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Population Pharmacokinetics of Hydroxychloroquine Sulfate in Healthcare Workers, Given for Prophylaxis Against Coronavirus Disease 2019 (COVID-19) in India

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Abstract

Healthcare workers (HCWs) and frontline workers were recommended hydroxychloroquine (HCQ) 400 mg twice a day on day 1, followed by 400 mg once weekly for the next 7 weeks, as prophylaxis against COVID-19. There was limited information on the population pharmacokinetics (popPK) of HCQ in an Indian setting when administered for prophylaxis against COVID-19, and hence this study was proposed. It was a multicentric prospective study conducted at 3 sites in India wherein HCWs who were already on HCQ prophylaxis, who were about to start prophylaxis or who had stopped the prophylaxis for any reason were enrolled. Each participant gave 2 to 6 blood samples at different time points and whole-blood HCQ concentrations were assayed using liquid chromatography with tandem mass spectrometry (LC MS/MS). popPK analysis was performed using PUMAS 1.1.0. A total of N = 338 blood samples from N = 121 participants were included in the popPK analysis. A 2-compartment structural model with linear elimination was able to explain the observed data. Body weight was found to be a significant covariate influencing drug clearance. The final model was assessed using goodness-of-fit plots, a visual predictive check and a bootstrap, all of which confirmed that the model was appropriate. Simulations based on the current regimen showed that trough values were below the half-maximal effective concentration (EC50) of 0.7 μmol against COVID-19. A new weight-based dosage regimen was proposed to maintain the trough concentration above the EC50 threshold.

Keywords

COVID-19, hydroxychloroquine sulfate, pharmacometrics, population pharmacokinetics, prophylaxis

Coronavirus disease 2019 (COVID-19), first reported in Wuhan, China, in December 2019,[1,2] spread rapidly to many nations as a deadly pandemic,[3] forcing researchers to explore prevention and treatment options. As the health crisis was looming all over the world, with thousands reported to have contracted the disease, some of whom died, the search for treatment or prevention options from among existing drugs took great priority. Accordingly, the repurposing of old and approved drugs such as azithromycin, chloroquine, doxycycline, hydroxychloroquine (HCQ), and ivermectin, among others, were explored for use in the prevention or treatment of COVID-19.[4] Among all these old drugs, HCQ garnered greater attention for large-scale use based on available in vitro data regarding its usefulness in COVID-19 prevention/treatment, ease of availability, known safety profile, and low cost.[5]

Although the mechanism of action of HCQ in the management of COVID-19 is not very clear, it is believed that HCQ, being a weak base, increases the pH in the endo-lysosome and prevents the activity of lysosomal protease from releasing the virus into the cytoplasm.[6] Two in vitro studies have demonstrated

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that HCQ inhibits severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[7,8] Therefore, the government of India advocated HCQ for chemoprophylaxis in healthcare workers (HCWs) on 21 March 2020.[9] Asymptomatic HCWs involved in the care of suspected or confirmed cases of COVID-19 were recommended 400 mg twice a day on day 1, followed by 400 mg once weekly for the next 7 weeks, to be taken with meals.[9] Subsequently, some observational studies showed benefit,[10] whereas randomized controlled trials did not show benefit.[5] Given this background, it was envisaged that a population pharmacokinetics (popPK) study would help guide appropriate dosing. A total of 7 HCQ popPK models are available in the literature,[11–16] 3 of which were developed using whole-blood concentrations: 1 each in patients with rheumatoid arthritis (RA),[12] cutaneous lupus erythematosus (CLE),[13] and COVID-19.[16] The other models were based on plasma concentrations,[11,14,15] or on merged blood and plasma concentrations,[13] using data from studies conducted on chemoprophylaxis against malaria, bioavailability in healthy volunteers, RA in pregnancy, and patients with CLE, respectively.

As there was limited information on popPK of HCQ in Indian settings and for the use of HCQ as prophylaxis against COVID-19, the objective of this study was to assess the pharmacokinetics of HCQ using sparse sampling in HCWs prescribed this drug for prophylaxis.

Methods

Ethics

The study was conducted in accordance with the principles of Good Clinical Practice, which is based on the Declaration of Helsinki, the National Guidelines for Ethical Research in Human Participants (Indian Council of Medical Research Guidelines, 2017), and the New Drugs and Clinical Trials rules (Central Drugs Standard Control Organisation, 2019) of India. The Institutional Ethics Committee approval from all the 3 sites was obtained before the commencement of the study and the study was registered in the Clinical Trials Registry of India (CTRI/2020/05/025242) before the recruitment of the first participant. Written informed consent was also obtained from all participants.

Study Design and Eligibility Criteria

This was a multicentric prospective interventional study conducted in 3 tertiary care teaching hospitals located in the Indian cities of Mumbai, Hyderabad, and Chandigarh, between June and November 2020. All consenting asymptomatic HCWs of any sex, aged between 18 and 65 years, actively on duty (not retired), and who were already on HCQ prophylaxis, about to start prophylaxis, or had stopped the prophylaxis either after completion of the 7-week regimen or before, for any reason, were included in the study. HCWs who showed symptoms suggestive of COVID-19 or were positive for COVID-19 at the time of enrollment, women of childbearing potential who are pregnant, lactating, or not willing to use adequate contraception and those with a history of HCQ intake for any other indication in the past 1 year were excluded from the study.

Methodology and Procedures

The HCWs who were actively involved in the screening, triage, diagnostics, and/or management of suspected or lab diagnosed patients testing positive for COVID-19 were counseled for the study. A thorough medical history and medication history was obtained to confirm eligibility. Subsequently, 6 mL of blood was collected. Based on the convenience of the participants, additional blood samples of 6 mL were collected at suitable time points (see below) to be representative of the entire dose–concentration curve. The samples were collected any time during the entire HCQ prophylaxis regimen, provided the date and time of all prior HCQ doses were reliably known with a precision of <1 hour. Also, there was no restriction on food and water consumption prior to sampling. At all subsequent visits, a thorough medication history and COVID-19-related history was obtained.

Blood Sampling Time Points

The time points chosen for blood collection in participants who were either already on HCQ prophylaxis or started HCQ prophylaxis after enrolling in this study were: (i) on the day of enrollment in the study (spot); (ii) a total of 3–5 hours after dosing to capture the maximum plasma concentration (C_{max}), based on the available literature; (iii) just before the next dose (trough); (iv) in between the trough and C_{max} ("other"), where the day of collection was different from that of the spot sample (eg, with once-weekly dosing, if the spot sample was collected on a Tuesday, the "other" sample was collected on days of the week other than a Tuesday); and (v) before the first dose in the case of participants who were HCQ naïve (baseline).

No more than 2 trough samples and no more than a total of 6 samples per participant were collected. When the first sample on the day of consent corresponded to a baseline, trough, or C_{max} sample, the sample was reported under the respective timeline and the spot sample was collected on any other day between the C_{max} and the next trough. For participants who had either completed the HCQ prophylaxis regimen or stopped in between, for any reason, a maximum of 3 blood samples were collected on different days of a week at the rate of 1 sample per calendar week.

Blood Sample Processing and Analysis

After sample collection, the whole blood was stored at –80°C in a deep freezer until analysis. The drug concentration analysis was performed with whole blood within 45 days of sample collection, using liquid chromatography with tandem mass spectrometry (LC MS/MS) and positive electrospray ionization (ESI), which was developed and validated by the analytical facility (Cliantha Research, Ahmedabad, Gujarat, India). After solid-phase extraction, HCQ (the parent analyte) and HCQ-d4 (the internal standard) were separated on a C18 column. HCQ and HCQ-d4 were detected by positive ESI followed by multiple reaction monitoring (MRM) of the transition at m/z 336.2 → 247.2 and 340.2 → 247.2, respectively. The method was linear for HCQ in the whole-blood concentration range 2–500 ng/mL. The limit of detection (LOD) was 0.1 ng/mL and the lower limit of quantification (LLOQ) was 2.0 ng/mL.

Sample Size Considerations

No formal sample size calculation was performed for the popPK analysis, in accordance with draft guidance to industry for popPK issued by the US Food and Drug Administration.[17] It is stated that "a large number of patients included in population PK analysis may improve the precision of the estimated effect of the factors that affect drug exposures and confirm which factors do not change drug exposures."[17] Variability, expressed in terms of the coefficient of variation between subjects on parameters like clearance and volume of distribution, must be accounted for in these models. If the expected variability is greater than or equal to 75%, a sample size of 100 is recommended.[18] However, in order to account for analytical errors, loss during shipment, and participants' inability to provide more than 1 sample, given the pandemic situation, it was decided to enroll as many participants as possible, but no more than N = 200 participants.

Data Management

All relevant data for each participant were recorded in a specially designed case record form (CRF). Data entry was performed with Microsoft Excel (Microsoft Corporation, Redmond, Washington). Data analysis was performed using R 4.0.3 (RStudio, Boston, Massachusetts),[19] and population pharmacokinetic analysis was performed with PUMAS 1.1.0 (PUMAS-AI, Centreville, Virginia).[20]

popPK Modeling

A nonlinear mixed-effects modeling approach was used for the popPK analysis. The population mean values for apparent clearance (Cl/F), apparent volume of distribution in central compartment (Vc/F), apparent volume of distribution in peripheral compartment (Vp/F), and apparent intercompartmental clearance (Q/F) were estimated from the observed data. First-order conditional estimation with interaction was used for the estimation of popPK parameters. All 1-, 2-, and 3-compartment models with first-order and zero-order kinetics were tested for the selection of the structural model. Between-subject variability (BSV) of the parameters was assumed to be normally distributed with a mean of zero and was modeled using an exponential model. Residual unexplained variability (RUV) was tested using additive, proportional, and combined error models. With minimal data to explain the absorption phase of the drug, the absorption rate was fixed at 1.15 hours based on published literature.[11] Allometric scaling was incorporated and tested using standard coefficients as well as estimating the coefficients in apparent clearance and apparent volume of distribution parameters to account for variability arising from body weight.

Age, sex, body weight, and body mass index (BMI) were tested as potential model covariates using univariate analysis based on forward addition and backward elimination process, and the significant covariates were retained in the final model. Although HCQ is primarily excreted by the kidneys, creatinine clearance was not included as one of covariates in popPK development as creatinine clearance is expected to be normal in healthy individuals with no history of renal abnormalities. Various models were tested to identify the best structural model, significant covariates, and best error model. In each step of model development the better model was selected based on the likelihood ratio test, which follows a chi-square distribution, with $P < .05$ indicating statistical significance.

Model Diagnosis and Evaluation

The final model was subsequently evaluated by visual inspection of goodness-of-fit plots. Population and individual predicted concentrations (PRED and IPRED, respectively) were plotted against observed concentrations. Further, conditional weighted residuals (CWRES) were plotted against time and PRED of HCQ. The visual predictive check (VPC) was also performed using 1000 simulations with stratification based on weight to confirm the validity. A bootstrap analysis was also performed using resampled 1000 bootstrap datasets created from the original dataset based on random sampling to evaluate the robustness and stability of the final model by comparing the estimates of the original dataset with their corresponding 95% confidence intervals of the estimates from bootstrap analysis.

Simulations

Simulations were performed for the current dosing recommendations using the final model parameter

Table 1. Demographic Characteristics

Characteristics (N = 121)	Median (Minimum, Maximum)	Mean (SD)
Age (years)	31 (22, 57)	31 (9.6)
Height (cm)	161 (139, 190)	162 (8.9)
Weight (kg)	64 (45, 104)	65.93 (11.24)
BMI (kg/m^2)	24.84 (17.7, 36.2)	25.02 (3.5)

BMI, body mass index; SD, standard deviation.

estimates.[9] For these simulations, participants with different body weights corresponding to the lowest value, first quartile value, median value, third quartile value, and highest value from the observed data were used. The lower limit of the prophylactic dose concentration range in blood for prophylaxis against COVID-19 was set at 0.7 μmol (235.13 ng/mL), based on the half-maximal effective concentration (EC50) estimated from in vitro studies.[7,8] The upper limit was set at 2.0 μmol (671.8 ng/mL), anticipating that gastrointestinal side effects would be minimal at that cut-off value.[21] Alternative optimal dosing regimens for different body weights were proposed by simulations using the final model parameter estimates, considering the reference value of 0.7–2.0 μmol (235.13–671.8 ng/mL).

Results

A total of N = 159 participants were recruited across the 3 sites in India. popPK modeling was carried out with data from N = 121 participants (male = 49; female = 72). The reasons for exclusion include withdrawal of consent (N = 2), HCQ concentration below lower limit of quantification (N = 33), and abnormal time after dose values/outliers (N = 3). The baseline demographic characteristics of the participants included in the final analysis are summarized in Table 1. The total number of blood samples collected from these N = 121 participants was 338. There were only 2 participants during the study period who tested positive for COVID-19. Both tested positive more than 7 days after their previous maintenance dose of HCQ.

A 2-compartment structural model with linear elimination (Figure 1) was able to explain the observed data better compared with the other models attempted. RUV was accounted for using a proportional error model. The final model structure is presented in Table 2. Body weight was identified as a statistically significant covariate (P = .023) that influences the pharmacokinetics of HCQ. Allometric scaling of apparent clearance with estimated coefficient and volume of distribution parameters with standard coefficient of 1 explained the model better. No other covariates tested were able to improve the model fit and were not included in the final model. The goodness-of-fit plots, depicted in Figure 2, showed that the PRED or IPRED versus blood concentrations of HCQ generally matched well with the observed HCQ concentrations, indicating a good correlation between the observed and predicted concentration for both population prediction and individual prediction. In addition, the CWRES were distributed in a well-balanced manner and were centered at zero over PRED of blood and time. The VPC is depicted in Figure 3, indicative of most observed concentrations falling within the model-predicted range. The uncertainty of the parameter estimates was represented as percentage relative standard error (RSE%) for fixed effects and percentage

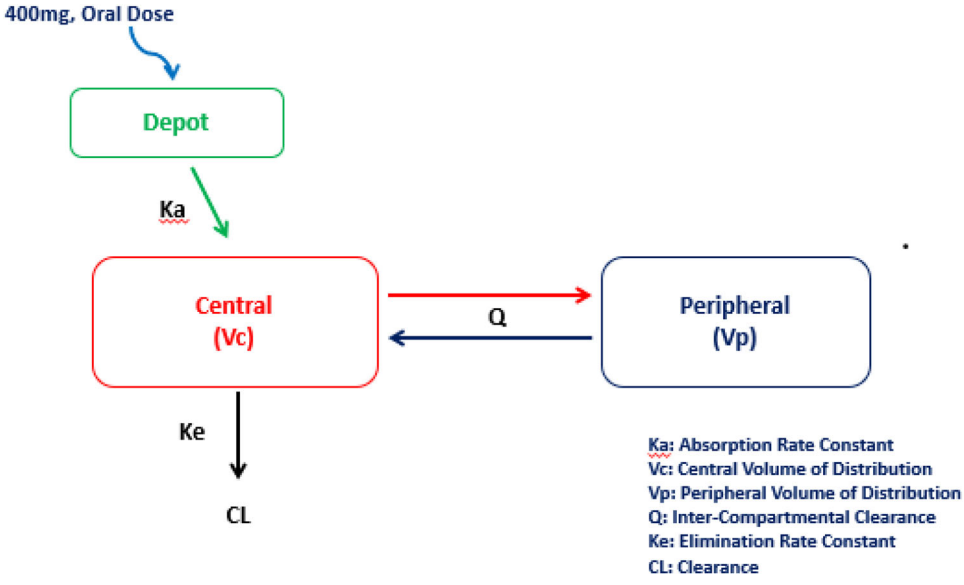

Figure 1. Model structure: 2-compartment oral dosing with first-order elimination.

Table 2. Model Structure

Absorption rate constant (Ka)	= tvka
Apparent clearance (Cl/F)	= tvcl * (WT/WTmed)$^{\Theta}$ * exp(BSV on Cl)
Apparent central volume of distribution (Vc/F)	= tvvc * (WT/WTmed)^{1} * exp(BSV on Vc)
Apparent peripheral volume of distribution (Vp/F)	= tvvp * (WT/WTmed)^{1} * exp(BSV on Vp)
Apparent intercompartmental clearance (Q/F)	= tvq * exp(BSV on Q)

BSV, between-subject variability; θ; effect of weight on clearance; tv, typical values of the population, also known as population mean; WTmed, median weight of the population (64 kg).

Table 3. Parameter Estimates and Bootstrap Confidence Intervals

Parameter (Units)	Model Estimate	Bootstrap Estimate	Bootstrap 95%CI
Fixed effects (%RSE)			
Ka (hour)	1.15[a]	1.15	Not applicable
Cl/F (L/h)	13.44 (6.31)	13.37	11.82–15.06
Vc/F (L)	723.50 (8.06)	724.36	618.16–843.00
Vp/F (L)	3395.57 (15.14)	3422.47	2438.24–4516.92
Q/F (L/h)	5.53 (22.14)	5.75	3.59–8.56
Weight on Cl/F	0.34 (76.91)	0.36	0.00–0.93
Random effects (%CV)			
BSV Cl/F (%)	0.18 (42.87)	0.18	0.10–0.26
BSV Vc/F (%)	0.35 (59.52)	0.35	0.24–0.49
BSV Vp/F (%)	0.29 (54.26)	0.28	0.07–0.57
BSV Q/F (%)	0.71 (84.47)	0.70	0.18–1.25
RUV (%)	0.17 (41.76)	0.17	0.14–0.21

BSV, between-subject variability; Cl/F, apparent clearance; CV, coefficient of variation; Ka, absorption rate constant; Q/F, apparent intercompartmental clearance; RSE, relative standard error; RUV, residual unexplained variability; Vc/F, apparent central volume of distribution; Vp/F, apparent peripheral volume of distribution. The values of Cl/F, Vc/F, Vp/F, and Q/F are the estimates of a typical individual, weighing 64 kg.
[a] Fixed.

Table 4. Proposed Weight-Based Dosage Regimen

Body Weight (kg)	Loading Dose (mg)	Initiation of Maintenance Dose After Loading Dose (Hours)	Initial Maintenance Dose (mg)[a]	Continuation Maintenance Dose (mg)[a]
45–54 kg	400 mg (single dose)	36th hour	200 mg (every 36 hours × 6 doses)	200 mg (every 36 hours × 19 doses)
55–74 kg	400 mg (single dose)	36th hour	300 mg (every 36 hours × 6 doses)	300 mg (every 48 hours × 19 doses)
75–94 kg	400 mg (single dose)	24th hour	300 mg (every 36 hours × 6 doses)	300 mg (every 36 hours × 19 doses)
95–104 kg	400 mg and 2nd dose at 12th hour	48th hour	300 mg (every 36 hours × 6 doses)	300 mg (every 36 hours × 18 doses)

[a] Maintenance dose regimen described here is for a period of 7 weeks, like the national advisory, where the initial maintenance is prescribed immediately after the loading dose and is followed subsequently by the continuation maintenance dose.[9] The maintenance dose would need to be continued beyond 7 weeks to maintain the plasma concentration for a prolonged duration.

coefficient of variation (%CV) for the dispersion of random effects, and the final model parameter estimates with bootstrap estimates are presented in Table 3. Simulations with current dosing regimen showed that the trough concentrations were falling below the threshold range (0.7–2.0 μmol) in all weight categories. The alternate weight-based dosing regimens, such that the whole-blood HCQ concentration remains within the reference COVID-19 prophylactic range, are summarized in Table 4 and the PK profile simulations are presented in Figures S1–S4. Each participant who falls within the respective weight band is expected to take a loading dose followed by maintenance dose regimens 1 and 2, sequentially.

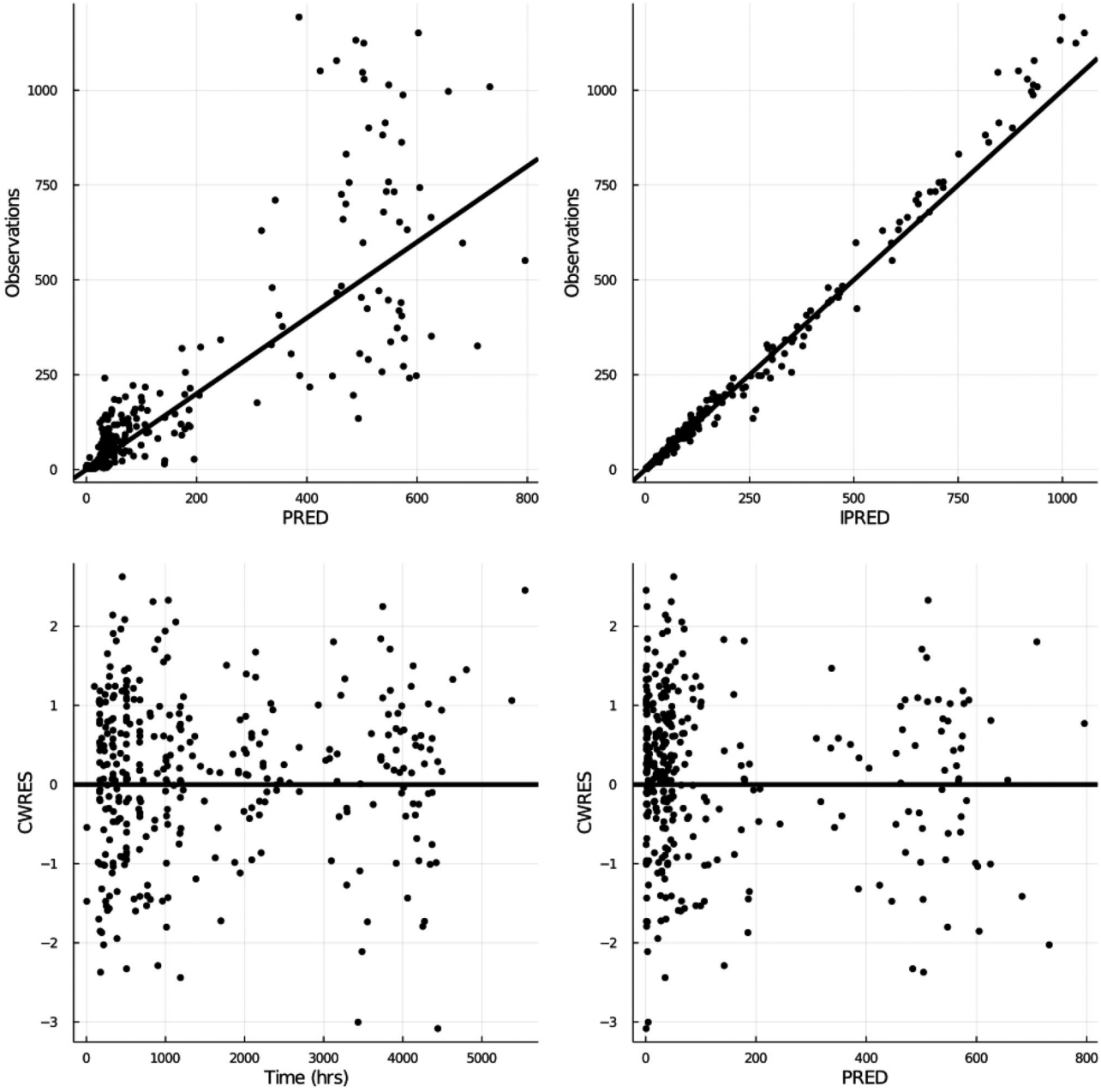

Figure 2. Goodness-of-fit plots.

Discussion

We report that popPK of blood HCQ concentrations in an Indian population prescribed for COVID-19 prophylaxis (400 mg, 2 doses, 12 hours apart on day 1, followed by 400 mg once a week for 7 weeks) were well described by the 2-compartment structural model with linear elimination. Body weight had a significant impact on the pharmacokinetic parameters of HCQ and hence weight-based dosing regimens are likely to prevent therapeutic failure or adverse events.

Several popPK models have been published for HCQ and the recent work reported by Themans et al focused on dose optimization of HCQ in COVID-19 patients.[22] Among the identified literature, models were developed using whole-blood concentrations, plasma concentrations, and both plasma and whole blood obtained from patients being treated for various conditions. Plasma concentrations of HCQ are more variable and it has been recommended that HCQ whole-blood concentrations should be preferred over plasma.[14,23]

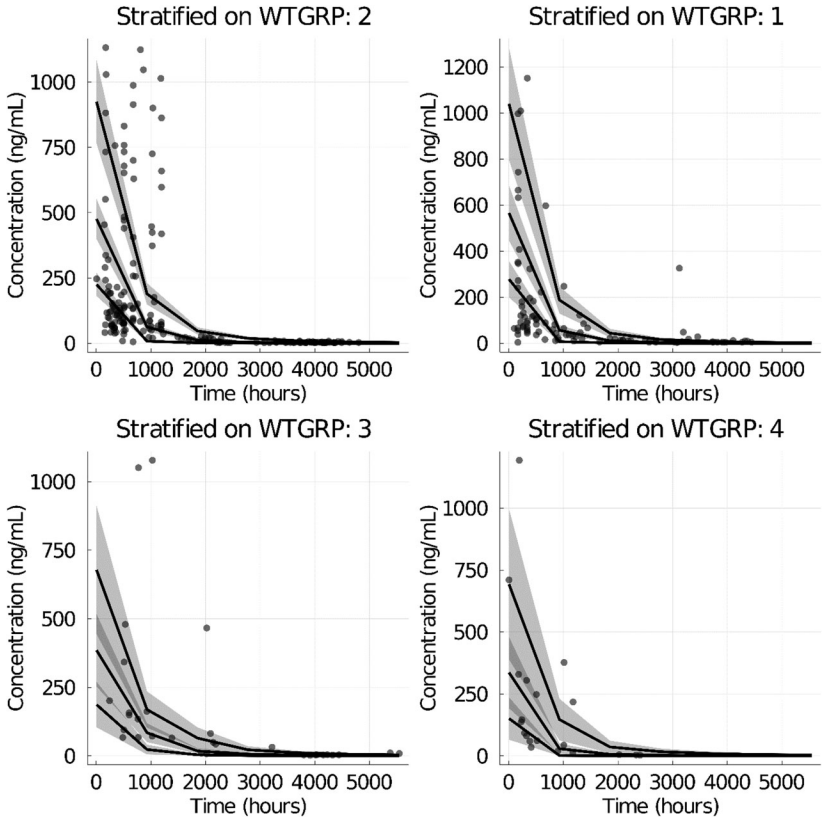

Figure 3. Visual predictive checks.

Hence, our model was also based on HCQ whole-blood concentrations.

Previously rated absorption rate constant have ranged from 0.5 to 1.3.[21,25–28] In the current model the absorption rate constant is fixed to 1.15 hours based on the study by Lim et al,[11] because of the lack of adequate data in the absorption phase for estimation. Apparent clearance was estimated to be 13.44 L/h from the final model, which is comparable with the values reported in published literature of 9–12 L/h.[11,24,25] When compared with other similar studies that used a 2-compartment model,[11,24–26] the apparent volume of distribution parameters were on the higher side, similar to that reported by Haas et al.[27] This could be explained by the fact that HCQ in general has a higher volume of distribution as a result of wider tissue distribution.[28] Further, we also report higher BSV and RUV. This is probably because information regarding factors such as renal function, protein binding, inhibitors, and inducers of HCQ metabolizing enzyme were not available in the current study. Body weight was found to exert a significant influence on drug clearance. This is in accordance with the findings reported by Themans et al,[22] and by Morita et al.[13] Thus, weight-based dosage regimens would help maintain blood concentrations within the desired range. In the published simulation-based studies,[29,30] the proposed dosing regimen for the prevention and treatment of COVID-19 was based on a previously developed model for HCQ in patients with malaria.

The simulation-based dosing recommendation using the final model is in agreement with the treatment suggested by Karataza et al,[29] who recommended a higher initial dose followed by lower sparse maintenance doses.

The strengths of our study are that a reasonable number of participants (N = 121) were enrolled in the study and the number of blood samples that formed the basis for the popPK analysis. To the best of our knowledge this is the first HCQ popPK model performed for an Indian population, and the first of its kind globally, for a prophylactic regimen against COVID-19. The limitations of our study are that a concentration–COVID-19 outcome model that could be used in conjunction with this model to characterize the efficacy of HCQ in the prevention COVID-19 could not be developed, as the number of participants who tested positive for COVID-19 positive was very small. Most potential participants who had completed their 7-week regimen but who tested positive for COVID-19 were unable to be included in the study as they received the treatment regimen of HCQ while they were ill, and this was one of our exclusion criteria. Further, the EC50

value was based on in vitro experiments that have their own limitations. EC50 values derived from preclinical and clinical studies would have added greater credibility to our predictions.

Conclusions

The once-weekly HCQ 400 mg maintenance dose was unable to maintain blood levels of HCQ above the in vitro EC50 recommended for prophylaxis against SARS-CoV-2. Weight-based and more frequent dosing might have helped to maintain the plasma concentrations above the proposed EC50 threshold against SARS-CoV-2. Further research is warranted to develop concentration–efficacy and concentration–safety models to determine the clinical applicability of the use of HCQ in prophylaxis against COVID-19, as new variants continue to cause multiple waves of the pandemic. These models would also aid in a better understanding of our results when used in conjunction. Although the use of HCQ in prophylaxis against COVID-19 is currently not recommended, the findings from this study would still be useful in the future if HCQ is explored as an option for prophylaxis in any future pandemics/epidemics caused by other viral illnesses, as has been done in the past.

Clinical Trials Registry of India (CTRI): CTRI/2020/05/025242. Registered prospectively on 19 May 2020.

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Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

Data Sharing

The datasets generated during and/or analyzed during the current study are available from the corresponding author, upon reasonable request.

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Author Contributions

JPR, NJG, and NAK conceived the idea. JPR, NJG, NAK, and SM designed the study and drafted the study protocol. All authors except NAK, SM, and APR were involved in data collection and data entry. APR and SM performed the popPK analysis. All authors contributed equally to the interpretation of the results. The first draft of the manuscript was written by JPR and APR, which was then critically revised by the rest of the authors. The final version of the article has been approved by all of the authors and all authors take responsibility for the accuracy of the published work.

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Supplemental Information

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