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REVIEW ARTICLE

Review: Hydroxychloroquine and Chloroquine for Treatment of SARS-CoV-2 (COVID-19)

Katelyn A. Pastick,[1] Elizabeth C. Okafor,[1] Fan Wang,[2] Sarah M. Lofgren,[1] Caleb P. Skipper,[1] Melanie R Nicol,[2] Matthew F. Pullen,[1] Radha Rajasingham,[1] Emily G. McDonald,[3] Todd C. Lee,[3] Ilan S. Schwartz,[4] Lauren E. Kelly,[5] Sylvain A. Lother,[6] Oriol Mitjà,[7] Emili Letang,[8,9] Mahsa Abassi,[1] and David R. Boulware[1]

[1]Department of Medicine, University of Minnesota, Minneapolis, Minnesota, [2]Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, Minnesota, [3]Clinical Practice Assessment Unit, Department of Medicine, McGill University Health Centre, Montreal, Canada, [4]Division of Infectious Diseases, Department of Medicine, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta, [5] Department of Pediatrics and Child Health, Department of Pharmacology, University of Manitoba, Winnipeg, Canada, [6]Department of Medicine, Sections of Critical Care and Infectious Diseases, University of Manitoba, Winnipeg, Canada, [7]Fight AIDS and Inf Dis Foundation, Hospital Germans Trias i Pujol, Barcelona, Spain, [8]Department of Infectious Diseases, Hospital del Mar/Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain, and [9]ISGlobal, Barcelona Institute for Global Health, Universitat de Barcelona, Barcelona, Spain

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a rapidly emerging viral infection causing coronavirus disease 2019 (COVID-19). Hydroxychloroquine and chloroquine have garnered unprecedented attention as potential therapeutic agents against COVID-19 following several small clinical trials, uncontrolled case series, and public figure endorsements. While there is a growing body of scientific data, there is also concern for harm, particularly QTc prolongation and cardiac arrhythmias. Here, we perform a rapid narrative review and discuss the strengths and limitations of existing *in vitro* and clinical studies. We call for additional randomized controlled trial evidence prior to the widespread incorporation of hydroxychloroquine and chloroquine into national and international treatment guidelines.

Key words. "chloroquine;" "clinical trials;" "coronavirus;""COVID-19;""Hydroxychloroquine;" "SARS-CoV-2."

The first report of the novel coronavirus, SARS-CoV-2, causing coronavirus disease 2019 (COVID-19) originated in Wuhan, China, in early December 2019. Since then, the virus has spread across national borders, now affecting more than 200 countries and territories, with over 1 million confirmed cases and 56 000 confirmed deaths as of April 4th, 2020 [1]. Accounts of limited personal protective equipment, lack of critical care resources such as ventilators, and healthcare worker shortages have become unfortunate daily realities as researchers scramble to identify strategies to both interrupt transmission and treat the disease. To date, there are more than 300 ongoing clinical research trials investigating potential therapeutic options for the prevention and/or treatment of COVID-19 [2].

Hydroxychloroquine and chloroquine have been labeled as potential "game-changers" in the popular press for COVID-19 [3]. In this rapid review, we provide an overview of these medications, their pharmacology, the possible mechanisms of action against SARS-CoV-2, and appraise the body of evidence of *in vitro* and clinical studies that have been published to date. We discuss their strengths and limitations, and we call for additional large scale randomized clinical trials adequately powered to show a demonstrable impact on meaningful clinical outcomes, before national and international guidelines endorse the widespread use of hydroxychloroquine/chloroquine for COVID-19.

PHARMACOLOGY OF HYDROXYCHLOROQUINE AND CHLOROQUINE

Chloroquine was first synthesized in 1934 and has been prescribed extensively for the prevention and treatment of malaria as well as the treatment of autoimmune conditions, such as rheumatoid arthritis and systemic lupus erythematosus [4, 5]. Hydroxychloroquine was later introduced in 1955 and quickly became favored due to its superior safety profile [4]. The mechanism of action of these drugs against *Plasmodium* parasites is believed to be partly related to its interaction with DNA and through inhibition of the polymerization of heme [6, 7]. The immunomodulatory activity of hydroxychloroquine is related to a broad spectrum of immunoregulation networks discussed extensively in other work [5, 8, 9]. In addition to activity against rheumatic diseases, the two antimalarial agents have also shown therapeutic activity or immune modulatory effects in a wide range of other diseases including antiphospholipid syndrome, amebiasis, HIV/AIDS, and some cancers [10–13].

These medicines are manufactured in tablet form for oral administration as chloroquine phosphate 500 mg (equivalent to 300 mg chloroquine base) and hydroxychloroquine sulfate

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Correspondence: Katelyn A Pastick, 689 23rd Ave SE, Minneapolis, MN 55455 USA (past0064@umn.edu).

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200 mg (equivalent to 155 mg hydroxychloroquine base) active drug per tablet, respectively. Dosage varies by treatment indication (Table 1 and Supplemental Table 1) [4, 6, 7, 14–19]. Doses as high as 2000 mg hydroxychloroquine and chloroquine have been used for the acute treatment of malaria. Both hydroxychloroquine and chloroquine are notable for their long terminal and elimination half-lives of 22 and 20–60 days respectively [6, 20]. In the urine, hydroxychloroquine has been detectable up to three months from time of last dose [6]. Hydroxychloroquine has been shown to reach peak plasma concentration within three-four hours [6], whereas chloroquine can reach its peak plasma concentration in half an hour [20].

ADVERSE EVENTS

The most common adverse events of hydroxychloroquine and chloroquine are gastrointestinal upset along with nausea, vomiting, and diarrhea [14–16]. In a study evaluating the use of chloroquine, nearly 24% of patients reported nausea/abdominal cramps and 17% diarrhea as side effects [17]. Up to 50% of patients receiving hydroxychloroquine report some gastrointestinal effect; this appears to be dose-dependent and most often occurs with loading doses of 800 mg or higher [21]. Retinopathy is one of the most frequently observed, severe, and irreversible side effect associated with high-dose (>5 mg/kg) and long-term use (>5 years) [19]. Chloroquine has a higher risk of retinopathy than hydroxychloroquine [22]; however, this is not a concern with short term dosing [23]. The most severe and life-threatening complications from use of hydroxychloroquine and chloroquine include QTc prolongation and the resultant risk of ventricular arrhythmias [4].

The incidence of QTc prolongation in this setting of chloroquine and hydroxychloroquine use is largely unknown, as it is highly dependent on baseline EKG findings, with risk exacerbated by the use of concomitant QTc-prolonging medications. In a study of healthy participants, 600 mg chloroquine was associated with an average QTc increase of 16ms (95% CI: 9-23ms), while 1500 mg chloroquine was associated with a 28ms increase (95% CI: 18-38ms), with the most significant QTc prolongation four hours after the second dose [24]. Studies related hydroxychloroquine and QTc prolongation are largely limited to case reports of chronic use [25, 26]. EKG monitoring is not part of standard practice for malaria treatment nor for rheumatology use, when used as monotherapy.

Significant drug interactions with chloroquine and hydroxychloroquine that should be avoided or require additional monitoring include digoxin, antiepileptics, antacids, cyclosporine, amiodarone, azithromycin, moxifloxacin, insulin and antidiabetic agents, tamoxifen, and praziquantel [27–28]. The combination of azithromycin with hydroxychloroquine frequently prolongs the QTc interval in a clinically significant manner, increasing over time. In an 84 patient consecutive cohort, 18% of patients' QTc increased by 40–60 ms, and 12% increased by >60 ms with 11% overall having QTc >500 ms [29]. Other adverse effects from acute use of hydroxychloroquine and chloroquine include, but are not limited to: hypoglycemia in diabetic patients; neurotoxicity in the form of tinnitus, headaches, and changes in mood; and hemolytic anemia in those with G6PD deficiency [6, 27].

HYDROXYCHLOROQUINE, CHLOROQUINE, AND SARS-COV-2

The mechanism of action of hydroxychloroquine/chloroquine against SARS-CoV-2 has yet to be fully elucidated. Chloroquine was first studied in SARS-CoV [30], which was responsible for the 2002–2003 SARS coronavirus epidemic. SARS-CoV shares 79% genetic sequence similarity to SARS-CoV-2, but is thought to result in more severe infection with a case fatality rate of 10% vs. 3% for SARS-CoV-2 [31, 32]. Based on studies initially performed on SARS-CoV, it is believed that SARS-CoV-2 enters cells by binding to the angiotensin-converting enzyme 2 (ACE-2) receptor, and that chloroquine may prevent the virus from binding to this receptor by inhibiting terminal glycosylation [30]. New research has proposed that hydroxychloroquine may additionally prevent SARS-CoV-2 from binding with gangliosides, which in turn may inhibit virion contact with the ACE-2 receptor [33]. Both hydroxychloroquine and chloroquine additionally can incorporate into endosomes and lysosomes, resulting in an increased pH of intracellular compartments. These organelles normally require an acidic environment for homeostasis. Ultimately, this increase in pH results in their dysfunction, leading to defective protein degradation, endocytosis, and exocytosis needed for viral infection, replication, and propagation [34]. Prior work has also demonstrated that coronaviruses can use proteins on the surface of endosomes and endolysosomes for viral entry into host cells [35]. Entry into the endolysosome may be necessary for the viral genome to be released into the cytoplasm of infected host cells [36]. However, it remains unclear how changes in the endosomal environment, particularly changes in pH, may affect the integrity of the SARS-CoV-2 viral genome. Overall, hydroxychloroquine/chloroquine are capable of affecting several cellular pathways and therefore may have several mechanisms of action against SARS-CoV-2.

HYDROXYCHLOROQUINE, CHLOROQUINE, AND SARS-COV-2 IN VITRO DATA

Prior to the 2019 SARS-CoV-2 pandemic, *in vitro* studies investigated the ability of chloroquine to inhibit SARS-CoV viral replication. In these studies, researchers discovered that by pretreating cells with chloroquine at a concentration of 10μM, chloroquine inhibited SARS-CoV viral replication as determined by indirect immunofluorescence [30]. When tested as a potential post-exposure treatment, the 50% maximal effective

Table 1. Hydroxychloroquine Indications and Dosing

Clinically available form	Indications [6]	Dosage [6]	Clinical side effects	Warnings on FDA-approved drug label (PLAQUENIL®) [6]
Hydroxychloroquine sulfate (200 mg/tablet, oral administration)	**Malaria**: Treatment or prophylaxis of uncomplicated malaria, HCQ/CQ-nonresistant strains of *Plasmodium* species	Prophylaxis– once weekly on the same day of each week, starting 2 weeks before exposure, and continued for 4 weeks after leaving the endemic area. Adults: 400 mg Weight-based dosing in adults and pediatric patients: 6.5 mg/kg (not to exceed 400 mg) Treatment of Uncomplicated Malaria Adults: 800 mg followed by 400 mg at 6 hours, 24 hours, and 48 hours after the initial dose (total 2000 mg). Weight-based dosage in adults and pediatric patients: 13 mg/kg (not to exceed 800 mg) followed by 6.5 mg/kg (not to exceed 400 mg) at 6 hours, 24 hours and 48 hours after the initial dose.	**Most common:** Gastrointestinal upset (nausea, vomiting, diarrhea) [14–16]. **Most severe:** Retinopathy. High-dose (>5 mg/kg base) and long-term (>5 years) use are risk factors related to retinopathy [19]. Rare but severe: Atrioventricular block and Cardiomyopathy under long-term treatment [4].	The following warnings indicate potential side effects based on observation of individual cases: Irreversible retinal damage Cardiomyopathy and QTc prolongation Worsening of psoriasis and porphyria Proximal Myopathy and Neuropathy Neuropsychiatric events Hypoglycemia
	Lupus Erythematosus Treatment of chronic discoid and systemic lupus erythematosus in adults	Adults: 200 to 400 mg daily (in a single dose or two divided doses)		
	Rheumatoid Arthritis: Treatment of acute and chronic rheumatoid arthritis in adults	*Initial adult dosage:* 400 mg to 600 mg daily (in a single dose or two divided doses) *Maintenance adult dosage:* When a good response is obtained, the dosage may be reduced by 50 percent and continued at a maintenance level of 200 mg to 400 mg daily (in a single dose or two divided doses), not exceed 600 mg or 6.5 mg/kg per day, whichever is lower, as the incidence of retinopathy has been reported to be higher when this maintenance dose is exceeded.		

HCQ: hydroxychloroquine, CQ: chloroquine.

dose (ED_{50}) of chloroquine was determined to be 4.4 ± 1μM [30]. Another study demonstrated that a chloroquine concentration of 8.8 ± 1.2μM could inhibit viral replication by 50%, though the exact infectious viral dose used in this study was unclear [37]. In 2006, Biot et al. demonstrated that chloroquine was more potent than hydroxychloroquine *in vitro* at inhibiting SARS-CoV replication in a Vero cell model (EC50 6.5 ± 3.2μM vs 34 ± 5μM, respectively) [38].

Since the current 2019 outbreak, researchers have built upon this knowledge to assess whether or not chloroquine may inhibit SARS-CoV-2. Earlier this March, Yao et al. published results of an antiviral assay using SARS-CoV-2 infected Vero cell lines. In contrast to the above findings with SARS-CoV, hydroxychloroquine was found to be more potent against SARS-CoV-2 [39]. This experiment demonstrated that hydroxychloroquine was more effective at impairing viral replication compared to chloroquine when given post-infection, with a 48-hour EC_{50} of 0.72μM and 5.47μM for hydroxychloroquine and chloroquine respectively [39]. Moreover, hydroxychloroquine was more effective than chloroquine at impairing SARS-CoV-2 viral replication when given prophylactically; the 48-hour EC_{50} for hydroxychloroquine and chloroquine were 5.85μM and 18.01μM respectively [39]. Additional work focused solely on chloroquine denoted similar *in vitro* antiviral findings [40, 41].

To identify a potential drug regimen for use in humans, physiologically based pharmacokinetic modeling was employed by Yao et al. to consider drug administration route, physiological parameters (i.e., intestinal absorption and lung tissue penetration), and drug biochemical properties. The publication reported simulated lung fluid concentrations but did not provide all the details used in the model [39]. The most promising regimen for the treatment of COVID-19 based on this modeling was an initial dose of 400 mg hydroxychloroquine twice daily and a maintenance dose of 200 mg twice daily for four days [39]. A 95% confidence interval for the estimate of the EC_{50} was not provided, and thus this dosing regimen should be interpreted with caution as it may be an inaccurate estimate.

Using a similar antiviral assay at four different multiplicities of infection as previously described, Liu et al. demonstrated that chloroquine was more potent than hydroxychloroquine at impairing viral replication at all multiplicities of infection tested. However, the effect was only statistically significant at multiplicities of infection of 0.01 and 0.2 [42]. To assess SARS-CoV-2 specific virion entry into the endosome lysosome degradation pathway, Liu et al. used a colocalization immunofluorescence assay. They determined that cells treated with hydroxychloroquine or chloroquine had significantly more virion localized to the early endosomes and fewer localized to endolysosomes when compared to untreated viral infected cells [42]. Together, these findings suggest that hydroxychloroquine and chloroquine are effective at impairing SARs-CoV-2 replication *in vitro*.

EVIDENCE FOR HYDROXYCHLOROQUINE AND CHLOROQUINE USE IN THE TREATMENT OF COVID-19

As of April 6, 2020, the published evidence of the effectiveness of hydroxychloroquine or chloroquine for the prevention and treatment of COVID-19 in humans is limited to five small studies and one subjective report (Table 2) [43–48]. In early March, Chen et al. published the results of the first hydroxychloroquine study in patients with COVID-19 [43]. In this small, 30-person inpatient, randomized controlled trial comparing hydroxychloroquine to the standard of care, researchers found no statistically significant differences in time to viral clearance by day seven between those who received hydroxychloroquine (87% clearance) versus those who did not (93%, $P > .05$). They also did not identify any difference in clinical outcomes (i.e., duration of fever, changes in lung imaging). While they did not comment on the severity of illness of those enrolled, those in the hydroxychloroquine and control arms had symptoms for approximately seven and six days respectively. At two weeks, all patients had negative viral nucleic acid tests. On March 16, 2020, Gao et al., extracted data from 100 patients with confirmed COVID-19 from ongoing inpatient studies in China and reported patient improvement with the use of chloroquine [48]. The authors claimed that chloroquine was superior to standard of care treatment in helping reduce time to clinical recovery and improving lung imaging findings; however, no data supporting these findings were published, and no clinical information, including the severity of illness and outcomes, nor statistical analyses were presented in this brief report [48].

On March 20, 2020, Gautret et al. reported results from a non-randomized, open-label study in France, with absent blinding, assessing hydroxychloroquine compared to standard of care treatment, garnering much attention [44]. Twenty-six hospitalized patients were treated with hydroxychloroquine (600 mg for ten days), six of whom received azithromycin (500 mg, followed by 250 mg for a total of five days). Sixteen patients who did not meet study inclusion criteria served as study controls. At the time of enrollment, nearly 17% of all patients were asymptomatic, 61% had upper respiratory symptoms, and 22% had pneumonia or bronchitis-like symptoms. In unadjusted analyses, the authors found significantly reduced viral titers in those who received hydroxychloroquine at day six compared to those who did not (70% vs. 12.5%, $P < .001$); however, six participants in the hydroxychloroquine treatment arm (23%) were excluded from analysis as they required intensive care admission, died, withdrew from the study, or were lost to follow-up. Given the small sample size of the overall study and the exclusion of these six participants in the absence of an intention to treat analysis,

Table 2. Hydroxychloroquine and Chloroquine SARS-CoV-2 (COVID-19) Clinical Studies

Reference	Overall Findings	Limitations	Study design	Number of patients		Treatment regimen	Severity of illness (As reported)	Location	Outcomes
				HCQ	Control				
	Hydroxychloroquine (HCQ)								
Chen J, et al [43].	No statistically significant differences in conversion rate by day 7 (86.7% vs. 93.3%, $P > .05$). No difference in clinical outcomes between groups.	Full article only available in Chinese. Not peer-reviewed. Small sample size.	Randomized controlled trial	15	15	400 mg HCQ for 5 days	Unknown severity; patients had symptoms for 6–7 days	Shanghai, China	At two weeks, all patients had negative viral nucleic acid tests.
Gautret et al [44].	In unadjusted analyses, there were significantly reduced viral titers in the HCQ arm at day 6 (70% compared to 12.5% PCR negative, $P < .001$). All six patients receiving HCQ and azithromycin were SARS-CoV-2 negative on day 6.	Study design. Small sample size/underpowered. Exclusion of six patients from analysis (no intention to treat analyses). Lack of long-term outcomes.	Non-randomized, non-blinded, open-label trial	26	16	600 mg HCQ for 10 days	17% were asymptomatic 61% had upper respiratory symptoms 22% had chest CT confirmed pneumonia	Marseille, France	Six patients in the treatment arm were excluded from analysis (one died, three required ICU admission, one withdrew, one was lost-to-follow-up).
Chen Z, et al [45].	Time to clinical recovery and cough remission were shortened in the HCQ group; resolution of pneumonia was higher in the HCQ group (80.6% vs. 54.8%) Two HCQ patients had mild adverse reactions (rash, headache).	Small sample size. Not peer-reviewed.	Randomized, parallel-group trial	31	31	400 mg HCQ for 5 days	Mild illness (PaO2/FiO2 > 300 mmHg) with chest CT confirmed pneumonia	Wuhan, China	Four patients in the control group developed severe illness (not defined).
Molina et al [46].	8/10 had positive nasopharyngeal swabs at days 5–6 (80%, 95% CI: 49–94).	Small sample size. Not peer-reviewed.	Prospective open-label study	11	0	600mg HCQ for 10 days + azithromycin 500 mg x1, then 250 mg	10/11 were receiving supplemental O2	Paris, France	One patient died, two were transferred to the ICU, one had medications stopped secondary to QTc prolongation.
Gautret et al [47].	Reduced nasopharyngeal viral titers at day 7 (83% negative) and 8 (93%). Mean length of hospitalization of 5 days.	Study design. Small sample size. Short follow-up time. Not peer-reviewed.	Non-randomized, non-blinded, open-label trial	80	0	600 mg HCQ for 10 days + 500 mg, followed by 250 mg azithromycin	5% were asymptomatic 54% had pneumonia 92% of patients had a low national early warning score (NEWS) and mild disease	Marseille, France	Sixty-five (81.3%) patients survived to hospital discharge. Three patients required ICU admission and one died.
	Chloroquine (CQ)								
Gao J et al [48].	CQ was stated to be superior to standard of care treatment in preventing exacerbation of pneumonia, reducing days to conversion rate, and shortening time to clinical recovery.	Combined patients from various ongoing studies. No statistical methodology.	Interim report	100	0	Not reported, likely varied from trial to trial.	NA	Qingdao, China	NA

HCQ: hydroxychloroquine, CQ: chloroquine, NA: not available; ICU: intensive care unit.

the results are significantly biased, and no definitive conclusion should be drawn. Long term outcome data from this study were not available, and the reported outcome of viral clearance may not be a good surrogate for important patient-centered outcomes, such as the need for mechanical ventilation, or mortality. Of the six patients who received azithromycin in addition to hydroxychloroquine, all patients had a negative SARS-CoV-2 PCR test by day 6 without comparison to adequate controls.

Gautret et al. also recently released results of another open-label unblinded study, assessing the combination of hydroxychloroquine and azithromycin in 80 hospitalized patients using the same dosing regimen as previously described [44, 47]. Six patients included in this analysis were included in the original study. Nearly 58% of these patients had at least one underlying chronic health condition. Four patients were asymptomatic at baseline, whereas 41% had upper respiratory tract symptoms, and 54% had pneumonia or bronchitis-like symptoms. Approximately 92% of patients had a low national early warning score, indicating that the overall severity of illness in this population was mild. They reported 83% of patients having undetectable nasopharyngeal viral loads on day seven; however, there was no comparison group and thus, the results are nearly impossible to interpret. In this study, 81% (65/80) of patients survived to hospital discharge, whereas three patients required intensive care, one died, and eleven were still hospitalized.

On March 31, 2020, Chen et al. published results of a randomized parallel-group trial, where 62 hospitalized participants were randomized to receive either 400mg hydroxychloroquine for five days in addition to standard of care or standard of care alone [45]. No placebo was utilized in this study. Standard of care was defined as oxygen, antiviral agents, antibacterial agents, and immunoglobulin, with or without corticosteroids. Only patients with chest CT confirmed pneumonia and mild illness with SaO2/SpO2 >93% or PaO2/FiO2 > 300 mmHg were allowed to enroll. While the researchers found a more substantial proportion of those receiving hydroxychloroquine had clinical improvement of pneumonia (80% vs. 55%, $P < .04$) as determined by day zero to day six chest CT, the methodology was not described. Outcomes were likely based on subjective individual clinician opinion, which may not have been blinded to the treatment allocation. Decreased duration of cough (2.0 vs. 3.1 days, $P < .001$), and shortened time to clinical recovery were also reported for those receiving hydroxychloroquine when compared to the controls; however, only 48% (15/31) of those randomized to hydroxychloroquine and 71% (22/31) of controls had cough at baseline and it is unknown for what duration. Four patients in the control group were said to have progressed to severe illness, though severe illness was not defined. No underlying patient comorbidities were examined, and this may have been an overall confounding variable. Additionally, the specific antiviral and antibacterial medications utilized as a part of standard of care treatment were unspecified and may have affected the results. Overall, similar to prior studies, serious limitations exist, and the results of this non-peer reviewed study should be interpreted with appropriate caution.

On April 3rd, Molina et al. reported outcomes of a prospective cohort of 11 hospitalized patients in response to Gautret et al.'s work [46]. These patients all received the same dose and duration of hydroxychloroquine and azithromycin as the Gautret study. Eight patients enrolled had significant comorbidities, and at the time of enrollment, 10/11 were receiving oxygen supplementation. They found that 80% (95% CI: 49–94%) of patients alive by days five-six still had SARS-CoV-2 RNA positive nasopharyngeal swabs, in contrast to other findings that suggested reduced viral titers. In this small cohort, two patients were transferred to intensive care, one patient died, and one had the medications stopped due to QTc prolongation. Unlike the Gautret study, these sicker patients were included in the analysis.

Importantly, all six of these studies have several important limitations that preclude their incorporation into clinical guidelines. All studies had small sample sizes (<100 participants) and were underpowered to demonstrate a clinical or statistical difference in outcomes. Only two studies were randomized controlled trials, while two were a non-randomized non-blinded open-label study, one was a prospective cohort, and one included a combination of patients from ongoing clinical trials without any statistical assessment available. Four of the five hydroxychloroquine studies shared the same dosing regimen (400 mg hydroxychloroquine for five days); but based on *in vitro* data, doses as high as 800 mg, if not higher, followed by 400 mg for several days, may be required for effective viral clearance in humans [39]. Only one of the six studies was officially peer-reviewed, though concerns have been raised about this article [49]. These studies should, therefore, be interpreted as solely hypothesis-generating and should not serve as supporting evidence for the widespread inclusion of hydroxychloroquine/chloroquine in clinical guidelines. Given that all of these studies reported differences in the disease severity, the overall patient populations may not be comparable. Moving forward, researchers should report the severity of COVID-19 illness based on symptom duration as well as using a systematic, severity scale.

Despite this dearth of evidence of efficacy, in light of the pressure that COVID-19 has posed on national health systems, several official guidelines have already incorporated hydroxychloroquine and chloroquine into the suggested treatment of patients with COVID-19 [28, 50, 51]. A subsequent surge in prescriptions has caused widespread shortages of hydroxychloroquine, which has threatened the supply of this medication for patients with autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. The incorporation of these medications into national treatment guidelines has several consequences. While hydroxychloroquine and chloroquine are seemingly safe medications, they are not benign and

there are important side effects. Although side effects are not common with these medicines, rare adverse effects of a medication that is prescribed commonly without a rigorous evidence base can be dangerous on a population level. Bluntly stated, any significant side effect is not justifiable if the drug is not effective. Given these potentially deleterious consequences of widespread use of these drugs in the absence of robust data, the European Medicines Agency has refused to approve chloroquine for COVID-19 and has restricted its use to solely clinical trials or through national emergency use programs [52].

To date, there are no studies of hydroxychloroquine as treatment of COVID-19 that are adequately powered to demonstrate efficacy or the absence of harm. Presently over 1 million cases of COVID-19 have been identified. If all patients had received hydroxychloroquine or chloroquine, even if, for example, QTc prolongation and arrhythmia are seen in less than 0.1% of the population, this would equate to 1000 adverse events, which is arguably not acceptable if the medication is not effective. Of note, Gautret and Molina examined the treatment of COVID-19 with combination hydroxychloroquine and azithromycin, which many clinicians have now started to prescribe together, without evidence, in the outpatient setting. In these studies, this combination was utilized in an inpatient environment, presumably with some degree of cardiac monitoring. Both azithromycin and hydroxychloroquine, alone, but especially in combination, can increase the risk of QTc prolongation, and lead to malignant arrhythmias [4, 6]. In a retrospective population study of 60 000 patients receiving hydroxychloroquine for rheumatic disease, researchers found an increased risk of cardiovascular mortality when hydroxychloroquine was used in combination with azithromycin (50 deaths) when compared to hydroxychloroquine and amoxicillin (25 deaths) (Hazard Ratio: 2.19, 95% CI: 1.22–3.94); however, all-cause mortality was the same (Hazard Ratio 1.34) [53]. This risk can be significantly increased when considering other commonly prescribed medications with the potential for QTc prolongation like selective serotonin reuptake inhibitors, tricyclic antidepressants, antipsychotics, and various antimicrobials. These small studies demonstrate insufficient evidence to therefore support the routine use of hydroxychloroquine and azithromycin outside of a clinical trial with adequate cardiac monitoring.

As a final consideration, lopinavir/ritonavir, a protease inhibitor broadly available for treating HIV infection with *in vitro* activity against SARS-CoV infection, has been recommended by the Chinese authorities to treat COVID-19. A recent open-label randomized trial of 14 days of lopinavir/ritonavir therapy among severely ill patients hospitalized with COVID-19 showed no clinical improvement and no reduction in SARS-CoV-2 viral load beyond standard care [54]. Despite these negative results, lopinavir/ritonavir is still commonly used to treat COVID in some settings, often concomitantly with hydroxychloroquine or chloroquine. Importantly, lopinavir/ritonavir can potentially increase chloroquine plasma levels by inhibition of cytochrome P450 CYP2D6 enzyme metabolism, therefore increasing the risk for malignant arrhythmias. Moreover, other factors such as myocarditis and myocardial ischemia, reported in the context of COVID-19 [55], or hypoxia and electrolyte abnormalities, often seen in the acute phase of severe COVID-19, can further contribute to the development of acute arrhythmias [56]. Thus, this calls for prudent and well-informed use of lopinavir/ritonavir and QTc-prolonging medications, like hydroxychloroquine and azithromycin, to treat COVID-19.

CONCLUSION

Additional studies examining hydroxychloroquine and chloroquine in preventing and treating COVID-19 are desperately needed. Given the weak evidence available, larger controlled trials are needed to more thoroughly assess if hydroxychloroquine/chloroquine have a clinical benefit in COVID-19. Several ongoing randomized clinical trials are actively recruiting participants to better address this question. These randomized trials are powered to show a reduction in meaningful clinical outcomes such as the development of COVID-19 in prevention trials or the need for hospitalization, critical care, or death in treatment trials. The results of these trials will be instrumental in determining whether or not these two antimalarial medications are at all efficacious, and if so, at what dose and for what duration they should be safely recommended in guidelines.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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websites. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report - 75. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200401-sitrep-72-covid-19.pdf?sfvrsn=3dd8971b_2. Accessed 4 April 2020.
2. NIH U.S. National Library of Medicine. Available at: https://clinicaltrials.gov/. Accessed 4 April 2020.
3. Kim AHJ, Sparks JA, Liew JW, et al. A rush to judgment? Rapid reporting and dissemination of results and its consequences regarding the use of hydroxychloroquine for COVID-19. Ann Intern Med **2020**; *In Press*.
4. Tönnesmann E, Kandolf R, Lewalter T. Chloroquine cardiomyopathy - a review of the literature. Immunopharmacol Immunotoxicol **2013**; 35:434–42.
5. Ben-Zvi I, Kivity S, Langevitz P, Shoenfeld Y. Hydroxychloroquine: from malaria to autoimmunity. Clin Rev Allergy Immunol **2012**; 42:145–53.
6. Concordia Pharmaceuticals Inc. Plaquenil Hydroxychloroquine Sulfate Tablets, USP; **2017**.
7. Sanofi-Aventis US. LLC. Aralen Chloroquine Phosphate, USP; **2013**.
8. Koranda FC. Antimalarials. J Am Acad Dermatol **1981**; 4:650–5.
9. Katz SJ, Russell AS. Re-evaluation of antimalarials in treating rheumatic diseases: re-appreciation and insights into new mechanisms of action. Curr Opin Rheumatol **2011**; 23:278–81.
10. De Carolis S, Botta A, Salvi S, et al. Is there any role for the hydroxychloroquine (HCQ) in refractory obstetrical antiphospholipid syndrome (APS) treatment? Autoimmun Rev **2015**; 14:760–2.
11. Belizna CC, Richard V, Thuillez C, et al. Insights into atherosclerosis therapy in antiphospholipid syndrome. Autoimmun Rev **2007**; 7:46–51.
12. Savarino A, Shytaj IL. Chloroquine and beyond: exploring anti-rheumatic drugs to reduce immune hyperactivation in HIV/AIDS. Retrovirology **2015**; 12:51.
13. Rebecca VW, Amaravadi RK. Emerging strategies to effectively target autophagy in cancer. Oncogene **2016**; 35:1–11.
14. Drent M, Proesmans VLJ, Elfferich MDP, et al. Ranking self-reported gastrointestinal side effects of pharmacotherapy in sarcoidosis. Lung **2020**; 198:395–403.
15. Srinivasa A, Tosounidou S, Gordon C. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: a brand-related issue? J Rheumatol **2017**; 44:398.
16. Tétu P, Hamelin A, Lebrun-Vignes B, et al. [Prevalence of hydroxychloroquine-induced side-effects in dermatology patients: a retrospective survey of 102 patients]. Ann Dermatol Venereol **2018**; 145:395–404.
17. Arnaout A, Robertson SJ, Pond GR, et al. A randomized, double-blind, window of opportunity trial evaluating the effects of chloroquine in breast cancer patients. Breast Cancer Res Treat **2019**; 178:327–35.
18. Global Health, Division of Parasitic Diseases and Malaria. Choosing a drug to prevent malaria. **2020**. Available at: https://www.cdc.gov/malaria/travelers/drugs.html. Accessed 6 April 2010.
19. Jorge A, Ung C, Young LH, et al. Hydroxychloroquine retinopathy - implications of research advances for rheumatology care. Nat Rev Rheumatol **2018**; 14:693–703.
20. Ducharme J, Farinotti R. Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. Clin Pharmacokinet **1996**; 31:257–74.
21. Furst DE, Lindsley H, Baethge B, et al. Dose-loading with hydroxychloroquine improves the rate of response in early, active rheumatoid arthritis: a randomized, double-blind six-week trial with eighteen-week extension. Arthritis Rheum **1999**; 42:357–65.
22. Kobak S, Deveci H. Retinopathy due to antimalarial drugs in patients with connective tissue diseases: are they so innocent? A single center retrospective study. Int J Rheum Dis **2010**; 13:e11–5.
23. Marmor MF, Kellner U, Lai TY, et al.; American Academy of Ophthalmology. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy (2016 Revision). Ophthalmology **2016**; 123:1386–94.
24. Mzayek F, Deng H, Mather FJ, et al. Randomized dose-ranging controlled trial of AQ-13, a candidate antimalarial, and chloroquine in healthy volunteers. PLoS Clin Trials **2007**; 2:e6.
25. O'Laughlin JP, Mehta PH, Wong BC. Life threatening severe QTc prolongation in patient with systemic lupus erythematosus due to hydroxychloroquine. Case Rep Cardiol **2016**; 2016:4626279.
26. Morgan ND, Patel SV, Dvorkina O. Suspected hydroxychloroquine-associated QT-interval prolongation in a patient with systemic lupus erythematosus. J Clin Rheumatol **2013**; 19:286–8.
27. U.S. Food and Drug Administration. EUA Chloroquine Phosphate Health Care Provider Fact Sheet April 3, **2020**.
28. U.S. Food and Drug Administration. EUA Hydroxychloroquine sulfate Health Care Provider Fact Sheet March 28, **2020**.
29. Chorin E, Dai M, Shulman E, et al. The QT interval in patients with SARS-CoV-2 infection treated with hydroxychloroquine/azithromycin. medRxiv **2020**; 2020.04.02.20047050.
30. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J **2005**; 2:69.
31. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet **2020**; 395:565–74.
32. Chen J. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes Infect **2020**; 22:69–71.
33. Fantini J, Scala CD, Chahinian H, Yahi N. Structural and molecular modeling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. Int J Antimicrob Agents **2020**; 105960.
34. Al-Bari MA. Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. J Antimicrob Chemother **2015**; 70:1608–21.
35. Burkard C, Verheije MH, Wicht O, et al. Coronavirus cell entry occurs through the endo-/lysosomal pathway in a proteolysis-dependent manner. PLoS Pathog **2014**; 10:e1004502.
36. Mingo RM, Simmons JA, Shoemaker CJ, et al. Ebola virus and severe acute respiratory syndrome coronavirus display late cell entry kinetics: evidence that transport to NPC1+ endolysosomes is a rate-defining step. J Virol **2015**; 89:2931–43.
37. Keyaerts E, Vijgen L, Maes P, et al. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun **2004**; 323:264–8.
38. Biot C, Daher W, Chavain N, et al. Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. J Med Chem **2006**; 49:2845–9.
39. Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis **2020**:ciaa237.
40. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res **2020**; 30:269–71.
41. Cortegiani A, Ingoglia G, Ippolito M, et al. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care **2020**; *In Press*.
42. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov **2020**; 6:16.
43. Chen J, Liu D, Liu L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). Journal of Zhejiang University **2020**; *In Press*.
44. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents **2020**; 105949.
45. Chen Z, Hu J, Zhang Z, et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. medRxiv **2020**.
46. Molina JM, Delaugerre C, Goff JL, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Med Mal Infect **2020**; *In Press*.
47. Gautret P, Lagier JC, Parola P, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: an observational study. Available at: https://www.mediterranee-infection.com/wp-content/uploads/2020/03/COVID-IHU-2-1.pdf.
48. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends **2020**; 14:72–3.
49. Voss A. Statement on IJAA paper. Available at: https://www.isac.world/news-and-publications/official-isac-statement. Accessed 5 April 2020.
50. [Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia]. Zhonghua Jie He He Hu Xi Za Zhi **2020**; 43:185–8.
51. Agencia Española de Medicamentos y Productos Sanitarios. Tratamientos disponibles para el manejo de la infección respiratoria por SARS-CoV-2. Available at: https://www.aemps.gob.es/laAEMPS/docs/medicamentos-disponibles-SARS-CoV-2-28-3-2020.pdf. Accessed 6 April 2020.
52. European Medicines Agency Science Medicines Health. COVID-19: chloroquine and hydroxychloroquine only to be used in clinical trials or emergency use programmes. Available at: https://www.ema.europa.eu/en/documents/press-release/

covid-19-chloroquine-hydroxychloroquine-only-be-used-clinical-trials-emergency-use-programmes_en.pdf. Accessed 6 April 2020.
53. Lane JWJ. Safety of hydroxychloroquine, alone and in combination with azithromycin, in light of rapid wide-spread use for COVID-19: a multinational, network cohort and self-controlled case series study. Available at: https://data.ohdsi.org/Covid19EstimationHydroxychloroquine/. Accessed 6 April 2020.
54. Cao B, Wang Y, Wen D, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. N Engl J Med **2020**; *In Press*.
55. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med **2020**; 8:420–2.
56. Lakkireddy DR, Chung MK, Gopinathannair R, et al. Guidance for cardiac electrophysiology during the coronavirus (COVID-19) pandemic from the Heart Rhythm Society COVID-19 task force; electrophysiology section of the American College of Cardiology; and the electrocardiography and arrhythmias committee of the council on clinical cardiology, American Heart Association. Heart Rhythm **2020**.