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REVIEW

Chloroquine and hydroxychloroquine in the context of COVID-19

Ashutosh M Shukla MD[1,2], Lennox K Archibald MD[1,2], Aparna Wagle Shukla MD[3], Hiren J Mehta MD[2], Kartikeya Cherabuddi MD[2]

[1]North Florida South Georgia VHS, Gainesville, FL, USA; [2]Department of Medicine, University of Florida, Gainesville, FL, USA; [3]Department of Neurology, University of Florida, Gainesville, FL, USA

Abstract

Chloroquine and closely related structural analogs, employed initially for the treatment of malaria, are now gaining worldwide attention due to the rapidly spreading pandemic caused by severe acute respiratory syndrome-coronavirus-2, named coronavirus disease (COVID) of 2019 (COVID-19). Although much of this attention has a mechanistic basis, the hard efficacy data for chloroquine/hydroxychloroquine in the management of the clinical syndrome of COVID-19 have been limited thus far. This review aims to present the available *in vitro* and clinical data for the role of chloroquine/hydroxychloroquine in COVID-19 and attempts to put them into perspective, especially in relation to the different risks/benefits particular to each patient who may require treatment.

Keywords: anti-inflammatory, antimalarial, antiviral, chloroquine, COVID-19, hydroxychloroquine, immunomodulatory, pandemic, SARS-CoV-2.

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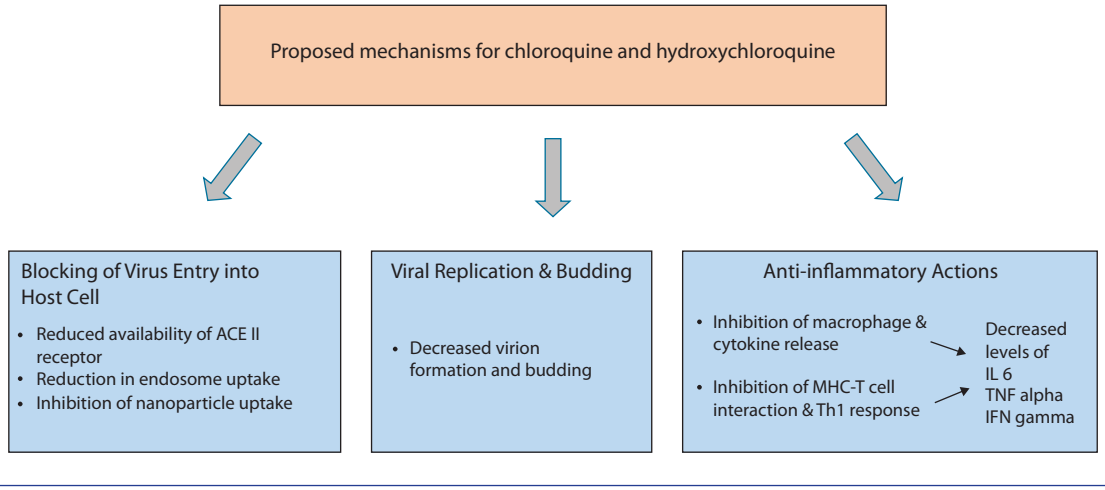
Chloroquine and related drugs were initially developed as antimalarial agents. During the Second World War, many clinicians made serendipitous observations that these drugs could be beneficial for treating rheumatological and dermatological conditions.[1–3] Since then, several well-designed studies have established their efficacy in the chronic management of connective tissue disorders, including systemic lupus erythematosus and rheumatoid arthritis,[4] and there has been a growing list of indications to support their therapeutic potential in varied diseases of oncology, cardiology, and nephrology.[1] These agents have also shown a promising role in viral infections, and with the recent declaration on March 12th, 2020, by the World Health Organization that coronavirus disease (COVID) of 2019 (COVID-19) is a pandemic, these compounds have rapidly gained worldwide attention for their ability to control the causative virus, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). In this narrative review, the authors discuss these medications from a mechanistic perspective with specific insights into their potential role in controlling viral infections. We discuss *in vitro* and *in vivo* evidence for their emerging therapeutic role in COVID-19 with due concerns to safety when applying the known facts to large populations increasingly affected with COVID-19.

Mechanistic rationale

Both chloroquine and hydroxychloroquine (HCQ) are weak bases that exist in the extracellular environment mostly in a protonated form with a positive charge. This positive charge makes them incapable of crossing the plasma membrane. The non-protonated portion that enters a cell is quickly protonated and concentrated in the acidic, low-pH organelles such as endosomes, Golgi vesicles, and lysosomes.[5] The antimalarial actions of these compounds are related to a heavy accumulation of these drugs in the acidic lysosomes of the parasites, which leads to a neutralizing, 'lysosomotropic' effect that prevents the detoxification of ingested 'heme' moiety, resulting in lysis of the malarial parasite.[1] Additionally, investigators have identified a series of immunomodulatory and anti-inflammatory effects for these agents.[1] These include inhibition of ligand-based toll-like receptor stimulation, inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) pathways in macrophages with resultant reduction in the generation of pro-inflammatory cytokines, reduced processing of the endogenous and exogenous ligands through lysosomes and endosomes with resultant reduction in the availability of processed antigens for presentation to the major

Figure 1. Hypothetical immunological and antiviral model.



histocompatibility complex–T cell receptor interactions, and downstream activation of cellular immunity.[6–9] Together, these properties lay the foundation of their use in several rheumatological, cardiovascular, and dermatological diseases.

These pleiotropic actions, seen in a variety of chronic diseases, have also guided their exploration for the control of viral infections. To date, these agents have been explored in Ebola virus disease,[10] human immunodeficiency virus (HIV) infection,[11,12] Middle East Respiratory Syndrome (MERS),[13] and SARS-CoV-1 infection,[5,14] and their promising action against SARS-CoV-1 has provided the basis for their putative benefits in treating SARS-CoV-2 infection.[15] However, the exact mechanism by which chloroquine/HCQ may be of benefit in COVID-19 is largely speculative and appears to be related to a series of actions demonstrated in alternate, yet similar, disease models (Figure 1).

HCQ is known to have significant effects on many mechanisms that drive the viral entry into the host cells. Most prominent among them are its actions on the angiotensin converting enzyme (ACE) II receptors. The original experiments during the SARS epidemic suggested that SARS-CoV-1 binds to ACE II receptors, primarily present in the lung, heart, kidney, and intestine for its entry into the host system. More specifically, SARS-CoV-1 binds to the sialic acid moiety of the ACE II receptors.[15,16] Chloroquine inhibits the intracellular glycosylation of the ACE II, and thus inhibits the addition of sialic acid moiety, which then leads to reduced ligand recognition and internalization of the virus.[17] Phylogenetic analysis of SARS-CoV-2 has shown about 80% nucleotide homology with SARS-CoV-1,[18] prompting the evaluation of these drug compounds for COVID-19. Once the virus is bound to the cell membrane, endosomes play an important role in the fusion of viral particles and their internalization. Thus, neutralization of the acidic pH of the endosome by chloroquine or HCQ may prevent the fusion of SARS-CoV-2 with the host cell inhibiting the primary entry.[19]

An alternate mechanism hypothesized to inhibit the uptake of the virus into the host cell is based on the ability of chloroquine to be a broad inhibitor of nanoparticle endocytosis by resident macrophages.[20] At the concentrations achieved in routine clinical dosing, chloroquine reduces *in vitro* and *in vivo* accumulation of synthetic nanoparticles.[21,22] It also reduces the expression of phosphatidylinositol-binding clathrin assembly protein,[22] required for clathrin-mediated endocytosis of nano-sized structures. The ultrastructural studies of SARS-CoV-2 show that these virions fall within the same size (60–140 nm) and shape (spherical) range[18] as the commonly studied synthetic nanoparticles.[23,24] Thus, these actions against SARS-CoV-2 may be applicable at early stages before viral replication,[20] which requires further experimental confirmation. Alternatively, these compounds may act at later stages by inhibiting specific enzymes needed for assembly of virion and budding of the virions from the cell membrane. These drugs may accomplish these goals without disruption of the viral particle and liberation of viral nucleic acid and enzymes that are necessary for viral replication from a lysosome.[25,26]

Finally, these compounds have been shown to have a profound effect on the inflammatory cascade. SARS-CoV, through its ACE II receptor attachment, infects the type 2 pneumocytes in the alveolar epithelium. This results in a local inflammatory reaction with resident neutrophils and macrophage activation as well as activation of the cellular immunity arm with T helper 1 (Th1)-type response. The resultant cytokine storm and alteration in epithelial permeability lead to the development of acute respiratory distress syndrome and associated morbidity and mortality related to COVID-19.[5] Chloroquine/HCQ reduces the secretion of the proinflammatory cytokines, in particular the Th1 cytokines, namely interleukin (IL)-1, IL-6 tumor necrosis factor-α (TNFα), and interferon-gamma (IFNγ),[27] by the alveolar macrophages, and thus may have a role in reducing the peak inflammatory response in COVID-19. These antiviral effects

combined with immunomodulatory properties are promising, and thus over the last 3 months, multiple studies have been launched to leverage these benefits in the clinical setting.

In vitro evidence of chloroquine/HCQ on SARS-CoV-2

A majority of the putative antiviral effects of chloroquine/HCQ molecules on the SARS-CoV-2 are the result of the indirect inferences drawn from the data available on SARS-CoV-1 or from an alternate viral model, that is, Epstein–Barr virus (EBV), HIV, and so forth. Led by its putative effects on the endosomal function disruption in one of the earliest *in vitro* studies, the Centers for Disease Control (CDC) studied the effects of chloroquine in primate Vero E6 cells (African green monkey kidney cells). The investigators found that chloroquine was effective against SARS-CoV-1, and the inhibitory effects were equally potent whether the primate cells were treated before or after exposure to the virus, suggesting both prophylactic and therapeutic applications. They further showed that addition of ammonium chloride to raise the endosomal pH also had a similar inhibitory effect on the viral replication, suggesting that these effects, that is, inhibition of cellular entry as well as postentry viral replication and assembly may be affected by the alterations in the pH of the intracellular organelles.[17]

In line with the findings from the CDC study, an investigative team in Wuhan, China, performed a similar *in vitro* time-of-addition assay involving Vero E6 cells for SARS-Cov-2. In a physiology-based pharmacokinetic model, chloroquine/HCQ concentrations in lung fluid were simulated under five different dosing regimens to explore the most potent and safe regimen. For 'entry' treatment, the drugs were added to the cells for 1 hour before viral attachment. For the 'post-entry' experiment, drugs were added at 2 hours post infection and maintained until the end of the experiment. Virus yield in the infected cell supernatants was quantified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Similar to the CDC experiments, the investigators found that these molecules affected the control of virus at both entry and at post-entry stages.[28] The investigators further found that among the two, HCQ was more potent than chloroquine as the effective concentration for a half-maximal response (EC_{50}) was much lower (0.72 µM) for HCQ than for chloroquine (5.47 µM). Based on these experiments, the investigators suggested to treat SARS-CoV-2 infection with a loading dose of 400 mg twice daily of HCQ sulfate to be given orally on day 1, followed by 200 mg given twice daily for 4 more days. In another study from China,[29] the investigators found the EC_{90} value of chloroquine against the SARS-CoV-2 in Vero E6 cells was 6.90 µM. The investigators recommended that these concentrations, as evidenced through the pharmacokinetic studies for these molecules performed in the plasma of rheumatoid arthritis patients who received chloroquine at 500 mg per day dose, were clinically achievable.[30]

Clinical evidence of chloroquine/HCQ on SARS-CoV-2

While the *in vitro* experiments provide a rationale for their use in COVID-19, there is limited evidence on the clinical efficacy of these compounds for treating COVID-19. At the time of writing this manuscript, we found there were over 50 randomized studies registered with clinicaltrials.gov for the use of HCQ in COVID-19. The vast majority were still in the pre-recruitment phase, and a few were in the early stages of recruitment, but none of the larger studies had published their results. The *in vivo* or clinical evidence presented in this review is derived mainly from the small-cohort, observational, and randomized studies (available for review at the time of manuscript preparation) on the clinical outcomes (Table 1).

In one of the first published pieces of evidence (published as a letter), Gao and colleagues from China drew attention to the potential role of chloroquine for treating COVID-19. Based on the clinical data collected from ten hospitals in China (Wuhan, Jingzhou, Guangzhou, Beijing, Shanghai, Chongqing, and Ningbo) involving >100 patients, the authors proposed that chloroquine was a promising therapeutic drug. They reported that the patients who received chloroquine phosphate had lower rates of pneumonia exacerbation, greater improvement in lung imaging findings, higher rates of conversion to the virus-negative state, and shorter disease course compared to those who did not receive the drug.[31] Unfortunately, the report did not publish specific details about the patient population, response pattern, or analysis of outcomes.

In another observational, single-arm, open-label clinical trial from Marseille, France,[32] the investigators reported clinical outcomes based on virological clearance from nasopharyngeal secretions. They enrolled 42 patients; over 80% of patients either had no symptoms (16.7%) or had upper respiratory tract symptoms (61.1%). Only a minority (22.2%) had lower respiratory tract diseases, including bronchitis and pneumonia. The investigators compared this with a control untreated group (n=16) consisting of patients who were either treated at a nearby facility without HCQ or who refused participation. Twenty-six patients received HCQ (200 mg three times daily for 10 days) of which six received it in conjunction with azithromycin (500 mg on day 1 followed by 250 mg daily for 4 days). The inclusion of azithromycin was rationalized on the basis of its *in vitro* activity against Zika and Ebola viruses[33,34] and on the clinical evidence for its ability to prevent severe respiratory tract infections in patients suffering from viral infection.[35] The primary outcome for the study was the virological clearance from nasopharyngeal secretions at day 6 after receiving the drug. The investigators found that patients treated with HCQ alone or in combination with azithromycin had significantly greater virological clearance (p=0.001) at day 6 (57.1 and 100%, respectively), compared to those in the control group (12.5%). Although encouraging, this ongoing study, as published, had several

Table 1. Important completed and planned clinical studies exploring the efficacy of chloroquine/hydroxychloroquine in the management (treatment/prevention) of COVID-19.

Study	No. of participants	Design	Dose	Cotherapy	Outcome
Available reports					
Gao et al.[31]	>100	Clinical cohort	Unknown	Unknown	Benefits in clinical parameters
Gautret et al.[32]	42	Observational prospective cohort study	200 mg three times a day for 10 days	Azithromycin in 6 out of 26 patients, 500 mg on day 1 and 250 mg on day 2–5	Virological clearance
Molina et al.[36]	11	Prospective cohort study	200 mg three times a day for 10 days	Azithromycin in 6 out of 26 patients, 500 mg on day 1 and 250 mg on days 2–5	Virological clearance 20% at day 5–6
Zhaowei et al.[37]	62	Randomized controlled study	200 mg twice a day for 5 days	None	Improvements in clinical and radiological parameters
Chen et al.[38]	30	Randomized controlled study	400 mg daily for 5 days	None	No improvements in virological clearance or clinical or radiological parameters
Important upcoming studies*					
Solidarity study**	40,000	Randomized controlled study	800 mg × 2 loading and 400 mg × 2 daily for 10 days	None Multiple comparison arms	Clinical parameters Hard outcome measures Biological markers
HERO-HCQ	15,000	Randomized controlled study	600 mg twice on day 1 and 400 mg daily for 29 days	None	Preventive study with rates of turning viral screening positive

*At the time of this manuscript, there are over 50 different clinical trials registered with the clinicaltrials.gov. The prospective list is aimed to highlight the largest therapeutic (Solidarity study) and preventive studies (HERO-HCQ) registered.

**Solidarity study is a pragmatic RCT with planned enrollments across many nations based on the local resources and the needs. Thus, the treatment regimen, therapeutic agent, and the outcome measures are finalized by the local study team. The current regimen and the outcome measures are depictive of the Solidarity's Norway arm of the study.

concerns to allow for strong recommendations in favor of or against HCQ. Six of the 26 patients in the treatment group were lost to follow-up and were not included in the analysis, with three transferred to intensive care unit (ICU), one death, and two withdrawals, limiting the interpretation of the data. Furthermore, the criteria for the selection of patients receiving additional azithromycin were not specified. Finally, the study had a very low proportion of patients with lower respiratory tract pathology, and outcomes of the clinical parameters including effects on hemodynamic stability, ventilator parameters, length of ICU and hospital stay, and mortality have not been published. Nonetheless, early strong indications for the efficacy of this paper prompted another group in France to prospectively study 11 consecutive COVID-19 patients with high comorbidity burden (in 8 out of 11) admitted under their care to be treated with the combination regimen of HCQ and azithromycin, in doses similar to those used in the Marseille study. Serious adverse events (one death, two transfers to the ICU, and one drug discontinuation due to prolongation of QTc interval) were reported in 4 out of 11 patients, and 8 out of the remaining 10 had persistently positive nasopharyngeal swab PCR after 5–6 days of therapy, prompting investigators to conclude that there is no evidence of rapid clearance with this therapy.[36]

Findings from two additional randomized control studies recently became available for review. In the first one, investigators from Wuhan, China (Renmin Hospital of Wuhan University),[37] evaluated the effects of a 5-day course of HCQ (200 mg twice-a-day regimen) in addition to a 'standard treatment' comprising oxygen therapy, antibiotics, and immunoglobulin, with or without corticosteroids compared with 'standard treatment' alone. The study utilized clinical measures, such as the return of body temperature and

persistent improvement of cough symptoms lasting >72 hours, as the primary outcomes. The results showed that the HCQ treatment group had a significantly shorter time to reach afebrile status (2.2±0.4 *versus* 3.2±1.3 days, $p<0.0008$) and had a significantly shorter time to cough relief (2.0±0.2 *versus* 3.1±1.5 days, $p<0.0016$) compared to the standard treatment group. The investigators also found that the rates of radiological improvements on chest computed tomography (CT) were higher in the HCQ treatment group (80.6%, 25 of 31 patients) compared to the control group (54.8%, 17 of 31 patients). While these findings are encouraging, another study from the Shanghai Public Health Clinical Center in China[38] did not find similar benefits with HCQ. In this study, 30 patients with confirmed COVID–19 were randomized to either receive conventional treatment only or conventional treatment with the addition of HCQ (400 mg daily for 5 days). The investigators found that the viral clearance, as judged by the detection of the viral nucleic acid in the pharyngeal swab on day 7 after treatment initiation, was not different for the two groups. They also found that the difference in the median time to achieve afebrile status and in the radiological findings of pneumonia was not different for the two groups.

Pharmacokinetics and safety concerns

Details of the pharmacokinetics and safety profiles for chloroquine/HCQ were recently reviewed in this journal and are available.[1] We have more than seven decades of clinical experience with these agents, and overall, the safety of these agents is well established. HCQ is the structural analog of the chloroquine molecule with the addition of a β-hydroxyl moiety at one end. Although this imparts HCQ a comparable clinical efficacy, it has been noticed to do so by providing a better safety profile.[1] In modern medicine, HCQ is a more commonly used formulation for most non-malarial indications. Both these agents are cheap, safe, and well tolerated by most patient populations, including pregnant women and those with chronic diseases or immunocompromised status. They are administered orally and have a near-complete absorption from the gastrointestinal tract with about 75% bioavailability.[39] Peak drug concentrations are achieved in about 4–12 hours after oral administration and are excreted principally through the kidneys, the process facilitated by acidification of the urine. Small quantities are also excreted through the bile, sweat, and saliva. They are widely distributed throughout the body, including the lung, [40] and have a large volume of distribution with significant intracellular sequestration, allowing them to have a long functional half-life (40–50 days) and achieve stable plasma levels usually after 4–6 weeks of regular daily dosing.[27] Unfortunately, these kinetics have been studied with chronic use of chloroquine/HCQ, and its applicability, especially in short courses, concerning alveolar concentration is not known.

The most frequent adverse effects related to acute use comprise gastrointestinal intolerance, concerns for acute anemia – especially in patients with pre-existing glucose-6-phosphate dehydrogenase (G6PD) deficiency, and flashing lights as an acute manifestation of retinopathy. While the risk of retinopathy is a major limiting factor for chronic use at higher doses, this is likely to be less of a concern with acute short-term doses recommended for COVID-19, especially for HCQ that has a faster clearance from retinal pigment cells, compared to chloroquine.[41] Another concern particularly relevant to the current pandemic is the possibility of myocardial toxicity, QTc interval prolongation, and the possibility of cardiac arrhythmias. Several anecdotal reports and cohorts have raised concerns for chloroquine/HCQ -induced cardiomyopathy.[42] However, a detailed review of the data suggests that these concerns are significant largely in patients prescribed high doses of these agents.[43] Recent reports have also suggested that COVID-19 itself may cause myocardial injury, which in and of itself is associated with the higher incidence of adverse outcomes.[44] It is noteworthy that the risk of adverse outcomes, and hence the need for therapy, is higher in the elderly COVID-19 population with multiple chronic diseases, the exact population at higher chances of having comorbidities or medications causing QTc prolongation. In this regard though, retrospective analyses of the rheumatological cohorts have shown that the incidence of cardiac arrhythmias is lower in patients on therapy with these agents compared to those not on treatment.[45] Although reassuring, these mutually contradictory data demand additional caution, as the doses of HCQ recommended for the treatment of COVID-19 are higher than those used conventionally for chronic low-dose therapy, and their interplay with direct toxicity of SARS-CoV-2 is not known. Thus, especially for the high-risk population with significant comorbidity burden, it may be prudent to obtain a routine electrocardiogram prior to initiating HCQ therapy. As azithromycin is also known to prolong the QTc interval, patients with prolonged QTc (i.e. ≥450–500 msec) may be better served by avoiding a combination regimen or with ongoing telemetry monitoring for the occurrence of arrhythmias.

Conclusions

The available data taken together show that chloroquine/HCQ appears to have a potential role in the management of the clinical syndrome of the COVID-19. However, the level of preclinical and clinical evidence is not robust and must be backed by a higher level of data. Unfortunately, lack of alternative therapy, high rate of infectiousness and mortality, and the rapidity of spread elevate the nature of public health hazards related to COVID-19. The pandemic has many health and non-health ramifications, including the global recession. Thus, a rising number of regulatory healthcare agencies from across the globe, including China, Italy, France, Europe, Canada, and the USA – list not exhaustive, have included these compounds in their guidelines for treating COVID–19.[46] At the same time, there are also over 50 studies of varying enrollment targets, and a variety of

clinical, biological, and mortality-related outcome measures registered with the clinicaltrials.gov. Prominent among these are two major studies that aim to evaluate the effect of these agents on therapy (WHO-sponsored pragmatic randomized study; Solidarity trial)[47] and the prophylaxis (National Institutes of Health [NIH]-sponsored study evaluating the preventive aspects in the healthcare workers – The Healthcare Worker Exposure Response & Outcomes-HCQ [HERO-HCQ]).[48] Pending the availability of such confirmatory studies, the use of these agents in COVID-19 should be viewed as experimental at this stage, and it should adhere to local, regional, or national ethics and research guidelines.

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Correspondence: Ashutosh M Shukla, Division of Nephrology, Hypertension and Transplantation, 1600 SW Archer Road, PO Box No., Gainesville, FL, 32610, USA. ashutosh.shukla@medicine.ufl.edu

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