



REVIEW

The Pharmacokinetic and Pharmacodynamic Properties of Hydroxychloroquine and Dose Selection for COVID-19: Putting the Cart Before the Horse

Taylor Morrisette · Thomas P. Lodise · Marc H. Scheetz ·
Srijib Goswami · Jason M. Pogue · Michael J. Rybak

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ABSTRACT

Coronavirus disease 2019 (COVID-19), caused by the 2019 novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is currently responsible for a global pandemic. To date, only remdesivir and dexamethasone have demonstrated a positive response in a prospective,

randomized trial for the treatment of patients with COVID-19. Hydroxychloroquine (HCQ) is an agent available in an oral formulation with in vitro activity against SARS-CoV-2 that has been suggested as a potential agent. Unfortunately, results of randomized trials evaluating HCQ as treatment against a control group are lacking, and little is known about its pharmacokinetic/pharmacodynamic (PK/PD) profile against SARS-CoV-2. The objective of this review was to describe the current understanding of the PK/PD and dose selection of HCQ against SARS-CoV-2, discuss knowledge gaps, and identify future studies that are needed to

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T. Morrisette · M. J. Rybak (✉)
Anti-Infective Research Laboratory, Department of
Pharmacy Practice, Eugene Applebaum College of
Pharmacy and Health Sciences, Wayne State
University, Detroit, MI, USA
e-mail: m.rybak@wayne.edu

T. P. Lodise
Albany College of Pharmacy and Health Sciences,
Albany, NY, USA

M. H. Scheetz
Department of Pharmacy Practice, Chicago College
of Pharmacy, Midwestern University, Downers
Grove, IL, USA

M. H. Scheetz
Pharmacometrics Center of Excellence, Midwestern
University, Downers Grove, IL, USA

M. H. Scheetz
Department of Pharmacy, Northwestern Memorial
Hospital, Chicago, IL, USA

M. H. Scheetz
Department of Pharmacology, College of Graduate
Studies, Midwestern University, Downers Grove, IL,
USA

S. Goswami
InsightRX, San Francisco, CA, USA

J. M. Pogue
Department of Clinical Pharmacy, University of
Michigan College of Pharmacy, Ann Arbor, MI, USA

M. J. Rybak
Division of Infectious Diseases, Department of
Medicine, Wayne State University, Detroit, MI, USA

M. J. Rybak
Department of Pharmacy, Detroit Medical Center,
Detroit, MI, USA

optimize the efficacy and safety of treatments against COVID-19.

Keywords: COVID-19; Hydroxychloroquine; Pharmacodynamics; Pharmacokinetics

Key Summary Points

Information regarding pharmacokinetic/pharmacodynamic (PK/PD) properties of hydroxychloroquine (HCQ) against SARS-CoV-2 are lacking.

This review describes the current understanding of PK/PD properties and dose selection of HCQ against SARS-CoV-2.

This review also discusses knowledge gaps that are needed to optimize efficacy/safety of treatments against COVID-19.

INTRODUCTION

The 2019 novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for coronavirus disease 2019 (COVID-19), was first identified in a group of patients that had contracted pneumonia-like illness of unknown etiology in Wuhan, China [1]. Despite variations in symptom severity, a subset of patients have been shown to progress to acute respiratory distress syndrome, often leading to mechanical ventilation and death [2]. Despite an impressive response by the medical community to identify therapies, the clinical efficacy of most potential antivirals remains uncertain [3]. To date, remdesivir has shown a moderate response effect (faster time to recovery) for patients with COVID-19 in a double-blind, randomized, placebo-controlled clinical trial (effect size greatest in those hospitalized patients requiring any supplemental oxygen); however, remdesivir is currently only available in limited rations and as an

intravenous formulation, which may pose challenges [4]. Furthermore, dexamethasone (preliminary, unpublished data) has been shown to reduce 28-day mortality compared to usual care in hospitalized COVID-19 patients receiving oxygen or invasive mechanical ventilation. Theoretically, use of interleukin 6 (IL-6) inhibitors as immunomodulators and other immunomodulatory agents may be promising in severe cases, but supportive care currently remains one of the cornerstones of therapy for COVID-19 [5].

Hydroxychloroquine (HCQ) is an anti-malarial agent with in vitro antiviral activity, anti-inflammatory effects, and immunomodulatory actions [6–8]. Given its wide availability and in vitro activity against SARS-CoV-2, HCQ became one of the earliest and most studied therapies for the treatment of and prophylaxis against COVID-19. Unfortunately, little is known about the appropriate dosage regimen(s) of HCQ to optimize efficacy and safety against COVID-19, as current knowledge is primarily derived from HCQ use in healthy volunteers and other non-COVID-19 disease states (e.g., malaria, rheumatoid arthritis, etc.) [9–13]. This is further highlighted by the fact that current enrolling clinical trials are utilizing different HCQ dosing regimens. Furthermore, there is considerable heterogeneity among studies regarding the pharmacokinetics (PK) sampling matrix (i.e., important because of varying concentrations depending on the matrix [blood versus plasma/serum]) and patient populations that have attempted to characterize HCQ's PK/pharmacodynamic (PK/PD) properties against SARS-CoV-2.

Although studies completed thus far show variable results, Arshad and colleagues performed a large multicenter, retrospective, observational analysis that evaluated patients hospitalized because of a COVID-19-related admission receiving HCQ 400 mg twice daily on day 1, followed by HCQ 200 mg twice daily on days 2 to 5 [14–17]. When comparing HCQ monotherapy ($n = 1202$) versus no treatment ($n = 409$), in-hospital mortality was significantly lower in the HCQ monotherapy group (13.5% vs. 26.4%). Importantly, there was also significantly more steroid usage in the HCQ

monotherapy group compared to the no treatment group (78.9% vs. 35.7%), which may have contributed to this mortality difference [14]. Furthermore, the World Health Organization discontinued the HCQ arm in the Solidarity trial because it showed “little or no reduction in the mortality of hospitalized COVID-19 patients when compared to standard of care” (HCQ dosed 800 mg twice daily on day 1, followed by HCQ 400 mg twice daily for a total of 10 days), and the Food and Drug Administration revoked the emergency use authorization to utilize HCQ for the treatment of COVID-19 [16–18].

Given these critical gaps and inconsistencies in the literature, the objective of this review was to describe the current understanding of the PK/PD of HCQ for the treatment of COVID-19 as it relates to the efficacy and safety of dosage regimens. The focus of this review is on initial therapy of HCQ based on the most common treatment durations for COVID-19 (when available). Given the scarcity of literature on HCQ and similarities in mechanisms of action and chemical structure, data describing chloroquine *in vitro* activity will also be reviewed. We also discuss gaps in the literature and considerations going forward regarding *in vitro* and *in vivo* analyses of HCQ and other COVID-19 treatment options and identify future PK/PD studies that are needed to optimize the efficacy, if possible, and safety against COVID-19. It is important to note that the authors’ primary goal was to summarize and evaluate the PK/PD of HCQ to help clinicians in this challenging time. The summaries described are not meant to advocate for or endorse the widespread use of HCQ for the treatment of COVID-19. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

PHARMACOKINETIC PROPERTIES OF HYDROXYCHLOROQUINE

Absorption

Hydroxychloroquine is currently only available as an orally administered tablet, available in

200 mg as the sulfate salt form (equivalent to 155 mg base) [19]. There are numerous PK studies on the bioavailability of the oral tablet. However, most are in healthy volunteers or in patients with disease states other than COVID-19 [9–13]. Most studies have concluded that HCQ peak concentrations are estimated to be observed within 3–5 h [11,19,20]. In healthy males who received a single HCQ 200 mg oral dose, a mean peak blood HCQ concentration of 0.1296 mcg/ml was achieved in 3.26 h, while a peak plasma HCQ concentration of 0.0503 mcg/ml was achieved in 3.74 h. Tett and colleagues performed a randomized, crossover study in which the HCQ 155 mg oral tablet was compared to an intravenous infusion of racemic HCQ 155 mg to evaluate the absolute bioavailability of the commercially available HCQ tablet. These authors concluded that the mean (\pm SD) fraction of the oral dose absorbed (estimated from urine and blood) was 0.74 (\pm 0.13), while a wide range of absorption was calculated from plasma data [11,19].

Despite the lack of data and recommendations, current centers around the world have described crushing the HCQ tablets into suspensions and administering them via feeding tubes in patients otherwise unable to take oral medications, despite the Institute for Safe Medication Practices (ISMP) listing HCQ film-coated tablets on the “Do Not Crush” medication list [[21], direct communications]. Although this approach is commonplace in some inpatient centers among COVID-19 patients, there are no data on the impact of crushing HCQ tablets, administration via feeding tubes, and overall bioavailability or the timing of absorption. Given the uncertainty in PK with this approach, this further emphasizes the importance of understanding and optimizing PK and PD of HCQ against SARS-CoV-2.

Protein Binding

Most studies have shown that the binding of HCQ to protein is moderate (\sim 40%) [9,22]. Albumin and α_1 -acid glycoprotein have been the two proteins associated with the majority of HCQ binding. HCQ exists as (R)-

and (S)-enantiomers, and stereoselective protein binding has been documented [22].

Tissue Distribution

It has been shown that HCQ exhibits linear PK [23]. Due to HCQ's sequestration in deep tissues, the volume of distribution (Vd) that HCQ displays is extremely large. Tett and colleagues reported a blood and plasma Vd of 5522 l and 44,257 l, respectively, following intravenous HCQ infusion in healthy volunteers [23]. HCQ and chloroquine, which show similar patterns of tissue distribution, have been shown to concentrate quite highly in the lungs, kidney, liver, and spleen in animal models [24]. Maisonnasse and colleagues found that HCQ concentrations in the lung were higher than in plasma, with lung:plasma ratios ranging from 27 to 177 in macaques [25]. Notably, these data may very well be quite different in humans, and lung:plasma ratios could be lower because of differences in the metabolic composition and lower drug recovery rates.

Metabolism and Transport Mechanisms

CYP enzymes catalyze the dealkylation of HCQ to pharmacologically active metabolites, and HCQ/chloroquine has been documented to be metabolized through CYP 3A, 2D6, and 2C8 systems. The metabolism of HCQ leads to the three active metabolites, desethylhydroxychloroquine, desethylchloroquine, and bisdesethylhydroxychloroquine, although they have been shown to increase more significantly following chronic administration. It is anticipated that there are lower levels of these active metabolites present in the initial days of therapy for COVID-19 patients, and it is unclear how the various concentrations of active compounds translate to overall activity against SARS-CoV-2. Limited research has been conducted on investigating the association between genetic polymorphisms in CYP 3A, 2D6, and 2C8 and HCQ drug concentration levels [26–30].

Little is known about the role of membrane transporters on HCQ PK/PD. There is literature

that suggests that HCQ inhibits uptake activity of human organic anion transporting polypeptide 1A2 [31]. Also, HCQ/chloroquine has been shown to be an inhibitor of p-glycoprotein [32].

Excretion

Reports have described a median of ~ 20% of HCQ being excreted renally as unchanged drug in humans [33,34]. Urinary elimination of HCQ as metabolites and unchanged drug has been reported to be between 6% and 60% [9,29,34]. Lim and colleagues reported HCQ clearance to be 15.5 l/h (two-compartment model best described these data). Most reports have described a terminal elimination half-life of 30–60 days (in contrast to a terminal blood half-life reported by Carmichael and colleagues of 43.3 h) [10,13]. Using plasma data following administration of the oral HCQ tablet, Tett and colleagues calculated a mean (\pm SD) terminal elimination half-life of 32 (\pm 9) days [11].

Hydroxychloroquine/Chloroquine Mechanism, Concentration, and In Vitro Inhibitory Activity AGAINST SARS-CoV-2

Although the mechanism of action of hydroxychloroquine and chloroquine against SARS-CoV-2 has not been fully elucidated, it is thought that these agents may prevent terminal glycosylation of its functional entry receptor (angiotensin-converting enzyme 2 receptor), thus inhibiting viral entry [6,7]. Furthermore, it has been shown that these agents can alkalinize intracellular compartments through incorporation of lysosomes and endosomes, leading to inhibition of viral replication and infection [8].

The majority of in vitro analyses of HCQ and chloroquine have utilized Vero cell lines (Table 1). Liu and colleagues evaluated the antiviral effects of HCQ against SARS-CoV-2 compared to chloroquine at four different multiplicities of infection (MOI) 48 h post-infection. At MOIs (0.01, 0.02, 0.2, and 0.8), the half maximal effective concentration (EC_{50}) values for chloroquine (2.71, 3.81, 7.14, and 7.36 μ M) and HCQ (4.51, 4.06, 17.31, and 12.96 μ M), respectively, were determined.

Table 1 Summary of in vitro EC₅₀ values reported for hydroxychloroquine/chloroquine from selected studies

Author	Medication	Cell line	MOI	Time-point ^a	EC ₅₀ ^b
Liu et al. [[35]]	HCQ (chloroquine)	VeroE6	0.01	48 h	4.51 (2.71)
			0.02		4.06 (3.81)
			0.2		17.31 (7.14)
			0.8		12.96 (7.36)
Wang et al. [[36]]	Chloroquine	VeroE6	0.05	48 h	1.13
Yao et al. [[37]]	HCQ (chloroquine)	Vero ^c	0.01	24 h	6.14 (23.90)
				48 h	0.72 (5.47)
Maisonasse et al. [25]	HCQ	VeroE6	0.01	48 h	2.19 ^d
				72 h	4.39 ^d

^a Post-infection^b Expressed in μM ^c Not stated in article if VeroE6 was the lineage utilized^d IC₅₀ (not EC₅₀)

Furthermore, the half cytotoxic concentration (CC₅₀) values were not found to be statistically significant from each other (chloroquine: 273.20 μM vs. HCQ: 249.50 μM) [35].

Wang and colleagues evaluated chloroquine against SARS-CoV-2 (nCoV-2019BetaCoV/Wuhan/WIV04/2019) and demonstrated potent in vitro activity in Vero E6 cells, which were infected at a MOI of 0.05. At 48 h, chloroquine was shown to potently inhibit SARS-CoV-2 with a EC₅₀ of 1.13 μM and CC₅₀ > 100 μM . This evaluation also showed that chloroquine worked at the “entry” and “post-entry” stages of SARS-CoV-2 infection [36].

Yao and colleagues performed an in vitro evaluation of HCQ and chloroquine against SARS-CoV-2-infected Vero cells at a MOI of 0.01 for 2 h, followed by treatment concentrations for 24 or 48 h. The authors concluded that HCQ was more potent than chloroquine, with EC₅₀ values of 6.14 μM and 23.90 μM , respectively, at 24 h and EC₅₀ values of 0.72 μM and 5.47 μM , respectively, at 48 h. Furthermore, HCQ was shown to exhibit a superior antiviral effect compared to chloroquine when cells were pretreated prior to viral challenge [37].

An evaluation from Maisonasse and colleagues analyzed the in vitro activity of HCQ against SARS-CoV-2 in VeroE6 cells (MOI: 0.01) and a model of reconstituted human airway epithelium (MOI: 0.1). In VeroE6 cells at 48 and 72 h, the half maximal inhibitory concentration (IC₅₀) values were 2.19 μM and 4.39 μM , respectively. However, HCQ at 1 μM or 10 μM was not shown to significantly reduce viral titers in the reconstituted human airway epithelium at 48 h compared to untreated control [25].

Despite these in vitro evaluations, it is important to note that very little is known regarding the relevance of in vitro EC₅₀ values in optimizing the PK/PD of HCQ in humans. Furthermore, depending on testing conditions, the reported studies have reported a ~ 24-fold range in EC₅₀ values. Finally, it is currently unknown which cell line is optimal for showing activity, and the discordant results between different cell lines introduce additional uncertainty in the relevance of these values. These limitations further emphasize the need to define the “optimal target” and how to correlate this target to efficacious HCQ exposures.

PHARMACOKINETIC/ PHARMACODYNAMIC ANIMAL STUDIES

Maisonnasse and colleagues evaluated the antiviral effect of HCQ both alone and in combination with azithromycin compared to placebo in SARS-CoV-2 infected macaques [10^6 PFU SARS-CoV-2 (BetaCoV/France/IDF/0372/2020) via combined intra-nasal and intra-tracheal routes]. The authors analyzed the PK of HCQ and stated that exposures in plasma were comparable to HCQ in humans at a dose of 200 mg three times daily at days 3 to 5. Neither the high- [90 mg/kg [human equivalent dose (HED) = 29.0 mg/kg] on day 1, followed by 45 mg/kg/day (HED = 14.5 mg/kg/day); $n = 5$] nor low-dose [30 mg/kg (HED = 9.7 mg/kg) on day 1, followed by 15 mg/kg/day (HED = 4.8 mg/kg/day); $n = 4$] regimens of HCQ accelerated the time to viral clearance compared to controls, despite showing that HCQ blood and lung concentrations of HCQ were higher than the EC_{50} values identified in VeroE6 cells. This lack of antiviral effect was further shown in the HCQ and azithromycin combination arm [25].

PHARMACOKINETIC/ PHARMACODYNAMIC IN SILICO STUDIES FOR TREATMENT IN HUMANS

To date, limited PK/PD profiling studies have been performed to identify optimal dosage regimens against COVID-19 in humans, and the exposures targeted in these assessments are of questionable relevance. In a study conducted by Garcia-Cremades and colleagues, translational PK/PD modeling was used to propose optimized HCQ dosage regimens for initial treatment to ensure the highest likelihood of success (predict viral decline and risk of QTc prolongation). A published two-compartment population PK model for HCQ was used to predict plasma concentrations for different dosing regimens of HCQ (200 mg, 400 mg, 600 mg, and 800 mg twice daily for 5, 7, and 10 days, with and

without various loading doses, and all simulations included 100 virtual patients that were simulated 1000 times). Evaluating the same EC_{50} range as previously published (0.72–17.31 μM), the authors of this study performed simulations by fixing one of the reported EC_{50} values, with each HCQ regimen simulated with each value 500 times, to determine HCQ's effect on viral replication. The authors found that the extrapolated HCQ plasma concentration for the 50% viral inhibition value following simulations was 4.7 μM , and the authors' targets were HCQ concentrations "close or above the clinical EC_{50} values and below 7.5 μM " for each simulation. Importantly, however, the authors do not provide sufficient support that these exposures would lead to the proposed results, and the data do not support $> 7.5 \mu\text{M}$ as a toxic target. A mechanistic PK/virologic/QTc model for HCQ was developed, and a simulation study was performed to predict viral decline and QTc prolongation. The authors concluded that HCQ regimens > 400 mg twice daily for at least 5 days were predicted to rapidly decrease viral loads, while regimens > 600 mg twice daily were predicted to prolong QTc intervals (because of levels $> 7.5 \mu\text{M}$) [38].

Yao and colleagues used a physiologically based PK (PBPK) model for HCQ to simulate lung fluid HCQ concentrations under five HCQ dosing regimens to evaluate the most effective and safe regimen. Quantitative markers were ratios of estimated free lung tissue trough concentration to EC_{50} ($R_{L\text{TEC}}$) based on human PK data and rat lung penetration data, which were compared to the "efficacious" chloroquine 500 mg twice daily that was reported to improve outcomes by Gao and colleagues [37,39]. Importantly, whether or not chloroquine 500 mg twice daily is an efficacious regimen is unclear as these data remain unpublished, and a recent report suggested high-dose chloroquine may be associated with increased toxicity and mortality [40]. The targeted HCQ $R_{L\text{TEC}}$ values were based on $R_{L\text{TEC}}$ values of 2.38, 5.92, and 18.9 on days 1, 3, and 5, respectively, simulated from the chloroquine 500 mg twice-daily regimen. All HCQ regimens produced $R_{L\text{TEC}}$ values higher than target $R_{L\text{TEC}}$ values for each day

evaluated; however, the authors recommended HCQ 400 mg twice daily on day 1, followed by 200 mg twice daily for 4 days while “considering efficacy, safety, and patient compliance.” Furthermore, the $R_{L_{TEC}}$ values of HCQ were found to be higher than those of chloroquine on days 1, 3, 5, and 10. It is important to note that the HCQ EC_{50} values were $\sim 7.6 \times$ less than those of chloroquine, which plays a role into HCQ’s higher $R_{L_{TEC}}$ values, and also that this enhanced potency is contrary to other in vitro analyses performed [37,41].

Perinel and colleagues performed a prospective analysis to characterize the PK of HCQ in patients admitted to the intensive care unit with laboratory-confirmed SARS-CoV-2 infection. Patients included in this analysis received initial HCQ regimens of 200 mg orally three times per day [42]. Based on previously published literature, HCQ trough values > 1 mcg/ml and < 2 mcg/ml were considered to “optimize efficacy and safety,” respectively [37,43]. It is important to note that there is no evidence in support of these values for optimal treatment against COVID-19. Thirteen patients were included in this analysis (12 of which were mechanically ventilated, 85% male), with a median age of 68 years (38–82 years), body weight of 82.7 kg (63.0–117.0 kg), and renal function of 79.6 ml/min (12.0–118.0 ml/min) as estimated via the CKD-EPI formula. Based on the HCQ initial regimen (200 mg orally three times/day), only 61% of patients achieved “therapeutic” levels (> 1 mcg/ml) at a mean of 2.7 days (1.0–4.5 days), while 15% of patients achieved what the authors classified as “toxic” levels (> 2 mcg/ml). To more clearly understand the variability of HCQ PK parameters and assess an optimal dosing regimen, a simulation study was performed across various dosing regimens. The authors proposed that a HCQ 800 mg loading dose on day 1 followed by 200 mg twice daily for 7 days provided “optimal” exposures (targeting levels > 1 mcg/ml and < 2 mcg/ml) compared to the other HCQ regimens simulated [42].

PHARMACOKINETIC/ PHARMACODYNAMIC IN SILICO STUDIES FOR PROPHYLAXIS IN HUMANS

Al-Kofahi and colleagues performed simulations to model and identify possible pre- and post-exposure HCQ dosage regimens that would achieve target exposure over the SARS-CoV-2 EC_{50} and help guide clinicians in dose selection for COVID-19. Of note, the population PK parameters were derived from HCQ plasma concentrations from healthy volunteers and malaria patients, not patients with diagnosed COVID-19. The authors of this study chose to evaluate the percentage of patients with trough concentrations above the SARS-CoV-2 EC_{50} values of 0.72 μ M and 1.44 μ M (to account for plasma protein binding). These EC_{50} values were chosen as targets as lower EC_{50} values are typically associated with lower MOIs, and the authors presumed that post-exposure prophylaxis may be associated with lower viral loads compared to acute infection with SARS-CoV-2; however, there is no evidence to support this claim [44].

Regimens simulated included post-exposure prophylaxis one: no loading dose, followed by 400 mg daily for 5 days; post-exposure prophylaxis two: 800 mg loading dose, followed by 400 mg daily for 3 days; post-exposure prophylaxis three: no loading dose, followed by 200 mg three times daily for 6 days; post-exposure prophylaxis four: 800 mg loading dose, followed by 600 mg daily for 3 days; post-exposure prophylaxis five: 800 mg loading dose, followed by 600 mg daily for 5 days; and post-exposure prophylaxis six: 800 mg loading dose, followed by 600 mg daily for 7 days. With the exception of post-exposure prophylaxis regimen one, all other regimens had between 89 and 94% troughs above target on day 1, with a median time above target being 16–23 h. Post-exposure prophylaxis regimens three, five, and six maintained the optimal PD targets compared to the other regimens at day 14 [44]. Despite these results, it is currently unclear if the target chosen by the authors is, in fact, an appropriate target. Importantly, Boulware and colleagues

conducted a randomized, double-blind, placebo-controlled trial assessing the effect of HCQ for post-exposure prophylaxis and found no significant difference in the incidence of new COVID-19 illness [45].

LIMITATIONS OF PHARMACOKINETIC/PHARMACODYNAMIC IN SILICO STUDIES FOR TREATMENT IN HUMANS

Overall, there are notable and important limitations across the highlighted studies. The primary limitation is that a proper exposure-response analysis was not performed in any of the human simulation studies. Targeted exposures were all related to published EC_{50} values to predict PD response, despite an absence of data to support this strategy. It is currently unknown if EC_{50} values are meaningful values and what exposures relative to these values would be associated with an antiviral effect. Furthermore, if EC_{50} values prove to be an effective target, it would be of importance to determine what conditions and cell line are associated with a reproducible and meaningful value. It warrants mention that the only true PD study currently available (Maisonasse et al. [25] macaques study described above) failed to demonstrate an antiviral effect with HCQ despite having serum and lung concentrations above the EC_{50} . Furthermore, if a target is identified, there is likely to be significant variation in PK in patients infected with COVID-19 that is not properly accounted for in the simulation studies published. When considering PK, it is also likely important to consider HCQ intracellular concentrations, as HCQ has been shown to accumulate in cells through “lysosomal trapping” [7]. Overall, there is a clear need for clinical data that links HCQ dose, PK, response, and safety in patients with COVID-19.

TOXICODYNAMICS

Although the most common adverse effects of HCQ are gastrointestinal in nature, ocular and

cardiac toxicities are arguably the most concerning [46–49]. Ocular adverse effects associated with HCQ include retinopathy, corneal changes, and decreased visual acuity; however, these are most commonly associated with long-term use and would likely not be of principal concern with the short durations of HCQ utilized for COVID-19 [50]. Nevertheless, a recent report associated both maximum ($p = 0.0340$) and mean ($p = 0.0124$) HCQ levels to predict HCQ-induced retinopathy. In this report of 537 patients, the overall frequency of retinopathy was 4.3%. HCQ-induced retinopathy was found in 1.2%, 4.8%, and 6.7% of patients with a maximum HCQ blood concentrations of 0.0–1.2 mcg/ml, 1.2–1.8 mcg/ml, and 1.8–6.3 mcg/ml, respectively, and found in 1.2%, 3.7%, and 7.9% of patients with mean HCQ blood concentrations of 0.0–0.74 mcg/ml, 0.74–1.2 mcg/ml, and 1.2–3.5 mcg/ml, respectively. Notably, risk of retinopathy increased with HCQ duration, with only 1% of patients experiencing this adverse effect within the first 5 years of HCQ use [41].

The most immediate life-threatening adverse effect of HCQ is ventricular arrhythmias arising from QTc prolongation. Unfortunately, to our knowledge, there has been no evaluation linking HCQ exposure to cardiac toxicity. Evaluating a dose-response relationship with cardiac toxicities, including QTc prolongation, cardiomyopathy, torsades de pointes, and ventricular arrhythmia, is of utmost importance in future studies. It is also important to note that, although cardiac issues can arise with HCQ monotherapy, most cardiotoxicity is likely in combination with other drugs (e.g., azithromycin) or those with baseline pre-disposing conditions, such as baseline prolonged QTc and/or electrolyte abnormalities (although a dose-dependent effect with HCQ monotherapy is possible). In the evaluation conducted by Perinel and colleagues, QTc prolongation occurred in two patients receiving HCQ 200 mg orally three times/day, with QTc values elevating from 381 to 510 ms and 432 to 550 ms with HCQ levels of 0.03 mcg/ml and 1.74 mcg/ml, respectively. Given these large variations in HCQ levels, baseline comorbidities or concomitant QTc-prolonging medications could

have contributed to QTc prolongation; however, these potential confounding variables were not reported by the authors. A limitation of this study was that there were limited HCQ exposures that were correlated to toxicities. This evaluation utilized a “toxic” level of > 2 mcg/ml, which was derived as “...a number of arguments suggest that a concentration of 2 mcg/ml should not be exceeded to avoid ocular toxicity” [42]. Despite this basis, HCQ-induced ocular toxicity is most commonly associated with prolonged exposures and will likely be of less concern with the short durations utilized for COVID-19; cardiotoxicity is of much more concern during acute ingestion of HCQ [41,50]. Indeed, Mercurio and colleagues conducted a retrospective, observational study evaluating hospitalized COVID-19 patients and found that those receiving HCQ monotherapy had a median (IQR) change in QT interval of 5.5 [– 15.5 to 34.3] ms [51]. Also important to note, given the large Vd and high tissue sequestration of HCQ, it is possible that patients may remain at risk for QTc prolongation after HCQ discontinuation. Baseline and periodic QTc monitoring should be used to help guide the safe use of HCQ in patients receiving this medication for COVID-19.

GAPS IN THE LITERATURE AND SUGGESTIONS FOR FUTURE STUDIES

Despite the work completed to date, many data need to be considered for the future testing of HCQ and other COVID-19 therapies to determine optimal dose(s) of these therapies. Antiviral “activity” in vitro and in vivo needs to be adequately defined (EC_{50} , EC_{90} , etc.) against SARS-CoV-2 in the most pertinent cell line. The previously cited in vitro studies display a broad range of EC_{50} values that were determined utilizing different MOIs and applying different time periods utilized for their samplings, despite the uncertainty of whether or not EC_{50} values are meaningful targets [35–37]. Further studies need to assess exposures in animal models in both blood and lung that are related to COVID-19 PD response (e.g., viral load reductions,

resolution of symptoms, survival, etc.). Studies also need to further examine the penetration of HCQ into relevant tissues, taking into account intracellular concentrations (macrophages, neutrophils, etc.) due to “lysosomal trapping” associated with HCQ. When studying intracellular concentrations of HCQ, macrophage concentrations in humans need to be determined as concentrations will need to be corrected for if serum to macrophage concentrations differ between species. Following the optimal determination of the PD “target,” serum exposures in blood/plasma/serum should be correlated with lung concentrations throughout in vivo evaluations and then which dose(s) this translates to in humans needs to be determined. Following all of these considerations, these dosage regimens should be evaluated for efficacy and safety in well-designed, randomized clinical trials. Also, the current ongoing clinical trials will need to be evaluated for possible efficacy and safety signals associated with different dosage regimens.

Current dosing protocols and guidelines for HCQ are a result of extrapolating data and findings from other disease states and/or pharmacology simulation studies performed. Given the “cart before the horse” approach with HCQ in response to COVID-19, it will be important to examine the results of ongoing clinical trials to determine whether dose response(s) were observed (if any). It will be important to analyze possible differences in outcomes in those with mild versus moderate versus severe disease and whether combination therapy may be an effect modifier. COVID-19 is likely to be a clinical issue for the foreseeable future. If HCQ is shown to fail in clinical trials, this emphasizes the problem with relying on in vitro activity of antimicrobials to translate to improved patient outcomes. While the initial pandemic may not have afforded the opportunity for robust pre-clinical work to inform agent selection and dosing, this should not be the case moving forward. We have been able to work towards improving outcomes in patients with COVID-19 with non-pharmacologic approaches and (possibly) some potential treatment options in the interim; however, we as clinicians should learn from the mistakes made and attempt to

place the horse before the cart as we proceed with future therapies.

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