Pharmacokinetics and Pharmacological Properties of Chloroquine and Hydroxychloroquine in the Context of COVID-19 Infection

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Chloroquine and hydroxychloroquine are quinoline derivatives used to treat malaria. To date, these medications are not approved for the treatment of viral infections, and there are no well-controlled, prospective, randomized clinical studies or evidence to support their use in patients with coronavirus disease 2019 (COVID-19). Nevertheless, chloroquine and hydroxychloroquine are being studied alone or in combination with other agents to assess their effectiveness in the treatment or prophylaxis for COVID-19. The effective use of any medication involves an understanding of its pharmacokinetics, safety, and mechanism of action. This work provides basic clinical pharmacology information relevant for planning and initiating COVID-19 clinical studies with chloroquine or hydroxychloroquine, summarizes safety data from healthy volunteer studies, and summarizes safety data from phase II and phase II/III clinical studies in patients with uncomplicated malaria, including a phase II/III study in pediatric patients following administration of azithromycin and chloroquine in combination. In addition, this work presents data describing the proposed mechanisms of action against the severe acute respiratory distress syndrome coronavirus–2 and summarizes clinical efficacy to date.

Since coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory distress syndrome (ARDS) coronavirus–2 (SARS-CoV-2), first emerged in late 2019, there has been a widespread search for effective preventive and treatment options to ameliorate the devastating pandemic. As vaccine availability is likely 12 or more months away, there is an immediate need to identify therapeutic options. Early interest, experimental usage, clinical studies, and emergency use authorizations have focused on several potential therapies including antivirals such as remdesivir or antimalarial agents such as chloroquine[1] and hydroxychloroquine[2] with or without the antibacterial agent azithromycin,[3] which has been previously used in the treatment of chloroquine-resistant malaria given reported *in vitro* synergy.[4]

With the recent results from the Randomised Evaluation of COVID-19 Therapy (RECOVERY) trial (NCT04381936) as well as the announced suspension of hydroxychloroquine arms in several other trials, there is decreasing support for its use in hospitalized or severely ill patients. However, many trials, particularly in prevention or early treatment settings, or in specific subpopulations, are ongoing. This publication seeks to summarize the current state of knowledge for both chloroquine and hydroxychloroquine, including pharmacokinetic (PK) properties, known pharmacology, and evidence related to use in treatment of COVID-19. Prior to the current pandemic, more studies had been reported with use of chloroquine than hydroxychloroquine; thus there is notably more information available for chloroquine regarding metabolism, safety, and overall use.

CHEMICAL STRUCTURES

Both chloroquine and hydroxychloroquine are orally administered 4-aminoquinoline antimalarial compounds. The chemical structures of chloroquine and hydroxychloroquine are shown in **Figure 1**. Chloroquine is administered as chloroquine phosphate and has a molecular weight of 515.9 g/mol (base: 319.9 g/mol). Hydroxychloroquine is administered as hydroxychloroquine sulfate and has a molecular weight of 433.95 g/mol (base: 335.9 g/mol).

CLINICAL PHARMACOLOGY SUMMARY

Table S1 summarizes limited information on the clinical PK of chloroquine and hydroxychloroquine that is available from Aralen (chloroquine phosphate, USP)[5] and Plaquenil (hydroxychloroquine sulfate tablets, USP)[6] US product labels.

Information on the PK of pharmacologically active metabolites of chloroquine and hydroxychloroquine is discussed in multiple

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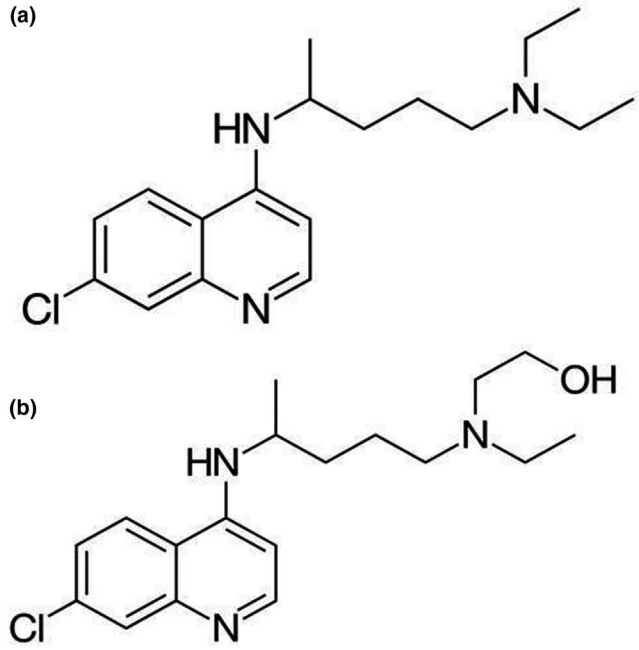

Figure 1 Chemical structure of chloroquine **(a)** and hydroxychloroquine **(b)**.

publications.[7-21] A summary of the compiled information is provided below.

Absorption

Chloroquine. The majority of the available PK data in literature are from healthy volunteers receiving a single oral dose containing 300 mg or 600 mg chloroquine base (**Table 1**). Cross study comparisons of exposure parameter estimates (maximum concentration (C_{max}), area under the concentration-time curve (AUC), and time to reach C_{max} (T_{max})) were not pursued, as different formulations were used across the different studies. Gustafsson *et al*. reported the mean (± SD) bioavailability of chloroquine from oral tablet as 89 ± 16%.[22] T_{max} ranged from 1 to 6 hours across studies, and this variability could be due to *in vivo* PK variability, formulation differences, and/or administration conditions. In addition, despite being in use for years, the available PK data on the effect of food on chloroquine absorption are limited.

Hydroxychloroquine. Most of the available PK data in literature are from healthy volunteers receiving a single oral dose of 155 mg hydroxychloroquine base (**Table 2**). Cross-study comparisons of exposure parameter estimates (C_{max}, AUC, and T_{max}) were not pursued, as the exact information on different formulations that were used across the different studies was not available. Tett *et al*. reported the mean (± SD) fraction of the oral dose absorbed as 0.74 (± 0.13) based on the blood and urine PK data.[16] McLachlan *et al*. reported similar findings with the reported mean fraction absorbed as 0.79 in nine patients with rheumatoid arthritis who received two doses of 155 mg racemic hydroxychloroquine base each, as a tablet and by intravenous infusion.[25] In a separate study, McLachlan *et al*. reported the lack of food-effect on the extent of absorption of hydroxychloroquine, except that the absorption lag-time appeared to be significantly prolonged in the presence of food.[26] Tett *et al*. evaluated between-subject and within-subject PK variability in six healthy volunteers and reported that the between-subject variability (BSV) in relative bioavailability was (27–38%) higher than the within-subject variability (11–16%).[27] Based on these findings, Tett *et al*. suggest a need for individualization of dosing to target concentrations associated with optimal outcomes and may minimize variability in response.[27] The available PK data for hydroxychloroquine are limited to ascertain PK linearity.

McLachlan *et al*. evaluated blood and plasma concentrations of the enantiomers of hydroxychloroquine in a separate study and reported that (R)-hydroxychloroquine had higher blood (ratio = 2.2, range = 1.6–2.9) and plasma (ratio = 1.6, range = 1.2–1.9) concentrations compared with the (S)-enantiomer.[30] Ducharme *et al*. noted similar findings with reported 62 ± 3% (mean ± SD) of the AUC of rac-hydroxychloroquine AUC for R(-)-hydroxychloroquine.[17]

Distribution

Chloroquine. Estimates reported for apparent volume of distribution (V/F) for chloroquine are relatively large and with notable variation (**Table 1**). Edwards *et al*. reported that chloroquine concentrations in packed cells were higher than the concentrations in plasma with a median ratio (cell:plasma) between 3 and 4. Similar estimates were reported by Gustafsson *et al*. with a mean ratio (cell:plasma) of 4.8 (range: 2–5).[22] Preclinical studies indicate it is widely distributed in the liver, spleen, kidney, and lung, with concentrations several hundred-fold above plasma concentrations.[5] Walker *et al*. evaluated plasma protein binding for chloroquine using equilibrium dialysis and reported mean protein binding as 61 ± 9% (range 46–74%) in plasma from healthy subjects and 64 ± 7% (range 55–79%) in plasma from patients with rheumatoid arthritis.[31] Ofori-Adjei *et al*. also evaluated protein binding of chloroquine enantiomers using equilibrium dialysis and reported mean protein binding estimates of 59% for chloroquine that is within the range reported by Walker *et al*.[32] The findings reported by Ofori-Adjei *et al*. suggest chloroquine protein binding is concentration independent over the range of 25–400 ng/mL and (S)-chloroquine binds more to plasma (~ 67%) than (R)-chloroquine (~ 49%). Chloroquine is also reported to transfer via placenta and into milk.[33,34]

Hydroxychloroquine. The reported mean (± SD) blood-to-plasma hydroxychloroquine concentration ratio is 7.2 (± 4.2).[15] Estimated protein binding is between 30% and 50%, and hydroxychloroquine is reported to bind to both albumin and alpha-1-acid glycoprotein.[35,36] The findings reported by McLachlan *et al*.[35] suggest hydroxychloroquine demonstrates stereoselective protein binding, with (S)-hydroxychloroquine binding more to plasma (~ 64%) than (R)-hydroxychloroquine (~ 37%), resulting in approximately 52% average protein binding

Table 1 Mean ± SD pharmacokinetic estimates for chloroquine[a]

CQ base dose	Study population (Age range in years)	N (nF)	C_{max}, μg/mL	T_{max}[b], h	AUC (Time Duration[c]), h*μg/mL	$T_{1/2}$, Days	CL	Vd	Bioanalytical method type: reported LLOQ	References
Following administration via IV infusion										
15 mg/kg as 4-hour IV infusion	HV (18–46)	10 (2)	P: 0.9 ± 0.5	NA	NA	NA	P: 36 ± 14 L/h	P: 132 ± 50 L/kg	HPLC: NA	23
	Malaria Patients (15–35)	9 (2)	P: 1.7 ± 0.6	NA	NA	NA	P: 32 ± 15 L/h	P: 136 ± 64 L/kg	HPLC: NA	
300 mg as 12–24 min IV infusion	HV (20–36)	11 (0)	P: 0.8 ± 0.3	NA	P: 7.5 ± 2.4 (168 h)	P: 12	P: 43 ± 10 L/h	P: 204 ± 86 L/kg	HPLC: 1 ng/mL	22
Following single dose administration via oral route										
150-mg tablet	HV (37–42)	5 (1)	NA	NA	B: 21.6 P: 1.9 (225 days)	B: 27 P: 33	B: 0.1 L/kg/h P: 1.1 L/kg/h	B: 79 L/kg P: 869 L/kg	HPLC: 1.5 Nmol/L in blood, 0.5 Nmol/L	21
300-mg tablet			NA	NA	B: 29.6 P: 5.2 (225 days)	B: 53 P: 63	B: 0.1 L/kg/h P: 0.8 L/kg/h	B: 185 L/kg P: 882 L/kg		
600-mg tablet			NA	NA	B: 92.1 P: 9.4 (225 days)	B: 52 P: 58	B: 0.1 L/kg/h P: 0.9 L/kg/h	B: 81 L/kg P: 710 L/kg		
300-mg tablet	HV (20–36)	11	P: 0.08 ± 0.01	P: 1–6	P: 6 ± 1.3 (168 h)	P: 12	NA	NA	HPLC: 1 ng/mL	22
300 mg in solution			P: 0.07 ± 0.01	P: 1–6	P: 5 ± 0.9 (168 h)	P: 9	NA	NA		
300-mg tablet	HV (23–30)	8 (3)	P: 0.4 ± 0.1	P: 1–6	NA	P: 4.4 ± 0.4	P: 5 ± 0.4 L/kg/h	P: 16 ± 3 L/kg	HPLC: 1 ng/mL	8
600-mg tablet	HV with history of CQ-induced pruritus (19–23)	8 (0)	P: 0.4 ± 0.2	P: 4–6	P: 19 ± 2 (168 h)	NA	P: 16 ± 5 L/h[d]	NA	HPLC: 10 ng/mL	13
	HV without history of CQ-induced pruritus (19–23)	6 (0)	P: 0.2 ± 0.06	P: 2–6	P: 17 ± 2 (168 h)	NA	P: 17 ± 5 L/h[d]	NA		
600-mg tablet	HV (20–47)	16 (12)	P: 0.3 (0.1–0.5[c])	P: 1–6	P: 15 (11–25[b]) (∞)	P: 6 (3–14)	P: 40 (25–57[b]) L/h	P: 7,600 (4,450–12,400)l	HPLC: 5 ng/mL	14
Following multiple doses administration via oral tablets										
300-mg dose/ week for 3 weeks	HV (41[e])	5 (2)	NA	NA	P: 25 (42 Days)	P: 16 ± 5[f]	P: 0.4 ± 0.1 L/kg/h	P: 250 ± 116 L/kg	HPLC: 1 ng/mL	24
200-mg dose twice/ week for 3 weeks	HV (31[e])	4 (0)	NA	NA	P: 14 (42 Days)	P: 16 ± 6[f]	P: 0.6 ± 0.1 L/kg/h	P: 302 ± 102 L/kg		
50-mg dose/day for 3 weeks	HV (30[e])	5 (0)	NA	NA	P: 13 (42 Days)	P: 20 ± 14[f]	P: 0.6 ± 0.1 L/kg/h	P: 283 ± 112 L/kg		

AUC, area under the concentration-time curve; B, blood; CL, apparent clearance; $C_{max,}$ maximum plasma concentration; CQ, chloroquine; h, hours; HPLC, high-performance liquid chromatography; HV, healthy volunteers; IV, intravenous; LLOQ, lower limit of quantification; min, minutes; N, number of subjects/patients; NA, information not available/reported; nF, number of female subjects/patients; P, plasma; SD, standard deviation; $T_{1/2}$, elimination or terminal half-life; T_{max}, time to reach C_{max}; Vd, apparent volume of distribution.

[a]Estimates were adjusted for unit uniformity and rounded. [b]Range reported. [c]If specific time duration for AUC is not reported, approximate time duration reported based on sampling duration or reported last concentrations. [d]Renal clearance estimates. [e]Mean age reported. [f]AUC estimates corrected to a single 600-mg dose.

Table 2 Mean ± SD pharmacokinetic parameter estimates for hydroxychloroquine[a]

HCQ dose	Study population (Age range in years)	N (nF)	C_{max}, μg/mL	T_{max}[b], h	AUC (Time Duration[c]), h*μg/mL	$T_{1/2}$, Days	CL (L/h)	Vd	Bioanalytical method type: Reported LLOQ	References
Following administration via IV infusion										
155 mg: IV infusion over 0.5 h	HV (19–27)	5 (3)	P: 0.8 (0.4–1.1[b])	NA	NA	P: 26 ± 10	P: 50 ± 23	P: 36757 ± 11102	HPLC: 1 ng/mL	15
			B: 1.9 (1.1–2.4[b])			B: 44 ± 12	B: 6 ± 1	B: 5791 ± 2566		
310 mg: IV infusion over 0.5 h		4 (NA)	P: 1.7 (1.4–2.4[b])			P: 53 ± 22	P: 30 ± 6	P: 51757 ± 30311		
			B: 3.3 (2.3–4.2[b])			B: 43 ± 22	B: 6 ± 1	B: 5254 ± 2021		
Following single dose administration via oral route										
155-mg tablet	HV (19–27)	5 (3)	P: 0.05 (0.03–0.08[b])	2–4.5	NA	P: 32 ± 9	NA	NA	HPLC: 1 ng/mL	16
			B: 0.2 (0.2–0.4[b])			B: 50 ± 16				
155-mg tablet with food	HV (26.1 ± 6.9[d])	9 (4)	B: 0.2 (0.1–0.3[b])	B: 2.5–6	NA	NA	NA	NA	HPLC: NA	26
155 mg in aqueous solution with food,			B: 0.2 (0.07–0.4[c])	B: 2.3–5.2						
155-mg tablet	HV (20–36)	24 (0)	B: 0.1 ± 0.04	B: 3–4	B: 6.4 ± 0.5 (168 h)	NA	B: 2.7 ± 1.6[d]	NA	HPLC: 5 ng/mL	17
155-mg tablet	HV (20–48)	10 (4)	B: 0.2 ± 0.01	B: 1.5–4.6	B: 1.8 ± 0.9 (32 h)	NA	NA	NA	HPLC: 1 ng/mL	28
155 mg in aqueous solution			B: 0.2 ± 0.01	B: 0.5–5.7	B: 1.7 ± 1 (32 h)					
155 mg two different tablet formulation	HV (21–29)	20 (0)	P: 0.03 ± 0.001	P: 4 ± 1[d]	P: 1.8 ± 0.4 (∞)	P: 11 ± 3	NA	NA	LC-MS/MS: 0.2 ng/mL	29
			P: 0.04 ± 0.03	P: 4 ± 1[d]	P: 2 ± 0.4 (∞)	P: 12 ± 4				

AUC, area under the concentration-time curve; B, blood; CL, apparent clearance; $C_{max,}$ maximum plasma concentration; CQ, chloroquine; h, hours; HCQ, hydroxychloroquine; HPLC, high-performance liquid chromatography; HV, healthy volunteers; IV, intravenous; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLOQ, lower limit of quantification; N, number of subjects/patients; NA, information not available/reported; nF, number of female subjects/patients; P, plasma; SD, standard deviation; $T_{1/2}$, elimination or terminal half-life; T_{max}, time to reach C_{max}; Vd, apparent volume of distribution.
[a]Estimates were adjusted for unit uniformity and rounded. [b]Range reported. [c]If specific time duration for AUC is not reported, approximate time duration reported based on sampling duration or reported last concentrations. [d]Renal clearance estimates.

for racemic mixture. Hydroxychloroquine is reported to transfer via placenta and into milk.[19,37-39]

Metabolism and drug interaction

Chloroquine. Information on the metabolism and excretion of chloroquine is scarce in the literature, including information on specific metabolism pathways. Ducharme *et al*. discussed the chloroquine metabolism data from literature and concluded that cytochrome P450 3A isozymes (CYP3As) and CYP2D6 are two enzymes affected or involved in chloroquine metabolism.[40] Projean *et al*. concluded based on the investigations of chloroquine metabolism in human liver microsomes and recombinant human CYP450s that chloroquine would be metabolized into N-desethylchloroquine primarily via CYP2C8 and CYP3A4.[41] The same study noted that at low chloroquine concentrations, CYP2D6 may also play a significant role. Based on literature review, it is proposed that the coadministration of drugs that modulate CYP2C8, CYP3A4, and CYP2D6 could have potential effects on the PK of chloroquine.[42] Based on *in vitro* studies, chloroquine is a weak inhibitor of CYP2D6 but is unlikely to have a significant effect on the PK of other CYP2D6 substrates in humans.[42] Additional information on the potential of drug–drug interaction with chloroquine as object and precipitant is detailed by Kiang *et al*.[43-45] **Table 3** presents results of clinical drug interaction studies involving chloroquine as the precipitant and as the object.

Hydroxychloroquine. Similar to chloroquine, information on the metabolism and excretion of hydroxychloroquine is scarce in the literature, including information on specific metabolism pathways. Hydroxychloroquine demonstrates similar PK properties to chloroquine. Hydroxychloroquine's major metabolite is desethylhydroxychloroquine due to its metabolism by cytochrome P450 enzymes CYP2D6, 2C8, 3A4, and 3A5.[53] Hydroxychloroquine is primarily eliminated through the kidneys. Rainsford, *et al*. discuss the information on hydroxychloroquine metabolism pathway and potential drug interactions from the literature.[19] In this review, the most significant drug interactions of relevance noted are with methotrexate. Based on the findings from the two studies,[28,50] Rainsford, *et al*. concluded coadministration of hydroxychloroquine with methotrexate causes reduced C_{max} while delaying T_{max} and lack of any significant drug interactions related to effects on the PK of hydroxychloroquine by methotrexate. In addition, hydroxychloroquine is considered a weak inhibitor of CYP2D6, and 400 mg daily hydroxychloroquine dose increased metoprolol by 50.7%.[54,55] **Table 4** presents results of clinical drug interaction studies involving hydroxychloroquine as the precipitant and the object, respectively.

Excretion

Chloroquine. The estimates for elimination half-life for chloroquine vary significantly (**Table 1**). Following multiple doses, chloroquine elimination half-life is reported to range from 30 to 60 days.[57,58] Krishna *et al*. concluded that due to the large V/F (= 100 L/kg), distribution rather than elimination processes determine the blood concentration profile of chloroquine in patients with acute malaria.[57] Other potential reasons for high variability in elimination half-life for chloroquine could be differences in PK follow-up duration, improvement in sensitivity of analytical methods over time, and/or different methodologies of calculations. Regarding chloroquine apparent clearance (CL/F), the reported mean estimates ranged from 16 to 40 L/hour (**Table 1**). Estimated urinary recovery of chloroquine is reported to be 46% and 55%.[20,22] Augustijns *et al*. evaluated whole blood PK of chloroquine enantiomers in humans after a single oral dose of the separate enantiomers and reported that the total body clearance was lower for the (R)-enantiomer (8 ± 2 L/hour) than for the (S)-enantiomer (14 ± 4 L/hour).[10] The study also reported that terminal half-life and mean residence time were longer for (R)-chloroquine (12 days and 16 days, respectively) than for (S)-chloroquine (10 days and 11 days, respectively).

Hydroxychloroquine. Similar to chloroquine, information on the metabolism and excretion of hydroxychloroquine is scarce in the literature including information on specific metabolism pathways. Fan *et al*. reported the mean estimated elimination half-life for hydroxychloroquine to be 11 to 12 days when estimated with hydroxychloroquine plasma concentration over 62 days and 1 to 2 days when estimated with hydroxychloroquine plasma concentration over 72 hours.[29] These findings suggest the impact of PK follow-up duration on the reported high variability in elimination half-life for hydroxychloroquine across the studies.

The reported mean amount of hydroxychloroquine excreted unchanged in the urine ranges between 23–27%.[15,16,69] McLachlan *et al*. evaluated renal clearance of the hydroxychloroquine enantiomers and reported that (S)-hydroxychloroquine had a mean (± SD) renal clearance from blood of 41 ± 11 mL/min, approximately twice that of (R)-hydroxychloroquine.[30] Ducharme *et al*. note similar findings with the reported total urinary excretion of (S)-hydroxychloroquine to be higher than that of (R)-hydroxychloroquine.[17] In the same study, the estimated elimination half-life of (S)-hydroxychloroquine (19 ± 5 days) was significantly shorter than that of (R)-hydroxychloroquine (22 ± 6 days), partly due to its faster urinary excretion and hepatic metabolism. Its renal clearance was twice that of (R)-hydroxychloroquine (4.61 ± 4.01 vs 1.79 ± 1.30 L/hour).

POPULATION PHARMACOKINETIC MODELS

With respect to the use of population PK (POP-PK) analysis approach, three studies for chloroquine[60-62] and four studies for hydroxychloroquine were identified during the literature search.[18,63-65] These studies are summarized in **Table 5**, and key information is summarized below. It is noteworthy that the intensity of the PK sampling, sensitivity of bio-analytical method used, and/or sampling duration may affect the PK model selection process. In addition, the lack of variability in intrinsic and extrinsic factors within the patient/subject population, whose PK data are being utilized for POP-PK analysis, may affect the ability to identify relationships between covariates and PK.

Table 3 Drug–drug interaction studies involving chloroquine as the object and the precipitant

Object/Precipitant	Object/Precipitant dose	Change in AUC (%)	Chloroquine dose	References
Object				
Acetaminophen (paracetamol)	1.5 g	21.7	250 mg chloroquine phosphate IM (150 mg chloroquine base) single dose	46
Levonorgestrel	150 µg	56.7	300 mg (formulation not mentioned) single dose	47
Primaquine	30 mg (base)	21	1,000 mg chloroquine phosphate (600 mg base) single dose	14
Tafenoquine	450 mg	23.5	1,000 mg chloroquine phosphate q.d. (600 mg base) for 2 days	48
Ethinylestradiol	30 µg	−4.6	300 mg (formulation not mentioned) single dose	47
Azithromycin	1,000 mg	3.0	1,000 mg chloroquine phosphate (600 mg base) q.d. on Days 1 and 2, 500 mg chloroquine phosphate (300 mg base) on Day 3	49
Precipitant				
Acetaminophen (paracetamol)	500 mg single dose	23.9	600 mg (formulation not mentioned) single dose	50
Cimetidine	400 mg q.d. for 12 days	113	chloroquine sulfate (600 mg base)	8
Metamizole (dipyrone)	500 mg single dose	22.9	600 mg (formulation not mentioned) single dose	8
Acetylsalicylic acid (aspirin)	325 mg single dose	0.9	600 mg (formulation not mentioned) single dose	8
Azithromycin	1,000 mg q.d. for 3 days	−3.8	1,000 mg chloroquine phosphate (600 mg base) q.d. on Days 1 and 2, 500 mg chloroquine phosphate (300 mg base) on Day 3	50
Methylene blue	130 mg b.i.d. for 3 days	−17.9	Males: 1,000 mg chloroquine phosphate q.d. (600 mg base) on Days 1 and 2 and 500 mg (300 mg base) on Day 3 Females: 750 mg chloroquine phosphate (450 mg base) on Days 1 and 2 and 375 mg (225 mg base) on Day 3	51
Primaquine	30 mg (base) single dose	6.7	1,000 mg chloroquine phosphate (600 mg base) single dose	48
Tafenoquine	450 mg q.d. 2 days	−4	1,000 mg chloroquine phosphate (600 mg base) q.d. on Days 1 and 2, 500 mg chloroquine phosphate (300 mg base) on Day 3	49
Tafenoquine	450 mg single dose	−3.7	1,000 mg chloroquine phosphate (600 mg base) q.d. for 2 days	49
Tafenoquine	450 mg q.d. Days 2 and 3	5.6	1,000 mg chloroquine phosphate (600 mg base) q.d. on Days 1 and 2, 500 mg chloroquine phosphate (300 mg base) on Day 3	49
Roselle (*Hibiscus sabdariffa*) Sudanese beverage	300 mL single dose	−71.3	600 mg (formulation not mentioned) single dose	52
Sweet lemon (*Citrus limetta*) Sudanese beverage	300 mL single dose	−68	600 mg (formulation not mentioned) single dose	52
Tamarind (*Tamarindus indica*) Sudanese beverage	300 mL single dose	−65.4	600 mg (formulation not mentioned) single dose	52

All doses administered orally unless otherwise stated.
Precipitant term refers to the drug that causes an effect on the substrate drug by inhibiting or inducing enzymes.
Object term refers to the drug whose exposure may or may not be changed by a precipitant drug.
AUC, area under the concentration-time curve; IM, intramuscular; q.d., once daily.
Source: UW Drug Interaction Database (DIDB), Copyright University of Washington, accessed: April 6–14, 2020.

Table 4 Drug–drug interaction studies involving hydroxychloroquine as the object and the precipitant

Object/ Precipitant	Object/ Precipitant dose	Change in AUC (%)	HCQ dose	HCQ interval	References
Object					
Methotrexate	15 mg	51.8	200 mg	Single dose	28
Metoprolol	100 mg	50.7	400 mg (8 days)	Twice daily	55
MK-2206	200 mg (21-day cycles)	16.4	400 mg (21-day cycles)	Twice daily	56
MK-2206	150 mg (21-day cycles)	31.7	200 mg (21-day cycles)	Twice daily	56
MK-2206	200 mg (21-day cycles)	56.2	200 mg (21-day cycles)	Twice daily	56
MK-2206	135 mg (21-day cycles)	92.2	400 mg (21-day cycles)	Twice daily	56
Precipitant					
Methotrexate	15 mg (single dose)	−6.8	200 mg (single dose)		28

Precipitant term refers to the drug that causes an effect on the substrate drug by inhibiting or inducing enzymes.
Object term refers to the drug whose exposure may or may not be changed by a precipitant drug.
AUC, area under the concentration-time curve; HCQ, hydroxychloroquine.
Source: UW Drug Interaction Database (DIDB), Copyright University of Washington, accessed: April 6–14, 2020.

Obua *et al*. report that the two-compartmental PK model best described the chloroquine pharmacokinetics based on a POP-PK analysis that utilized sparse PK data obtained from finger prick sampling in pediatric malaria patients.[59] The same study reports that no correlation was identified between body weight or age with the PK model parameters. Höglund *et al*. report that for adult malaria patients, the disposition of chloroquine was also adequately described by the two-compartment model without

Table 5 Population pharmacokinetic studies for chloroquine and hydroxychloroquine

Study population (Age)	N (nF)	Treatment	PK matrix	Reference
Chloroquine				
Pediatric malaria patients (range: 6 months–5 years)	83 (34)	Age 6–24 months: CQ (75 mg base/day for 3 days) + sulfadoxine/ pyrimethamine (250 mg/12.5 mg single dose) Age 25–60 months: CQ (150 mg base/day for 3 days) + sulfadoxine/ pyrimethamine (500 mg/25 mg single dose)	Finger prick/dried blood spot sampling	60
Pregnant and nonpregnant women (mean age: 26 years)	60 (60)	450 mg CQ base for 3 days + single dose of 1500 mg sulfadoxine and 75 mg pyrimethamine	Plasma	61
Malaria patients (range: 17–52 years)	75 (39)	Multiple doses: 10 and 5 mg/kg CQ base at 0 hours and 6–12 hours on day 0, and 5 mg/kg each on day 1 and day 2 + 15 mg/kg/day primaquine base for 14 days starting from the second day (day 1) of CQ	Plasma	62
Hydroxychloroquine				
Rheumatoid arthritis patients (range: 20–81 years)	123 (88)	HCQ dosing varied: Single dose: 155 mg HCQ base orally or via 30 min IV infusion Multiple doses: 155 mg/day or 310 mg/ day HCQ base dose orally with or without methotrexate	Whole blood	63
Healthy adults and malaria patients (approximate mean age: 27 years)	91 (21)	Healthy adults: Single oral dose of 310 mg HCQ base or 310 mg/day HCQ base dose/week Malaria patients: 620 mg HCQ base + 310 mg HCQ base at 6, 24, and 48 hours	Plasma	18
Patients with CLE or SLE (mean age: 42.5 years)	90 (66)	Multiple doses: 155–310 mg HCQ base/day	Blood and Plasma	64
Pregnant women with rheumatic diseases (median age: 31 years)	50 (50)	Multiple doses: 310 mg/day HCQ base dose for most subjects with or without other concomitant prescription medications	Serum	65

CLE, cutaneous lupus erythematosus; CQ, chloroquine; HCQ, hydroxychloroquine; IV, intravenous; N, number of subjects/patients; nF, number of female subjects/patients; NA, information not available/reported; PK, pharmacokinetic; SLE, systemic lupus erythematosus.

the use of any covariates on the model parameters.[61] A POP-PK approach was also used to assess differences in the PK of chloroquine in pregnant and nonpregnant women, and findings are discussed in Special Populations: Pregnancy section of this manuscript.[60] Within the POP-PK analysis literature on chloroquine discussed here, the reported BSV (coefficient of variation (CV%)) for CL/F estimate was ~ 30% (Relative standard error (RSE): 24–35%). For the V/F estimates for the central compartment, reported BSV (CV%) ranged from 40–57% (RSE: 22–67%). For hydroxychloroquine, Carmichael *et al*. report that one compartment PK model adequately described its pharmacokinetics based on pooled PK data from several pharmacokinetic studies in patients with rheumatoid arthritis.[62] The same study notes that the limited number of data points per individual resulted in a one-compartment PK model selection instead of a multicompartment model as reported in the literature. Lim *et al*. report that in healthy adults and adult malaria patients, hydroxychloroquine pharmacokinetics were best described by a two-compartment PK model with first-order absorption with absorption lag time without any covariates.[18] However, Morita *et al*. report that for patients with cutaneous lupus erythematosus or systemic lupus erythematosus, a one-compartment model with first-order absorption and absorption lag time adequately described hydroxychloroquine PK and body weight was identified as a significant ($P < 0.001$) covariate.[63] Balevic *et al*. report the use of POP-PK analysis to assess the effect of pregnancy on the PK of hydroxychloroquine in patients with rheumatic diseases and note a lack of significant changes in hydroxychloroquine exposure due to pregnancy in a small cohort of patients.[64] The study reports that a one-compartment PK model best described hydroxychloroquine PK and body weight was found to be a significant covariate on V/F. Within the POP-PK literature on hydroxychloroquine discussed above, the reported BSV (CV%) in CL/F estimates ranged from 16–44% (RSE: 4–41%). For V/F estimates for the central compartment, BSV (CV%) ranged from 16–23% (RSE: ~58%).

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING AND TISSUE PARTITIONING

SARS-CoV-2 is a virus with a predilection for the respiratory system resulting in COVID-19 in susceptible individuals, leading to ARDS. The target protein and cofactors for viral attachment and uptake are expressed in the lung and bronchial branches.[66] Several researchers have proposed that lung tissue and/or lung-substructure concentrations of drugs may be used for dose selection in clinical trials for COVID-19 and ultimately understanding exposure–response against the virus or against the inflammatory response to the virus.

Physiologically-based pharmacokinetic (PBPK) models leverage characteristics of individual organs and tissues, physicochemical properties, absorption, distribution, metabolism and excretion, and tissue partition coefficients characteristics to allow prediction of systemic and tissue concentrations of parent and/or metabolite over time under various conditions and for various populations.[67] This approach to model-informed drug development can provide greater insights into local concentration–effect relationships than can empiric-based PK modeling.

Several PBPK models for hydroxychloroquine have been developed. One was developed to better understand drug concentrations in the lysosome under varying pH conditions as well as various tissues.[68] The other was developed to project optimized dosing of hydroxychloroquine for the treatment of SARS-CoV-2 by simulating plasma, blood, and lung concentrations and comparing simulated concentrations with inhibitory concentrations determined in *in vitro* SARS-CoV-2 growth assays (as described in a subsequent section on the *In Vitro* data for SARS-COV-2).[2]

The same investigators have also modified a compound file of chloroquine to model systemic and pulmonary concentrations of chloroquine. The models were verified with observed PK data compared with concentrations predicted *in silico* in blood and plasma. The tissue partition coefficients were extrapolated from published tissue concentrations in rats.

The PBPK models and simulations for hydroxychloroquine and chloroquine reiterated prior knowledge that blood concentrations for both exceed that of plasma. More importantly, the full PBPK models indicated that hydroxychloroquine and chloroquine both have a predilection for lung. The model-predicted lung concentrations exceed plasma concentrations at steady state by more than 400-fold. In addition, if anti-inflammatory effects and prevention of a cytokine storm are instrumental in treating disease, then drug concentrations in the lung may provide a beneficial effect.

In addition, PBPK modeling and simulation have been utilized to inform dosing decisions for COVID-19 clinical trials. Ultimately, as efficacy and safety data from multiple COVID-19 prevention and treatment trials accrue, a more precise estimation across studies will help inform on effective doses and exposures over time.

TOXICITIES (ADVERSE EFFECTS)

In both chloroquine and hydroxychloroquine use, gastrointestinal discomfort is the most commonly described side effect manifesting primarily as nausea, vomiting, diarrhea, and stomach pain. These toxicities are more common at higher doses.[69] Hypoglycemia may occur and can be enhanced in patients receiving concurrent hypoglycemic drugs. Rash and itching are well described, and due to the long half-lives, they may last for prolonged periods after discontinuation. Hemolytic anemia is a serious adverse effect in individuals with glucose-6-phosphate dehydrogenase deficiency who receive chloroquine or hydroxychloroquine.[5,6] Retinal toxicity is a well-described adverse effect of hydroxychloroquine; however, it is primarily a concern with chronic and cumulative doses of = 1,000 grams.[70]

Effects of chloroquine and hydroxychloroquine on QTc prolongation

Chloroquine. The effects of chloroquine alone as well as chloroquine and azithromycin on corrected QT interval (QTc) prolongation were investigated in a previously unpublished single-center, open-label, placebo-controlled, randomized, multiple-dose healthy volunteer study (N = 119 total; 24 per group except for the chloroquine + 1,500-mg azithromycin group where 23 subjects were enrolled. Three subjects withdrew from the chloroquine + 1,500-mg azithromycin group during

the treatment phase of the study due to adverse events (one due to diarrhea, one due to loss of appetite, and one due to nausea, diarrhea, and vomiting) and thus were not included in the pharmacokinetic or QTc analyses). A parallel group design was utilized given the long half-lives of both chloroquine and azithromycin. Subjects were assigned to one of the following five treatment groups:

- Placebo once daily (q.d.) × 3 days
- 1,000 mg chloroquine phosphate (600 mg base) q.d. × 3 days
- 1,000 mg chloroquine phosphate plus 500 mg azithromycin q.d. × 3 days
- 1,000 mg chloroquine phosphate plus 1,000 mg azithromycin q.d. × 3 days
- 1,000 mg chloroquine phosphate plus 1,500 mg azithromycin q.d. × 3 days

Triplicate 12-lead electrocardiograms (ECGs) were serially obtained on Day −1 (baseline), Day 1, Day 2, and Day 3, including at 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours on Day 3. Serial plasma samples were obtained for chloroquine and azithromycin quantitation on Days 1–3 including at times immediately following ECG assessments on Day 3. QT interval data were corrected for heart rate using Fridericia's method (QTcF). In all analyses, the means of the ECG parameters within a triplicate were used as the observations for a subject at a nominal timepoint. The primary end point was change of QTcF from baseline at each nominal timepoint on Day 3. Comparisons were made between treatments vs. placebo and the chloroquine alone group using an analysis of variance model. For each comparison the model-based mean and 90% confidence interval were reported. No adjustments were made for multiplicity. The mean age, weight, and body mass index were 35.5 years, 83.3 kg, and 26.6, respectively.

In comparison with QTcF values in the placebo group, the chloroquine increased mean time-matched QTcF values ranging from 18.4 to 35 milliseconds on Day 3 in the chloroquine alone group (**Table 6**). The maximum time-matched difference in QTcF occurred at 10 hours post dose on Day 3. Maximum observed plasma chloroquine concentrations on Day 3 were similar for all groups (mean 0.335 (CV 29%) μg/mL) and generally occurred between 6 hours and 7 hours post dose. These results are consistent with the large magnitude of effect of chloroquine on QTcF in previous studies.[49,71]

It should be noted that the high chloroquine dose (600 mg twice daily for 10 days or total dose 12 g) of a recent phase IIb trial of patients with SARS in Manaus, Brazilian Amazon was stopped because of safety. There was a trend for higher lethality compared with the lower dose and one quarter of the high-dose patients developed QTc = 500 milliseconds.[72] All patients in this study also received 5 days of azithromycin, also a QTc-prolonging drug.

Hydroxychloroquine. The effect of hydroxychloroquine on QTc is less well studied than the effect of chloroquine. Case reports have noted prolonged QTc values with hydroxychloroquine toxicity.[73-78] In a study of 85 patients with connective tissue disorders who were receiving hydroxychloroquine, the investigators concluded that QT intervals did not appear to be different from what would be considered normal in these patients.[79]

Recently there are emerging QT data from 84 patients with SARS-CoV-2 infection treated with a combination of hydroxychloroquine and azithromycin. QTc values were determined for subjects at baseline (before administering hydroxychloroquine and azithromycin) and while on drug, but no reference was given regarding doses administered, the timing of QTc assessment with respect to dose, or the formula used to correct QT for heart rate. QTc values were prolonged maximally (28 milliseconds) from baseline between Days 3 and 4. In 30% of patients, QTc increased by greater than 40 milliseconds. In 11% of patients, QTc increased to = 500 milliseconds, representing high-risk group for arrhythmia. Development of acute renal failure but not baseline QTc was a strong predictor of greater QTc prolongation.[80]

Table 6 Analysis of mean change from time-matched baseline in QTcF repeated measures ANOVA

Time (hour post dose)	ΔQTcF (milliseconds)		
		90% Confidence interval	
	Mean	Upper limit	Lower limit
0	18.4	13.3	23.5
1	22.2	17.0	27.3
2	25.7	20.6	30.8
3	27.6	22.5	32.7
4	31.3	26.1	36.4
5	29.2	24.1	34.3
6	29.9	24.8	35.1
8	31.5	26.3	36.6
10	35.0	29.9	40.2
12	32.4	27.3	37.6

Calculation of ΔQTcF: The primary end point was change of QTcF (the corrected QT interval by Fridericia) from Day 1 baseline at each nominal timepoint on Day 3, using Fridericia's method correcting for heart rate effects on the QT interval. Comparisons were made (on day 3 of a 1,000 mg chloroquine phosphate q.d. regimen) between chloroquine vs. placebo cohorts using an ANOVA (analysis of variance) model.

SPECIAL POPULATIONS

Special populations: Renal/Hepatic impairment

Chloroquine. No dose adjustment is suggested by the manufacturer for patients with renal impairment. No dose adjustment is suggested by the manufacturer for those with hepatic impairment; however, caution should be used in patients with hepatic disease and/or alcoholism, as the drug concentrates largely in the liver. **Table S1** summarizes the language provided in both the chloroquine and hydroxychloroquine labels regarding both renal and hepatic impairment.

Hydroxychloroquine. No dose adjustment is suggested by the manufacturer for patients with renal impairment. Jallouli, *et al.* evaluated three patients with systemic lupus erythematosus

receiving long-term dialysis for hydroxychloroquine blood concentrations before and after dialysis. Patients received either 200 mg/day ($N = 2$) or 400 mg/day ($N = 1$) of hydroxychloroquine, and blood concentrations did not change significantly before or after dialysis.[81] Hydroxychloroquine was not detected in the dialysis bath of all three patients (< 50 ng/mL). This suggests hydroxychloroquine is not dialyzable. No dose adjustment is suggested by the manufacturer for those with hepatic impairment; however, caution should be used in patients with hepatic disease and/or alcoholism, as the drug concentrates largely in the liver. **Table S1** summarizes the language provided in both the chloroquine and hydroxychloroquine labels regarding both renal and hepatic impairment.

Special populations: Age

Geriatric Population: Typical adult dosing is recommended for patients greater than 65 years of age. Hydroxychloroquine and chloroquine clinical trials did not include a sufficient number of geriatric subjects to determine whether they respond differently than younger subjects. However, given both drugs are significantly renally cleared and elderly patients are more likely to have decreased renal function, consideration should be given to dose reduction in this population.

Pediatric Population: Both hydroxychloroquine and chloroquine are preferably dosed based on actual body weight in the pediatric population. The American Academy of Ophthalmology recommends keeping daily doses ≤ 6.5 mg/kg for hydroxychloroquine-sulfate (≤ 5.0 mg/kg base) and ≤ 3.8 mg/kg chloroquine-phosphate (≤ 2.3 mg/kg base) for all patients to reduce the risk of retinopathy and permanent vision loss, though this risk is linked to duration of use over years.[82] Approved pediatric doses are above this threshold for treatment of acute uncomplicated malaria.

Chloroquine. In infants, children, and adolescents, the highest approved dose of chloroquine is for the treatment of acute malarial attack. The highest recommended chloroquine dose for the treatment of acute, uncomplicated malarial attack is 10 mg base/kg orally followed by 5 mg base/kg at 6, 24, and 48 hours after initial dose for a total of four doses.[83] Doses for chemoprophylaxis are suggested to be lower.[84] Several fatalities have been reported due to accidental ingestion at even lower doses (0.75–1 g chloroquine-phosphate in one 3-year-old child).[5] The injection label notes that in no instance should a single intramuscular or subcutaneous dose exceed 6.25 mg (5 mg base) per kg of body weight, since children are especially sensitive to the effects of the 4-aminoquinolines. Severe reactions and sudden death have been reported following parenteral administration in children.[5,85]

Hydroxychloroquine. In infants, children, and adolescents, 10 mg base/kg followed by 5 mg base/kg hydroxychloroquine at 6, 24, and 48 hours after initial dose is the highest recommended dose for the treatment of acute uncomplicated malarial attack.[83] Doses for chemoprophylaxis, juvenile rheumatoid arthritis, and systemic lupus erythematosus are suggested to be lower.[84]

Special populations: Pregnancy

Chloroquine. Based on World Health Organization (WHO) guidelines for the treatment of malaria, chloroquine is considered safe in the first trimester of pregnancy and has been studied in combination with azithromycin for intermittent preventive treatment in pregnancy as a fixed dose combination. Chloroquine and metabolites (desethylchloroquine) can be detected in the cord and urine of newborn infants.[33,86-89] Karunajeewa, *et al*. performed a population PK analyses of chloroquine and its active metabolite, monodesethylcholorquine in pregnancy. Pregnancy was found to have a significant effect on chloroquine and desethylchloroquine disposition after conventional doses of chloroquine. Exposures of both analytes were found to be significantly lower in pregnant patients (AUCs for chloroquine and desethylchloroquine saw reductions of 25% and 45%, respectively) in comparison with nonpregnant patients.[61]

Ogunbona *et al*. investigated the excretion of chloroquine and desethylchloroquine in breast milk in lactating mothers following a single 600 mg (chloroquine base) oral dose. The maximum daily dose an infant could receive from breastfeeding was ~ 0.7% of maternal starting dose.[90]

Hydroxychloroquine. The American Academy of Pediatrics (AAP) considers use of hydroxychloroquine usually compatible with breastfeeding, and hydroxychloroquine is considered appropriate treatment for systemic lupus erythematosus during pregnancy by the American College of Rheumatology.[91,92] Unless pregnancy is planned, fetal exposure cannot be avoided by discontinuing hydroxychloroquine at the time pregnancy is discovered, as hydroxychloroquine is stored in the liver with an extended half-life. Hydroxychloroquine can be detected in cord blood in concentrations similar to those in postpartum maternal serum[93] and crosses the placenta. One study saw no differences when comparing patients with systemic lupus erythematosus with no hydroxychloroquine exposure ($N = 163$), continuous hydroxychloroquine use ($N = 56$), and those discontinuing hydroxychloroquine during the first trimester ($N = 38$) when assessing for miscarriages, stillbirths, pregnancy losses, and congenital abnormalities.[94] Another study by Costedoat-Chalumeau *et al*. demonstrated no difference in growth rate and no evidence of visual, hearing, or developmental abnormalities in the mean 26-month follow-up of children between the hydroxychloroquine and the control group.[95] Hydroxychloroquine has been shown to be transferred to human breast milk. Breastfed infants are exposed to ~ 2% of maternal dose on a body-weight basis.[37]

Special populations: Critically ill

Considering the adverse event profile and elimination pathway of both hydroxychloroquine and chloroquine, strong consideration to alternative therapies should be made in patients exhibiting these comorbidities. Arentz *et al*. recently published a case series report discussing characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. Of the 21 patients with polymerase chain reaction–confirmed SARS-CoV-2 infection by nasopharyngeal sample, 18 were identified to have comorbidities.

Chronic kidney disease (N = 10) and congestive heart failure (N = 9) were the most common comorbidities.[96]

PROPOSED MECHANISM OF ACTION FOR COVID-19 DISEASE

Direct antiviral

The presumed mechanism of antiviral activity of chloroquine and hydroxychloroquine is increasing the pH of the endosome that is required for viral/cellular fusion. Using flow cytometry and sorting of SARS-CoV spike glycoprotein and angiotensin-converting enzyme 2 (ACE2) on Vero E6 cells, Centers for Disease Control and Prevention (CDC) investigators also found that chloroquine impairs the glycosylation of ACE2, thereby impairing the binding of the virus to its cellular receptor.[97] To date, there are no data on stereospecific antiviral effects of chloroquine or hydroxychloroquine.

Immunomodulatory

In addition to direct antiviral activity, chloroquine and hydroxychloroquine have known immunomodulatory and anti-inflammatory effects. The anti-inflammatory effects of hydroxychloroquine were first discovered through the serendipitous observation that World War II soldiers receiving chloroquine for malaria prophylaxis noted improvements in skin rashes and arthritis, ultimately leading to the development of this drug as a treatment for systemic lupus erythematosus and other immunologic conditions such as rheumatoid arthritis.[98,99] Immunomodulatory effects of chloroquine as well as hydroxychloroquine include inhibition of antigen presentation to dendritic cells, reduced cytokine production in macrophages, and reduced signaling of both B and T cells. Therefore, in addition to direct antiviral effects, chloroquine and hydroxychloroquine may play a role in reducing the cytokine storm associated with COVID-19 progression to ARDS. Although these drugs are generally considered more immunomodulatory than immunosuppressive, it is worth pointing out that in early infection, the role of the innate immune system is a critical factor in preventing COVID-19 progression to serious disease. As our understanding of COVID-19 pathogenesis evolves, whether these immunomodulatory effects are beneficial or detrimental will need to be elucidated.

IN VITRO DATA FOR SARS-COV-2

Chloroquine

Antiviral activity of chloroquine and hydroxychloroquine against COVID-19 has been mostly investigated in *in vitro* studies. Following the outbreak of the 2003 SARS virus, several compounds were screened for *in vitro* activity against SARS-CoV. *In vitro* hydroxychloroquine and chloroquine drug sensitivity data for SARS-CoV-2 are reported as percent inhibition and are most often obtained from 24-hour and 48-hour experiments in Vero or Vero E6 cells derived from African green monkey kidney epithelium.[2,100-102] CDC investigators reported that, when added post infection, chloroquine concentrations as little as 0.1 μM (32 ng/mL) reduced viral spread by 50% while chloroquine concentrations of 100 μM (32,000 ng/mL) reduced viral spread by up to 94%.[97] The half-maximal inhibitory effect was estimated to occur at 4.4 μM ± 1.0 μM (1,408 ± 320 ng/mL). Pretreatment with chloroquine was found to have an even more potent viral inhibition with 0.1, 1, and 10 μM (32,320, and 3,200 ng/mL), reducing infectivity by 28, 53, and 100% respectively, which may suggest lower drug exposures required for prophylaxis than treatment.

Following the COVID-19 outbreak, Wang *et al*. tested the *in vitro* inhibitory activity and cytotoxicity of a number of potential therapeutics against SARS-CoV-2, including chloroquine in Vero E6 cells.[100] Of the seven drugs tested, chloroquine (along with remdesivir) had one of the highest selectivity indexes with a half maximal effective concentration (EC_{50}) of 1.13 μM (361 ng/mL) and half maximal cytotoxic concentration > 100 μM (= 32,000 ng/mL). (**Table 7**).

Hydroxychloroquine. Similar to chloroquine, hydroxychloroquine has also been tested in a number of *in vitro* experiments for activity against SARS-CoV-2. A wide range of EC_{50} values has been reported under various conditions (**Table 7**), with some comparative studies finding greater potency with hydroxychloroquine[2] and some less potent.[101] Below is a summary of the various experiments, including experimental characteristics and reported EC_{50} values, as shown in **Table 7**.

CLINICAL STUDIES FOR COVID-19

For clinical use in COVID-19, the CDC provides anecdotal dosing suggestions, but explicitly states that optimal dosing and duration of hydroxychloroquine for COVID-19 are unknown.[103] Translational pharmacokinetic/pharmacodynamic modeling is one approach to propose optimized hydroxychloroquine dosing regimens which ensure the highest likelihood of success as COVID-19 treatment. With lack of known exposure correlates for efficacy to date, several groups have attempted to integrate available pharmacological data and mechanistic knowledge related to COVID-19, including *in vitro* data for SARS-CoV-2, historical data on population pharmacokinetics, safety data of hydroxychloroquine from large patient cohorts, and newly emerging clinical pharmacokinetic/pharmacodynamic data from patients with COVID-19.[2,104-106] Early characterization of the clinical exposure–response relationship between hydroxychloroquine and SARS-CoV-2 viral load suggests that the hydroxychloroquine doses needed to cause more rapid viral clearance compared with standard of care could be much higher than those currently being studied for COVID-19 patients in any setting and these doses may lead to substantial risk of cardiac toxicity.[104-106] As these investigations have pointed out, when making *in vitro–in vivo* extrapolations to optimize dosing, there are many considerations to be made, including (i) the compartment to be targeted (e.g., plasma, intracellular vs. extracellular lung tissue, lung fluid, and other organs), (ii) free vs. total concentrations, (iii) plasma vs. serum vs. whole blood, and (iv) EC_{50} vs. 90% maximal effective concentration or magnitude above these targets.

Initial support for the use of chloroquine and hydroxychloroquine came from a published report which states that results from 100 patients across multiple institutions found that for the

Table 7 ***In vitro*** **data for SARS-COV-2**

Cell type	Viral input MOI	Drug incubation (h)	CQ EC_{50} μM (ng/mL)	HCQ EC_{50} μM (ng/mL)	References
Vero E6	0.05	48	1.13 (361)	N/A	100
Vero	0.01	24	23.90 (7,646)	6.14 (2,062)	2
Vero	0.01	48	5.47 (1,750)	0.72 (242)	2
Vero E6	0.01	48	2.71 (867)	4.51 (1,515)	101
Vero E6	0.02	48	3.81 (1,219)	4.06 (1,364)	101
Vero E6	0.2	48	7.14 (2,284)	17.31 (5,814)	101
Vero E6	0.8	48	7.36 (2,354)	12.96 (4,353)	101
Vero E6	0.001	48	N/A	4.17 (1,401)	102

CQ, chloroquine; EC_{50}, half maximal effective concentration; HCQ, hydroxychloroquine; MOI, multiplicity of infection; SARS-COV-2, severe acute respiratory distress syndrome coronavirus–2.

treatment of COVID-19, chloroquine was superior to standard of care in preventing exacerbation of pneumonia, reducing days to conversion rate and shortening time to clinical recovery.[1] To date, this study has not been published, and data regarding magnitude of benefit, specific dosing or timing of treatment initiation, observed toxicities, and treatments received by the control group are not available. Other data with chloroquine, as mentioned above, is from one 81-person trial in Brazil, comparing 600 mg chloroquine base twice daily for 10 days (12 g total) with 450 mg × 2 on day 1, then 450 mg daily for 4 additional days (2.7 g total). This study did not have a placebo arm and was halted prior to reaching enrollment goals because higher mortality was observed in the high-dose chloroquine arm.[72] One additional small study ($n = 22$) showed no difference between chloroquine and lopinavir/ritonavir for viral clearance.[107]

Clinical studies of hydroxychloroquine are heterogeneous in terms of hydroxychloroquine regimen, outcomes measurements (clinical, SARS-CoV-2 viral load), and severity of illness.[108,109] Only one nonrandomized study has reported a significant reduction in time to viral clearance among patients with mild disease who received hydroxychloroquine or hydroxychloroquine with azithromycin, and this small study had significant limitations, including a control arm with significantly higher viral loads compared with those who received treatment.[110] The largest well-characterized observational cohorts of hospitalized patients, hydroxychloroquine with or without azithromycin were not associated with a mortality benefit.[111-112]

Only two randomized clinical trials have been published in hospitalized COVID-19 patients, one of which found that high-dose hydroxychloroquine (1,200 mg/day for 3 days followed by 800 mg/day for 2–3 weeks) given to mild or moderately ill patients did not improve time to negative polymerase chain reaction test nor time to alleviation of symptoms.[113] A small randomized study in China reported no apparent clinical benefit of 400 mg daily for 5 days compared with placebo in COVID-19 patients, the majority of whom had mild disease.[114] Recently, the RECOVERY trial (NCT04381936) stopped enrollment into the hydroxychloroquine arm of their trial in hospitalized COVID-19 patients, reporting no difference in mortality, hospital stay, or other outcomes, between 1,542 subjects on hydroxychloroquine and 3,132 subjects on standard care. Subsequently, the WHO-sponsored SOLIDARITY trial and an National Institutes of Health (NIH)-sponsored trial have suspended enrollment of their hydroxychloroquine arms, reporting no benefit observed, although the data have not yet been released.

Hydroxychloroquine has also been proposed for use in prophylactic settings. The first randomized clinical trial published in an outpatient setting found that high-dose hydroxychloroquine (800 mg loading dose followed by 600 mg/day for 4 days) given within 4 days of a high-risk exposure did not reduce the likelihood of acquiring COVID-19.[115] Studies in high-risk healthcare workers evaluating a role for preexposure prophylaxis are currently underway.

DISCUSSION

In summary, to date, there is a lack of evidence from controlled, randomized clinical trials powered for efficacy end points (the gold standard for evidence-based medicine) for use of chloroquine or hydroxychloroquine to treat COVID-19. Increasingly, evidence points to a lack of benefit in hospitalized and severely ill patients; whether a role exists in early treatment or prevention remains to be determined. Although chloroquine and hydroxychloroquine are very similar in their pharmacologic properties, there are subtle differences of which clinicians and scientists should be aware. The Infectious Disease Society of America released guidelines for the treatment of COVID-19, recommending hydroxychloroquine only in the context of a clinical trial.[116] While the NIH guidelines state there are insufficient clinical data for or against chloroquine or hydroxychloroquine, they do specifically recommend against the use of high-dose hydroxychloroquine (defined as 600 mg twice daily for 10 days) or hydroxychlroquine in combination with azithromycin.

SUPPORTING INFORMATION

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CONFLICT OF INTEREST

M.L.R. and P.E.S. are employees of Merck & Co Inc. D.W. is an employee of Certara. J.C. is an employee of Pfizer Inc. M.R.N. and R.M.S. are

coinvestigators on separate clinical trials evaluating hydroxychloroquine. All other authors declared no competing interests for this work.

DISCLAIMER

A.J. and J.H.Z. are employees of the US Food and Drug Administration (FDA). This article reflects the views of the authors and should not be construed to represent the FDA's views or policies.

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