REVIEW

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Natural and semisynthetic candidate molecules for COVID-19 prophylaxis and treatment

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Abstract

Coronaviruses (CoVs) represent a family of viruses that have numerous animal hosts, and they cause severe respiratory, as well as systemic and enteric infections, in humans. Currently, there are limited antiviral strategies for treating patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The lack of specific antiviral medicines and SARS-CoV-2 vaccines continues to aggravate the situation. Natural product-based antiviral drugs have been used in the two previous CoV outbreaks: Middle East respiratory syndrome coronavirus (MERS-CoV) and the first SARS-CoV. This review emphasizes the role of natural and semisynthetic candidate molecules for coronavirus disease 2019 (COVID-19) prophylaxis and treatment. The experimental evidence suggests that nature could offer huge possibilities for treatment of the COVID-19 pandemic.

Keywords: COVID-19, hydroxychloroquine, azithromycin, hesperidin, rosocyanin.

Background

Coronaviruses

Coronaviruses (CoVs) represent a family of viruses that have numerous animal hosts (cattle, pigs, cats, horses, dogs, camels, bats, rodents, ferrets, palm civets, mumps, rabbits, snakes, and avian species) that cause severe respiratory infections in humans, as well as systemic and enteric infections. The CoVs family consists of four main groups [1, 2]:

(*i*) Alpha-CoVs, which infect bats, pigs, cats, dogs, and humans (causing rhinopharyngitis).

(*ii*) Beta-CoVs, which mainly circulate in bats, but can also affect cattle, horses, pigs, dogs, mice, rats, hedgehogs, and other mammals. Two species cause rhinopharyngitis in humans: human HKU1 and OC43 CoVs. Two other species cause very severe respiratory syndromes in humans: Middle East respiratory syndrome coronavirus (MERS-CoV) and the first severe acute respiratory syndrome coronavirus (SARS-CoV) in Asia. Recently, at the end of December 2019, SARS-CoV-2 (a new coronavirus) was identified in Wuhan, China, which is highly infectious, easily transmitted from human to human and it causes severe pneumonia.

(*iii*) Gamma-CoVs are mainly avian viruses, but they also infect dolphins.

(*iv*) Delta-CoVs are mainly avian viruses but can also infect pigs.

Two potentially dangerous zoonotic CoVs have emerged over the past two decades: SARS-CoV, originating in China (Guangdong Province), that caused an outbreak from 2002 to 2003, and MERS-CoV, which caused an outbreak in 2012. Like most CoVs in the beta group, the hosts of MERS-CoV, SARS-CoV and SARS-CoV-2 are bats, probably the natural reservoir of these viruses; infected dromedaries are only intermediate hosts of MERS-CoV, and palm civets are intermediate hosts for SARS-CoV. There have been many attempts to identify the intermediate host for SARS-CoV-2, yet it has still not been detected [3–5]. Currently, no specific antiviral drugs or vaccines have been approved to combat SARS and MERS. In order to stop the SARS pandemic in 2002–2003, several restrictions on travel and patients' isolation were applied, as well as measures of conventional control [1, 6].

The new SARS-CoV-2 coronavirus

Official name

The *International Committee on Taxonomy of Viruses* (ICTV) stated that the new virus is named SARS-CoV-2 (February 11th, 2020). At the same time, the *World Health Organization* (WHO) announced that the name of the new disease caused by SARS-CoV-2 is coronavirus disease 2019 (COVID-19), and subsequently, on March 11th, 2020, *WHO* declared a pandemic for SARS-CoV-2 [7, 8].

Mechanism of action

SARS-CoV-2 encodes 29 proteins; four make up the virus's actual structure, including the spike ('S') protein. One group of the remaining 25 coronavirus proteins coordinates how the virus assembles copies of itself and how it sneaks past the host's immune system [9]. SARS-CoV-2 has the following structural proteins: 'S' glycoprotein, which causes the virus to attach to the membranes of human host cells; 'E' glycoprotein, which

is required for the initial uptake of the virus by the host cell membranes; 'M' protein of the viral membrane, playing a role in viral assembly; and 'N' protein of the nucleocapsid, involved in the regulation of viral ribonucleic acid (RNA) synthesis [7, 8, 10, 11].

Until recently, SARS-CoV-2 infection was thought to only occur through binding to a widespread human cell receptor, angiotensin-converting enzyme 2 (ACE2) [12]. Thus, the human cells are infected with SARS-CoV-2 by the binding of the surface 'S' glycoprotein to the human ACE2, the main host cell receptor for the virus. However, the existence of only one virus entry receptor was not sufficient to explain the high infectivity and severity of COVID-19 disease. Recent research has shown that another human host cell receptor, the extracellular matrix metalloproteinase [cluster of differentiation 147 (CD147)] inducer, also known as basigin (BSG) or extracellular matrix metalloproteinase inducer (EMMPRIN), makes an important contribution to "opening the door" for SARS-CoV-2 infection [13]. Recent evidence suggests that SARS-CoV-2 may be using a co-receptor when entering the cells, the same one used by MERS-CoV, namely the dipeptidyl peptidase-4 (DPP-4)/CD26 receptor. New studies have shown that SARS-CoV-2 binds to DPP4/CD26 when entering the cells of the respiratory tract [14–16]. Probably, there are other receptors, still undiscovered, which will have major significance in the fight against COVID-19 [17].

SARS-CoV-2 is 1000 times more aggressive than SARS-CoV, having a "double attack" approach, by binding to the human cell membrane, using both a host cell enzyme (furin) and ACE2 as receptors. Early indications are that SARS-CoV-2 also requires ACE2 and transmembrane protease serine 2 (TMPRSS2) to enter cells [18]. As a result of the "double attack", the 'S' glycoprotein from SARS-CoV-2 has significantly less free energy than SARS-CoV, which suggests that, at the same temperature, SARS-CoV-2 is more stable and has a capacity of infection greater than SARS-CoV. Moreover, SARS-CoV-2 may survive high temperatures, becoming infectious when temperatures are low (during wintertime). This did not happen with SARS-CoV, which, after 2003, did not withstand high temperatures and disappeared [19].

Epidemiology, prevention, and control of SARS-CoV-2

Respiratory CoVs are likely to spread in a manner similar to that of rhinoviruses, through direct contact with an infected person, infected secretions, with large aerosol drops or even through fecal–oral transmission. COVID-19 is transmitted from symptomatic persons to other persons to which they are in close contact by respiratory drops, by direct contact with infected persons or by contact with contaminated objects or surfaces. COVID-19 is more pronounced in the upper respiratory tract (nose and throat) earlier, meaning during the first three days following the symptoms' debut. It is not yet clear whether the virus can spread before the manifestation of specific symptoms. Immunity develops soon after the infection, but gradually fades over time. If the SARS-CoV-2 infection manifests as a common flu or common cold, there is a possibility that the number of infections may decrease as the weather gets warmer. Another possibility is that SARS-CoV-2 supports mutations in a potentially beneficial way, making humans' infection with this virus more difficult. For example, in 2002, SARS-CoV underwent a random genetic mutation (as is usually the case with viruses), and the infection became more severe but more difficult to be transmitted to other humans. However, even if SARS-CoV-2 could become a seasonal disease, such as influenza or colds, it may have a lower impact during subsequent years, as many people have already acquired immunity, although it is not clear if people can become infected with this virus more than once [7, 20–22].

At present, there is no evidence that SARS-CoV-2 has undergone any significant mutation. The available viral sequences are almost identical, making it difficult to determine how the virus will behave. *WHO* insists that "there is no evidence that the virus has changed", although a report by Chinese researchers on genetic analysis among 103 patients infected with SARS-CoV-2, states that the virus has evolved into two major types (designated "S" and "L"). The "S" type (~30%) is the initial, less aggressive virus, and the "L" type (~70%) has become detached from the "S" type, being more aggressive and spreading much faster. This opinion is not shared by the *WHO*, nor by American virologists, and further research will clarify whether these genetic mutations exist. A recent study includes convincing data showing a change of amino acids within the 'S' protein of the virus (D614G). This change occurred early during the pandemic and the viruses containing this change are now stated to be prevalent in many places around the world [23].

The latest epidemiological studies have shown that the distribution of the incubation period and the distribution of the infection interval are pretty much the same [24, 25]. From 30% to 50% of transmissions are suggested to occur from pre-symptomatic persons. Considering the SARS-CoV-2 infectiousness and the high proportion of transmissions from pre-symptomatic persons, pandemic control by preventing manual contact is not feasible [26]. The incubation period for SARS-CoV-2, meaning the period from a person's exposure to the virus and the symptoms debut is about 5–6 days on average, but it can also be up to 14–16 days. During the incubation (pre-symptomatic) period, some infected persons could be contagious. Subsequently, the transmission starting from a pre-symptomatic case could take place before the symptoms debut [22, 27]. Thus, the virus could be transmitted by the persons infected with SARS-CoV-2 before significant symptoms appear. During pre-symptomatic transmission, infectious drops and contaminated surfaces are the main factors responsible for the virus spreading [28, 29].

Clinical manifestations and diagnosis of SARS-CoV-2 infection

In most cases, SARS-CoV-2 infection is associated with the triggering of a true cytokine "storm": interleukins (IL-2, IL-6, IL-7 and IL-10), interferon (IFN)-γ-inducible protein 10, granulocyte/macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein-1 alpha (MIP-1α), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α). In those who survive intensive care, immunity will increase excessively, and its responses will lead to lung damage and/or pulmonary

fibrosis in the long-term, resulting in a reduced quality of life. Signs and symptoms of SARS-CoV-2 may occur from day 2 to day 14 after exposure and they can include cough, fever, or shortness of breath. SARS-CoV-2 symptoms can range from very mild to very severe. Older people or people with chronic diseases (cardiovascular, diabetes, cancer) may experience acute manifestations of this disease. There is increasing data that SARS-CoV-2 infection occurs more frequently in men than in women, probably because of the ACE2 receptor, which is dependent on the "loading" of the body with sex hormones. At hospitalization, lymphocytopenia was observed in 83.2% of patients, leukopenia in 33.7% and thrombocytopenia in 36.2% of patients [30].

Confirmation of cases suspected of SARS-CoV-2 infection is done using mainly molecular [reverse transcription–polymerase chain reaction (RT-PCR)] and serological [enzyme-linked immunosorbent assay (ELISA)] tests. A comparison between the molecular and serological tests showed that the molecular test is more significant, having much better sensitivity and specificity. The evaluation of the sensitivity and specificity of both molecular and serological assays, as well as rapid test methods, remains a high priority to bring the COVID-19 pandemic under control [31, 32]. Currently, seven potential diagnostic kits are available on the market for SARS-CoV-2. A new Cochrane review assessed how accurate the tests for detecting the COVID-19 antibodies of current and past SARS-CoV-2 infection [33]. Respiratory tests were found to be positive for the virus, while the blood tests were negative in the early period of infection. It has also been suggested that in the first few days of the disease, patients have high levels of the virus, despite mild symptoms [34, 35].

Treatment of SARS-CoV-2 infection: essential overview

Presently, there are no specific vaccines or antiviral drugs for CoVs, although several molecules have been tested against CoVs, even for SARS-CoV-2, with over 3500 publications about the results of these tests. The main drugs used in the clinical studies are intended to restrict the components of the SARS-CoV-2 infection life cycle. These components may include: the viral access into the hosting cell [blocked by IFN, chloroquine (CQ), or umifenovir], the viral replication [blocked by darunavir/cobicistat or lopinavir/ritonavir, both inhibiting the 3C-like protease ($3CL^{pro}$)] and the viral RNA synthesis (stopped by favipiravir, remdesivir, ribavirin, emtricitabine/tenofovir or alafenamide). Currently, commonly used antiviral drugs, including neuraminidase inhibitors (oseltamivir, peramivir, zanamivir, etc.) are not effective for COVID-19 because CoV does not produce neuraminidase. Ganciclovir, acyclovir, ribavirin, and other medicines have little effect and are not recommended for clinical application. Drugs that have been shown to be effective in current studies include: lopinavir/ritonavir or rudecvir combined with recovery plasma, IFN-β and monoclonal antibodies [36–42].

Out of the molecules tested, we selected those that gave positive results for COVID-19 prophylaxis and treatment: CQ, niclosamide and ivermectin, remdesivir, hesperidin, curcumin–boron complexes, and blockers of the angiotensin II type 1 (AT1) receptor ("sartans").

CQ, a known antimalarial agent, may have effects against many viruses, such as hepatitis B, human immunodeficiency virus (HIV) type 1 and human coronavirus-229E (HCoV-229E), and may be immunomodulatory and anti-inflammatory as well. It is postulated that CQ works by modifying ACE2 glycosylation and altering the endosomal pH. Its anti-inflammatory properties can be beneficial for the prevention and treatment of SARS. According to a paper issued by the *International Journal of Antimicrobial Agents*, on February 11th, 2020, French researchers stated that CQ had shown its *in vitro* efficacy against a wide spectrum of viruses, including SARS, during the 2003 epidemic. At the same time, this drug, tested *in vitro* on SARS-CoV-2, has shown efficiency in blocking the replication of this virus. Thus, the Chinese authorities and the researchers who conducted the studies concluded that CQ phosphate has potential to combat COVID-19 and recommended it be mentioned in the new "Guide for the prevention, diagnosis and treatment of pneumonia caused by COVID-19", published by the *National Health Commission* of the People's Republic of China [43–46]. However, using hydroxychloroquine (HCQ) for humans, for which significant *in vitro* antiviral properties have been demonstrated, is still a topic much debated with controversial results [47–49]. There has been widespread interest in HCQ as both a preventive measure and for treating patients with COVID-19. However, another larger scale clinical trial has shown HCQ is not actually effective against SARS-CoV-2 infection. While *WHO* has halted its clinical trials, around the world there are more than 200 other trials currently underway [50]. Our opinion and that of many scientists is that the information war over HCQ has slowed down the knowledge of COVID-19.

Niclosamide is a drug known and used mainly as an anthelmintic, having been shown to inhibit SARS-CoV-2 replication. Reduction of virus yield in infected cells was dose-dependent [51–53].

Recently, an *in vivo* study demonstrated the ability of ivermectin to reduce viral angiotensin receptor blocker (ARB) up to 5000 times after 48 hours of SARS-CoV-2 infection. Ivermectin is currently used as an antiparasitic for animals. In the future, further clinical studies are required in order to prove whether ivermectin is efficient to cure COVID-19 [24, 54].

Remdesivir (GS-5734) is a novel nucleoside analog and a broad-spectrum antiviral drug. In contrast to fapilavir and ribavirin, *in vitro* and animal experiments have confirmed that remdesivir has strong antiviral activity against human CoV infection and various CoVs isolated from bats. In theory, remdesivir is currently the most promising drug for SARS-CoV-2. The results of animal experiments showed that compared to the control group, remdesivir can effectively reduce the virus concentration in the lungs of MERS-CoV-infected mice, improving the functionality of the damaged tissue. Also, early treatment with remdesivir during infection has a clear benefit for *Rhesus* macaques infected with SARS-CoV-2 [55]. For humans, its efficacy is better than the combination of lopinavir/ritonavir/IFN-β. Recently, the *New England Journal of Medicine* issued a report about a USA patient infected with SARS-CoV-2 who was treated and cured using remdesivir [56–58]. However, there are three recent

studies showing potential contradictory findings on the use of remdesivir against SARS-CoV-2 in humans. The most recent reported remdesivir utilization has not shown a clinical improvement, and the results are not statistically significant, while side effects are being reported as well. Subsequently, remdesivir was stopped during the clinical trial due to the side effects [59].

Hesperidin, a natural polyphenol found in *Citrus* spp. [60], binds to the receptor for ACE2, the gateway to SARS-CoV-2 into the human cell. Hesperidin administration significantly suppressed the expression of the ACE2 receptor protein, with inhibitory effects on virus entry into the host cells [48, 61, 62].

Rosocyanin, a curcumin–boron complex, inactivates viral proteases. The high affinity of rosocyanin for proteases has been known for a long time [63, 64].

AT1 receptor blockers ("sartans", such as valsartan, irbesartan, losartan, candesartan, telmisartan, and eprosartan) have been widely applied since the 1990s to control high blood pressure. Currently, complete information on the proportion of hypotensive patients among SARS-CoV-2 patients that were hospitalized is not available; it is, therefore, too early to predict the number of patients with SARS from the ongoing epidemic who could be cured with AT1 blockers, with no risk of hypotension exacerbation. The initiative of applying telmisartan and losartan (AT1 antagonists) as anti-SARS-CoV-2 therapeutic agents in order to treat patients before the occurrence of the acute respiratory syndrome, is still unproven until more tests are done. Therefore, the quickest approach to assess the feasibility of "sartans" is an analysis of patients' clinical data and the extent to which patients treated with AT1 antagonists before diagnosis (for diabetes, hypertension, kidney disease, etc.) have a good response acquiring the viral disease. There is only one study that showed that blocking AT1 receptors with losartan and inhibiting ACE2 with enalapril reduced the proportion of macrophages induced by Dengue fever virus (DENV2), a SARS-CoV-2-like RNA virus, suggesting a decrease in cell penetration by the virus and a role of ACE2 in Dengue virus infection [65–67]. The use of ACE inhibitors (ACEIs)/ARBs for COVID-19 patients does not lead to harmful outcomes and may even provide benefits and decrease mortality from COVID-19 [68].

Next, we will investigate the most scientifically debated semisynthetic molecules (HCQ and azithromycin) and two natural compounds or derivatives (hesperidin and rosocyanin).

Efficacy and safety of chloroquines for COVID-19 treatment

Chloroquines

In Germany, the well-known Bayer drug manufacturer synthesized CQ, an amine form of quinine, for the first time in 1934. Initially, CQ was used as a substitute for natural quinine (a molecule identified in the bark of the *Cinchona* trees found in Peru), which was originally chosen as a prophylactic against malaria. Subsequently, CQ became a first-line drug for the prophylaxis and treatment of malaria, becoming one of the most prescribed drugs. CQ and HCQ belong to the same family of molecular entities; HCQ is different from CQ through the hydroxylation of the *N*-ethyl substituent. HCQ is available as a phosphate or sulfate for oral administration. The pharmacokinetics of HCQ and CQ are similar, having gastrointestinal absorption and rapid renal elimination. Unfortunately, the efficiency of CQ against malaria has progressively decreased due to the continued evolution of CQ-resistant strains of *Plasmodium falciparum*. CQ can also be administered in the treatment of autoimmune diseases and to control inflammatory processes, and it has a wide spectrum of use against some bacterial, fungal, and viral infections [69, 70].

Antiviral properties of chloroquines: mechanism of action and toxicity

Studies have highlighted the antiviral effects of CQs through the following mechanisms: (*i*) It alters the pH value of endosomes, which triggers an important inhibitory effect on viral infections that invade the cells through the endosomal pathway; (*ii*) It influences viral replication by inhibition of virus genes expression; (*iii*) It interferes with ACE2 receptor glycosylation thus preventing the binding of SARS-CoV-2 to human host cells; (*iv*) It interferes with the infection and virus replication by affecting autophagy; (*v*) It influences the 'M' protein by proteolytic processing and hinders the assembly of the virion; (*vi*) It works as an immunomodulatory agent capable of mediating the body's anti-inflammatory response in the following diseases: lupus erythematosus, rheumatoid arthritis, and sarcoidosis [45, 46].

Recent research showed that pneumonia caused by CoVs may be closely linked to the abnormal metabolism of hemoglobin in humans [71]. CQ is a drug commonly used to treat porphyria or 'vampire disease' (a hereditary disease caused by heme synthesis disorder). Therefore, the combination of surface glycoproteins of SARS-CoV-2 with human porphyrins causes a series of pathological reactions (decreased hemoglobin), preventing the formation of the heme and practically reducing the number of red blood cells with major effects on lung oxygenation. HCQ competes with porphyrin and binds to the viral protein, thus inhibiting its attack on the heme or its binding to porphyrin [72]. The number of red blood cells is a significant biochemical indicator, and it is different between men and women. The number of erythrocytes in healthy men is considerably higher than in healthy women. This could be one reason why men are more likely to be infected with the new SARS-CoV-2 [45, 46].

Although the prolonged use of CQ as an antimalarial drug has confirmed its safety in humans, a low risk of macular retinopathy (depending on the cumulative used dose) and cardiomyopathy cannot be ignored. However, in general, CQ and HCQ are considered to be safe, having mild and transient side effects [73–76].

Possible antiviral effects of chloroquine *versus* SARS-CoV-2

In vitro

CQ is a versatile bioactive agent, with antiviral activity observed against RNA viruses: Chikungunya, Crimean hemorrhagic fever, Dengue, Ebola, Hendra, hepatitis A, hepatitis C, HIV, influenza A and B, influenza A H5N1,

Lassa, Nipah, polio, rabies and Zika, as well as against various deoxyribonucleic acid (DNA) viruses, such as hepatitis B and *Herpes simplex*. The *in vitro* properties of HCQ were occasionally proven during the treatment of infected patients, but there was no general confirmation, with differences regarding the disease, the dosage, the treatment duration and the clinical team responsible for the study [77, 78].

In vivo

Based on *in vitro* proof, a recent clinical trial recommended the use of CQ phosphate for 10 days (500 mg twice a day) for cases with pneumonia (severe, moderate and mild) due to SARS-CoV-2, in which there were no contraindications to CQ phosphate. Several precautions were suggested: (*i*) Blood analysis to avoid anemia, leukopenia and thrombocytopenia development, and other renal and liver dysfunctions/disorders; (*ii*) Routine electrocardiography to preclude bradycardia or the extension of the QT interval [79].

Recently, the *National Center for Biotechnological Development*, under the authority of the *Ministry of Science and Technology of China*, stated that CQ is one of the three promising drugs to fight the new SARS-CoV-2, with 23 clinical trials underway. CQ has been used in hospitals in Beijing, central and southern China (Guangdong Province). According to preliminary reports, Chinese authorities suggest that about 100 patients infected with the new CoV have been treated with CQ, and they have recorded a faster decrease in fever and improved computed tomography scans of the lungs, and the patients had a shorter recovery time in comparison with the control groups and with no obvious major adverse effects. Accordingly, the *Chinese Medical Advisory Committee* recommended the inclusion of CQ in the treatment of COVID-19 [79, 80].

In a document published on its own website, the *Dutch Center for Disease Control* suggested that CQ could be used to treat severe infections that require hospitalization and oxygen therapy. The suggested prescription for adults consists of the administration of 600 mg HCQ (six tablets A-CQ© 100 mg) followed by 300 mg after 12 hours on the first day, then 300 mg ×2/day on the second day, up to the fifth day. This document emphasized the need to stop treatment after the fifth day in order to diminish the risk of adverse effects, given the half-life of the drug (30 hours). It is also necessary to distinguish between the doses of CQ phosphate and HCQ, since 500 mg of CQ phosphate is equivalent to 300 mg of HCQ [79].

Another study from the *Italian Society of Infectious and Tropical Diseases* (Lombardy Section) recommends the administration for 10 days of CQ (500 mg ×2/day) or HCQ (200 mg ×2/day), although the treatment may be varied between the fifth and twentieth day depending on the clinical gravity. The target category consists of people with mild respiratory manifestations and comorbidities, as well as cases with severe respiratory deficiency [79].

Just recently (March 20th, 2020), a clinical trial coordinated by French professor Didier Raoult confirmed that HCQ is significantly associated with a reduction or disappearance of viral load in patients with COVID-19, the effect being reinforced by the simultaneous administration of an antibiotic (azithromycin). Thus, an acute viral disease can be successfully treated with two cheap and widely available drugs [79, 80].

Hydroxychloroquine therapy for COVID-19

The above-mentioned clinical trial showed that HCQ sulfate is stronger than CQ for inhibition of SARS-CoV-2 [43], being administered at a dose of 400 mg ×2/day on the first day, then 200 mg ×2/day for the next four days, to which 500 mg of azithromycin was added on the first day, followed by 250 mg daily for the next four days [81]. Comparing the effect of the treatment with HCQ with the effect of the combined treatment of HCQ and azithromycin on day 6 after initiation, it was found that 100% of the patients treated with the combined treatment (HCQ and azithromycin) were cured, as opposed to 57.1% of the patients treated only with HCQ and 12.5% of the control group (p<0.001) [44, 82]. However, several studies have shown that the high CQ dose scheme, administered for 10 days, was not entirely safe and had no clinical advantages. It remains for future clinical trials to prove the efficiency of the CQ treatment [51, 83].

A recent observational study showed that HCQ has not been associated with and has not considerably decreased the risk of death during the treatment [49]. Other studies showed that HCQ, ivermectin and azithromycin, if simultaneously administrated, seemed to potentially act against infection with the new virus. However, their efficacy should be studied individually and in combination [84]. At the same time, a combination of zinc sulfate and CQ may play a role in the therapeutic management of COVID-19, suggesting a potential synergic mechanism of these substances if used early during the disease course [85]. Other studies showed that the azithromycin/nitazoxanide combination could be a more effective treatment than the HCQ/azithromycin combination as the standard treatment for early COVID-19 [54].

CQ and HCQ are known to cause heart rhythm problems that could be exacerbated if the treatment is combined with other drugs, such azithromycin, with similar effects on the heart [86]. Recent studies have reported very serious heart rhythm problems, sometimes fatal. Physicians are recommending close monitoring of COVID-19 patients that are receiving CQ and HCQ, watching for the possibility of side effects at higher doses and to be more careful when the treatment is combined with other drugs like azithromycin that can have similar side effects on the heart [87]. Short-term treatment with CQ is safe, but in combination with azithromycin, there may be a synergic effect on QT length, resulting in heart failure and cardiovascular mortality [88].

Recently, the *Indian Council of Medical Research* recommended a starting dose for HCQ of 400 mg twice on the first day, continued with 400 mg daily as chemoprophylaxis and treatment in patients with COVID-19 [89]. It may be necessary to administrate a loading dose followed by a maintenance dose in order to achieve optimal treatment [43, 90].

The discrepancies in the use of HCQ in clinical management among intensive care unit patients with severe COVID-19 requires extensive research. The lack of evidence barely justifies the widespread use of HCQ for prophylaxis. Long-term use of HCQ for the treatment

of malaria indicates their safety during acute human administration. However, the minor risk of macular retinopathy and a prolonged QT interval in arrhythmic, renal, and hepatic impaired patients, cannot be ignored. The number of USA patients receiving prescriptions for HCQ, CQ and azithromycin increased dramatically as the COVID-19 began to unfold, according to new data. The report was issued a month after the United States *Food and Drug Administration* (FDA) revoked the emergency use authorization for HCQ and CQ to treat COVID-19 amid continuing concerns about the safety of widespread use of the drugs to treat the infection. Large, well-conducted, randomized clinical trials are needed to determine whether HCQ and CQ have preventative or efficacy against COVID-19 and acceptable safety. At this time, their use outside of clinical trials is not warranted. There is insufficient evidence to support the efficacy or safety of HCQ and CQ for the treatment of COVID-19 in patients [76, 91]. Some studies show that the treatment is not harmful, but it also does not offer any benefit. The findings indicate that HCQ is not an effective treatment during hospitalization of patients with COVID-19 but does not address its use as prophylaxis or in patients with less severe community-transmitted SARS-CoV-2 infection. COVID-19 treatment with HCQ and CQ has been recommended in many treatment guidelines, including in Brazil, China, France, Italy, the Netherlands, South Korea, and the United States [73, 92]. However, recently, a multicenter study of patients hospitalized with COVID-19 in Madrid with prednisone-associated HCQ reported promising results [92].

Azithromycin as a possible anti-COVID-19 drug (prophylactic and therapeutic)

Azithromycin

Erythromycin A and its derivatives have been widely used as oral therapy in the ambulatory treatment of respiratory diseases in humans, including in children. Azithromycin (9-deoxy-9*a*-aza-9*a*-methyl-9*a*-homoerithromycin A) is a structural derivative of erythromycin A, modified by the insertion of a nitrogen atom into the macrolide nucleus. This compound is stable at gastric pH, having a high affinity for the lung tissue, due to the presence of tertiary amino groups that enhances its amphiphilic properties [93, 94].

Azithromycin was introduced on the market in 1988, and after 2000, it became a leader in the field of antibiotics used to fight respiratory tract infections. The use of azithromycin in respiratory infections is due to its location in certain potential areas of infection of human lung tissue, presenting the following maximum concentrations, after a single oral dose of 500 mg: 23 mg/L in alveolar macrophages, 3.9 mg/L in bronchial mucosa, 2.2 mg/L in epithelial mucosal fluid, and 1.6 mg/L in sputum. Worldwide, azithromycin use has led to considerable stimulation of the development of bacterial and viral infection therapy [93].

Mechanisms of action

The discovery of macrolides with antibacterial, antiviral, and anti-inflammatory properties is a step forward in designing new generations of pharmacological therapies against asthma and exacerbations of chronic obstructive pulmonary disease (COPD) [95].

Antibacterial and antiviral properties

Macrolide antibiotics, such as azithromycin, exhibit bacteriostatic activity by disrupting ribosomal activity during bacterial division and by subsequent interruption of protein synthesis [95].

Macrolides have shown effects against viruses causing respiratory infections, such as influenza virus, rhinovirus, and respiratory syncytial virus (RSV). Azithromycin can prevent extracellular viruses from invading the host cell, but it does not affect the attachment of viruses to the cell surface. Therefore, azithromycin can interrupt the internalization of the virus into the host cells, in the early stage of the infection process. At the same time, due to its unique mechanism of inhibition, azithromycin acts before and after infection with the influenza virus [96, 97].

Azithromycin is also an interesting and promising molecule for therapy against infections with the Zika virus. Moreover, its administration is already approved in humans, including pregnant women. Recent studies confirmed the *in vitro* activity of azithromycin against Zika virus. Because there are no specific drugs or vaccines available for the Zika virus, azithromycin may be the first compound used to prevent and treat infections with this virus, with the benefit of being an approved, safe, usable and pregnancy-friendly drug. In addition, azithromycin also targets the newly developed downstream viruses released from the host cells, inactivating their endocytic penetration capacity [98–100].

Anti-inflammatory properties

Azithromycin has marked immunomodulatory effects, which gives it efficacy in reducing COPD exacerbations. It is known that azithromycin regulates the inflammatory cascade, attenuates excessive cytokine production in viral infections and may limit their exacerbation. Furthermore, azithromycin influences the activity of phagocytes, altering their functions (chemotaxis, phagocytosis, oxidative 'explosion', bactericidal effect, and cytokine production) [101–104].

Toxicity

In vivo tests have shown that azithromycin has a much lower toxicity than erythromycin A: for erythromycin A, median lethal dose (LD_{50}) i.v./*p.o.* is 360/4000 mg/kg, while for azithromycin, LD_{50} i.v./*p.o.* is 825/10 000 mg/kg. Therapy with high-dose macrolides has been correlated with reversible hearing loss. However, the incidence of this long-term treatment effect during low-dose macrolide therapy is not well-characterized [105, 106].

Azithromycin in the prophylaxis and treatment of COVID-19

It is known that azithromycin stops the release of cytokines, which trigger lung inflammation that can kill patients with coronavirus. Azithromycin has also been shown to inhibit the production of viruses, demonstrating that a combination of HCQ and azithromycin has a syner-

gistic *in vitro* effect on SARS-CoV-2 [107]. Azithromycin seems to reduce virus entry into cells. Moreover, it can augment the response of the immune system against viruses through several actions. Azithromycin regulates the production of type I and III IFNs (especially IFN-β and IFN-λ) and genes involved in virus recognition, such as melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I). Azithromycin regulates and/or decreases the production of IL-1β, IL-6, IL-8, IL-10, IL-12 and IFN-α. The inhibitory effect of azithromycin on IL-8 may also be of interest in COVID-19 therapy. Another property of azithromycin is its antibacterial effect, which may be most interesting to prevent or treat co-infections of bacteria and SARS-CoV-2 [108].

So far, a clinical trial has studied azithromycin given in combination with HCQ for the treatment of COVID-19. This combination has a synergistic, strong inhibitory effect on SARS-CoV-2, being recommended as follows: HCQ at a dose of 400 mg ×2/day, on the first day, then 200 mg ×2/day, for a further four days, adding azithromycin at a dose of 500 mg ×1/day, followed by 250 mg ×1/day for the next four days. The HCQ–azithromycin combination can act both as an antiviral therapy in COVID-19 cases and also prophylactically, to counter secondary bacterial infections and the spread of the virus [108].

A recent study in Kerala (India) proved that, in a strictly monitored protocol-driven in-hospital setting, treatment with HCQ alone and HCQ + azithromycin was associated with a significant reduction in mortality among patients hospitalized with COVID-19 [109]. The study showed major gains from HCQ and azithromycin in the first 500 cases. However, the mean age of the patients was 34, while the average age of patients in India is 37 and 63 in Italy. Also, the study had a far smaller proportion of patients with moderate to severe symptoms. In summary, there is currently no preclinical evidence or clinical care results supporting benefits from azithromycin treatment [110].

Additional studies are required. Currently, given the promising results of the above-mentioned pharmacological combination, decision makers at the international level can combat this emerging viral infection in real time, to limit the expansion of COVID-19 globally. Subsequently, other strategies and research, together with vaccine development, may also be efficient, but only after an unknown period of time [81, 82, 111].

Hesperidin: natural candidate molecule for COVID-19 prophylaxis

Hesperidin

Hesperidin (3',5,7-trihydroxy-4'-methoxy-flavanon-7-O-ramnoglucoside) is a polyphenol/flavanone glycoside composed of a hesperetin/aglycone part and a glycosidic part (rutinose – a disaccharide composed of rhamnose and glucose). It was first isolated in 1828 by the French chemist Lebreton from the spongy inner portion (*albedo*) of the orange pericarp (the shells). Hesperidin is also found in the pericarp of lemon and other *Citrus* spp. fruits; the highest amounts were found in sweet orange (15.25± 8.21 mg/100 g fresh fruit of *C. sinensis*) [112, 113].

Pharmacological action

Effects on the cardiovascular system

Hesperidin (as 'vitamin P' of capillary permeability) supplementation has been used in patients with vascular disorders, and this effect has attracted considerable interest in the last decade. Due to its hyaluronidase inhibitory activity, daily administration of hesperidin (30 mg) is recommended to increase capillary resistance [114]. The antihypertensive effects of hesperidin have been extensively studied in various pharmacodynamic models. Short-term administration of hesperidin and its aglycone (hesperetin) to hypertensive rats led to a reduction in dose-dependent systolic blood pressure. In addition, an enalapril–hesperidin combination significantly lowered blood pressure due to nitric oxide-mediated vasodilatation. However, no final conclusions about its clinical applications can be drawn from these studies. Further research should confirm whether the results obtained on animal models can be valid in humans [115].

Anti-hypercholesterolemic properties

Hesperidin significantly diminishes low-density lipoproteins (LDLs), cholesterol, total lipid, and triglyceride levels, beneficially increasing the concentration of high-density lipoproteins (HDLs) [116, 117].

Anti-inflammatory and analgesic properties

Numerous studies have indicated that hesperidin and hesperetin are capable of reducing various inflammatory markers. Thus, *Citrus* spp. fruits consumption is associated with a lower risk of acute coronary events or stroke. Hesperidin has significant anti-inflammatory and analgesic effects; it is especially recommended for patients with hypersensitivity to non-steroidal anti-inflammatory drugs. Although recent research has shown the anti-inflammatory effects of *Citrus* spp. juices, the anti-inflammatory mechanism of hesperidin is not yet fully known; further studies are needed to elucidate the molecular inhibitory mechanisms of hesperidin [118].

Antioxidant properties

Hesperidin exhibits antioxidant effects specific to polyphenols. Along with another polyphenol (diosmin), hesperidin may act as a strong antioxidant; this explains the therapeutic benefits of this combination in chronic venous insufficiency, involving oxidative stress as a pathological mechanism [119].

Antibacterial properties

Hesperidin has a protective role against bacterial and fungal infections, being active *in vitro* against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus hemolyticus*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Salmonella typhi*, *Shigella dysenteriae*, and *S. flexneri* [112].

Antiviral properties

In vitro, in cell culture, hesperidin is active against the bladder stomatitis virus. The antiviral action of hesperidin is mediated by its anti-hyaluronidase effect. At the same time, hesperidin has been proven to be

effective against *Herpes simplex*, influenza, human RSV and poliovirus type 1 (PV1), in animal models. Recently, hesperidin has been proven to have a good effect against rotavirus infections, which cause diarrhea in young children and infants [120].

Toxicity

The LD_{50} for hesperidin was found to be 3000 mg/kg body weight, which is 180 times higher than the daily therapeutic dose. Thus, hesperidin isolated from *Citrus* spp. fruits shows a good safety profile in pharmacodynamic and clinical studies [121].

Hesperidin for COVID-19 prophylaxis

Currently, there are limited antiviral strategies for treating patients that contract COVID-19; the lack of specific antiviral medicines and SARS-CoV-2 vaccines continues to aggravate the situation. Natural compounds are candidates to produce cheaper and safer drugs. Natural product-based antiviral drugs have also been used in two previous CoV outbreaks (MERS-CoV and SARS-CoV); this suggests that nature could offer huge potential for the approach taken against the COVID-19 pandemic. Flavonoids represent a group of polyphenolic compounds well known for their many pharmacological actions (*e.g.*, antiviral activity) [122].

Anti-CoV therapies have as main pharmacological mechanisms: (*i*) The prevention of viral RNA synthesis, by acting on the genetic material of SARS-CoV-2; (*ii*) Inhibition of viral replication by acting on critical SARS-CoV-2 enzymes; (*iii*) Blocking the virus from binding to the receptors of human cells; (*iv*) Inhibition of the viral process of assembly and infection, by acting on structural proteins [52, 122–124].

In a few mathematical simulations, *in silico*, hesperidin exhibited the highest binding energy to the 'S' active site of SARS-CoV-2 compared to other natural or synthetic compounds by disrupting the interaction with ACE2 (in fact this enzyme is the virus receptor on the host cell) with the receptor-binding domain (RBD) of the virus. The ACE2 enzyme has been proven to be a receptor for SARS-CoV-2, interacting with the viral 'S' glycoprotein on the 'S1' domain. Thus, to facilitate therapeutic interventions, the SARS-CoV-2 'S' glycoprotein may be considered a selective target, and the action of hesperidin targeting the binding of the virus 'S' RBD protein to the human ACE2 enzyme may block infection. Also, recent *in silico* research suggests that hesperidin may bind to three key proteins of the SARS-CoV-2: the 'S' surface glycoprotein, the extracellular peptidase (PD-ACE2) and M^{pro} or $3CL^{pro}$ (SARS CoV-2 protease, responsible for viral replication). Four other flavonoids isolated from *Citrus* spp. fruits (tangeretin, hesperetin, nobiletin and naringenin), which are abundantly found in orange and lemon pericarp, have a remarkable affinity for the receptors of the three key proteins of SARS-CoV-2. This fact suggests that all of the natural flavonoids isolated from *Citrus* spp. fruits may contribute to the inhibition of viral infection and replication [62, 123–126].

These studies indicate that polyphenols may yield a noticeable and well-demonstrated action against coronaviruses, at least *in vitro*, in addition to their previously established antiviral activity *in vivo*. The research proves that the main mechanisms of action underlying this promising effect of polyphenols are the reduction of SARS-CoV-2 titer and the inhibition of nucleocapsid protein expression. Considering their anti-inflammatory effects, the polyphenols could be used for the treatment of respiratory complications of COVID-19. Proving the antiviral effect of polyphenols in humans requires a randomized controlled clinical trial with measurable, reproducible, and relevant clinical results. Other flavonoids, coexisting with hesperidin in *Citrus* peels, have also shown good binding to one or more targets, especially hesperetin (hesperidin aglycone) and naringin. The latter flavonoid also showed the ability to restrict the pro-inflammatory overreaction of the immune system, which could help fight severe forms of COVID-19 [127].

Rosocyanin: a promising boron–curcumin candidate compound for COVID-19 prophylaxis

Background

Curcumin (antibacterial and antiviral) activity has been studied by various scientists. Curcumin has been proven to be effective against numerous human pathogens, such as: *Staphylococcus* spp., *Pseudomonas* spp. and *Streptococcus* spp., the hepatitis C virus, influenza virus and HIV. Curcumin alleviates the lung inflammation and macrophage activation generated by influenza virus by restricting the nuclear factor-kappa B (NF-κB) signaling pathway. Despite its impressive potential, curcumin has not yet been recognized as an antiviral therapeutic drug. The specific interaction of curcumin with integrase and protease, viral proteins playing central roles in virus replication, might represent the essential mechanism for this antiviral potential [63]. On the other hand, recently, the HIV protease was tested with a boronic compound showing sub-picomolar effects two orders of magnitude stronger than darunavir (the current HIV-approved protease inhibitor) [128].

Recently, *in silico*, it has also been shown that curcumin together with HCQ can destabilize the structural integrity of SARS-CoV-2 surface proteins, resulting in a proposal for *in vivo* combined therapy of curcumin together with HCQ [129].

Over the years, curcumin solutions or turmeric extracts have been used as coloring reagents in order to assess the amount of boron in the soil, water and foods [130].

Curcumin could be found in two tautomeric conformations (keto- and enol-), with many *cis* and *trans* isomers that may vary depending on the pH, solvent polarity, temperature, and aromatic rings substitution. The above-mentioned conformations play a very important role in the curcumin antioxidant activities, as well as in their physical and chemical properties [131]. Curcumin is insoluble in water at neutral pH, so it has a low bioavailability, and it also decomposes and/or crystallizes in acidic and alkaline pH solutions. When it is taken orally, most of the curcumin never reaches the blood, being scarcely absorbed from the gut, is rapidly metabolized and excreted in the feces.

Rosocyanin

When combining curcumin with boric acid, its esters form a complex called rosocyanin, which is regularly used to assess the amount of boron in various organic and inorganic matrices [132].

The use of rosocyanin for the development of antibacterial, anticancer, and antifungal drugs has led to the increase of a boron presence in the pharmaceutical industry [133].

At pH 2, rosocyanin proves a charged structure because of the two OH^+ functional groups of the benzene ring, whereas at pH 3–5 it has a nonionic structure [134].

Using the boron-based compounds reaction with curcumin, we enhanced its *in vivo* stability without bioactivity loss (*i.e.*, antiproliferation and antioxidant effects). Recently, one study showed that a bortezomib drug co-administered with curcumin has synergistic and therapeutic activity for multiple myeloma treatment. Furthermore, the combination of 2-aminoethyl diphenyl borate with curcumin was shown to increase the *in vitro* and *in vivo* stability of curcumin [135].

Rosocyanin restricts HIV-1 and HIV-2 protease activity. Simple changes of the curcumin structure unfavorably raised its half-maximal inhibitory concentration (IC_{50}) value. However, the reaction of the central dihydroxy groups of curcumin with the boron atom favorably reduced the IC_{50} to a level of 6 μM by activation of the *α,β*-unsaturated carbonyl functional group of curcumin. Subsequently, the HIV-1 and HIV-2 inhibition was increased more than 10-fold when rosocyanin was used. These effects show that the curcumin moiety is very important to enhance the rosocyanin biological activity [64].

A recent *in silico* analysis, by using molecular docking, showed that curcumin can inhibit SARS-CoV-2 in a range of 3–10 μM. This concentration is similar to that of nelfinavir (an important antiviral drug) [136].

As a result, boron compounds with curcumin began to be increasingly investigated, discovering remarkable biological properties (antitumor, antiviral, antioxidant, and antithrombotic). It is believed that in the future these compounds could be used as drugs in the prophylaxis of pandemic viral attacks, such as SARS-CoV-2, as well as to improve blood circulation in the body. Considering the scientific arguments presented above and also the ability of curcumin to form boron complexes, several boron-based compounds could be prepared for the prevention of COVID-19 [137].

Conclusions and future perspectives

There is insufficient preclinical and clinical evidence on HCQ effectiveness combined with azithromycin in COVID-19 treatment. Presently, there are a few scientific data that clarify how to prevent SARS-CoV-2 infection, but some conclusions can be drawn to support the positioning of azithromycin as a new active substance in the fight against COVID-19: (*i*) Macrolides in general and azithromycin in particular are molecules with important anti-inflammatory, antibacterial and antiviral action; (*ii*) The HCQ–azithromycin combination represents a pharmacological therapy that should be urgently studied; (*iii*) In the future, azithromycin should be considered for ambulatory administration, both in children and adults, for prophylactic purposes, in order to prevent COVID-19. Moreover, *Citrus* spp. fruits could be approached as the best natural resources for the prophylaxis and treatment of SARS-CoV-2 infection. Hesperidin is available as a dietary supplement, a natural compound isolated from the sweet orange pericarp (*C. sinensis*). Because it "sticks" to the viral 'S' glycoprotein and interferes with its replication, hesperidin inhibits SARS-CoV-2. Hesperidin may also be used as an adjuvant in the treatment of high blood pressure. As well, rosocyanin, which actually is a curcumin–boron complex, has been shown to be an extremely effective inhibitor of viral proteases (HIV1 and HIV2) [64] and in addition a potent chelator of free iron [134]. It is believed that in the future these compounds could be used as drugs for the prophylaxis of pandemic viral attacks, such as SARS-CoV-2.

Conflict of interests

The authors declare that they have no conflict of interests.

Ethical standards

The manuscript does not contain clinical studies or patient data.

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