

Controlling COVID-19 in Humans

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Abstract

The surprising pandemic of the respiratory syndrome disease COVID-19 caused by the new coronavirus SARS-CoV-2 has forced scientists, clinicians and/or health professionals to rapidly dedicate themselves to study with celerity and depth not only on the causes of the disease and pandemic but also how to address antiviral strategies, applying the best knowledge available to avoid contagion, decrease transmission, identify the subsequent phases of the infection, and avoid the most serious pathological consequences of this disease: respiratory and cardiovascular distress, multiorgan failure and death. On these bases, in the present work we wanted to review and to discuss on the CoV-host interactions, commenting possible ways to control COVID-19 in humans from the perspective of an integrated use of crossing strategies: sanitary control, direct antiviral treatment, use of antioxidants, anti-inflammatories and other agents, with micronutritional support of infected patients, as a challenging contribution to handling and control COVID-19 infections.

Keywords: SARS-CoV-2; COVID-19; Virus-Host Interactions; Antiviral Treatments; Disease Control Strategies

Abbreviations

ACE: Angiotensin Converting Enzyme; ACE2: Angiotensin Converting Enzyme 2; ARDS: Acute Respiratory Distress Syndrome; CAT: Catalase; COPD: Chronic Obstructive Pulmonary Disease; CD4⁺T: Cluster of Differentiation 4 T-Helper Lymphocytes; CoVs: Coronavirus; COX-2: Cyclooxygenase-2; FDA: Food Drug Administration; G6PD: Glucose 6 Phosphate Dehydrogenase; GPx: Glutathione Peroxidase-1; GSH: Glutathione; GST: Glutathione S-Transferase; HO-1: Heme Oxygenase; iNOS: Inducible Nitric Oxide Synthase; IFN- γ : Interferon Gamma; IL-1 β : Interleukin-1 Beta; IL-4: Interleukin-4; IL-5: Interleukin-5; IL-10: Interleukin-10; IL-12: Interleukin-12; IL-6: Interleukin-6; IL-8: Interleukin-8; LPS: Lipopolysaccharide; MCP-1: Monocyte Chemoattractant Protein-1; MERS: Middle East Respiratory Syndrome; MMP-9: Matrix Metalloprotease-9; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; NF- κ B: Nuclear Factor Kappa B; NQO-1: NADPH Quinone Oxidoreductase-1; Nrf2/ARE: Nuclear Factor Erythroid 2-Related Factor 2/Antioxidant Responsive Element; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; nsps: Nonstructural Proteins; RBD: Receptor-Binding Domain; RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species; SARS: Severe Acute Respiratory Syndrome; SFN: Sulforaphane; SOD: Superoxide Dismutase; STAT3: Signal Transducer and Activator of Transcription 3; TCM: Traditional Chinese Medicine; TLR4: Toll-Like Receptor 4; TMPRSS2: Transmembrane Protease Serine 2; TNF- α : Tumor Necrosis Factor Alpha; TXNRD: Thioredoxin Reductase; UDPGT: Uridine diphosphate Glucuronosyltransferase; uPA: Cytokine-Regulated Urokinase-Type-Plasminogen-Activator; VEGF: Vascular Endothelial Growth Factor.

Introduction

Coronaviruses (CoVs) are membrane-enveloped, positive-stranded RNA viruses with nucleocapsid, and a crown-like appearance under an electron microscope due to the presence of spike (S) glycoproteins anchored to the envelope. CoVs have been the major pathogens responsible for viral infection diseases in humans this century. The most recent outbreak of the viral respiratory disease COVID-19 is attributed to the novel SARS-CoV-2 [1]; it belongs to the same β -genus coronaviruses family as SARS-CoVs and share genomic similarity with this virus [2]. COVID-19 disease has appeared less deadly but more transmissible and spread than the predecessors SARS-CoV and MERS-CoV diseases [3]. Initial observations pointed to the respiratory tract as a major site of CoVs disease morbidity, the main symptoms of COVID-19 infections being: fever (87.9%), dry cough (67.7%), fatigue (38.1%), sputum production (33.4%), shortness of breath (18.6%), sore throat (13.9%), headache (13.6%), myalgia or arthralgia (14.8%), chills (11.4%), nausea or vomiting (5.0%), nasal congestion (4.8%), diarrhea (3.7%), hemoptysis (0.9%), and conjunctival congestion (0.8%) [4]. Many of the symptoms caused by SARS-CoV-2 are similar to those caused by the previous SARS-CoV [2], although SARS-CoV-2 lead to a more severe acute disease particularly in aged patients with chronic diseases [1]. The clinical spectrum of COVID-19 varies from asymptomatic or paucisymptomatic forms to clinical conditions characterized by respiratory lung complications combined with severe inflammation, edema, vascular congestion and multiorgan failure [5]. COVID-19 became a pandemic difficult to control, and the purpose of the present study was to briefly analyze and discuss the biology of SARS-CoV-2-host-interactions in their different steps of the infection in human hosts aiming to get insights that may help us to improve control strategies against COVID-19 infections and disease.

Discussion

COVID-19 epidemiology

COVID-19 infections are very contagious from human-to-human and quickly spread out [1]. Epidemiological data indicate that mortality (%) doubles for each group of age being higher in the older age groups whereas it is very low in infants and young people below 15 years of age. The fatal cases were primarily elderly patients, in particular those aged > 80 years (about 15%) and 70 to 79 years (8.0%). About half (49.0%) of the critical patients and those affected by preexisting comorbidities such as cardiovascular disease, diabetes, chronic respiratory disease and oncological diseases, died; overall case-fatality rate is about 3.4% [4]. Children seem to be infected at significantly lower rates than adults, and those who have been infected experience milder symptoms [6]. Nonetheless, the population below 15 years of age has to be under vigilance since it is considered that they might be potentially asymptomatic transmitters. It is not yet known why the response to COVID-19 infection is different between adults and children, and perhaps it might be linked to factors related to their younger immune systems, different levels of receptors for the virus, etc. [7,8]. It is important to be vigilant also for relapses, and the type of patients in which regression occurs. Many different sanitary recommendations have been world-wide given to populations to avoid contagion and prevent transmission; it is agreed that the best weapon to fight this virus is to reduce the spread of the infection [9].

Immunological peculiarities in COVID-19 infections

Viral respiratory infections are characterized by aberrant production of cytokines, among which TNF- α , IL-1 β , IL-12, IFN- γ , IL-6, IL-8, chemokines, VEGF, COX-2, iNOS, MMP-9, uPA, etc. being secreted by pulmonary alveolar macrophages and other cells as defense mechanisms [10]. In COVID-19 infections there is an excess production of many different types of pro-inflammatory cytokines, causing severe damage to tissues, particularly in lung tissue [11]. COVID-19 infections may also affect primarily T lymphocytes, particularly CD4⁺T cells, resulting in their significant decrease in number as well as in IFN- γ production, which may be associated with disease severity [12]. Together with the clinical characteristics, early immunologic indicators such as diminished T lymphocytes and elevated cytokines may serve as potential markers for prognosis in COVID-19.

Treatment

Till now, there is no specific antiviral treatment officially recommended for COVID-19, and no vaccine is currently available. Therapeutic strategies for most milder cases are only supportive. For most other cases the treatment is symptomatic; oxygen therapy represents major intervention for patients with severe infection [1]. In severe pneumonia imaging abnormalities are seen with rapid evolution from focal unilateral to diffuse bilateral ground-glass opacities in the lung [13]; also, pulmonary thromboembolism, a known common cause of mortality in viral pneumonia patients may occur [14]. To maintain the respiratory function in these patients, it is necessary to: (i) rapidly implement oxygenation; (ii) lower inflammation with strong anti-inflammatory agents; (iii) rapidly resolve thromboembolism with anti-coagulants. Many laboratories in the world are trying to develop anti-SARS-CoV-2 vaccines on the basis of the available genomic and immunological information for this coronavirus. On the other hand, previously approved pharmacological drugs for other diseases are being screened and tested to see if they can interfere COVID-19 infections. By using this strategy, two drugs have been found so far to be promising, the antiviral remdesivir (Gilead Sciences) and the anti-malaria chloroquine [15]. Meanwhile, hundreds of thousands of new COVID-19-infected patients appearing every day in many countries use first-hand over-the-counter products and traditional natural treatments as urgent and immediately available options trying to stop or sooth the consequences of a progressing illness. Usually, people are traditionally accustomed to treat common viral infection symptoms for colds and coughs with NSAIDS, herbal infusions and syrups, balms, etc., treating fatigue with vitamin supplements. Common over-the-counter products for fever, sore throat, and cough treatments usually contain pain relievers (analgesic), anesthetic and antibacterial agents. People in many different countries usually appreciate the soothing properties of honey and infusions with honey, lemon (*Citrus*) and/or ginger to relief initial symptoms. Honey, has been reported to be very effective on sore throats in children; evidence-based research has found that honey acts through modulation of multiple molecular signaling pathways and targets in infections exhibiting significant antiviral, antibacterial, antioxidant, and anti-inflammatory properties [16].

Rationale for different treatments

Lowering viral load

Viral load varies among patients. The best way to suppress viral loads is to treat patients with antivirals having direct activity on the virus to rapidly counteract RNA replication and multiplication as well as functional viral proteins involved in host invasion. SARS-CoV targets include ORFs (such as ORF7a, etc.), nonstructural proteins (nsps) such as Nsp1, Nsp2, Nsp3 (Nsp3b, Nsp3c, PLpro, and Nsp3e), Nsp5 (proteinase 3CLpro), Nsp7_Nsp8 complex, Nsp9-Nsp10, Nsp11, Nsp13 (helicase), Nsp14-Nsp16, structural protein such as E (envelope membrane protein channel), M (membrane protein), N (nucleocapsid protein), S (the spike glycoprotein), RdRp (RNA-dependent RNA polymerase) and several other encoded components. In addition, at least two host enzymes are considered key targets for virus cell infection: the angiotensin converting enzyme 2 [ACE2] (a SARS-CoV-2 host receptor for the virus spike (S) glycoprotein) and the transmembrane protease serine 2 [TMPRSS2] (performing a prefusion proteolytic action on the spike (S) glycoprotein) [17,18].

Virus attachment to host cells

Similar to other SARS-CoV (S) viruses, SARS-CoV-2 attach to human host cells when its envelope-anchored spike (S) glycoprotein binds to the host membrane-bound ACE2 cell receptor; upon binding, the spike (S) glycoprotein changes conformation while primed by the host TMPRSS2 protease and the viral and host membranes fuse. A defined receptor-binding domain (RBD) of the spike (S) glycoprotein specifically recognizes ACE2 [17].

The ACE2 host cell receptor

The angiotensin converting enzymes (ACEs) are key catalytic components of the renin-angiotensin system (RAS), mediating precise regulation of blood pressure. The angiotensin-I-converting enzyme (ACE) converts the hormone angiotensin I to the active vasoconstrictor

tor angiotensin II. On the other hand, the homologous carboxymonopeptidase ACE2 catalyzes the conversion of angiotensin II (which promotes oxidative stress, inflammation, endothelial dysfunction, thrombosis) to angiotensin 1 - 7 (opposing the effects of Ang II) [19]. ACE2 is a negative regulator of the renin-angiotensin system (RAS), and it is involved in multiple functions such as regulating myocardium contractility, it has a protective role in cardiovascular diseases, diabetes, it is active on vasoactive peptides, controls intestinal inflammation and diarrhea, and regulates the gut microbiome [20-22]. Maintaining the ACE2/ACE balance is important to inhibit cell lung apoptosis, as seen in acute pulmonary thromboembolism (apoptosis might be responsible for severe tissue damage in lung respiratory dysfunctions) [23]. Indeed, the ACE2/ACE ratio is positively correlated with the anti-apoptotic Bcl2 protein levels and the Bcl2/Bax ratio; ACE2 is hence a central and fundamental regulator placed at the crossroad of numerous physiological functions [21]. We shouldn't be surprised to know why the loss or downregulation of ACE2 may be also so detrimental in the progression of non-respiratory cardiac, vascular and renal pathologies caused by COVID-19 infections; and we can understand better how co-morbidities, such as hypertension, diabetes, cardiovascular disease, age (> 70), etc. make it worse SARS-CoV-2 infections [22].

The ACE2 host cell receptor and host susceptibility to infection by SARS-CoVs

ACE2 expression determines host susceptibility to infections by SARS-coronaviruses; ACE2 is not equally expressed in all hosts and in all tissues. Undifferentiated airway epithelial host cells expressing little ACE2 were poorly infected with SARS-CoV, while well-differentiated cells (expressing more ACE2 receptors) were readily infected [24]. Kuba, *et al.* [25] tested SARS-CoV infections in ACE2 knockout mice and control wild-type mice; this resulted in viral replication in the lungs and the recovery of large amounts of virus in wild-type mice whereas in the ACE2 knockout mice only a very low quantity of infectious SARS-CoV virus particles could be recovered, as well as greatly reduced copy numbers of SARS-CoV Spike RNA; pathologic alterations in lungs were also reduced in ACE2 knockout mice as compared to wild-type mice [25]. The injection of SARS-CoV spike (S) glycoprotein into SARS-CoV infected mice worsened acute lung failure that could be attenuated by blocking the RAS pathway with losartan (ACE2 receptor blocker), thus providing a molecular explanation why infections by SARS-CoV cause severe, often lethal, respiratory and other organ complications [25]. The susceptibility to infection by SARS-CoVs are also initially determined by the affinity between the spine (S) receptor binding domain (RBD) and the host ACE2. The SARS-CoV-2 (S) spike glycoprotein bound the ACE2 receptor with an affinity of ~ 15 nM, approximately 10 to 20 times greater than the binding affinity of SARS-CoV (S) glycoprotein to ACE2; the higher binding affinity of SARS-CoV-2 (S) glycoprotein to ACE2 may mean more severe pulmonary pathology in COVID-19 infections [1].

Interfering virus-ACE2 host cell attachment

According to the previous information, interfering the SARS-CoV (S) glycoprotein/ACE2 host interaction might represent a valid strategy to control SARS-CoVs infections, including SARS-CoV-2. However, as we have previously commented, ACE2 plays a central role in RAS and various other physiological systems, and if not well tuned, this inhibition might lead to serious secondary health effects. But we know that ACE2 is also a target for some anti-hypertensive and cardiac drugs [26] and it would be important to revise their molecular mechanism of action to find out how possible would be to inhibit ACE2 safely in a COVID-19 infection. Hoffmann, *et al.* [17] isolated SARS-CoV-2 from a patient and tested its entry into lung epithelial cells *in vitro* in the presence of camostat mesilate (a trypsin-like protease inhibitor); the drug blocked the entry of the virus into the lung epithelial cells [17,27]. These authors found also that serum from convalescent SARS-CoV patients cross-neutralized SARS-CoV-2 (S)-driven cell entry, suggesting that antibodies produced in another SARS-CoV infection against the SARS-CoV (S) spike glycoprotein might cross-protected against SARS-CoV-2 infections [17]. The inhibition of the membrane-bound ACE2 by a recombinant soluble human ACE2 protein exhibited beneficial effects on cardiovascular diseases [21]. This result suggests that transitory blocking of ACE2 should be possible. Natural small molecules may also interact with ACE2. A phytochemical called emodin (an anthraquinone) significantly blocked the interaction between the SARS-CoV (S) glycoprotein and ACE2 in a dose-dependent manner [28]. Emodine is found in the plant genera *Rheum* and *Polygonum* and it is the major bioactive component in rhubarb (*Rheum palmatum*). This

kind of compound should be tested as a potential natural therapeutic agent against COVID-19 infections. Other phytochemicals (from TCM herbs) able to target ACE2 have been identified by computational studies, opening the possibility to test experimentally them as natural blockers of the SARS-CoV-2 (S) glycoprotein attachment to the ACE2 receptor. Molecular docking studies performed by Chen and Du [29] identified five phytochemicals with high capacity (estimated as ΔG (kcal/mol) to bind ACE2: Baicalin (flavone glycoside), Scutellarin (flavone), Hesperetin (flavonoid), Glycyrrhizin (triterpenoid) and Nicotianamine (amino acid) (Table 1).

Interfering virus exit from infected cells

Recently, it was reported that the SARS-CoV-2 (S) glycoprotein has a PRRARS[V] amino acid sequence in its structure which is not present in the (S) glycoproteins of most other Betacoronavirus (e.g. SARS coronavirus); this sequence is susceptible to be cleaved by host furin proteases [22,30]. Furins are ubiquitous proprotein convertases that are abundant in the host respiratory tract that may be involved in viral and other pathogens propagation; the SARS-CoV-2 (S) glycoprotein can be cleaved by furin upon entrance or upon exit from infected epithelial cells to rapidly and efficiently infect new host cells, granting to SARS-CoV-2 a transmissibility higher than other SARS coronavirus [30]. There are potent furin inhibitors being tested as antivirals [31].

Interfering virus proteases and replication with synthetic drugs

During CoVs replication, viral proteases [main protease (Mpro), papain-like protease (PLpro), chymotrypsin-like protease (3CLpro)] produce cleavages in virus-translated polyprotein precursors to generate non-structural proteins (nsps); some nsps can block components of the host innate immune response to evade their antiviral activities [32]. 3CLpro has been proposed as a target for the development of antiviral therapeutic strategies based on its key role in viral replication during infection [33]. Anti-HIV-1 protease inhibitors that were approved by the US FDA for clinical applications were evaluated as potential anti-CoVs through molecular docking studies into an optimized CoV Mpro structure [34]; the inhibitory potencies of those inhibitors on the SARS-CoV Mpro structure were: LPV (lopinavir) > RTV (ritonavir) > APV (amprenavir) > TPV (tipranavir) > SQV (saquinavir). Lopinavir and saquinavir were the most and the least powerful inhibitors of coronavirus protease, respectively [34]. In similar studies, the HIV inhibitors lopinavir, ritonavir, and saquinavir showed strong interaction with the active site of the SARS-CoV-2 3CLpro [35]. As we can see, there is a reiterative result pointing to lopinavir as a stronger 3CLpro inhibitor. On the other hand, virtual docking studies applied to commercial medicines listed in the DrugBank database, identified 10 potential candidates able to bind the SARS-CoV-2 Mpro among antibiotics, antitumor, antifungal, and other products [36]. A screening among various drugs previously approved by the US FDA for other diseases, revealed that Remdesivir (Gilead Sciences) and Chloroquine (4-aminoquinoline) are effective inhibitors of the SARS-CoV-2 *in vitro* [13]. Chloroquine has been used for more than 70 years to prevent and treat malaria; it is also an effective anti-inflammatory agent for rheumatoid arthritis and lupus erythematosus [37]. It shows broad spectrum antiviral activities, being one of its mechanisms of action increasing the endosomal pH required for virus/cell fusion [38]. Double antiviral and anti-inflammatory activities of chloroquine may in part explain its reported antiviral benefits. In clinical assays, chloroquine phosphate or a derivative (i.e. hydroxychloroquine chloride) were claimed to be superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung images, promoting the negative conversion of the virosis and/or shortening the length of the disease [39], while some uncertainty remains about possible side effects associated with this drug. Hydroxychloroquine is being used together with the antibiotic azithromycin on COVID-19-infected patients in France [40] and Brazil; in USA a similar preparation is being used with added Zinc. On going clinical assays with these treatments are matter of controversial discussions about efficacy, benefits and/or side effects.

Interfering virus proteases and replication with natural substances

Cystatin D is a small salivary cysteine protease inhibitor that plays a protective role against proteases acting in the oral cavity; it is an inhibitor of secreted and lysosomal cysteine proteases, and alters gene expression involved in crucial cellular functions (cell adhe-

sion, cytoskeleton and RNA synthesis/processing); cystatin D reduces the secretion of several protumor cytokines, including pulmonary and activation-regulated chemokine/CCL18 [41]. Cystatins alters the intracellular proteolytic processing of precursor virus polyproteins and may cause reduction in virus yield; cystatin D has been reported to be a potent inhibitor of human CoVs replication [42]. Molecular docking studies have allowed to identify several phytochemicals with potential to inhibit the SARS-CoV-2 Mpro; these compounds were kaempferol, quercetin, luteolin-7-glucoside, dimethoxy curcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin, and epicatechin-gallate [43]. In other studies, the stilbene phytochemical resveratrol was shown to be a potent anti-MERS-CoV agent *in vitro* [44] (Table 1).

Compound	Source	Antiviral properties/Targets	References
Chloroquine (4-aminoquinoline)	Synthetic anti-malarial drug	Inhibitor of SARS infections and spreading. Interferes cell fusion by increasing endosomal pH.	[13,37,45]
Cystatin D	Oral cavity	Salivary cysteine protease inhibitor, potent inhibitor of CoVs.	[42]
Hydroxychloroquine (azithromycin)	Chloroquine derivative	Being tested on COVID-19-infected patients in open-label non-randomized clinical trials.	[40]
Lopinavir, Ritonavir	HIV protease inhibitors	Mixture of lopinavir and ritonavir exhibited effectiveness against the SARS-CoV 3CLpro.	[46]
Lycorine* (phenanthridine)	<i>Lycoris radiata</i> (Amaryllidaceae)	Inhibited <i>in vitro</i> SARS-CoV1 cytopathic effect in Vero cells-based assays [EC50 value of 15.7±1.2 nM].	[47]
Naphthofluorescein	Synthetic	Blocks furin cleavage of SARS-CoV-2 (S) glycoprotein.	[30]
Nelfinavir	Synthetic	Potential inhibitor of SARS-CoV-2 main protease.	[46]
Opinavir, Ritonavir	HIV protease inhibitors	Inhibited SARS-CoV-2 Mpro.	[36]
Opinavir, Ritonavir, Saquinavir	HIV protease inhibitors; other compounds	Computational approach of SARS-CoV-2 protease vs. HIV protease inhibitors; +20 other compounds with inhibiting potential.	[35]
Phytochemicals (molecular docking study)	Medicinal plants from TCM*	Baicalin (<i>Scutellaria baicalensis</i>), Scutellarin (<i>Scutellaria</i> spp.), Hesperetin (<i>Citrus</i> spp.), Glycyrrhizin (licorice root of <i>Glycyrrhiza glabra</i>), Nicotianamine (ubiquitous amino acid in higher plants).	[29]
Phytochemicals (molecular docking studies)	Various plant species families	Potential targeting of the Mpro SARS-CoV-2 protease by kaempferol, quercetin, luteolin-7-glucoside, demethoxycurcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin, epicatechin-gallate.	[43]
Remdesivir**	Gilead Sciences	Broad spectrum antiviral agent against multiple RNA viruses; superior activity than Opinavir and Ritonavir; for treatment and prophylaxis of COVID-19 infections.	[13]
Resveratrol	Stilbene	Effective inhibitor of MERS-CoV infections.	[44]

Table 1: Synthetic and natural antiviral compound against SARS-type coronavirus.

*TCM: Traditional Chinese Medicine; **Remdesivir and Chloroquine Effectively Inhibit SARS-CoV-2 *In Vitro* [13].

Decreasing ROS and oxidative stress

Reactive oxygen species (ROS) and oxidative stress plays a critical role in the viral life cycle as well as in the pathogenesis of the disease. In overwhelming viral infections there is massive ROS and lipid peroxidation production, causing rapid depletion of host antioxidants (GSH, antioxidant enzymes) and leading to a serious decrease in the host GSH/GSSG ratio, mitochondrial dysfunction, impaired antioxidant systems, or a combination of these factors resulting in oxidative stress. In respiratory virus infections a significant down-regulation of the airway antioxidant system is induced by the virus, contributing to the lung oxidative damage [48]. The antioxidant response to control these events would depend on the patients' capacity to dispose of enough GSH and antioxidant enzymes supplies. The antioxidant and detoxifying cytoprotective responses are regulated in our body by the Nrf2 transcription factor through the expression of the antioxidant response element (ARE)-dependent genes including HO-1, NQO-1, GST, UDPGT, SOD, CAT, G6PD, GPx and others [49]. Understanding of the interplay between virus-induced oxidative stress and antioxidative host response will aid in the selection of antiviral supplements for better management of the viral disease [49]. Consequently, modulation of the oxidative stress is imperative in SARS-CoV-2 infections to prevent host tissues damage. This might be better done by taking common and natural antioxidants like vitamin C (hydrosoluble), vitamins D and E (membrane antioxidants), Selenium (antiviral antioxidant, cofactor of antioxidant enzymes), carotenoids, lycopene, lutein, allyl sulfides, polyphenols (curcumin, EGCG, quercetin, resveratrol), etc. most of them inducers of glutathione (GSH) and antioxidant enzymes synthesis by stimulation of the Nrf2/ARE pathway, in particular the sulfur-containing phytochemical sulforaphane [50] (Table 2).

Compound	Source	Antiviral properties/Targets	References
N-Acetyl-L-Cysteine	(Synthetic) Precursor of GSH	Antioxidant, anti-inflammatory, decrease exacerbations of bronchiectasis, mucolytic	[51]
Catalase	Vegetables; endogenous synthesis	Acts on H ₂ O ₂ Our endogenous catalase declines with age.	[52]
Glutathione [GSH]	Synthesized by our own body; found in some dietary fruits and vegetables	Major soluble antioxidant in our body; acts on ROS control.	[53]
Antioxidant phytochemicals	Dietary vegetables, fruits and spices	Polyphenols (curcumin, EGCG, quercetin, resveratrol, curcumin), carotenoids (lycopene), xanthophyls (lutein), sulfur compounds (allyl disulfides, sulforaphane), etc.	[54]
Selenium	Metallic element	Essential cofactor of antioxidant selenoprotein enzymes (GPx, TXNRD)	[55]
Sulforaphane (sulfur phytochemical)	Dietary vegetables from the Brassicaceae family	Stimulate the Nrf2/ARE pathway to synthesize antioxidant enzymes; decrease inflammation and cellular toxicity, modify protease/anti-protease balance, reduce TMPRSS2 secretion, decrease viral host entry and downstream infection.	[50]
Volatile monoterpenes	Aromatic plant from essential oils	Powerful antioxidant and/or anti-inflammatory compounds: Eugenol (cloves), Carvacrol (organ), Thymol (<i>Thymus vulgaris</i>), Eucalyptol (<i>Eucalyptus globulus</i>), etc.	[56]

Table 2: Antiviral antioxidants.

SARS-induced inflammation

Coronavirus infection induces the production of ROS, which activate the NF-κB transcription factor (Nf-κB) for the secretion of pro-inflammatory cytokines, these cytokines are active in the innate immune response, such as, for instance IFN-λ in respiratory epithelial

cells [57]. In pulmonary CoV infections, the occurrence of spread cell damage, epithelial dysfunction, obstructive thromboembolism, and other virus-induced disturbances boost massive ROS production [58] and over-production of pro-inflammatory cytokines (“cytokine storm”) and chemokines, leading to acute respiratory distress syndrome (ARDS) and increased mortality [10,59].

Controlling inflammation

Anti-inflammatory agents are commonly used to inhibit COX-2 and iNOS enzyme activities, IL-6-STAT3, TNF- α -NF κ B and IFN- γ cytokine pathways [60]. Conventional anti-inflammatory NSAIDS drugs are commonly used as analgesic and/or anti-inflammatory drugs for many ailments, including respiratory diseases; and also corticosteroids, which are powerfully wide-range immunosuppressive agents. However, the use of these drugs in COVID-19 has generated controversies. On the other hand, a number of specific anti-cytokine approach treatments have proven to be effective for a variety of cytokine storm syndromes; these include drug targeting for IL-1, IL-6, IL-18, and IFN- γ . For instance, Tocilizumab anti-IL-6 receptor (IL-6R) antibody inhibits IL-6 activity and has been found therapeutically effective for patients with serious inflammatory diseases [61]. Blocking IL-6 (a main hallmark of inflammation) has been reported to be successful against COVID-19 infection [62]. We may use also natural anti-inflammatory and analgesic products; they usually work by downregulating the expression of the nuclear factor NF- κ B pathway genes [63]. Among these components, it worths to mention 1,8-cineol (eucalyptol), a volatile monoterpene that has proven to significantly attenuate pulmonary inflammatory responses caused by influenza virus in mice [64]. 1,8-Cineol significantly decreased the level of TNF- α and IL-1 β and increased the level of IL-10 in lung tissues after acute lung injury induced by LPS [65]. 1,8-Cineol efficiently decreased levels of IL-4, IL-5, IL-10, and MCP-1 in nasal lavage fluids, as well as the level of IL-1 β , IL-6, TNF- α and IFN- γ in lung tissues of mice infected with influenza virus [65]. 1,8-Cineol exhibit mucolytic and spasmolytic action on the respiratory tract, with proven clinical efficacy and therapeutic benefits in inflammatory airway diseases, such as asthma and COPD [66]. Other natural anti-inflammatory agents are Curcumin, α -Lipoic acid, and Resveratrol that have been used also in respiratory infections (Table 3).

Compound	Source	Model	Antiviral properties/Targets	References
1, 8 cineol (eucalyptol)	<i>Eucalyptus globulus</i> (Myrtaceae)	Lung tissues of mice with influenza virus	Downregulation: IL-1 β , IL-6, TNF- α , IFN- γ , p65 subunit of NF- κ B, ICAM-1, VCA M-1	[64]
1, 8 cineol (eucalyptol)	<i>Eucalyptus globulus</i> (Myrtaceae)	Bacterial LPS-injured lung tissue	Downregulation: TNF- α , IL-1 β , p65 of NF- κ B, TLR4, myeloperoxidase activity. Upregulation: IL-10	[65]
1, 8 cineol (eucalyptol)	<i>Eucalyptus globulus</i> (Myrtaceae)	Humans with respiratory diseases	Mucolytic and spasmolytic actions, reduce lung inflammations	[66]
Curcumin	<i>Curcuma longa</i> L. (Zingiberaceae)	Humans	Suppress oxidative stress and inflammation, downregulates NF- κ B, reduces blood sugar and levels of glycosylated haemoglobin, etc.	[67]
α -Lipoic acid	Vegetables (spinach), animals	Humans, experimental animals	Antioxidant, increases expression of antioxidant enzymes, recycles Vit C and E, anti-inflammatory, reduce inflammatory markers IL-6, ICAM-1	[68]
Resveratrol	<i>Vitis vinifera</i> , etc.	Humans	Inhibited cytokine release by alveolar macrophages in COPD*	[69]

Table 3: A few natural anti-inflammatory compounds against respiratory infections.

*COPD: Chronic Obstructive Pulmonary Disease.

The innate immune response in COVID-19

The innate immune system plays a fundamental role in the response against viruses [7]; in the lungs, the innate responses against incoming respiratory viruses are governed primarily by alveolar and interstitial macrophages, dendritic cells, airway epithelial cells, innate lymphocytes, neutrophils and plasma proteins [10]. Interferons (innate immune response components) trigger a general immune response that make cells in the body more resistant to viral infections, inhibiting the spread of viruses; nonetheless, interferon treatments can have severe side effects like inflammation, and it might not be convenient in excessively inflamed COVID-19 patients. It worths to notice that several SARS-CoV nonstructural proteins (nsps) antagonize the induction of interferon and are also involved in the avoidance of the Interferon Stimulated Gene (ISG) effector functions [70], thus manipulating the host innate immune response. Another component of the innate immune response is the mannose-bind lectin [MBL]; MBL is a collagenous C-type lectin that binds to mannose on the surface of a wide range of pathogens, including viruses, and resulting in the interference and aggregation of the viral particles. MBL is found in our plasma, but it can be also found in several dietary vegetables (for instance, *Allium porrum*) [71]. Zhou., *et al.* [72] demonstrated that MBL bound specifically to the SARS spike S1 protein, whereas Zhang., *et al.* [73] showed MBL binding to the spike S1 protein of the Infectious Bronchitis Virus (IBV) coronavirus blocking the attachment of this virus to IBV-susceptible cells in chicken tracheal tissues; MBL exhibited also direct antiviral activity against IBV when incubated with IBV virions [73]. It has been found that a substantial proportion of humans have MBL deficiency due to the existence of MBL polymorphisms, potentially conditioning the susceptibility to respiratory infectious diseases [74]; this observation invites to test COVID-19 patients for their MBL status.

Supporting patients with micronutritional supplements

Viral infection diseases lead to micronutrient deficiency which should be compensated by micronutrient supplementation. Vitamin C (ascorbic acid), Vitamin D (25-hydroxyvitamin D), Selenium (Se) and trace of key minerals have an important role in antioxidant defense, redox signaling, redox homeostasis and the immune system. In addition, Vitamin D reduces respiratory tract infections by maintaining tight junctions, by killing enveloped viruses, and reducing the production of pro-inflammatory cytokines of the innate immune response (reduces “cytokine storm” risks); patients with chronic diseases have significantly higher risk of death from COVID-19 infections than otherwise healthy people; raising serum 25-hydroxyvitamin D concentrations through Vitamin D supplementation and sun exposure may reduce the incidence, severity, and risk of death by these infections [75]. Selenium (Se) compounds exhibits strong radical-scavenging activity and it is also particularly implicated in viral infections, since its deficiency has been associated with several virus pathogenicity's; more importantly, the antioxidant enzymes GPx, and TXNRD are selenoproteins [55]. It is imperative to help COVID-19 patients having exhausted antioxidant and immune capacities with antioxidant and anti-inflammatory compounds such as glutathione (GSH), α -lipoic acid, polyphenols, antioxidant monoterpenes (eugenol, carvacrol, thymol), anti-inflammatory monoterpene (eucalyptol) [56] and antioxidant enzymes (catalase); dietary sulforaphane (Table 2) is able to induce the Nrf2/ARE signal transduction pathway to promote the synthesis of GSH and antioxidant enzymes [49,50].

Conclusion

Each step in CoV infections has its own peculiarities, from the initial virus invasion of the oropharyngeal region (upper respiratory tract) to invasion of the lower respiratory tract and/or invasion of other body tissues and organs, as increasingly being reported in many COVID-19 cases. The virus is initially confronted with our own individual higher or lower innate immune response capacity, and we know that it has mechanisms to manipulate and evade the innate immune response. A successful host cell invasion is followed by a rapid viral multiplication, excessive ROS liberation, unbalanced pro-oxidant/antioxidant ratio, oxidative stress, cell damage, apoptosis and high cytokine-mediated inflammatory responses due to uncontrolled cytokine production, these events affecting mostly aged patients and/or those having chronic diseases. To control COVID-19 infections, we think that it would be important to take, upon medical prescription, antivirals as soon as a contagion is confirmed, and/or from the beginning of the first symptoms trying to low initial viral loads; antioxi-

dants and anti-inflammatory agents should be taken all the way through the infection since they may help to restore redox equilibrium, fight the oxidative stress and control the unregulated immune response making it difficult the infection to progress. For severe critical cases, antibiotics and anticoagulants treatments would depend on the patient's pathology (respiratory, cardiovascular, renal, other), the clinical condition and criteria of the clinician. In this work, we just wanted to offer a series of opinions, suggestions and ideas for medical and research discussion on virus-host interactions and molecular targets in relation to possible treatments, hoping they could contribute to control COVID-19 infections.

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