# Therapeutic Preferences for Coronavirus 2(SARS-CoV-2) Patients

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**Research Article**

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# Therapeutic Preferences for Coronavirus 2 (SARS-CoV-2) Patients

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**ABSTRACT:**

**Background:**

Modern researches have focused the attention towards the potential advantage of chloroquine, a broadly used antimalarial drug, in the treatment of patients infected by the novel appeared coronavirus (SARS-CoV-2). Chloroquine/hydroxychloroquine has been frequently used in treating SARS-CoV-2 infection. Which is useful in controlling the cytokine storm that occurs late-phase in critically ill SARS-CoV-2 infected patients. The scientific community should consider this information in light of previous experiments with Chloroquine/hydroxychloroquine in the field of antiviral research.

**Methods:**

In the view of current situation efforts of international health professionals have since focused on rapid diagnosis and isolation of patients as well as the search for therapies able to counter the most severe effects of the disease. It is mandatory to investigate the possible effect of chloroquine/hydroxychloroquine against SARS-CoV-2. Since this molecule was previously described as a potent inhibitor of most coronaviruses, including SARS-CoV-1. Preliminary trials of chloroquine repurposing in the treatment of COVID-19 in China have been encouraging, leading to several new trials. Here we discuss the possible mechanisms of chloroquine interference with the SARS-CoV-2 replication cycle.

**Results:**

Chloroquine has been shown to be capable of inhibiting the in vitro replication of several coronaviruses. Recent publications support the hypothesis that chloroquine/hydroxychloroquine can improve the clinical outcome of patients infected by SARS-CoV-2.

**Keywords:** SARS-CoV-2, COVID-19, 2019-nCoV, Antiviral, Hydroxychloroquine.

## Background

Severe acute respiratory syndrome (SARS) is caused by a newly discovered coronavirus (SARS-CoV). No effective prophylactic or post-exposure therapy is currently available. Severe acute respiratory syndrome (SARS) is an emerging disease that was first reported in Guangdong Province, China, in late 2002. The disease rapidly spread to at least 30 countries within months of its first appearance, and concerted worldwide efforts led to the identification of the etiological agent as SARS coronavirus (SARS-CoV), a novel member of the family *Coronaviridae*. Complete genome sequencing of SARS-CoV confirmed that this pathogen is not closely related to any of the previously established coronavirus groups. Budding of the SARS-CoV occurs in the Golgi apparatus and results in the incorporation of the envelope spike glycoprotein into the virion. Due to the severity of SARS-CoV infection, the potential for rapid spread of the disease, and the absence of proven effective and safe *in vivo* inhibitors of the virus, it is important to identify drugs that can effectively be used to treat or prevent potential SARS-CoV infections. Many novel therapeutic approaches have been evaluated in laboratory studies of SARS-CoV: notable among these approaches are those using RNA, passive antibody transfer, DNA vaccination, vaccinia or parainfluenza virus expressing the spike protein, interferons, and monoclonal antibody to the S1-subunit of the spike glycoprotein that blocks receptor binding. This report, we describe that identification of chloroquine as an effective pre- and post-infection antiviral agent for SARS-CoV.

## Introduction

Antimalarial drugs with the 4-aminoquinoline scaffold such as the important drugs, chloroquine and hydroxychloroquine, have been used to prevent and treat malaria for many years. Chloroquine is an amine acidotropic form of quinine that was synthesized in Germany by Bayer in 1934 and emerged approximately 70 years ago as an effective substitute for natural quinine [1,2]. Quinine is a compound found in the bark of *Cinchona* trees native to Peru and was the previous drug of choice against malaria [3]. For decades, chloroquine was a front-line drug for the treatment and prophylaxis of malaria and is one of the most prescribed drugs worldwide [4]. Chloroquine and the 4-aminoquinoline drug hydroxychloroquine belong to the same molecular family. Hydroxychloroquine differs from chloroquine by the presence of a hydroxyl group at the end of the side chain: the *N*-ethyl substituent is β-hydroxylated. This molecule is available for oral administration in the form of hydroxychloroquine sulfate. Hydroxychloroquine has

pharmacokinetics similar to that of chloroquine, with rapid gastrointestinal absorption and renal elimination. However, the clinical indications and toxic doses of these drugs slightly differ. In malaria, the indication for chloroquine was a high dose for a short period of time (due to its toxicity at high doses) or a low dose for a long period of time. Hydroxychloroquine was reported to be as active as chloroquine against *Plasmodium falciparum* malaria and less toxic, but it is much less active than chloroquine against chloroquine-resistant *P. falciparum* owing to its physicochemical properties. What is advantageous with hydroxychloroquine is that it can be used in high doses for long periods with very good tolerance. Unfortunately, the efficacy of chloroquine gradually declined due to the continuous emergence of chloroquine-resistant *P. falciparum* strains [5]. Chloroquine is also utilised in the treatment of autoimmune diseases [6]. Yet the activity of the molecule is not limited to malaria and the control of inflammatory processes, as illustrated by its broad-spectrum activity against a range of bacterial, fungal and viral infections [7–10]. Indeed, in the mid-1990s, due to its tolerability, rare toxicity reports, inexpensive cost and immunomodulatory properties [11], chloroquine repurposing was explored against human immunodeficiency virus (HIV) and other viruses associated with inflammation and was found to be efficient in inhibiting their replication cycle [12]. Recently, a novel coronavirus emerged in the Chinese city of Wuhan in December 2019. After human coronavirus 229E (HCoV-229E) (classified in the genus *Alphacoronavirus*) and HCoV-OC43 (*Betacoronavirus* lineage 2a member) described in the 1960s, SARS-CoV-1 (*Betacoronavirus* lineage 2b member) that emerged in March 2003, HCoV-NL63 (*Alphacoronavirus* lineage 1b member) described in 2004, HCoV-HKU1 (*Betacoronavirus* lineage 2a member) discovered in 2005, and finally MERS-CoV that emerged in 2012 (classified in *Betacoronavirus* lineage 2c), the novel coronavirus is the seventh human coronavirus described to date as being responsible for respiratory infection. Evidence was rapidly reported that patients were suffering from an infection with a novel *Betacoronavirus* tentatively named 2019 novel coronavirus (2019-nCoV) [13,14]. Despite drastic containment measures, the spread of 2019-nCoV, now officially known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is ongoing. Phylogenetic analysis of this virus indicated that it is different (80% nucleotide identity) but related to SARS-CoV-1 [15]. Because the world is threatened by the possibility of a SARS-CoV-2 pandemic, the broad-spectrum antiviral effects of chloroquine warranted particular attention for repurposing this drug in the therapy of the disease caused by SARS-CoV-2, named coronavirus disease 2019 (COVID-19).

## Antiviral Role of Chloroquine

In vitro, chloroquine appears as a versatile bioactive agent reported to possess antiviral activity against RNA viruses as diverse as rabies virus [16], poliovirus [17], HIV [12,18–20], hepatitis A virus [21,22], hepatitis C virus [23], influenza A and B viruses [24–27], influenza A H5N1 virus [28], Chikungunya virus [29–31], Dengue virus [32,33], Zika virus [34], Lassa virus [35], Hendra and Nipah viruses [36,37], Crimean–Congo hemorrhagic fever virus [38] and Ebola virus [39], as well as various DNA viruses such as hepatitis B virus [40] and herpes simplex virus [41].The antiviral properties of chloroquine described in vitro have sometimes been confirmed during treatment of virus-infected patients but have not always been reproduced in clinical trials depending on the disease, the concentration of chloroquine used, the duration of treatment and the clinical team in charge of the trial. Regarding coronaviruses, the potential therapeutic benefits of chloroquine were notably reported for SARS-CoV-1 [11,42]. Chloroquine was also reported to inhibit in vitro the replication of HCoV-229E in epithelial lung cell cultures [43,44]. In 2009, it was reported that lethal infections of newborn mice with the HCoV-O43 coronavirus could be averted by administering chloroquine through the mother's milk. In vitro experiments also showed a strong antiviral effect of chloroquine on a recombinant HCoV-O43 coronavirus [45]. Although chloroquine was reported to be active against Middle East respiratory syndrome coronavirus (MERS-CoV) in vitro [46], this observation remains controversial [47].

## Action of chloroquine against SARS-CoV-2

Because of its broad spectrum of action against viruses, including most coronaviruses and particularly its close relative SARS-CoV-1, and because coronavirus cell entry occurs through the endolysosomal pathway [48], it made sense in a situation of a public-health emergency and the absence of any known efficient therapy to investigate the possible effect of chloroquine against SARS-CoV2. A recent paper reported that both chloroquine and the antiviral drug remdesivir inhibited SARS-CoV-2 in vitro and suggested these drugs be assessed in human patients suffering from COVID-19 [49]. Recently, the China National Center for Biotechnology Development indicated that chloroquine is one of the three drugs with a promising profile against the new SARSCoV-2 coronavirus that causes COVID-19. Chloroquine repurposing was investigated in hospitals in Beijing, in central China's Hunan Province and South China's Guangdong Province. According to preliminary reports [50,51] from the Chinese authorities suggesting that

approximately 100 infected patients treated with chloroquine experienced a more rapid decline in fever and improvement of lung computed tomography (CT) images and required a shorter time to recover compared with control groups, with no obvious serious adverse effects, the Chinese medical advisory board has suggested chloroquine inclusion in the SARS-CoV-2 treatment guidelines. As a result, chloroquine is probably the first molecule to be used in China and abroad on the front line for the treatment of severe SARS-CoV-2 infections. Although the long use of this drug in malaria therapy demonstrates the safety of acute chloroquine administration to humans, one cannot ignore the minor risk of macular retinopathy, which depends on the cumulative dose [52], and the existence of some reports on cardiomyopathy as a severe adverse effect caused by chloroquine [53,54]. A survey of SARS-CoV-2-infected patients for adverse effects of chloroquine therapy remains to be performed. However, chloroquine is currently among the best available candidates to impact the severity of SARS-CoV-2 infections in humans. Currently, at least ten clinical trials are testing chloroquine as an anti-COVID-19 therapy [55].

**Mechanism of action of Chloroqunion**

Chloroquine has multiple mechanisms of action that may differ according to the pathogen studied. Chloroquine can inhibit a pre-entry step of the viral cycle by interfering with viral particles binding to their cellular cell surface receptor. Chloroquine was shown to inhibit quinone reductase 2 [56], a structural neighbour of UDP-*N*-acetylglucosamine 2-epimerases [57] that are involved in the biosynthesis of sialic acids. The sialic acids are acidic monosaccharides found at the extremity of sugar chains present on cell transmembrane proteins and are critical components of ligand recognition. The possible interference of chloroquine with sialic acid biosynthesis could account for the broad antiviral spectrum of that drug since viruses such as the human coronavirus HCoV-O43 and the orthomyxoviruses use sialic acid moieties as receptors [58]. The potent anti-SARS-CoV-1 effects of chloroquine in vitro were considered attributable to a deficit in the glycosylation of a virus cell surface receptor, the angiotensin-converting enzyme 2 (ACE2) on Vero cells [59]. Chloroquine can also impair another early stage of virus replication by interfering with the pH-dependent endosome-mediated viral entry of enveloped viruses such as Dengue virus or Chikungunya virus [60,61]. Due to the alkalisation of endosomes, chloroquine was an effective in vitro treatment against Chikungunya virus when added to Vero cells prior to virus exposure [30]. The mechanism of inhibition likely involved the prevention of endocytosis and/or rapid elevation

of the endosomal pH and abrogation of virus–endosome fusion. A pH-dependant mechanism of entry of coronavirus into target cells was also reported for SARS-CoV-1 after binding of the DC-SIGN receptor [62]. The activation step that occurs in endosomes at acidic pH results in fusion of the viral and endosomal membranes leading to the release of the viral SARS-CoV-1 genome into the cytosol [63]. In the absence of antiviral drug, the virus is targeted to the lysosomal compartment where the low pH, along with the action of enzymes, disrupts the viral particle, thus liberating the infectious nucleic acid and, in several cases, enzymes necessary for its replication [64]. Chloroquine mediated inhibition of hepatitis-A virus was found to be associated with uncoating, thus blocking its entire replication cycle [22]. Chloroquine can also interfere with the post-translational modification of viral proteins. These post-translational modifications, which involve proteases and glycosyltransferases, occur within the endoplasmic reticulum or the trans-Golgi network vesicles and may require a low pH. For HIV, the antiretroviral effect of chloroquine is attributable to a post-transcriptional inhibition of glycosylation of the gp120 envelope glycoprotein, and the neosynthesised virus particles are noninfectious [19,65]. Chloroquine also inhibits the replication Dengue-2 virus by affecting the normal proteolytic processing of the flavivirus prM protein to M protein [32]. As a result, viral infectivity is impaired. In the herpes simplex virus (HSV) model, chloroquine inhibited budding with accumulation of non-infectious HSV-1 particles in the trans-Golgi network [66]. Using non-human coronavirus, it was shown that the intracellular site of coronavirus budding is determined by the localization of its membrane M proteins that accumulate in the Golgi complex beyond the site of virion budding [67], suggesting a possible action of chloroquine on SARS-CoV-2 at this step of the replication cycle. It was recently reported that the C-terminal domain of the MERS-CoV M protein contains a trans-Golgi network localization signal [68]. Besides affecting the virus maturation process, pH modulation by chloroquine can impair the proper maturation of viral protein [32] and the recognition of viral antigen by dendritic cells, which occurs through a Toll-like receptor-dependent pathway that requires endosomal acidification [69]. On the contrary, other proposed effects of chloroquine on the immune system include increasing the export of soluble antigens into the cytosol of dendritic cells and the enhancement of human cytotoxic $CD8^+$ Tcell responses against viral antigens [70]. In the influenza virus model, it was reported that chloroquine improve the cross-presentation of non-replicating virus antigen by dendritic cells to $CD8^+$ T-cells recruited to lymph nodes draining the site of infection, eliciting a broadly protective immune response [71]. Chloroquine can also act

on the immune system through cell signalling and regulation of pro-inflammatory cytokines. Chloroquine is known to inhibit phosphorylation (activation) of the p38 mitogen-activated protein kinase (MAPK) in THP-1 cells as well as caspase-1 [72]. Activation of cells via MAPK signalling is frequently required by viruses to achieve their replication cycle [73]. In the model of HCoV-229 coronavirus, chloroquine-induced virus inhibition occurs through inhibition of p38 MAPK [44]. Chloroquine is a well-known immunomodulatory agent capable of mediating an anti-inflammatory response [11]. Therefore, there are clinical applications of this drug in inflammatory diseases such as rheumatoid arthritis [74– 76], lupus erythematosus [6,77] and sarcoidosis [78]. Chloroquine inhibits interleukin-1 beta (IL-1β) mRNA expression in THP-1 cells and reduces IL-1β release [72]. Chloroquine-induced reduction of IL-1 and IL-6 cytokines was also found in monocytes/macrophages [79]. Chloroquine-induced inhibition of tumor necrosis factor-alpha (TNFα) production by immune cells was reported to occur either through disruption of cellular iron metabolism [80], blockade of the conversion of pro-TNF into soluble mature TNFα molecules [81] and/or inhibition of TNFα mRNA expression [72,82,83]. Inhibition of the TNFα receptor was also reported in U937 monocytic cells treated with chloroquine [84]. In the Dengue virus model, chloroquine was found to inhibit interferon-alpha (IFNα, IFNβ, IFNγ, TNFα, IL-6 and IL-12 gene expression in U937 cells infected with Dengue-2 virus [33].

**Methods:**

In the view of current situation efforts of international health professionals have since focused on rapid diagnosis and isolation of patients as well as the search for therapies able to counter the most severe effects of the disease. It is mandatory to investigate the possible effect of chloroquine/hydroxychloroquine against SARS-CoV-2. Since this molecule was previously described as a potent inhibitor of most coronaviruses, including SARS-CoV-1. Preliminary trials of chloroquine repurposing in the treatment of COVID-19 in China have been encouraging, leading to several new trials. Chloroquine is effective in preventing the spread of SARS CoV in cell culture. Favorable inhibition of virus spread was observed when the cells were either treated with chloroquine prior to or after SARS CoV infection. In addition, the indirect immunofluorescence assay described herein represents a simple and rapid method for screening SARS-CoV antiviral compounds. Here we discuss the possible mechanisms of chloroquine interference with the SARS-CoV-2 replication cycle.

**Results:**

Chloroquine has strong antiviral effects on SARS-CoV infection of primate cells. Chloroquine has been shown to be capable of inhibiting the in vitro replication of several coronaviruses. Recent publications support the hypothesis that chloroquine/hydroxychloroquine can improve the clinical outcome of patients infected by SARS-CoV-2.

**Discussion**

Modern research have brought attention to the possible advantage of chloroquine, a broadly used antimalarial drug, in the management of patients infected by the novel developed coronavirus (SARS-CoV-2) [85]. The scientific community should consider this evidence in view of previous experiments with Hydroxychloroquine in the area of antiviral research. The sulfate and phosphate salts of chloroquine have both been available as antimalarial drugs. Hydroxychloroquine has also been used as an antimalarial, but in addition is now broadly used in autoimmune diseases such as lupus and rheumatoid arthritis. Although chloroquine and Hydroxychloroquine are considered to be safe and side-effects are generally mild and transitory [86]. Chloroquine and hydroxychloroquine use should therefore be subject to strict rules, and self-treatment is not recommended. The in vitro antiviral activity of hydroxychloroquine has been identified since the late 1960's [87] and the growth of many different viruses can be inhibited in cell culture by both chloroquine and hydroxychloroquine, including the SARS coronavirus [88]. However, chloroquine did not prevent influenza infection in a randomized, double-blind, placebo-controlled clinical trial [89], and had no effect on dengue-infected patient in a randomized controlled trial in Vietnam [90]. Hydroxychloroquine was also active ex vivo but not in vivo in the case of ebolavirus in mice [91], in ferrets. The case of chikungunya virus (CHIKV) is of specific interest: Hydroxychloroquine showed promising antiviral activity in vitro [92], but was shown to enhance alphavirus replication in various animal models, most probably because of the immune modulation and anti-inflammatory properties of Hydroxychloroquine in vivo. In a nonhuman primate model of CHIKV infection, Hydroxychloroquine treatment was shown to aggravate acute fever and delay the cellular immune response, leading to an incomplete viral clearance [93]. A clinical trial conducted during the chikungunya outbreak in 2006 in Reunion Island indicated that oral Hydroxychloroquine treatment did not recover the course of the acute disease and that chronic arthralgia on day 300 post illness was more frequent in treated patients than in the control group

[94]. Overall, the assessment of previous trials indicates that, to date, no acute virus infection has been successfully treated by Hydroxychloroquine in humans. Hydroxychloroquine has also been tested in chronic viral diseases. Its use in the treatment of HIV-infected patients has been considered inconclusive [95] and the drug has not been included in the panel recommended for HIV treatment. Recently FDA-approved drugs and two broad spectrum antivirals against a clinical isolate of SARS-CoV-2. One of their conclusions was that "Hydroxychloroquine (is) highly effective in the control of 2019-nCoV infection in vitro" and that it's "safety track record suggests that it should be evaluated in human patients suffering from the novel coronavirus disease". At least 16 different trials for SARS-CoV-2 already registered to use Hydroxychloroquine or Hydroxychloroquine in the treatment of COVID-19 "Chinese Clinical Trial Register" (ChiCTR). In a recent publication [96], researchers indicate that, "according to the news briefing", "results from more than 100 patients have established that Hydroxychloroquine phosphate is superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus negative conversion, and shortening the disease course". This would represent the first successful practice of Hydroxychloroquine in humans for the treatment of an acute viral disease, and is undoubtedly excellent news, since this drug is cheap and widely available. However, it should be considered carefully before drawing definitive conclusions, since no data has been provided yet to support this declaration. Results were produced in ten different hospitals and possibly from a number of different clinical protocols among those listed above, which include various designs for control groups (none, different antivirals, placebo, etc.) and various outcome primary indicators. The final interpretation is therefore technically demanding, and in the absence of published data, it is difficult to reach any secure conclusion. It will be of the greatest importance to know if the experiential efficacy is associated specifically with Hydroxychloroquine phosphate, or if this includes other salts (e.g., sulfate) of Hydroxychloroquine, and hydroxychloroquine. It is also compulsory to determine if the benefit of hydroxychloroquine therapy depends on the age class, the clinical presentation or the stage of the disease. In conclusion, the option of using hydroxychloroquine in the treatment of SARS-CoV-2 should be observed with attention in light of the recent promising announcements, but also of the potential detrimental effect of the drug detected in previous attempts to treat acute viral diseases.

## CONCLUSION:

Chloroquine has been shown to be capable of inhibiting the in vitro replication of several coronaviruses. Recent publications support the hypothesis that chloroquine and hydroxychloroquine can improve the clinical outcome of patients infected by SARS-CoV-2. The multiple molecular mechanisms by which chloroquine can achieve such results remain to be further explored. Since SARS-CoV-2 was found a few days ago to utilize the same cell surface receptor ACE2 (expressed in lung, heart, kidney and intestine) as SARSCoV-1 [96,97] (Table 1), it may be hypothesised that chloroquine also interferes with ACE2 receptor glycosylation thus preventing SARS-CoV-2 binding to target cells. Wang and Cheng reported that SARS-CoV and MERS-CoV upregulate the expression of ACE2 in lung tissue, a process that could accelerate their replication and spread [96]. Although the binding of SARS-CoV to sialic acids has not been reported so far (it is expected that *Betacoronavirus* adaptation to humans involves progressive loss of hemagglutinin-esterase lectin activity), if SARS-CoV-2 like other coronaviruses targets sialic acids on some cell subtypes, this interaction will be affected by chloroquine treatment [98,99]. Today, preliminary data indicate that chloroquine interferes with SARS-CoV-2 attempts to acidify the lysosomes and presumably inhibits cathepsins, which require a low pH for optimal cleavage of SARS-CoV-2 spike protein [100], a prerequisite to the formation of the autophagosome [60]. Obviously, it can be hypothesized that SARS-CoV-2 molecular crosstalk with its target cell can be altered by chloroquine through inhibition of kinases such as MAPK. Chloroquine could also interfere with proteolytic processing of the M protein and alter virion assembly and budding (Fig. 1). Finally, in COVID-19 disease this drug could act indirectly through reducing the production of proinflammatory cytokines or by activating anti-SARS-CoV-2 $CD8^+$ T-cells. As the world's heath experts race to find treatments and eventually, a cure for the novel coronavirus, two drugs have jumped to the front of the conversation chloroquine and hydroxychloroquine. The side effects include seizures, nausea, vomiting, deafness, vision changes and low blood pressure. Hydroxychloroquine has very noteworthy advantages as a prime candidate for antiviral prophylaxis against the current COVID-19 pandemic where no current vaccine or antiviral prophylaxis is in place. Its established mechanisms of action of preventing viral entry and fusion, sign of in vitro efficacy at clinically recommended doses, high tissue concentration as well as preliminary clinical proof of efficacy as treatment all provision its promising preventative part. Its safety record and low cost at doses we suggest indicate a high potential advantage to risk and

benefit to cost ratio when used for prophylaxis. We need relevant agencies to consider initiating trials as well as prepare for direct mass distribution of a hydroxychloroquine based COVID-19 preventative program without undue delay. One drug, lopinavir-ritonavir, did not show promise for treating Covid-19-related pneumonia in China. But another drug Remdesivir, has "broad antiviral activity,"

## Abbreviations

**SARS**: Severe acute respiratory syndrome
**CoV-2**: Coronavirus type 2
**COVID**: Coronavirus disease
**nCoV**: Novel Coronavirus
**P. falciparum**: Plasmodium falciparum
**HIV**: Human immunodeficiency virus
**HKU1**: Human coronavirus 1
**MERS**: Middle East Respiratory Syndrome
**UDP**: Uridine diphosphate
**ACE2**: Angiotensin-converting enzyme 2
**HSV**: Herpes simplex virus
**MAPK**: Mitogen-activated protein kinase
**TNF**: Tumor necrosis factor
**CHIKV**: Chikungunya virus

- **DECLERATION SECTION**

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# Figures

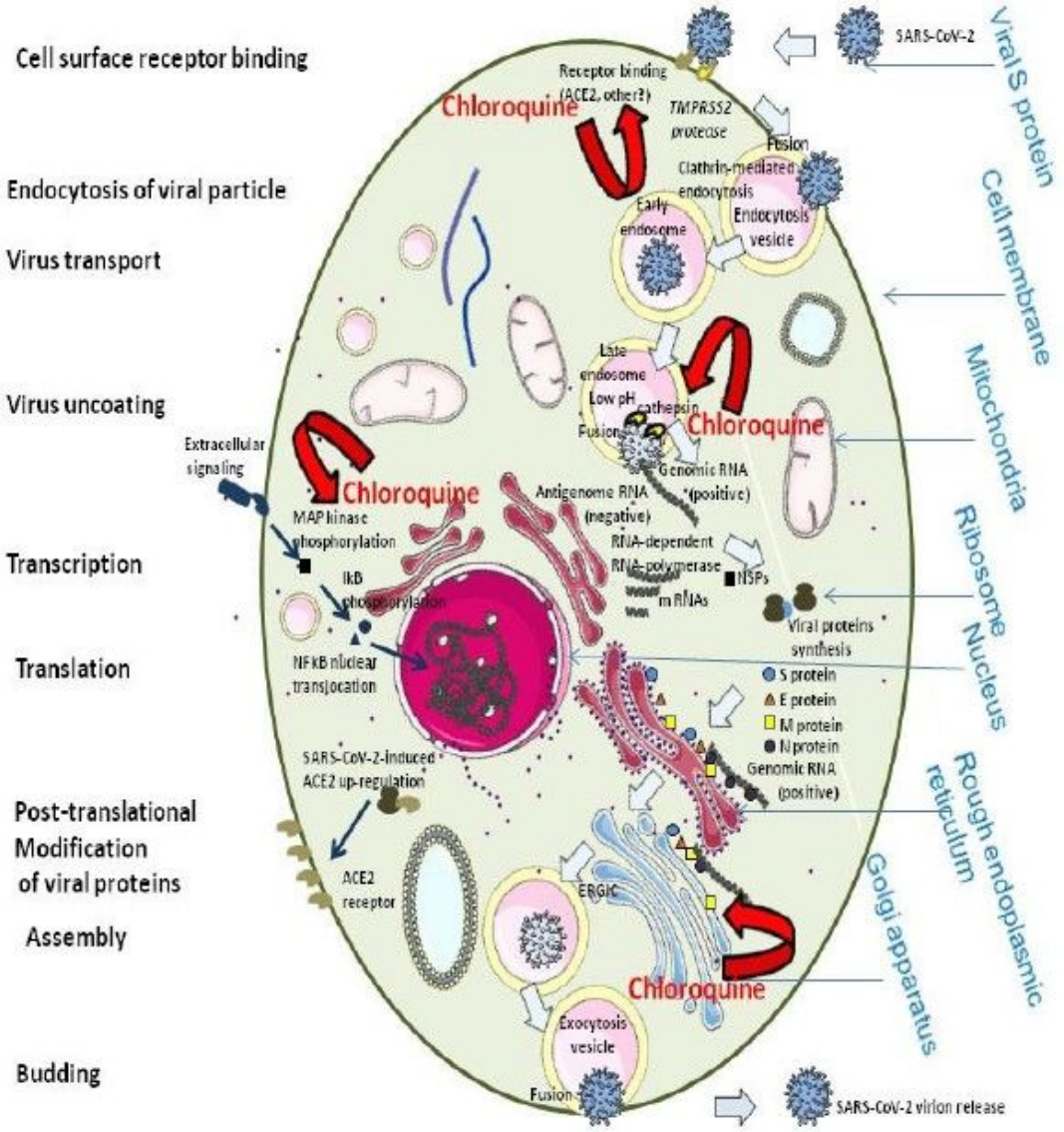


**Figure 1**

Schematic representation of the possible effects of chloroquine on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication cycle. SARSCoV2, like other human coronaviruses, harbours three envelope proteins, the spike (S) protein (180–220 kDa), the membrane (M) protein (25–35 kDa) and the envelope (E) protein (10–12 kDa), which are required for entry of infectious virions into target cells. The virion also contains the nucleocapsid (N), capable of binding to viral genomic RNA, and nsp3, a key component of the replicase complex. A subset of betacoronaviruses use a hemagglutinin-esterase (65 kDa) that binds sialic acids at the surface of glycoproteins. The S glycoprotein determines the host tropism. There is indication that SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) expressed on pneumocytes [110]. Binding to ACE2 is expected to trigger conformational changes in the S glycoprotein allowing cleavage by the transmembrane protease TMPRSS2 of the S protein and the

release of S fragments into the cellular supernatant that inhibit virus neutralisation by antibodies [111]. The virus is then transported into the cell through the early and late endosomes where the host protease cathepsin L further cleaves the S protein at low pH, leading to fusion of the viral envelope and phospholipidic membrane of the endosomes resulting in release of the viral genome into the cell cytoplasm. Replication then starts and the positive-strand viral genomic RNA is transcribed into a negative RNA strand that is used as a template for the synthesis of viral mRNA. Synthesis of the negative RNA strand peaks earlier and falls faster than synthesis of the positive strand. Infected cells contain between 10 and 100 times more positive strands than negative strands. The ribosome machinery of the infected cells is diverted in favour of the virus, which then synthesises its non-structural proteins (NSPs) that assemble into the replicase-transcriptase complex to favour viral subgenomic mRNA synthesis (see the review by Fehr and Perlman for details [112]). Following replication, the envelope proteins are translated and inserted into the endoplasmic reticulum and then move to the Golgi compartment. Viral genomic RNA is packaged into the nucleocapsid and then envelope proteins are incorporated during the budding step to form mature virions. The M protein, which localises to the trans-Golgi network, plays an essential role during viral assembly by interacting with the other proteins of the virus. Following assembly, the newly formed viral particles are transported to the cell surface in vesicles and are released by exocytosis. It is possible that chloroquine interferes with ACE2 receptor glycosylation, thus preventing SARS-CoV-2 binding to target cells. Chloroquine could also possibly limit the biosynthesis of sialic acids that may be required for cell surface binding of SARS-CoV-2. If binding of some viral particles is achieved, chloroquine may modulate the acidification of endosomes thereby inhibiting formation of the autophagosome. Through reduction of cellular mitogen-activated protein (MAP) kinase activation, chloroquine may also inhibit virus replication. Moreover, chloroquine could alter M protein maturation and interfere with virion assembly and budding. With respect to the effect of chloroquine on the immune system, see the elegant review by Savarino et al. [22]. ERGIC, ER

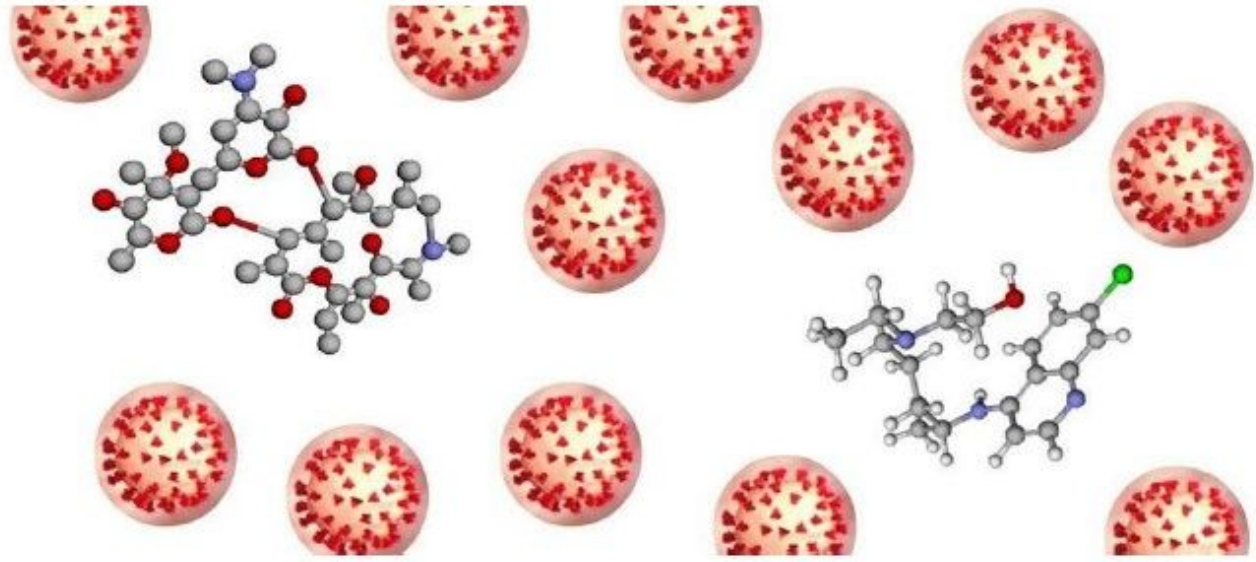

**Figure 2**

Hydroxychloroquine and azithromycin make SARS-CoV-2 undetectable in French clinical trial

# Supplementary Files

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