Chapter

Assembling an Anti-COVID-19 Artillery in the Battle against the New Coronavirus

Chanda Siddoo-Atwal

Abstract

The panic and confusion surrounding the pandemic caused by the novel coronavirus requires a systematic study of the disease (COVID-19) and the arsenal of weapons available to the biochemist in the fight against infection. When developing a particularly bad flu in January 2020 while in India after the visit of a friend, who had just travelled back from Wuhan (China), it gave me an early opportunity to study the tricky diagnosis of this dreaded disease first-hand. The somewhat unusual symptoms and a lingering weakness and malaise for months suggested that it was no ordinary influenza virus. Since that time, a baffling number of disparate symptoms have been ascribed to COVID-19 infection including respiratory, gastrointestinal, circulatory, urinary tract and nerve dysfunction that have even resulted in multi-organ failure in some cases. Naturally, an array of risk factors have also been identified ranging from age, sex, obesity, diabetes, and hypertension to cigarette smoking that can increase mortality rate dramatically. In the intervening period, much research has appeared on biochemical compounds that may help to prevent this infection and, possibly, aid in patient recovery. Among these bioactive molecules are certain anti-inflammatory substances such as vitamin D, zinc, chloroquine, soy isoflavones like genistein, and glycyrrhizic acid, some of which may be successful in attacking different biochemical processes of the new coronavirus and disarming its deadly artillery against the human host. In a few instances, the viral processes that are inhibited by these chemicals are essential for the replication and reproduction of this RNA virus thereby striking a lethal blow to its machinery. Thus, taken together, these compounds may form a worthy arsenal against a formidable foe in the absence of an effective vaccine, and, especially, if relapse or re-infection proves to be a common occurrence in recovered COVID-19 patients.

Keywords: novel coronavirus, COVID-19, influenza, obesity, diabetes, hypertension, cigarette smoke, vitamin D, zinc, chloroquine/quinine, soy isoflavones, genistein, glycyrrhizic acid, RNA virus replication

1. Life cycle of the novel coronavirus

Coronaviruses are large, enveloped, single-stranded, positive-sense RNA viruses with a genome of approximately 30 kilobases in length. The genus *Coronavirus* belongs to the family *Coronaviridae* in the order *Nidovirales*. They are classified into three groups. Group 1 contains various mammalian viruses including porcine epidemic diarrhea virus, porcine transmissible gastroenteritis virus, and human coronaviruses 229E and NL63. Group 2 includes canine respiratory coronavirus

among other mammalian viruses and human coronavirus OC43. Human severe acute respiratory syndrome coronavirus (SARS-CoV-1) is considered a distant relative of this group. Group 3 contains solely avian coronaviruses. Human coronaviruses (HCoVs) cause respiratory infections, mainly, but gastroenteritis and neurological disorders may also occur. So far, at least seven human coronaviruses have been described including SARS-CoV-2, which was just sequenced in 2020, and two of these coronaviruses (OC43 and 229E) are responsible for 10–30% of all common colds. HCoV-HKU1 is mostly associated with bronchiolitis and pneumonia [1–3].

The gross life cycle of the SARS-CoV-1 has been observed in Vero E6 cells (African green monkey kidney cells) following inoculation with the virus under an electron microscope. The SARS-CoV-1 enters the cells through membrane fusion. Then, the nucleocapsids are assembled in the rough endoplasmic reticulum (RER) and mature by budding into the smoothe vesicles derived from the Golgi apparatus. Finally, the smoothe vesicles fuse with the cell membrane and the mature virus particles are released [4]. SARS-CoV-2 displays a similar life cycle.

Recent molecular studies have revealed that in order to facilitate entry of the virus into a human cell, the "S" spike surface glycoprotein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE-2) cellular receptor. Binding of the virus occurs via the S1 subunit of the S protein to a receptor and entry requires S protein priming by the cellular serine protease in order to allow fusing together of viral and cell membranes, a process which is initiated by the S2 subunit [5]. Following the fusion of viral and plasma membranes, the virus RNA undergoes transcription and replication inside the cell cytoplasm. Viral proteins are synthesized and the new RNA genomes are assembled and packaged in the endoplasmic reticulum, in the Golgi apparatus, and in the endoplasmic reticulum-Golgi intermediate compartment prior to virion release in vesicles. In fact, the S protein of SARS-CoV-2 binds to ACE-2 receptors with an approximately 10–20 fold higher affinity than that of SARS-CoV-1 and this added feature may aid in the efficient spread of SARS-CoV-2 among human populations. However, SARS-CoV-2 does not employ the other usual CoV receptors such as aminopeptidase N and dipeptidyl peptidase 4 to enter human cells [6].

ACE-2 is a membrane-associated aminopeptidase that converts angiotensin II to angiotensin 1–7 and plays a role in the cleavage of peptides [3]. Expression of ACE-2 in human tissues correlates with known sites of SARS-CoV-1 infection including lungs (particularly airway epithelia), heart, kidneys, small intestine, testes, and vascular endothelia [7]. These same tissues also overlap with the sites of SARS-CoV-2 infection in humans due to ACE-2 receptor availability.

2. A personal experience

On a personal note, as a biochemist, I have been following every bit of new research on any chemical compound that might successfully combat the virus. Around January 6th, 2020, I developed a very bad flu while in India after meeting with a friend who had just travelled to Wuhan in China. Overnight, I got a sore-throat that lasted a few days followed by a severe head cold with sinus congestion and mucous and, finally, it developed into a dry cough. During this debilitating flu, I also had some loose bowel movements with mucous. In the aftermath of the flu that lasted around 14 days, I was plagued with dizziness and weakness for two more weeks.

Although we had heard of the novel coronavirus in China, there was no reason to believe that was what I had just experienced since there had been no unusual respiratory distress. So, it did not seem to overlap with the pneumonia-like symptoms of the new coronavirus from China. Moreover, the friend had returned at the beginning of January and, as far as we knew at that time, the virus had only appeared in

December. Therefore, it seemed unlikely that the friend had been exposed to any infected individuals while in China. Furthermore, the traveller from China never became sick (although one other person who attended the same meeting as myself developed a very bad flu within two weeks of coming in contact with this person). At the same time, there are also many seasonal flus like swine flu (H1N1) that are endemic in India, so there was no reason to consider that it was a coronavirus infection. Finally, the only medicines I took initially were some herbal Ayurvedic cold remedies mainly with a licorice-root base (a potent anti-inflammatory), aspirin at night, and an electrolyte solution to prevent dehydration from diarrhea. When I had a relapse of the gastrointestinal symptoms in March including stomach pain after I returned to Canada, a course of azithromycin helped to resolve the symptoms.

However, it was only when the weakness and malaise persisted for 3–4 months after the initial illness and new data started to emerge about the differing patterns of COVID-19 infection, that I started to consider another possible cause. Firstly, all my symptoms were consistent with the disparate effects of the novel coronavirus including the lingering apathy. Secondly, it became apparent that the new coronavirus had appeared in Wuhan some time before December. Thirdly, unlike other flu viruses, the phenomenon of asymptomatic spreaders became widely known. So, now, even though I had not been tested for the new virus or COVID-19 antibodies, I started to suspect that I could have experienced a form of coronavirus infection.

Finally, I had my COVID-19 test in August 2020 and, although it was negative, it did not preclude the possibility that I had the disease in January 2020 and that my body had formed and shed antibodies to the novel coronavirus (antibody testing was also negative). Since it is not known exactly how long antibodies persist following infection, even these may not be detected after a certain recovery period (there are recent reports antibodies decline after three months). Studies in rhesus monkeys show that re-infection does not occur in the recovered macaques up to 28 days after initial infection [8]. Nevertheless, prolonged inflammation and reports of re-infection in recovered humans are a surprising aspect of this virus. In my case, one additional negative C-reactive protein (inflammatory marker) test decisively clinched the matter.

3. Top COVID-19 risk factors

3.1 Internal risk factors

Some scientists have opined that COVID-19 is highly contagious and highly lethal to a small subset of the population, while it produces milder symptoms in most people. Although the SARS-CoV-2 virus infects people of all ages, the World Health Organization (WHO) has determined that the evidence to date suggests that older adults and adults with underlying medical conditions are at a higher risk of developing severe COVID-19 disease [9].

One large study out of New York State seems to indicate that obesity, high blood pressure, and diabetes are strong risk factors for COVID-19 [10]. It has also been observed that cardiovascular disease and respiratory diseases could greatly affect the prognosis [11]. In fact, in an interesting German study involving autopsies on 12 COVID-19 patients the results revealed that coronary heart disease and asthma were common comorbid conditions in 50% of the deceased [12].

Other research suggests that cancer patients are more vulnerable to COVID-19 infection. A multicenter study showed that patients with cancer had higher risks in all severe outcomes of the disease tested. Hematologic cancer, lung cancer, or metastatic cancer (stage IV) cases experienced the highest frequency of severe events, while nonmetastatic cancer cases experienced similar frequencies to patients without

cancer. Moreover, cancer patients who received surgery had higher risks of severe events than patients without cancer or those who underwent radiotherapy [13].

In addition, a surprising gender disparity appears to be present in relation to SARS-CoV-2 infection. Statistics from Australia, Belgium, Germany, Italy, the Netherlands, South Korea, Spain, the U.K and the US reveal that mortality rates from the virus are significantly higher in infected males than in infected females. In New York, approximately 60% of COVID-19-related deaths occurred in men. This may partly reflect biological characteristics since women produce stronger immune responses than men and are physically better at warding off viral and other types of infections. Nevertheless, biochemical differences in sex hormones are also likely to play a role in determining this dichotomy [14] and certain researchers have suggested it may be due to the presence of ACE-2 receptors in the testicles [15].

In the largest Chinese study to date assessing severity of coronavirus infection in smokers, it was found that higher percentages of current and former smokers needed ICU support or mechanical ventilation. Higher percentages of smokers among the severe cases also died [16]. Therefore, ultimately, the risk of any one individual is determined by the number of risk factors they display. For example, a ninety year old male smoker with diabetes and hypertension displaying five risk factors (age, gender, smoke inhalation, high blood pressure, and diabetes) would have an extremely high risk of contracting a terminal case of COVID-19.

However, genetic risk factors as a result of ethnic origin can only be considered once all these other significant risk factors have been taken into consideration. So far, despite attempts by various institutions to prove an ethnic link to COVID-19 infection, there is no compelling evidence to suggest that any one human group is genetically more susceptible to the novel coronavirus than any other beyond mitigating factors such as socioeconomic status or environmental conditions [17]. In order to establish a true genetic component, rigorous genetic testing must be undertaken to identify predisposing genes in susceptible ethnic groups. Prior to gene isolation and identification of a specific genetic polymorphism, a biochemical reaction resulting in a higher percentage of the disease is often demonstrated in a particular human population. As an example, the human sunburn cycle in response to UVA/B radiation only occurs in a minority of people with fair skin; however, most people simply tan when they are exposed to sunlight. In fact, these represent two separate physiological processes (burning and tanning). The former condition, scientific sunburn as a result of the human sunburn cycle, is mostly due to a genetic polymorphism involving the expression of very low levels of melanin in human skin since it can be corrected by wearing a sunscreen containing black sesame melanin [50 mg/ml] in a zinc oxide cream base [7.5%] [18–20]. It is also correlated with a high risk for skin cancer. Nonetheless, there may be other genetic factors like differences in DNA repair enzyme activity which can contribute to this unusual trait in certain individuals, as well [21].

Simultaneously, a surprising recent genetic association study has revealed that a major genetic risk factor for severe COVID-19 in humans may actually be inherited from Neanderthals. Outside the continent of Africa (0.3%), modern humans have inherited significantly more genetic material from other hominid species including Neanderthals (approximately 2%) and Denisovans [22]. Europeans and South Asians appear to have the greatest complement of Vindija Neanderthal genes from Croatia and a gene cluster on chromosome 3 inherited from this species has been identified as a risk locus for respiratory failure after infection with SARS-CoV-2. Among certain South Asian populations, up to 50% can carry at least one copy of this risk haplotype and the highest carrier frequency occurs in Bangladesh where 63% of the population carries it. In the UK it has been reported that individuals of Bangladeshi origin have roughly a two times higher risk of dying from COVID-19 than people of other nationalities [23].

3.2 External risk factors

Interestingly, there are high levels of air pollution in the two regions of China and Northern Italy that were hardest hit by the virus suggesting that environmental conditions can have an impact on the infectiousness of the disease [24]. Italian researchers have recently proposed an association between higher mortality rates in Northern Italy and peaks of particulate matter concentrations in this region. The most polluted northern provinces of Italy were found to have more infection cases than the less polluted southern provinces and this correlated well with ambient particulate matter concentrations that often exceeded the legal limit in these areas. All data for this study was collected prior to the lockdown [25]. Surprisingly, further research by the same group demonstrated that SARS-CoV-2 RNA was present on outdoor airborne particulate matter that was collected from an industrial site in Bergamo, Italy. This evidence suggests that, under the right atmospheric conditions, SARS-CoV-2 could create clusters with particulate matter and enhance persistence of the virus in the atmosphere by facilitating its capacity for diffusion. However, the vitality and virulence of the coronavirus diffused via this method remain to be confirmed [26].

This could have been a significant factor in the spread of the coronavirus in highly polluted and populated cities like Mumbai, India. Social conditions such as crowding in slums have also been considered contributory to dispersal of the virus in developing countries like Brazil and India. Proximity to infected individuals increases the risk of person-to-person transmission since the SARS-CoV-2 virus is spread mainly by respiratory droplets, but can be aerosolized, too [3].

No matter how healthy an individual may be, the more exposure they have to a particular virus, the greater risk they have of contracting the disease. The greater the number of particles of the virus one is exposed to, the greater the chance that they will overwhelm the body and immune responses. This is the reason that young doctors and other frontline healthcare workers are getting serious cases of COVID-19 and dying at a higher frequency than the general population.

View of Downtown Mumbai – December 2019

View of Mumbai Harbour – December 2019

4. An array of symptoms and complications

In general, COVID-19 infection is associated with the increased production of pro-inflammatory cytokines, C-reactive protein, increased risk of pneumonia, sepsis, acute respiratory distress syndrome, and heart failure [24]. In fact, a cluster of unexplained pneumonia cases were first reported in Wuhan, China in late December 2019. A few days later, the cause of this pneumonia was identified as a new member of the coronavirus family. Since then, the virus has spread throughout China and precipitated a global pandemic [6].

Early reports from China suggested the most common symptoms of COVID-19 infection were fever (88%) and dry cough (67.7%). Rhinorrhea (4.9%) and gastrointestinal symptoms (diarrhea 4–14%) were less common. At the same time, a majority of patients (81%) had only mild symptoms (no pneumonia or mild pneumonia). Among patients with more pronounced symptoms, 14% experienced severe symptoms while 5% were critically ill with respiratory failure, septic shock, or multiorgan dysfunction or failure [3].

Although the novel coronavirus preferentially infects cells in the respiratory tract, autopsy results from Germany showed that it can be detected in multiple organs. The highest levels of the virus were detected in the lungs and the respiratory tract, while lower levels were usually present in the heart, liver, brain, kidneys, and spleen. This data suggests that SARS-CoV-2 may spread via the bloodstream and infect other organs. It also appears that COVID-19 may predispose patients to venous thromboembolism in several different ways including via endothelial dysfunction and promotion of a procoagulatory state by tissue factor pathway activation. High plasma levels of proinflammatory cytokines were observed in a small subset of patients with severe COVID-19 and, therefore, direct activation of the coagulation cascade by a cytokine storm is also plausible [12].

In one study, it was found that 22% of critically ill patients experienced myocardial injury from the infection [3]. In another study, the incidence of thrombotic

complications in ICU patients with COVID-19 infections was reported to be 31%. It was concluded that COVID-19 may predispose to both venous and arterial thromboembolism due to excessive inflammation, hypoxia, immobilization, and diffuse intravascular coagulation [27].

In addition, the COVID-19 pandemic is surprisingly associated with neurological symptoms and complications including anosmia, hypogeusia, seizures, and stroke. Although statistics are not widely available at this point, the clinical course of COVID-19 is most severe in elderly male patients with comorbidities such as hypertension, diabetes, heart disease, and obesity which are all risk factors for stroke. There appears to be hypercoagulability associated with COVID-19 as a result of a "sepsis-induced coagulopathy" that may be a predisposing factor due to the formation of blood clots in the body [28]. COVID-19 complications in the brain can include delirium, inflammation, and encephalitis. A new study from UCL suggests that serious problems can occur even in individuals with mild cases of the virus [29].

A temporary loss of smell (anosmia) can be a consistent indicator of COVID-19 infection. An interesting finding is that the virus seems to change the sense of smell in patients by infecting and affecting the function of non-neural cells that support olfactory neurons. However, the neurons themselves do not appear to be infected as they do not express ACE-2 receptors [30].

Diabetes is already known to be a risk factor for COVID-19 and diabetics are more likely to die from the disease. Now, mounting evidence suggests that not only does diabetes make patients more vulnerable to the novel coronavirus, but the virus may actually trigger diabetes in some. Preliminary tissue studies indicate that the virus may act by damaging insulin-producing cells in the pancreas of affected individuals [31].

Even though, initially, children were thought to be unaffected by the novel coronavirus, a cluster of children with hyperinflammatory shock and features similar to Kawasaki disease and toxic shock syndrome was first reported in England. This hyperinflammatory condition could lead to severe illness, multiorgan failure, and even death in extreme cases. New reports out of the UK and US suggest that symptoms in young children (mainly toddler to elementary school age) can include inflammation of the blood vessels and coronary arteries. Almost all these pediatric cases had positive SARS-CoV-2 test results. As a result, this illness has been termed COVID-19-associated multisystem inflammatory syndrome [32].

5. The scope of coronavirus vaccines

Historically, there is no doubt that vaccines have provided a tremendous tool against infection for a variety of microbes including those causing small pox, tetanus, typhoid, cholera, and polio. Vaccines are an effective way for a population to achieve herd immunity (the concept that a pandemic will end once 60–70% of people become immune to any particular virus or microorganism). However, more recently, there are instances in which the production of viral vaccines has not been so successful as in the case of human immunodeficiency virus (HIV) and human coronaviruses (HCoVs) possibly due to their complex genomes. Virologists and immunologists maintain that it takes up to ten years to prepare a really good vaccine that has been properly tested. In fact, some of these specialists are skeptical about the race to find the first vaccine for the novel coronavirus within one year and often strike a cautionary note.

There are a number of things to consider in connection with a SARS-CoV-2 vaccine. Firstly, even if a safe and effective vaccine is made against the novel

coronavirus, it may not be widely available in time to make a significant difference to the pandemic. Secondly, no successful vaccine against *any* coronavirus has been produced so far despite seventeen years of research. Moreover in March, the British Society for Immunology published an open letter stating that it is unknown whether this virus will induce long-term immunity in affected individuals as other related viruses do not [33]. Thirdly, certain vaccines can protect against a disease, but not against infection, so vaccinated individuals could potentially become asymptomatic carriers of SARS-CoV-2. Fourthly, some vaccines developed against SARS-CoV-1 (a close viral relative of SARS-CoV-2) actually exacerbated the disease in mice. Fifthly, although the easiest way to make a vaccine is to inactivate the pathogen, there are new vaccines in current trials based on RNA from coronaviruses or other RNA viruses that have never before been approved or tested in humans. Therefore, there could conceivably be unintended or irreversible consequences. Finally, at least one of the novel coronavirus vaccines approved for clinical trials so far has caused severe adverse events in three of eight healthy, young individuals that were tested [34] and other trials have been suspended. Unfortunately, the contamination of vaccines which are mass produced for a burgeoning human population also seems to be a potential problem and an ideal tool for rival countries to conduct biological warfare upon each other. Oral or nasal vaccines may be safer in this respect [35]. In addition, there is a physical limit to the number of vaccines a person can safely receive as new and deadlier viruses arise in the environment.

6. Biochemical effects of special supplements

6.1 Vitamin D

The action of UVB radiation striking and reacting thermally with 7-dehydrocholesterol in human skin results in the production of Vitamin D3 in the human body. This form of Vitamin D is converted to the hormonal metabolite, calcitriol, in a set of biochemical reactions in the liver, kidneys, and other organs as required. Then, calcitriol binds with the nuclear vitamin D receptor, which is a DNA binding protein, that interacts directly with regulatory sequences near target genes and affects their transcriptional output.

Vitamin D also enhances cellular innate immunity partly through the induction of antimicrobial peptides, including human cathelicidin, and, defensins. Cathelicidins exhibit direct antimicrobial activities against a spectrum of microbes including many types of bacteria, enveloped and nonenveloped viruses, and fungi. The main action of these host-derived peptides is to kill the invading pathogens by perturbing their cell membranes. Moreover, vitamin D is effective in reducing concentrations of pro-inflammatory cytokines that produce the inflammation that injures the lining of the lungs leading to pneumonia during viral infections like COVID-19 and increasing concentrations of anti-inflammatory cytokines [24].

According to a recent clinical study with a large sample size taken from different countries around the world, vitamin D supplements were found to protect against respiratory tract infections including colds and influenza. The most benefit was observed in patients who were very vitamin D deficient. This protective effect is likely provided by the capacity of vitamin D to boost levels of antimicrobial peptides in the lungs [36].

Vitamin D deficiency is a world-wide problem, but is particularly pronounced in the elderly, who are at greatest risk of contracting severe COVID-19 infection. The release of pro-inflammatory cytokines is one of the major causative factors in serious COVID-19 infections. However, vitamin D modulates their presence in the

body by preventing macrophages from releasing too many inflammatory cytokines and chemokines. Calcitriol has also been found to exert an influence on ACE-2 receptors. Thus, it is not surprising that vitamin D deficiency has been correlated with COVID-19 cases and an increased risk of mortality in a European study [37].

6.2 Zinc

RNA synthesis occurs in the life cycle of the SARS-CoV-1 virus in order to reproduce its genetic material and is catalyzed by an RNA-dependent RNA polymerase, which is the core enzyme of a multiprotein replication/transcription complex. In the case of SARS-CoV-1, an excess of intracellular zinc ions has been found to efficiently inhibit the RNA-synthesizing activity of this replication and transcription multiprotein. Enzymatic studies *in vitro* have revealed that zinc directly blocks the activity of the RNA polymerase by inhibiting elongation and reducing template binding. This RNA polymerase core, which is a central component of the coronaviral replication/transcription machinery, is well conserved among the members of the coronavirus family including SARS-CoV-2 [38, 39]. Therefore, it is quite possible that zinc treatment would have a similar biochemical effect on SARS-CoV-2 and interfere with its ability to replicate.

Since current research indicates that the mineral, zinc, can inhibit the replication of coronavirus and a variety of other RNA viruses in cell culture, it has become a potentially important and interesting supplement to study at this time. In the human body, zinc performs a variety of vital antioxidant functions and is required for maintaining good health. Inside the cell, the harmful effects of free radicals are balanced by the action of antioxidant enzymes (such as copper-zinc superoxide dismutase) and non-enzymatic antioxidants (such as metallothioneins). As zinc cannot pass easily through membranes, zinc-transporting proteins, ZIPs (Zrt-Irt-like protein or Zinc Iron permease) and ZnTs (Zinc transporters) help to facilitate this process. Metallothionein also aids in the regulation of zinc levels and the distribution of this metal in the extracellular space. The presence of zinc within the cell causes an increase in metallothionein, which is the major zinc-binding protein, and together they form a thermodynamically stable complex [40, 41]. Thus, low risk ways of increasing zinc bioavailability in the body can be safely considered.

In rats, rice fortified with zinc oxide or zinc carbonate is a feasible vehicle for zinc absorption, although zinc oxide displays lower bioavailability than zinc carbonate [42]. In young adults, zinc absorption from supplemental zinc citrate is comparable with that from zinc gluconate, but higher than from zinc oxide [43]. It is already known that zinc can be absorbed from topical (non-nano) zinc oxide by human skin in small quantities (nano forms of zinc oxide are not associated with significant zinc absorption) [44]. One of our recent studies suggests that zinc is absorbed by the human body from our suncare products (all with the same basic formula containing a medicinal form of zinc oxide) in sufficiently large quantities with regular use [45].

So, recently, when our company received an inquiry from Health Canada regarding any innovations that may benefit Canadian health workers at this critical time during the novel coronavirus pandemic, the answer was that we do have a product that may be useful to medical professionals and health workers in the field. It is a natural, award-winning suncare product specially formulated to block apoptotic sunburn (Skin Protector Plus). Its active ingredient is a non-nano, medicinal form of zinc oxide. The novel thing about this product is that it appears to be an efficient delivery system for boosting zinc levels in the whole body in a relatively short period of time. There is no toxicity associated with this product due to the use of high grade zinc oxide and natural ingredients. Since it is so safe and contains no harsh chemicals (already tested on human volunteers), no pre-clinical trials would be required to test

its efficacy in protecting subjects from COVID-19 in a clinical study. The objective of such a comprehensive study would be to test and confirm the hypothesis outlined above, *in vivo*; namely, if maximum zinc levels are maintained in the human body via percutaneous zinc absorption from a topically applied zinc oxide cream, then it may provide one suitable defense against SARS-CoV-2 infection. Although oral supplementation is also an option, this type of topical application on the surface of the skin may be a faster method of ensuring even zinc distribution throughout the body and delivery to the various potential points of viral entry. Moreover, it may actually provide a physical barrier or blockade against entrance of the virus into the body by allowing suffusion and accumulation of zinc pools directly beneath the skin.

6.3 Chloroquine/quinine

Quinine, an alkaloid derived from the bark of the cinchona tree, is most commonly found in South America, Central America, the islands of the Caribbean, and parts of the western coast of Africa. It is an important antimalarial drug and a synthetic form with a similar mode of action is known as chloroquine [46]. Chloroquine has been reported to inhibit the SARS-CoV-1 virus in infected cell cultures *in vitro* at doses equivalent to those used in the treatment of acute malaria in humans. Its antiviral effect appears to depend on the fact that chloroquine is a weak base that increases the pH of acidic vesicles when added extracellularly. The nonprotonated portion of chloroquine enters the cell where it becomes protonated and concentrated in acidic, low-pH organelles such as endosomes, Golgi vesicles, and lysosomes. The subsequent antiviral activity of the chloroquine depends partly on the extent to which a particular virus utilizes endosomes for entry into the cell [47]. In addition, this drug appears to interfere with terminal glycosylation of the angiotensin-converting enzyme 2 (ACE-2) cellular receptor, which is engaged by the virus for extracellular binding. This step may have a negative effect on the ability of the virus to gain entry into the host cell and, therefore, to initiate its replication cycle. Thus, infection may be deterred at clinically admissible concentrations [48]. Chloroquine also displays an immunomodulatory activity by suppressing the production and release of tumour necrosis factor alpha and interleukin 6 [49].

Furthermore, chloroquine was demonstrated to have strong antiviral activity against HCoV-OC43 *in vitro*. The anticoronaviral properties of chloroquine were also tested against HCoV-OC43 infection in newborn mice *in vivo.* Treatment with daily doses of chloroquine were found to have a long-lasting protective effect against lethal coronavirus OC43 infection in the newborn mice [1].

These favourable results suggest that chloroquine may be considered for use at antimalarial doses in the prevention of infections caused by coronaviruses, particularly SARS-CoV-2, which utilizes ACE-2 receptors in order to gain entry into host cells like its close relative, SARS-CoV-1.

6.4 Glycyrrhizic acid

Licorice root has been a commonly used ingredient in both Ayurvedic and traditional Chinese medicine for centuries, particularly in cough and cold remedies. Twenty triterpenoids and nearly three hundred flavonoids have been isolated from this herb. Scientific studies have shown that these metabolites possess many pharmacological activities including antiviral, antimicrobial, anti-inflammatory, and anti-tumour properties. However, glycyrrhizic acid or glycyrrhizin (GL), 18β-glycyrrhetinic acid (GA), liquiritigenin (LTG), licochalcone A (LCA), licochalcone E (LCE) and glabridin (GLD) are the main active components which possess antiviral and antimicrobial activities [50].

It has been known for some time that glycyrrhizic acid extracted from licorice (*Glycyrrhiza glabra)* root is active against viruses. This chemical is able to disrupt the growth and cytopathology of several unrelated DNA and RNA viruses without harming the host cell or its ability to replicate. Glycyrrhizic acid has also been demonstrated to inactivate herpes simplex virus particles irreversibly [51].

In a more recent study, the anti-SARSCoV activity of 15 glycyrrhizic acid derivatives was tested. Glycyrrhizin was shown to inhibit SARS-CoV-1 replication *in vitro* [52]. GL has also been reported to act by inhibiting viral gene expression and replication, reducing adhesion force and stress, and reducing High mobility group box 1 protein (HMGB1) binding to DNA. In addition, GL can enhance host cell activity by blocking the degradation of IκB, activating T lymphocyte proliferation and/or suppressing host cell apoptosis [50]. Thus, the potential for this licorice root component (GL) against SARS-CoV-2 infection is plausible.

6.5 Genistein & soy isoflavones

Isoflavones and their related flavonoid compounds, particularly genistein, exert antiviral properties against a wide range of DNA and RNA viruses *in vitro* and *in vivo* [53]. The biological properties of the flavonoids are well studied, but the mechanisms of action underlying their antiviral properties are not fully understood. Isoflavones appear to have a combination of negative effects on viruses including affecting virus binding, entry, replication, viral protein translation and formation of certain viral envelope glycoprotein complexes. A variety of host cell signalling processes can also be affected by isoflavones including induction of gene transcription factors and secretion of cytokines. All these effects are dependent on dose, frequency of administration, and different combinations of isoflavones employed in bioassays *in vitro*. Genistein may be able to mimic the action of 17-beta-estradiol [E2] due to its similar structure or to act as an E2 antagonist and its activity as a broad-spectrum tyrosine kinase inhibitor may contribute to its ability to influence estrogen receptor-independent mechanisms [54]. Despite their unique effect on immune function and anti-inflammatory activity, there is still a lack of data confirming the antiviral efficacy of such soy isoflavones *in vivo* against coronaviruses and other viruses thereby forming a worthwhile subject for biochemical study.

7. Summary

At least seven human coronaviruses have been described to date including SARS-CoV-2, which is closely related to and resembles SARS-CoV-1 in many respects. Both viruses bind to ACE-2 receptors on human cells. ACE2 is a membrane-associated aminopeptidase that converts angiotensin II to angiotensin 1–7 and plays a general role in the cleavage of peptides. Expression of ACE2 in human tissues correlates with known sites of SARS-CoV-1 infection including lungs (particularly airway epithelia), heart, kidneys, small intestine, testes, and vascular endothelia. These same tissues overlap with known sites of SARS-CoV-2 infection in humans.

A cluster of unexplained pneumonia cases were first reported in Wuhan, China and, a few days later, the cause of this pneumonia was identified as a new member of the coronavirus family. SARS-CoV-2 infection appears to be associated with a puzzling array of symptoms and complications. The major symptoms noted in China were fever (88%) and dry cough (67.7%), while rhinorrhea (4.9%) and gastrointestinal symptoms (diarrhea 4–14%) were less common. A majority of patients

(81%) had only mild symptoms (no pneumonia or mild pneumonia). Among patients with more pronounced symptoms, 14% experienced severe symptoms while 5% were critically ill with respiratory failure, septic shock, or multiorgan dysfunction or failure.

New data suggests that SARS-CoV-2 may spread via the bloodstream to infect other organs. In addition to the lungs, other target organs can include the heart, liver, brain, kidneys, and spleen. It also appears that COVID-19 may predispose patients to venous thromboembolism in several different ways including via endothelial dysfunction and promotion of a procoagulatory state. In fact, it was found that a significant percent of critically ill patients experienced myocardial injury from the infection and it has been concluded that COVID-19 may predispose to both venous and arterial thromboembolism due to excessive inflammation, hypoxia, immobilization, and diffuse intravascular coagulation. The COVID-19 pandemic is associated with various neurological symptoms and complications including anosmia, hypogeusia, seizures, and stroke, as well. COVID-19 complications in the brain can include delirium, inflammation, and encephalitis. Despite initial reports that children were unaffected by the novel coronavirus, it has emerged that pediatric patients are susceptible to a COVID-19-associated multisystem inflammatory syndrome that can cause serious inflammation of the blood vessels.

Several internal risk factors have been identified for SARS-CoV-2 infection. The main ones include age (older adults are more vulnerable to serious infection by the virus), gender (the virus is significantly more deadly in men than in women), obesity, heart disease, diabetes, cancer status, and smoking. However, there is no convincing evidence to date that any particular ethnic group displays a stronger genetic susceptibility to the virus (although, there may be a possible link to an inherited Neanderthal gene locus). Nevertheless, specific genetic variants such as those for the gene that encodes a protein that interacts with the ACE-2 receptor may be involved in determining individual patient responses to the disease. Simultaneously, external risk factors like environmental pollution, social conditions such as crowding, and frequency of exposure to infected persons also seem to play an important role.

Reports of re-infection in recovered humans is a surprising aspect of this virus. Recently, a team from the University of Hong Kong reported the first case of re-infection of COVID-19 within a period of approximately four and a half months. Genomic analyses confirmed that the patient had re-infection instead of persistent viral shedding from first infection. Moreover, there was a difference of 24 nucleotides between both viruses that infected the patient suggesting two different viral strains were involved [55].

Even though the virus is associated with positive COVID-19/COVID-19 antibody and high C-reactive protein test results, antibody levels may decline soon after infection. Consequently, it is quite possible that a lasting resistance to the virus will not be achievable. In the event that long-term immunity cannot be induced to the novel coronavirus by a vaccine, an annual, bi-annual, or even tri-annual inoculation may be required (current data suggests that antibodies begin to decrease or disappear three months after infection). This means that other modes of protection and prevention like supplementation may be more relevant in this case. Some candidates include Vitamin D, zinc, chloroquine/quinine, glycyrrhizic acid, and genistein due to anti-viral properties such as the ability to inhibit replication and reproduction of coronaviruses.

Scientists have concluded that drastic social distancing, quick detection and isolation of infected individuals and travel restrictions were the most effective steps for containment of COVID-19 in China. Genome sequencing has also helped to track and control COVID-19 infections quickly. However, if people do not

continue to be careful, certain places may become vulnerable to further rounds of this disease. WHO recently reported that coronavirus infections among younger populations were skyrocketing. The proportion of cases in teens and young adults increased six-fold, while the proportion in young children and babies increased seven-fold by August. This may be attributable in part to the resurgence of large parties and social gatherings attended by young people following the relaxation of restrictions during the summer. Therefore, it seems very likely that the denouement of the COVID-19 story will be largely dictated by our social habits and ability to adapt to a new set of societal norms and conditions. This will include wearing face masks in public places, possibly, with a thin zinc coating along with a special zinc oxide crème formulation applied to the skin underneath [56].

Author details

Chanda Siddoo-Atwal[1,2,3]

1 University College London, London, United Kingdom

2 Simon Fraser University, Burnaby, Canada

3 President and Primary Biochemist of Moondust Cosmetics Ltd, West Vancouver, Canada

*Address all correspondence to: moondustcosmetics@gmail.com

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