

REVIEW

Rethinking the role of hydroxychloroquine in the treatment of COVID-19

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Abstract

There are currently no proven or approved treatments for coronavirus disease 2019 (COVID-19). Early anecdotal reports and limited in vitro data led to the significant uptake of hydroxychloroquine (HCQ), and to lesser extent chloroquine (CQ), for many patients with this disease. As an increasing number of patients with COVID-19 are treated with these agents and more evidence accumulates, there continues to be no high-quality clinical data showing a clear benefit of these agents for this disease. Moreover, these agents have the potential to cause harm, including a broad range of adverse events including serious cardiac side effects when combined with other agents. In addition, the known and potent immunomodulatory effects of these agents which support their use in the treatment of auto-immune conditions, and provided a component in the original rationale for their use in patients with COVID-19, may, in fact, undermine their utility in the context of the treatment of this respiratory viral infection. Specifically, the impact of HCQ on cytokine production and suppression of antigen presentation may have immunologic consequences that hamper innate and adaptive antiviral immune responses for patients with COVID-19. Similarly, the reported in vitro inhibition of viral proliferation is largely derived from the blockade of viral fusion that initiates infection rather than the direct inhibition of viral replication as seen with nucleoside/tide analogs in other viral infections. Given these facts and the growing uncertainty about these agents for the treatment of COVID-19, it is clear that at the very least thoughtful planning and data collection from randomized clinical trials are needed to understand what if any role these agents may have in this disease. In this article, we

Abbreviations: ACE-2, angiotensin-converting enzyme 2; ARDS, acute respiratory distress syndrome; BID, bis in die (twice per day); COVID-19, coronavirus disease 2019; CQ, chloroquine; CRP, C reactive protein; ECG, electrocardiogram; HCQ, hydroxychloroquine; ICU, intensive care unit; IVIG, intravenous immunoglobulin; MERS-CoV, Middle East respiratory syndrome coronavirus; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV, severe acute respiratory syndrome coronavirus.

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review the datasets that support or detract from the use of these agents for the treatment of COVID-19 and render a data informed opinion that they should only be used with caution and in the context of carefully thought out clinical trials, or on a case-by-case basis after rigorous consideration of the risks and benefits of this therapeutic approach.

KEYWORDS

coronavirus, COV-SARS-2, immunology, immune, SARS

1 | BACKGROUND

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has caused a global pandemic that is severely straining health systems everywhere.¹ COVID-19 has an estimated symptomatic case fatality rate of approximately 1.4% which is around 15 times greater than that estimated for seasonal influenza.²⁻⁴ The mortality rate rises dramatically for individuals with increasing age and comorbidities.⁵

There are currently no proven or approved treatments for this disease, though numerous therapeutic agents are under investigation. The illness course is variable; some individuals are asymptomatic, others experience a mild, self-resolving flu-like illness, and others still progress to moderate or severe disease.⁶ For those who progress to more severe disease, there are typically four phases of the illness course, see Figure 1. The first is the incubation period which lasts a median of 5.1 days, with a large range.⁷ The second is a mild symptomatic phase which lasts around 5 days and typically includes flu-like symptoms

including fever, cough, myalgias, and fatigue, though gastrointestinal symptoms like anorexia, nausea, vomiting, and diarrhea as well as anosmia can be prominent.^{1,6} This is followed by progression to a hyperinflammatory acute respiratory distress syndrome (ARDS).⁸ The onset of this third phase is typically marked by dyspnea, tachypnea, and progressive, sometimes silent hypoxemia. This phase is marked by high fevers, elevated inflammatory markers, and the progressive formation of bilateral diffuse pulmonary opacities on chest radiographs and associated respiratory failure. Some individuals develop multi-system organ failure with complications that can include micro and macro thromboses, myocarditis, elevated muscle enzymes suggestive of myositis, and kidney failure.⁹⁻¹¹

Because of the severity of the illness course in some cases of COVID-19, effective treatments are desperately needed. Unfortunately, few high quality randomized controlled treatment trials have been published to date for investigational agents for this disease. To date, hydroxychloroquine (HCQ) and chloroquine (CQ) have been widely used around the world for COVID-19 and previously for Ebola, H7N9 influenza and SARS virus infection, based on very limited data,

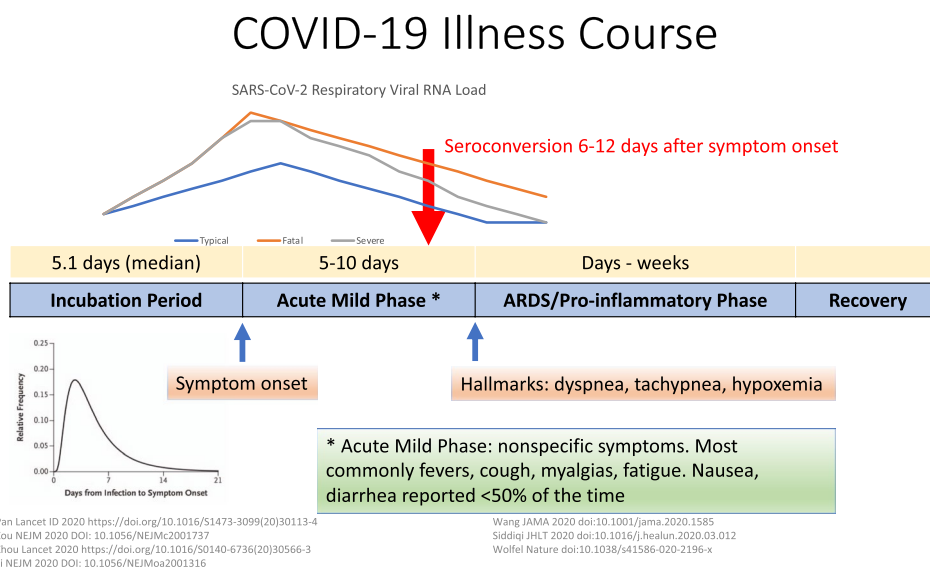


FIGURE 1 COVID-19 clinical course of illness. The first phase of COVID-19 infection involves an incubation period of variable duration, with a median of 5.1 days. The second is an acute mild phase that most commonly includes flu-like symptoms like cough, fevers, and myalgias, but can also include gastrointestinal symptoms. Some patients progress to an ARDS hyperinflammatory phase that is often marked by dyspnea, tachypnea, and hypoxemia. The respiratory viral load rises before the onset of symptoms and peaks around the onset of symptoms. It declines over the first week. Severe cases have higher viral loads compared with mild cases. Prolonged viral shedding in severe and mild cases is reported

though they remain unproven and of unknown benefit. The efficacy of HCQ may depend on the timing of administration, as it is being considered for post-exposure prophylaxis and all stages of infection. Additionally, certain safety concerns have been raised about these agents, not only because of their long half-life but also because of their potential adverse effects when combined with certain other drugs. Here we will review the known effects of HCQ on virus replication and the immune system and present the evidence to date and notable considerations for HCQ therapy in patients with COVID-19.

2 | MECHANISM OF ACTION

HCQ and CQ have been used for many years to treat a number of diseases, including auto-immune diseases like lupus and rheumatoid arthritis as well as for the prevention and treatment of malaria.¹² These agents are known to raise intracellular pH and, in particular, affect endosomal activity.^{13,14} This action has wide-ranging secondary effects, including potent immune modulation via specific mechanisms. In the context of COVID-19, limited data exist on the antiviral activity of these agents. Both antiviral and immune modulatory activities are discussed below, as are considerations for potential adverse reactions and drug interactions.

3 | PRE-CLINICAL DATA AND DATA FOR THE ANTIVIRAL ACTIVITY OF CQ AND HCQ INCLUDING AGAINST SARS-LIKE CORONAVIRUSES

Following the emergence of SARS, CQ was found to be a potent *in vitro* inhibitor of SARS-CoV. Keyaerts and colleagues showed that no significant viral replication was measured in Vero E6 cells inoculated with SARS-CoV in the presence of CQ.¹⁵ The authors did note that antiviral activity diminished as CQ was added later to the cells, suggesting that the mechanism of action may be earlier in the viral life cycle. Later, Vincent et al showed that CQ when added post-infection to Vero E6 cells could markedly decrease the number of infected cells.¹⁶ They suggested that the mechanism of action might include the elevation of endosomal pH, disruption of intracellular transport of the virus, as well as altered glycosylation of angiotensin-converting enzyme 2 (ACE-2) potentially reducing SARS-CoV-2 binding to ACE-2 and therefore preventing viral entry. Though it is unclear how CQ and HCQ may impact SARS-CoV-2 engagement with other surface-exposed proteins reported to interact with SARS-CoV and SARS-COV-2.¹⁷⁻²⁰

Similar work was performed after the emergence of a related virus called the Middle East respiratory syndrome coronavirus (MERS-CoV). While CQ was shown to have

anti-MERS activity in immortalized cell lines, later studies showed that MERS-CoV can rapidly infect certain antigen-presenting cells and that CQ did not inhibit the infection of or viral replication in these cells.^{21,22}

After the emergence of SARS-CoV-2, scientists explored drugs that might have efficacy against this novel virus. CQ was rapidly identified as having potent activity *in vitro* against the virus.²³ The mechanism of action was thought to be the same as was proposed for its activity against SARS-CoV. However, there are no published series or randomized clinical trials of patients with SARS-CoV or MERS-CoV treated with HCQ or CQ.

4 | PHARMACOLOGY AND SAFETY

HCQ has been used for a long time in diseases such as rheumatoid arthritis, systemic lupus erythematosus, and malaria. As noted previously, CQ and HCQ are efficiently absorbed, reach peak serum concentrations in 2-3.5 hours, have an elimination half-life of 22 to 45 days, and can reach serum concentrations of approximately 1.5 μM through the administration of 6.5 mg/kg/day.^{12,24-28} It is important to note that discrepancies in concentrations and half-life measurements can result from varied methods and source material for estimation, and that measurement in whole blood is preferred over serum or plasma.^{29,30} Administration of CQ and HCQ in animal models demonstrated splenic, renal, hepatic, and importantly lung accumulation levels 200-700 times greater than in plasma, and more recent studies also noted that melanin binding contributes to skin and eye accumulation.^{31,32} There are several well-described side effects with long-term use, such as cardiomyopathy and retinal toxicity. While short-term use of HCQ has a substantially lower risk of cardiomyopathy and retinopathy, there remain concerns related to QT prolongation, hypoglycemia, as well as side effects such as gastrointestinal disturbance.

Given the increase in the use of HCQ for COVID-19, a group recently published their analysis of the risk for adverse events associated with this medication.³³ They included more than 950 000 HCQ users of whom more than 320 000 had combination therapy with azithromycin. They found no elevated risk for adverse events for short term HCQ treatment (defined as within the first 30-days after starting therapy) compared with equivalent therapy for individuals with rheumatoid arthritis treated with sulfasalazine. However, they did find a 15%-20% increased risk of chest pain or heart failure and a twofold increased risk of cardiovascular mortality in the first month of treatment following the addition of azithromycin to HCQ. This finding highlights the concern that the combination of these two medications may place patients at increased health risk. Given the lack of evidence that HCQ is

beneficial for the treatment of COVID-19, these two agents should likely not be combined for COVID-19 outside of a clinical trial unless there are strong indications for each, no viable alternatives in individual patients and they are administered with appropriate counseling and close monitoring for adverse events.

5 | CLINICAL DATA FOR HCQ AND CQ IN COVID-19

Based on the initial *in vitro* data, there was early clinical interest in using CQ and HCQ for the treatment of COVID-19. A group from China reported an interim analysis of outcomes for more than 100 patients with COVID-19 treated with CQ and noted it was superior to the control. There were, unfortunately, no details about these patients in the report, though they reported that patients who received CQ had less severe pneumonia, improved lung imaging, earlier conversion of viral shedding, and a shorter disease course.³⁴ They also reported that this treatment was safe. This led to the release of an expert consensus in China that recommended high dose CQ phosphate at 500 mg twice per day (BID) for 10 days for patients with mild, moderate or severe COVID-19.³⁵

Since that time, several small studies have been released looking at HCQ and CQ in more detail. The first was a small randomized control trial of 30 patients with COVID-19 in China who were randomized 1:1 to receive HCQ or not, in addition to other antivirals that included interferon alpha, umifenovir, and lopinavir/ritonavir.³⁶ They found no difference in radiographic progress, time to becoming afebrile, or percent who had a negative throat swab at day 7. Shortly thereafter a small non-randomized clinical trial was released from France for hospitalized non-intensive care unit (ICU) patients which reported HCQ led to dramatically faster viral clearance from nasopharynx compared with control.³⁷ They reported that nearly 60% of patients on HCQ had day 6 viral clearance compared with under 15% of controls. In this methodologically flawed study, 26 patients were in the HCQ arm and 16 in the control arm. The analysis excluded six patients from the analysis who tended to be sicker. The study has since been widely criticized, and the society that published the study subsequently released a statement saying it did not meet their standards.³⁸

This same French group reported that among the six patients who received HCQ and azithromycin, viral clearance from nasopharynx was fastest; they suggested this might indicate synergy between the two agents.³⁷ They subsequently released another report of a non-randomized series of 80 patients with mild COVID who had been treated with HCQ and azithromycin.³⁹ Overall, the patients were admitted with an average of 5 days of symptoms, 93% had a day 8 negative reverse transcription-polymerase chain reaction (RT-PCR)

from the nasopharynx, and 81.3% were discharged at the time of writing the report with an average hospital length of stay of just over 4.5 days. They had no comparison arm in this second report.

A second French group studied the combination of HCQ and azithromycin in 11 patients with COVID-19.⁴⁰ They found that in the 10 patients who were alive at day 6, only two had viral clearance, casting further doubt on the initial report from the first French group, where reported viral detection rates at day 6 were so much lower.

Another small randomized controlled trial from China looked at HCQ vs no HCQ for mild COVID.⁴¹ They reported that the resolution of cough and fever was faster in the HCQ arm. However, there were some important limitations to the study. First, 80 patients were excluded from the study for unclear reasons. Second, the standard therapies that were allowed in each group included steroids, antivirals, and intravenous immunoglobulin (IVIG) but how these were distributed between the groups was not reported. There are no data about discharge, mortality, and viral clearance and therefore the endpoints they reported may not be meaningful.

In a preprint of a retrospective analysis of 181 hospitalized patients with COVID-19 in France, authors compared 84 patients who received HCQ (600 mg/day) within 48 hours of admission to 97 patients who did not.⁴² The baseline characteristics were similar between the groups. The patients included had symptoms for a median of 7 days before admission to the hospital. They were enrolled if they were admitted to the hospital but not in the intensive care unit and were on ≥ 2 L of supplemental oxygen. Although this was not a randomized trial, no benefit was found for the group that received HCQ and approximately 10% who received this medication needed it discontinued because of changes in their electrocardiograms (ECGs).

A further recent preprint from China reports the largest randomized controlled trial studying HCQ to date.⁴³ It included 150 hospitalized patients with COVID-19 and randomized them 1:1 to receive HCQ plus standard of care or standard of care in an open-labeled fashion. The vast majority of the patients had mild to moderate disease (99%) and the mean day from symptom onset to randomization was 16.6 days. HCQ was dosed as a loading dose of 1200 mg daily for 3 days followed by a maintenance dose of 800 mg daily for 2-3 weeks, with longer courses given for severe patients. The primary endpoint was conversion to negative SARS-CoV-2 polymerase chain reaction (PCR) from upper and/or lower respiratory tract samples by day 28. They found no difference in negative SARS-CoV-2 conversion by day 28 for each group with 85.4% negative in the HCQ arm and 81.3% negative in the standard of care arm, $P = .341$. Negative conversion rates were also similar at earlier time points. In the initial analysis there was no

TABLE 1 Summary of human studies with HCQ/CQ for COVID-19 to date

References	RCT?	Total population in the study	Outcome	Notes
34	No	>100	Report of superiority of CQ	No details about patients in the study
35	No	N/A	N/A	Expert consensus recommending CQ for all with COVID-19 in China
36	Yes	30	No difference	HCQ + standard of care (SOC) vs SOC
37	No	42	Report faster viral clearance with HCQ	Publisher has since said that the report did not meet their standards
39	No	80	No comparison group	
40	No	11	No evidence of fast viral clearance with HCQ	Casts further doubt on reports from Gautret et al
41	Yes	62	Faster resolution of cough and fever with HCQ	Not clear these endpoints matter
42	No	181	No benefit to HCQ	
43	Yes	150	Did not meet primary outcome, but CRP declined faster with HCQ	HCQ started late in disease course (mean 16.6 days after onset)
44	Yes	~80	Stopped early because high dose CQ led to more adverse events	

difference in symptom improvement by day 28; however, in a post hoc analysis controlling for confounding effects of other antivirals suggested improvement in symptoms in the HCQ group compared with the control group and the HCQ group did have a greater reduction in C reactive protein (CRP). The primary outcome for this trial was negative, and overall the trial did not support the use of HCQ late in the course of COVID-19. For a summary of all of the human data for HCQ and CQ for COVID-19, please see Table 1.

Finally, in a recent study in Brazil participants were randomized to receive high dose CQ (600 mg BID for 10 days) vs low dose CQ (450 mg BID \times 1 day then 450 mg daily \times 4 days).⁴⁴ Additionally, all patients were treated with ceftriaxone and azithromycin. More than 25% of patients in the high dose arm developed a prolonged QTc > 500 ms. The high dose group had an increased mortality rate of 17% vs 13.5%. There was no evidence for the rapid clearance of viral load on their testing. Because of concerns about safety and no clear benefit to the higher dose of CQ the study was stopped.

6 | HCQ AND OTHER VIRAL INFECTIONS

It is worth noting that HCQ and CQ have in vitro activity against a number of viruses, including influenza, but they have yet to show clinical benefit for viral infections.⁴⁵ In fact, a large clinical trial in Singapore sought to determine if HCQ could reduce influenza infection among 1500 patients randomized 1:1 who received CQ (500 mg/day for 1 week then once a week for 12 weeks) or matching placebo, monitoring for symptoms and laboratory evidence of influenza in each group.⁴⁶ There was no difference in the incidence of influenza and more patients in the CQ group reported adverse events (45% vs 33%) including headache, dizziness, nausea, and diarrhea most commonly.

7 | HCQ AND THE IMMUNE SYSTEM

The primary use of HCQ, beyond its well-established role as an antimalarial agent, is as an immunomodulator for

autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). In this context, HCQ disrupts lysosomal antigen processing by antigen-presenting cells and lowers T-cell recruitment and subsequent production of pro-inflammatory cytokines including IL-6 and TNF α .⁴⁷ While the ultimate effect of this modulation of the immune system is favorable for treating auto-immune conditions, it is not clear how these effects of HCQ may affect a person with acute COVID-19. It is important to note that as observed with SARS, patients with COVID-19 can exhibit leuko- and lymphopenia, that NK and CD8 T-cells exhibit markers (NKG2A, PD-1) and diminished function consistent with exhaustion.⁴⁸⁻⁵⁰ Further, the extent of functional exhaustion and reduced diversity may correlate with the risk of severe disease, while restoration of monocytes and lymphocytes is linked with improved viral clearance and recovery.⁵¹⁻⁵³ Key points to consider are summarized in Figure 2 and Table 2 and discussed in further detail below.

While it is a potent immune modulator, HCQ is not considered immunosuppressive and has not to date been associated with elevated infection risk.⁵⁴ In the context of its long-term use for rheumatologic disease, HCQ has also not

been associated with an increased the risk of infection.⁵⁵ A large study of more than 16 000 patients with rheumatoid arthritis (RA) found no increased risk for pneumonia for those on this agent.⁵⁴ Similarly, another cohort of more than 23 000 patients with RA found no elevated risk for infection requiring hospitalization for those on HCQ compared with other agents.⁵⁵ A third large cohort study of more than 24 000 patients with RA also found the risk of infection requiring hospitalization or outpatient parenteral antibiotics was less for those on HCQ compared with biologic disease-modifying antirheumatic drugs.⁵⁶ Additionally, a small case-control study of patients with SLE matched 65 patients that developed herpes zoster with 130 that did not and found a reduced the risk of Herpes zoster infection in those receiving HCQ.⁵⁷ Furthermore, there has been no signal of elevated infection risk or exacerbation of chronic infections for patients on HCQ and there is, therefore, no warning about infection risk on the FDA label on this drug.

HCQ affects and modulates the innate immune system and response to viral infections. In the context of SARS-CoV-2 infection, disruption of vesicle acidification by HCQ is postulated to have antiviral effects and reduce the

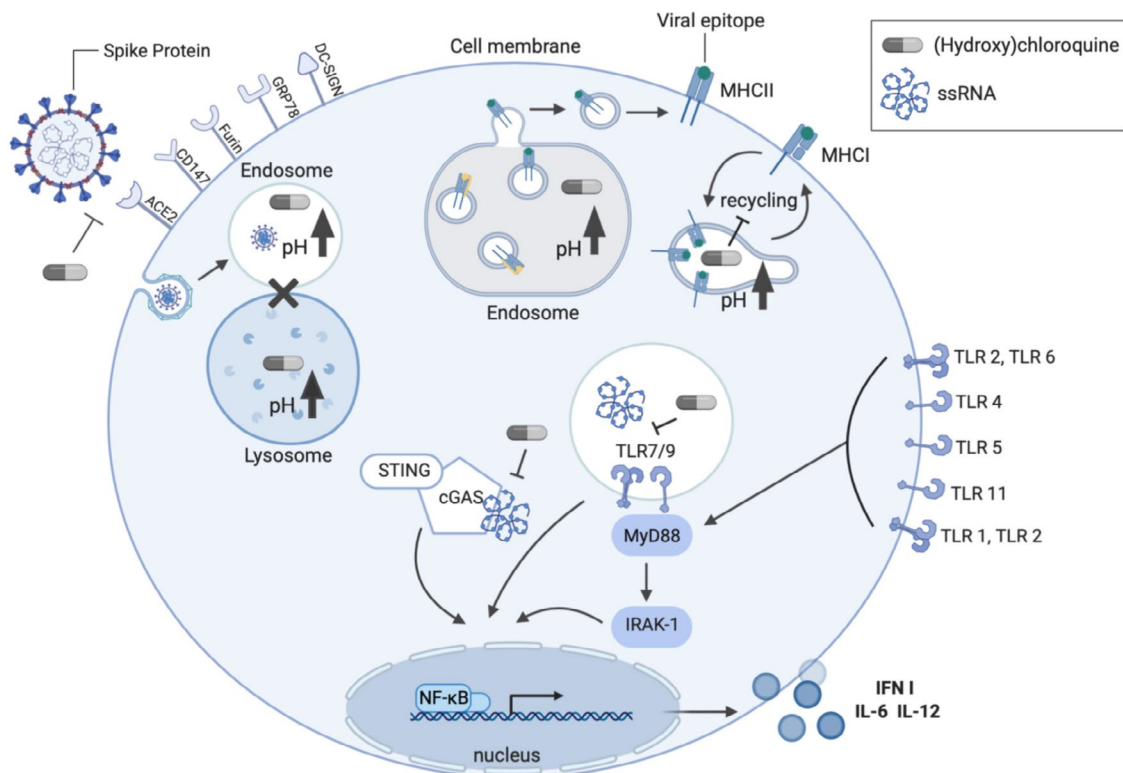


FIGURE 2 Schematic—proposed mechanisms of action of HCQ in SARS-CoV-2 infection. HCQ can limit coronavirus infection and reduce inflammatory and immune cell function. Treatment with HCQ alters the n-terminal glycosylation of ACE-2, which can reduce the affinity of ACE2-S1 (Spike) interactions, though the impact on the interaction of other relevant surface proteins is unclear. HCQ can also inhibit viral infection by disrupting endosomal acidification to interfere with viral fusion. Induction of cytokine expression resulting from innate immune signaling is also impacted by HCQ mediated reduction in DNA/RNA binding and activation of cGAS/STING signaling and altered endosomal pH also disrupts binding to TLR7/9. Elevated endosomal pH can also alter (cross-)presentation of antigen by MHC Class I and II, modifying the development and activation of antigen-specific T cell and B cell populations

TABLE 2 Impact of (hydroxy)chloroquine on major immune populations

Immune cell	Antiviral activity	Impact of (hydroxy)CQ
Plasmacytoid dendritic cells (pDC)	In response to viral infection pDCs are activated and produce high levels of IFN-I. Activated pDCs induce the activation of the adaptive immune response	Inhibits pDC maturation and IFN-I production
Macrophages	Activated through TLR3 binding of dsRNA, promoting macrophage secretion of pro-inflammatory cytokines	Reduces TNF- α , IL-1 β and IL-6 synthesis
Natural Killer cells	NK cells produce IFN- γ and TNF α in response to a viral infection. NK-cells recognize low MHC-I presentation on virus-infected cells and release perforin causing lysis of the target cell	Inhibiting the processing of perforin to its active form, consequently reducing NK cell cytotoxicity
CD4 T cells	Upon activation produce IFN- γ and IL-4. Regulates B- lymphocyte and CTL antiviral responses	Downregulated antigen presentation by MHC, limiting the stimulation of CD4 T-cells and its expression of CD154
CD8 T cells	Upon activation present cytotoxic activity against viral-infected cells	Inhibits cytotoxic activity by inhibiting lysosomal release
B Cells	Production of virus-specific antibodies	Altered endosomal pH modulates antigen presentation, biases selection of naïve antigen-specific B cells, reducing affinity maturation of previously engaged clones

overabundant production of cytokines. However, dysregulation of vesicular acidification by HCQ may have additional effects on innate and adaptive immune responses of patients with COVID-19 with unknown consequences. The typical innate immune response to SARS-CoV-2 is marked by suppressed type I interferon. TLR 7 plays an important role in the recognition of SARS-CoV-2 RNA and initiating the host innate immune response.⁵⁸ By increasing endosomal pH, HCQ reduces TLR 7 and TLR 9 affinity for viral RNA, thereby diminishing the induction of cytokines which may play a role in viral control including type I interferon, IL-6, and IL-12. It is important to note that MyD88-dependent signaling is not impacted by CQ, though it can be modulated by SARS-CoV and is necessary for the protection in a murine challenge model.⁵⁹ Furthermore, HCQ inhibits cyclic GMP-AMP synthase (cGAS) activity in host cells.⁶⁰ Activation of the cGAS/STING pathway by RNA/DNA-dependent mechanism promotes increased type I interferon (IFN β) production. It is known that SARS-CoV induces cGAS/STING and that SARS-CoV-2 is highly responsive to type I interferon.^{61,62} Additionally, HCQ reduces NK cell cytotoxic function by limiting perforin processing to its functional form.⁶³ Relevant to reducing cytokine levels, a study of patients receiving CQ for Early Persistent Musculoskeletal Pain and Arthritis who were also infected with Chikungunya Virus demonstrated that IL-6 and IL-13 levels remained strongly up-regulated throughout the study period.⁶⁴ The ultimate effects of HCQ on the innate immune response to SARS-CoV-2 remain unknown but are clearly important to consider in this clinical context.

In addition to attenuating innate immune signaling, HCQ also impacts the adaptive immune response. HCQ inhibition

of endosome acidification also affects antigen processing and presentation, which, in turn, alters both T-cell and B-cell responses. Treatment with CQ/HCQ reduces the number of rapidly proliferating T-cells and limits differentiation toward Th1 and Th17.^{65,66} Reduced antigen presentation limits the induction of CD4 helper T-cells, specifically the expression of CD154, reducing IL-6 and TNF α production.⁶⁷ In vitro inhibition of autophagy with CQ during T-cell activation rendered T helper cells hypo-responsive to re-stimulation with the antigen, reduced proliferation, and less IL-2 production.⁶⁸ In CD8 T-cells, HCQ limits degranulation and adversely impacts cytotoxic function in vitro, it though does increase cross-presentation and IFN γ production in memory CD8 cells re-stimulated with antigen (both in vitro and in vivo in humans).⁶⁹⁻⁷²

In a study of influenza vaccination in patients with SLE, HCQ did not adversely impact seroconversion.⁷³ However, it is important to note that HCQ does bias selection of antigen-specific B-cells toward naïve cells and away from affinity maturation, thereby reducing clonal expansion which may have important implications for the generation of neutralizing antibodies.^{74,75} This is particularly significant due to concerns related to antibody-dependent enhancement (ADE) of viral infection. ADE occurs when non-neutralizing antibodies bind the virus, thereby promoting entry through the FC-receptor, and is of particular concern when patients exhibit low levels of neutralizing antibodies.⁷⁶ Furthermore, though long-term administration does not appear to negatively impact vaccination to prevent bacterial and viral infections, antibody-mediated responses are diminished by concurrent vaccination and short-course treatment with HCQ.⁷⁷⁻⁸⁰ Taken together, these

TABLE 3 Comparison of reported in vitro inhibition of influenza and COV-SARS, COV-SARS-2

Treatment	CQ						HCQ	
	Virus	H1N1	H3N2	SARS-CoV-1	SARS-CoV-1	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2
IC50/EC50 (μ m)	3.6	0.84	4.4	8.8	5.47	2.71	0.72	4.51
Incubation	16-18hr	16-18hr	16-18 hr	3 days	48 hr	1 hr pre + 48 hr	48 hr	1 hr pre + 48 hr
Citations	81	81	16	15	82	25	82	25

observations highlight that the immunomodulatory effect of HCQ on COVID-19 remains unknown. HCQ may contribute to dampening an overly exuberant immune response during the inflammatory phase of the infection and enhance cross-presentation to CD8 T-cells and their IFN γ production. It is also possible that acute treatment with HCQ may weaken the innate immune response to the virus, impair adaptive immune responses, and could, in the worst case, alter the repertoire of T-cells and B-cells generated in response to SARS-CoV-2, potentially reducing the efficacy of recall responses to re-exposure or even put them at risk for antibody-mediated enhancement. The activity of HCQ in the context of virus-induced inflammation, innate immune activity, and nascent adaptive responses creates a complex milieu that will require careful study to decipher and discern which patients, dosing, and stage of the disease may benefit from this intervention.

8 | DISCUSSION

As hospitals around the globe have filled with patients with COVID-19, front line providers remain without effective therapeutic tools to directly combat the disease. The initial anecdotal reports out of China led to the initial wide uptake of HCQ and to a lesser extent CQ for many hospitalized patients with COVID-19 around the globe. As more data have become available, enthusiasm for these medications has been tempered. Well designed, large randomized controlled trials are needed to help determine what role, if any, these medications should have in treating COVID-19 moving forwards.

While HCQ has in vitro activity against a number of viruses, it does not act like more typical nucleoside/tide antiviral drugs. For instance, HCQ is not thought to act on the critical viral enzymes including the RNA-dependent RNA polymerase, helicase, or proteases. Despite in vitro activity against influenza, in a large high quality randomized controlled trial, it showed no clinical benefit, suggesting that similar discordance between in vitro and in vivo observations is possible for SARS-CoV and SARS-CoV-2⁷³ (Table 3).

Additionally, HCQ and especially CQ have cardiovascular and other risks, particularly when these agents are used

at high doses or combined with certain other agents. While large scale studies have demonstrated that long-term treatment with CQ or HCQ does not increase the incidence of infection, caution should be exercised in extrapolating safety from the studies of chronic administration to largely healthy individuals to estimate the risk associated with short-course treatment in acutely and severely ill patients. Furthermore, the immunologic actions that make HCQ an important drug for the treatment of auto-immune diseases might have unintended consequences when it is used for patients with COVID-19. The effects of this immune modulation on patients with COVID-19 are unknown at this time, including a potential negative impact on antiviral innate and adaptive immune responses which need to be considered and studied.

For all these reasons, and in the context of accumulating preclinical and clinical data, we recommend that HCQ only be used for COVID-19 in the context of a carefully constructed randomized clinical trial. If this agent is used outside of a clinical trial, the risks and benefits should be rigorously weighed on a case-by-case basis and reviewed in light of both the immune dysfunction induced by the virus and known antiviral and immune modulatory actions of HCQ.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

E.A. Meyerowitz, P.M. Reeves, and M.C. Poznansky designed the review; E.A. Meyerowitz, P.M. Reeves, J.A. Gelfand, A.G.L. Vannier, M.G.N. Friesen, M.V. Callahan, S. Schoenfeld, A.Y. Kim, M.C. Poznansky wrote, and reviewed the manuscript and performed a detailed literature research; E.A. Meyerowitz, P.M. Reeves, A.G.L. Vannier, M.G.N. Friesen, and M.C. Poznansky performed the literature

research; E.A. Meyerowitz, P.M. Reeves, A.G.L. Vannier, M.G.N. Friesen, assembled the tables; E.A. Meyerowitz created the illustration in Figure 1; M.G.N. Friesen created the illustration in Figure 2.

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