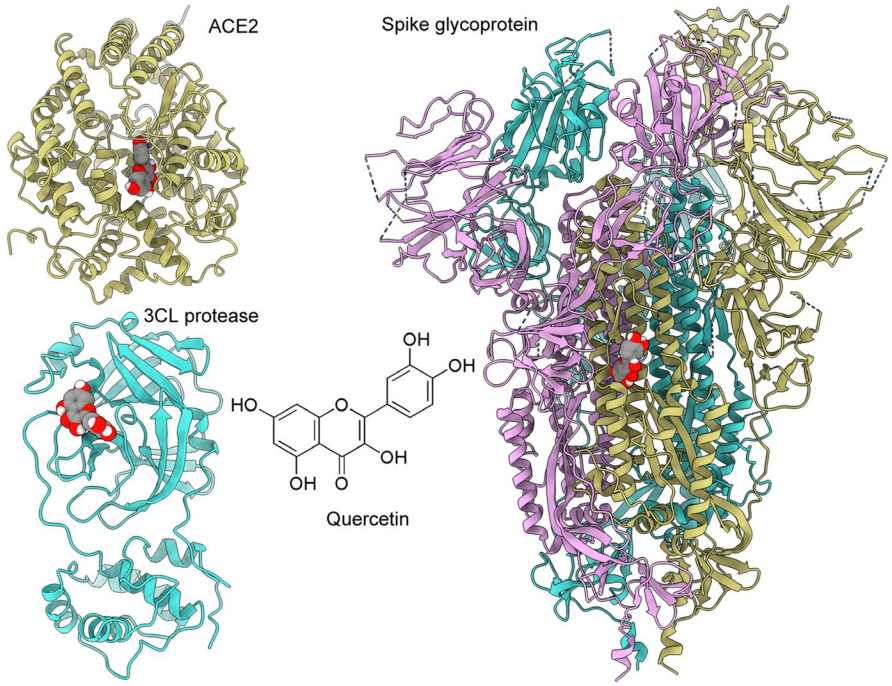

FIGURE 1 Structures of three quercetin complexes with two SARS-CoV-2 viral proteins, and the viral protein receptor ACE2. Quercetin is shown in a sphere model (the carbon, oxygen and hydrogen atoms are in grey, red, and white colours respectively). ACE2 (PDB 1R4L) and SARS-CoV-2 3CL protease (PDB 6M2N) are shown in khaki and cyan ribbons, respectively. The three chains (A, B, and C) of the SARS-CoV-2 spike glycoprotein (PDB 6VSB) are in khaki, cyan, and plum colours, respectively

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