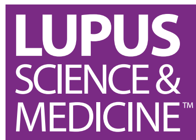

Pharmacokinetics of hydroxychloroquine in paediatric lupus: data from a novel, direct-to-family clinical trial

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ABSTRACT

Objective Determine the pharmacokinetics (PK) and exposure–response of hydroxychloroquine (HCQ) and desethylhydroxychloroquine (DHCQ) in paediatric SLE (pSLE).

Methods We conducted an exploratory phase 2, direct-to-family trial. Children enrolled in the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry with a diagnosis of pSLE were eligible if they were receiving HCQ as standard of care for ≥3 months. Biological samples were collected at up to four visits over a 6-month period. At each visit, plasma was obtained to measure the concentrations of HCQ and DHCQ, as well as cytokines. HCQ and DHCQ plasma PK data were analysed using a population PK modelling approach.

Results Twenty-five subjects provided a total of 88 plasma concentrations for PK analysis. There was a poor linear fit between HCQ concentrations and total body weight (R^2=0.03). There was a decline in both interferon (IFN)-alpha and IFN-gamma with higher concentrations of HCQ and DHCQ. Volume of distribution for HCQ in plasma was higher in children compared with published values in adults (73 000 L vs 44 000 L), but clearance values in children were similar to adults.

Conclusions We report the first population PK model for HCQ and DHCQ in children using data from a novel direct-to-family clinical trial. We observed high interindividual variability in HCQ PK and found that weight-based dosing for HCQ is poorly correlated with drug concentrations, suggesting the need to use therapeutic drug monitoring to individualise dosing. Furthermore, our results suggest that the current weight-based dosing paradigm for HCQ may result in suboptimal drug exposures, particularly for children with obesity. Accordingly, additional studies of HCQ are needed in pSLE to determine the optimal drug concentration and dosing to reduce disease activity and improve outcomes.

Trial registration number NCT04358302.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Virtually all children with SLE are treated with hydroxychloroquine (HCQ); however, the pharmacokinetics (PK) of HCQ in children with SLE are poorly understood, resulting in a dosing paradigm that is extrapolated from adults.

WHAT THIS STUDY ADDS

⇒ Weight-based dosing for HCQ is poorly correlated with drug concentrations, suggesting the need for alternative dosing strategies such as therapeutic drug monitoring.
⇒ The current weight-based dosing paradigm for HCQ may result in suboptimal drug exposures, particularly for children with obesity.
⇒ The data needed for PK studies, including biological samples, dosing information, and clinical covariates, can be obtained entirely outside of a brick-and-mortar research facility—such as a patient's home.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The developed population PK model can be used in the future to conduct dosing simulations to obtain target HCQ drug concentrations and optimise dosing in children.

INTRODUCTION

To control disease activity and prevent poor outcomes, virtually all patients with SLE receive treatment with hydroxychloroquine (HCQ), which can prevent disease flares, reduce cumulative organ damage and improve survival.[1–3] HCQ is metabolised to several metabolites, the major active metabolite being desethylhydroxychloroquine (DHCQ).[4] Despite widespread use in children, there are virtually no population pharmacokinetic/pharmacodynamic (PK/PD) studies to guide HCQ dosing in paediatric SLE (pSLE), and children are prescribed the same weight-based dosage as adults.[5] However, weight-based dosing may produce significantly different exposure in children compared with adults due to differences in the maturity of drug-metabolising enzymes,

renal function and other physiological changes.[6] Moreover, low systemic concentrations of HCQ are associated with increased disease activity and increased risk of disease flares in adults[7–11] and in children,[12 13] underscoring the need for PK studies to ensure children obtain therapeutic HCQ and DHCQ concentrations.

In addition to the physiological changes that may alter HCQ PK in children compared with adults, non-adherence to HCQ may result in subtherapeutic concentrations and treatment failure. In real-world settings, adherence to chronic medications such as HCQ is a significant barrier to treating SLE, with up to 30%–50% of patients having chronic poor adherence and low drug concentrations.[14 15] As a result, patients with inadequate exposure to HCQ may be 2.5 times more likely to have a severe lupus flare.[1] The risk of severe flares underscores the need to evaluate HCQ adherence in children in a real-world setting.

To overcome the gap in existing PK and exposure–response data in children, we conducted a novel, direct-to-family clinical trial in pSLE using an electronic pill bottle cap to improve adherence to HCQ. As a secondary outcome of the trial, we collected plasma samples to measure HCQ concentration and biomarkers of HCQ response including inflammatory cytokines. Accordingly, this analysis aimed to determine the population PK and exposure–response of HCQ and DHCQ in pSLE.

METHODS

Study design and eligibility

We conducted an exploratory phase 2, single-site, open-label, direct-to-family, adherence and exposure–response study (NCT04358302) as previously described.[16] Patients, parents, and other stakeholders were involved as previously described.[16] Children were eligible if they were enrolled in the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry with a diagnosis of SLE and were already receiving HCQ as standard of care for ≥3 months. Additionally, we employed several enrichment criteria. Participants were preferentially offered enrolment if they (1) had not initiated or changed doses of any concomitant glucocorticoids at their most recent CARRA Registry visit, and (2) had not initiated or changed doses of any concomitant synthetic or biological disease-modifying antirheumatic drugs (ie, methotrexate, leflunomide, azathioprine, sulfasalazine, belimumab) at their most recent CARRA Registry visit.

As a direct-to-family trial, consent/assent and all study visits were conducted entirely remotely in the comfort of the participant's homes.

HCQ dosing

Participants were not prescribed HCQ as part of the study; all participants were already taking HCQ sulfate for ≥3 months as standard of care and were assumed to be at steady state. HCQ dosing information was captured two ways. First, all participants were provided with the electronic pill bottle cap and bottles to use with their HCQ prescription at the start of the study. The date and time were electronically captured every time the pill bottle was opened. Second, study personnel recorded the participant's HCQ dosage and reported dosing interval. Because all patients were required to be on HCQ for at least 3 months prior to their first study visit, we assumed the first electronic dosing record was the same dosage regimen used during the 3 months prior to the first study visit.

Sample collection and analysis

Biological samples were collected at up to four study visits per participant: at baseline, day 14 (±5), day 30 (±5) and day 180 (±30). At each visit, plasma was obtained to measure the concentrations of HCQ and DHCQ. Briefly, the plasma samples were collected in K2 EDTA tubes, centrifuged at approximately 2200 g for 10–15 min, and stored between −20°C and −70°C until the time of analysis. Inflammatory biomarkers of HCQ response were measured in serum and included interferon-alpha (IFN-α), IFN-gamma (IFN-ϒ), interleukin-1 beta (IL-1β), IL-6 and tumour necrosis factor-alpha (TNF-α). Each participant also had samples for *CYP2D6* genotype collected during the study.

Plasma samples for HCQ and DHCQ were analysed using selected reaction monitoring turbo iron spray liquid chromatography and mass spectrometry by Q^2 Solutions (Valencia, California, USA). The injection volume was 20 µL and the analytical flow rate was 500 µL/min using Acquity UPLC BEH C18 (2.1×50 mm, 1.7 µm). Peak areas were integrated by SCIEX (V.1.6.2) and a weighted (1/×2) linear regression was performed using Watson V.7.4.2 (Thermo Fisher Scientific). All concentration calculations were based on the peak area ratio (PAR) of HCQ and DHCQ to their internal standards. Concentrations of the analyte in quality control samples were determined by back-calculation from the calibration curve. The calibration range was 1 ng/mL (lower limit of quantitation (LLOQ)) to 500 ng/mL (upper limit of quantitation) for HCQ and DHCQ. Mean inter-run precision (coefficient of variation percentage (%CV)) was 0.6%–4.5% for HCQ and 1.9%–3.7% for DHCQ. Only one study sample was diluted for re-assay. Intrarun precision (%CV) was 0.6%–5.3% for HCQ and 0.8%–4.8% for DHCQ. Six samples were analysed outside of the long-term storage stability of 199 days, the longest of which was 219 days.

IFN-ϒ, IL-1β, IL-6 and TNF-α were analysed using V-PLEX Human Proinflammatory Panel according to the manufacturer's specifications and IFN-α was analysed using the Simoa Advantage Kit. Values below the quantifiable limit were imputed to be one-half of the LLOQ. Variants in the *CYP2D6* gene were detected by single nucleotide primer extension after PCR amplification. The *CYP2D6* variants of interest were classified into phenotypes using diplotype tables from the Pharmacogenomic Knowledge Base.[17]

Population PK analysis

HCQ and DHCQ plasma PK data were analysed with Phoenix NLME (Certara, Princeton, New Jersey, USA, V.8.4) using a population PK analysis approach.[18–20] Initially, the first order conditional estimation with extended least squares (FOCE-ELS) algorithm was used for model fitting. However, when parameter precision could not be estimated using FOCE-ELS, the Quasi-Random Parametric Expectation Maximization algorithm was used. Parameter precision was reported as the relative SE, calculated as 100×SE/parameter value.[21] Several structural models for HCQ (eg, one and two compartments) were explored assuming linear PK.[22] To estimate the residual error, additive, multiplicative and combined (additive plus multiplicative) error models were evaluated. Similar to prior work, the absorption rate constant (K_a) was fixed to 1.15/hour and the lag time of absorption (Tlag) was fixed to 0.39 hour.[22] Once the final base model was developed for HCQ, compartments to describe the PK disposition of DHCQ were added to the optimal model for parent drug. Based on prior published literature for chloroquine, we assumed a fixed fraction of formation clearance (0.18) from HCQ to DHCQ and used this to estimate DHCQ apparent volume (V/F) and apparent clearance (CL/F)[23] (online supplemental figure 1). During simultaneous fitting, parent and metabolite concentrations were converted to molar units, using a molecular weight of 335.872 g/mol for HCQ and 307.82 g/mol for DHCQ.

Between-subject variability (BSV) for PK parameters was estimated using an exponential relationship (equation 1).[18–20]

$$\mathrm{PAR_a} = \theta_{\mathrm{Pop}} \times \exp\left(\eta_{\mathrm{a}}\right) \tag{1}$$

where $\mathbf{PAR_a}$ is the parameter estimate for individual 'a'; $\boldsymbol{\theta_{Pop}}$ denotes the population parameter value; and $\boldsymbol{\eta_a}$ is the individual's variability from the average population parameter value with a mean of zero and variance ω^2. BSV was reported as % coefficient of variation (%CV [100*square root of variance]), with additional calculations noted in the supplemental materials.

Equation 2 denotes the calculation for shrinkage, where $\mathbf{SD}$ $(\boldsymbol{\eta_a})$ is the SD of the ath ETA across all number of subjects, and $\boldsymbol{\omega a,a}$ is the population variance of the random effect.

$$Shrinkage = 1 - \frac{SD(\eta_a)}{\sqrt{\omega a,a}} \tag{2}$$

Multiplicative, additive and combined (additive plus multiplicative) residual error models were explored as shown in equations 3–5, respectively.[18–20]

$$C_{obs,\ a} = C_{pred,a} \times \left(1 + \varepsilon_{prop,a}\right) \tag{3}$$

$$C_{obs,a} = C_{pred,a} + \varepsilon_{add,a} \tag{4}$$

$$C_{obs,a} = C_{pred,a} \times \left(1 + \varepsilon_{prop,a}\right) + \varepsilon_{add,a} \tag{5}$$

where $\mathbf{C_{obs,a}}$ denotes the observed concentration for individual 'a'; $\mathbf{C_{pred,a}}$ denotes the predicted concentration for individual 'a'; $\boldsymbol{\varepsilon_{prop,a}}$ and $\boldsymbol{\varepsilon_{add,a}}$ represent random effects with a mean of zero and a respective variance of $\sigma_{prop,a}^{2}$ and $\sigma_{add,a}^{2}$.

Covariates were tested for model inclusion based on several factors, including physiological relevance, evaluation of scatter plots for continuous variables and box plots for categorical variables that compared individual changes of a PK parameter (ETAs) from the population-typical value against covariates.[18–20] A standard forward inclusion-backward elimination approach was used to include covariates in the population PK model. To judge inclusion of a covariate in the PK model, we used a p value of 0.05 with 1 df for forward inclusion, and a p value of 0.01 with 1 df for the backward elimination step.

We did not assume a priori covariates on CL/F or V/F. However, in a sensitivity analysis, we evaluated the impact of including weight (WT) on CL/F and V/F for both HCQ and DHCQ using both fixed exponents (1 on V/F, 0.75 for CL/F) and by directly estimating exponents. WT was normalised to a 70 kg adult, otherwise covariates were normalised to the median value in the study population as shown in equation 6 (continuous covariates) and equation 7 (categorical covariates).[18–20]

$$PAR_a = \theta_{Pop} \times \left(\frac{cov_i}{cov_m}\right)^{\theta_{covariate}} \tag{6}$$

$$PAR_a = \theta_{Pop} \times^{\theta_{covariate} \times VARIABLE} \tag{7}$$

where $\mathbf{PAR_a}$ is the parameter estimate for individual 'a'; $\boldsymbol{\theta_{Pop}}$ denotes the typical population parameter value; $\mathbf{cov_i}$ is the individual covariate value, $\mathrm{cov_m}$ is the population median covariate value, $\theta_{\mathbf{covariate}}$ is the covariate effect, and **VARIABLE** is a categorical variable that is dichotomised to a value of zero or one for each level of a categorical predictor.

Lean body weight was calculated using the James formula: $(1.1\times WT)-128\times(WT^2/H^2)$ for males and $(1.07\times WT)-148\times(WT^2/H^2)$ for females, where WT is weight in kilograms (kg) and H is height in centimetres (cm).[24] Creatinine clearance was estimated using the modified Schwartz formula: 0.413×height/serum creatinine.[25] Body mass index (BMI) was reported as the percentage of the 95th percentile using the Centers for Disease Control classification (95% BMI).[26] For continuous covariates that were missing <10% of values, the missing values were imputed using the median study value. Missing values for categorical covariates were not imputed.

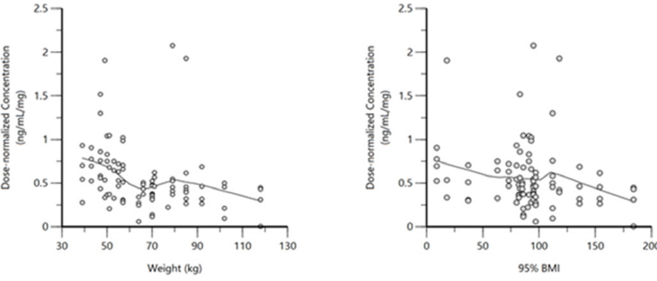
Population PK model evaluation and validation

During the PopPK model building process, we used standard model diagnostic methods to assess PK model performance, including: diagnostic plots, precision and plausibility of PK parameter estimates, successful minimisation, and Objective Function Value (OFV) and shrinkage values.[21] The following diagnostic plots were generated: observed data versus individual predictions (IPRED) and population predictions (PRED); conditional weighted

Table 1 Demographics and clinical characteristics

Clinical characteristics n=25 subjects	n (%) or median (range)
Age (years)	15 (9–17)
Female	21 (84)
Race	
White	6 (24)
Black	2 (8)
Asian	2 (8)
Other	14 (56)
Unknown	1 (4)
Ethnicity	
Hispanic	9 (36)
Not Hispanic	13 (52)
Unknown	3 (12)
Weight (kg)	66 (39–118)
Body mass index (kg/m^2) % of the 95th percentile	91 (9–184)
HCQ total daily dose (mg), per patient	300 (87.5–400)
CYP2D6 phenotype (genotype)	
Normal (*1/*1, *1/*17, *1/*41, *1/*9)	13 (52)
Intermediate (*1/*4, *1/*5, *5/*10)	9 (36)
Poor (*4/*5)	1 (4)
Missing	2 (8)

HCQ, hydroxychloroquine.



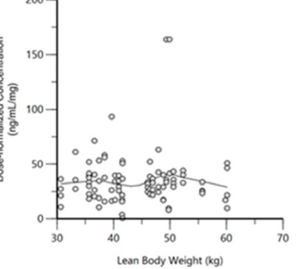


Figure 1 Relationship between dose-normalised hydroxychloroquine (HCQ) concentration and body size. Top left: dose-normalised HCQ concentrations (ng/mL/mg) versus weight (kg); top right: dose-normalised HCQ concentration versus the 95th percentile of body mass index; (95% BMI); bottom left: dose-normalised HCQ concentrations versus lean body weight (kg). The black line represents the locally estimated scatterplot smoothing fit.

residuals (CWRES) versus PRED and time; and CWRES versus IPRED.

We evaluated model performance by using the final population PK model to conduct Monte Carlo simulations and generate the 95% CIs for PK parameter estimates (100 replicates of non-parametric bootstrapping).[21] In addition, we conducted 1000 Monte Carlo simulation replicates and used proportional prediction-corrected visual predictive checks (pcVPCs) to compare observed versus simulated results.[21] We determined the Empirical Bayesian Estimates for V/F and CL/F from the final PK model.[21]

Exposure–response analysis

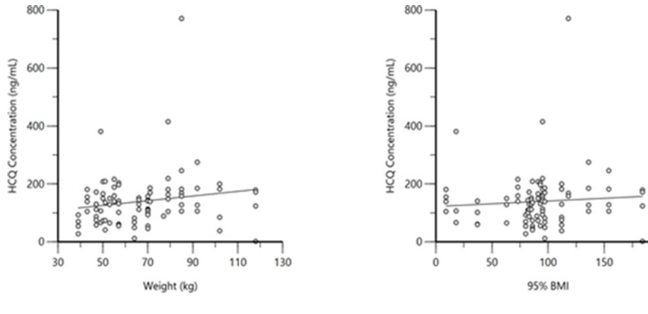
We graphically analysed the relationship between log HCQ and DHCQ concentrations and biomarkers of response, including IFN-α, IFN-ϒ, IL-1β, IL-6 and TNF-α. Due to the limited number of samples, a formal PK/PD model was not developed.

RESULTS

Twenty-six patients were recruited into the study; one subject dropped out before plasma samples were obtained and was excluded from the PK analysis. Accordingly, 25 patients provided a total of 88 plasma concentrations for HCQ and 88 concentrations for DHCQ (table 1). Patients were recruited across a total of 11 states in the USA. There were no concentrations below the quantifiable limit (BQL) for HCQ or DHCQ. Samples were obtained at a median (IQR) of 18.5 hours (8.31–22.6) after each dosage. The median (range) of concentrations for HCQ and DHCQ was 130 ng/mL (1.74–771) and 52.25 ng/mL (1.76–342), respectively. The median (range) molar ratio of HCQ to DHCQ was 1.99 (0.79–5.0) and there were heterogeneous fluctuations in the amount of HCQ relative to DHCQ (online supplemental figure 2) for each individual across the study. The ratio of HCQ to DHCQ was highest for the one patient who was a *CYP2D6* poor metaboliser (online supplemental figure 3).

HCQ concentrations and body size

Dose-normalised HCQ concentrations plotted against body size suggested that HCQ concentrations decreased as total body weight and BMI increased, but that relationship did not appear to hold for lean body weight (figure 1). Additionally, there was a very poor linear fit between HCQ concentrations and total body weight (R^2=0.03), the 95th percentile of BMI (R^2=0.005) and lean body weight (R^2=0.03) (figure 2). There was also a very poor linear fit comparing HCQ concentrations normalised by weight-based dosage and total body weight (R^2=0.02). Due to the very low within-day variation for HCQ, these plots did not account for time after dosage.[8 27]



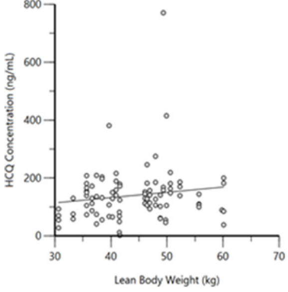
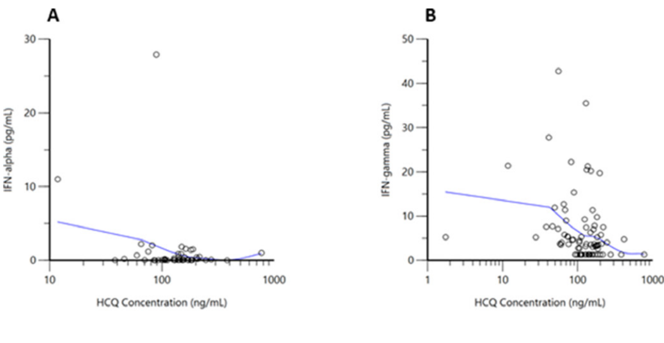


Figure 2 Relationship between hydroxychloroquine (HCQ) concentrations and body size. Top left: HCQ concentration (ng/mL) versus weight (kg); top right: HCQ concentration versus the 95th percentile of body mass index (95% BMI); bottom left: HCQ concentrations vs lean body weight (kg). The black line represents a linear fit.

Exposure–response

The relationship between HCQ and DHCQ and the inflammatory biomarkers of drug response are noted in figure 3 and online supplemental figure 4. Altogether, 80 of 87 concentrations for IL-1β were BQL, and therefore, IL-1β was not included in the exposure–response analysis. There was a decline in both IFN-α and IFN-ϒ with higher concentrations of both HCQ and DHCQ. Based on visual inspection of the exposure–response plots, the optimal HCQ concentration appeared to be ~200–250 ng/mL to reduce IFN-α and IFN-ϒ; however, there were few data points with HCQ concentrations at or above this target. Compared with HCQ, the relationship between DHCQ concentrations and each inflammatory cytokine was similar.



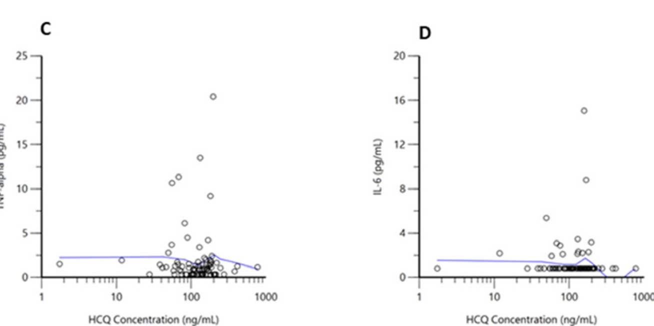


Figure 3 Relationship between inflammatory biomarkers and hydroxychloroquine (HCQ) concentrations: (A) interferon (IFN)-alpha; (B) IFN-gamma; (C) tumour necrosis factor (TNF)-alpha and (D) interleukin-6 (IL-6). The blue line represents a locally estimated scatterplot smoothing fit.

Population PK model development and evaluation

Base model development

As previously described, one and two-compartment base models using additive, multiplicative and combined additive plus multiplicative error models were explored. During base model development, no a priori covariates were included. A two-compartment base model with multiplicative (proportional) error had a slightly better overall fit and lowest −2 log-likelihood and Akaike Information Criterion (AIC) when compared with a one-compartment model. However, due to prior PK models for HCQ using a one-compartment model,[22 28 29] and to simplify the number of parameters when co-modelling parent and metabolite, we selected a one-compartment base model with multiplicative error. Additionally, model diagnostic plots (individual and population predictions vs observations, conditional weighted residuals vs time and predictions) were highly similar between the one-compartment and two-compartment models.

Initially, BSV was estimated on all parameters for the base 1 compartment model. Shrinkage was 24% for CL/F and 25% for V/F; therefore, the final base model for HCQ was a one-compartment base model with multiplicative error BSV for CL/F and V/F. Parameters for the final base model are noted in online supplemental table 1.

Metabolite base model development

The structural co-model included a one-way formation from HCQ to DHCQ using a fixed formation clearance ratio of 0.18 (see the Methods section), and estimation of DHCQ CL/F and V/F. Once DHCQ was added to the base model, the one-compartment model for the parent drug had optimal performance based on AIC and diagnostic plots compared with a two-compartment model with a distribution compartment for HCQ. Initially, BSV was estimated for all parameters; however, shrinkage was >40% on DHCQ V/F and was removed. The final parent-metabolite co-model was a one-compartment (for HCQ) model linked to a one-compartment metabolite compartment and BSV on HCQ CL/F and V/F, and DHCQ CL/F (online supplemental figure 1). Parameters for the final base co-model are noted in online supplemental table 2. In an exploratory analysis, we investigated a co-model where DHCQ V/F was fixed to that of HCQ V/F in order

to estimate DHCQ formation clearance; this model resulted in higher residual error and was not retained.

Covariate model development

Based on ETA versus covariate plots and physiological plausibility, we evaluated the following potential covariates for HCQ CL/F: creatinine clearance, total body weight (WT), concomitant medications belimumab, methotrexate, azathioprine and mycophenolate mofetil. WT was also evaluated as a covariate for DHCQ CL/F. The poor metaboliser phenotype also appeared to have a relationship with ETA CL/F for HCQ and DHCQ, but since there was only one patient with this phenotype, it was not evaluated for model inclusion.

During forward inclusion, no covariates were significant at a p value of 0.05. Based on prior publications that included WT as a covariate on HCQ CL/F[29] or desethylchloroquine CL/F,[23] we investigated a model that included WT/70 as a covariate for both HCQ and DHCQ CL/F. Directly estimating the exponential effect of WT/70 had better model performance, compared with using fixed exponential effects. After including WT/70, the BSV decreased from 37.6% to 34.8% for HCQ CL/F and from 57.3% to 47.9% DHCQ CL/F. There was also a reduction in AIC by 5.1 points and reduction in the −2 log-likelihood by 9.1 points. Accordingly, we retained WT/70 as a covariate on HCQ and DHCQ CL/F for the final model, despite WT/70 not being significant during forward inclusion and backward elimination.

Final PK model

The final PK model was a one-compartment model for HCQ linked to a metabolite compartment for DHCQ with BSV on HCQ CL/F, HCQ V/F and DHCQ CL/F; and WT/70 as exponential covariates on HCQ CL/F and DHCQ CL/F. Shrinkage estimates were ≤25% for all random effect parameters. Parameter estimates from the final population model are listed in table 2, and diagnostic plots are noted in online supplemental figures 5 and 6 and the pcVPC is noted in online supplemental figure 7.

The equations for the final PK model were:

$$Ka = tvKa \tag{8}$$

$$Tlag = tvTlag \tag{9}$$

$$\frac{V}{F}\left(HCQ\right) = \frac{tvV}{F} \times \exp\left(\eta_{\frac{V}{F}}\right) \tag{10}$$

$$\frac{V}{F}\left(DHCQ\right) = \frac{tvV}{F} \tag{11}$$

$$\frac{CL}{F}\left(HCQ\right) = \frac{tvCL}{F} \times \frac{WT_{individual}}{70}^{dCldWT} \times \exp\left(\eta_{\frac{CL}{F}}\right) \tag{12}$$

$$\frac{CL}{F}\left(DHCQ\right) = \frac{tvCL}{F} \times \frac{WT_{individual}}{70}^{dCldWT} \times \exp\left(\eta_{\frac{CL}{F}}\right) \tag{13}$$

where $\mathbf{K_a}$ is the absorption rate constant, **tv** is the typical population value of a parameter, **Tlag** is the lag time after oral administration, **η** is the deviation from the average population PK parameter value, **V/F** is apparent volume of distribution, **CL/F** is the apparent oral clearance, **WT** is the total body weight, and **dCldWT** is the exponential, allometric scaling coefficient of WT on **CL/F**.[21]

DISCUSSION

We conducted the first direct-to-family clinical trial in pSLE, quickly and efficiently enrolling 26 children

Table 2 Final PK model parameter estimates

Parameter	Estimate	RSE (%)	2.5th percentile	Bootstrap median	97.5th percentile
K_a (1/hour)	1.15 (fixed)	—	—	—	—
Tlag (hour)	0.39 (fixed)	—	—	—	—
CL/F (L/hour/70 kg)	60.6	20.7	29.3	61.5	73.9
V/F (L)	73 540	31.1	13 586	75 615	141 017
V/F_{DHCQ} (L)	6788	72.9	0.2	5850	14 902
CL/F_{DHCQ} (L/hour/70 kg)	22.2	19.5	9.9	22.2	29.5
Exponential scaling of weight/70 on HCQ CL/F	0.59	85.5	−1.1	0.6	1.2
Exponential scaling of weight/70 on DHCQ CL/F	1.20	45.4	−0.6	1.1	2.0
Between subject variability (%CV)					
CL/F	34.8	5.8	—	—	—
V/F	81.5	32.4	—	—	—
CL/F_{DHCQ}	47.9	9.6	—	—	—
Residual error					
Proportional error, HCQ (%)	39.3	22.2	0.3	0.4	0.5
Proportional error, DHCQ (%)	45.7	20.1	0.4	0.4	0.5

CL/F, apparent oral clearance; CV, coefficient of variation; DHCQ, desethylhydroxychloroquine; HCQ, hydroxychloroquine; K_a, absorption rate constant; PK, pharmacokinetics; RSE, relative SE; Tlag, lag time after oral administration; V/F, apparent volume of distribution.

from across the USA in only 10 days. We also developed the first population PK model for HCQ and DHCQ in children, which can be used to conduct simulations to obtain target drug concentrations and optimise dosing. Enrolment success was likely due in part to the family-centred design that minimised patient and parent burden and centred study visits around their schedule, as well as partnerships with key stakeholders including the CARRA Registry.[16] As a result, almost half of the patients contacted about the study wanted to participate. The enrolment success underscores the potential for direct-to-family trials as a novel PK study design in children with rare diseases. Due to the inherently low number of patients with rare diseases, the reduced patient burden could result in higher trial recruitment and retention rates.

HCQ is prescribed to children with SLE using a weight-based dosing of 5 mg/kg total body weight, not to exceed 400 mg/day. We found that there was poor correlation between HCQ concentrations and weight-based dosage, suggesting total body weight is not a good predictor of HCQ concentrations. Moreover, after accounting for dosage, we found that dose-adjusted HCQ concentrations declined as a function of increasing total body weight and BMI, but not with lean body weight. Accordingly, our results suggest that the current weight-based dosing paradigm for HCQ may result in comparatively lower drug exposures for patients with obesity. Since up to one in four children with SLE experience obesity,[30] it is possible that dosing based on 5 mg/kg of total body weight with a dosage cap may result in suboptimal HCQ exposure for a large number of children. Despite this observation, there were no other measures of body size that strongly correlated with HCQ exposure, suggesting therapeutic drug monitoring may be the optimal approach to individualise dosing.

We estimated steady-state PK parameters for HCQ CL/F, V/F and DHCQ CL/F with overall good precision, despite using a plasma matrix, although estimates were limited by the small sample size of this pilot trial. However, there was less precision surrounding DHCQ V/F and covariate parameter estimates. Although PK parameters for HCQ vary widely in adults,[29 31 32] we found that V/F for HCQ in plasma was higher in children compared with published values in adults (73 000 L vs 44 000 L), but with similar estimates of CL/F. The reason for potentially higher V/F could be due to different study populations, or limitations in the study design as discussed below. Additionally, although ETA versus covariate plots suggested several potential covariate effects, none reached statistical significance. Based on the PK and physiochemical properties of HCQ such as lipophilicity and renal excretion,[4] we expected to see relationships between WT on V/F and *CYP2D6* phenotype or creatinine clearance on CL/F, suggesting our study was likely underpowered to test for significant covariate relationships. Accordingly, larger studies in children may be needed to identify the covariates responsible for the high variability observed in HCQ PK.[32]

Regarding exposure–response, we noticed a visual relationship with higher HCQ and DHCQ concentrations and lower IFN-α and IFN-ϒ concentrations, although this relationship was influenced by a small number of data points. Because we did not have access to samples prior to HCQ treatment, we could not assess the impact of HCQ pretreatment and post-treatment on plasma cytokines. Nevertheless, SLE is an IFN-driven disease, and compared with healthy controls, patients with SLE have higher circulating serum IFN-α and IFN-ϒ, higher IFN gene expression, and correlation between IFN-α and IFN-ϒ expression and SLE disease activity scores.[33 34] Moreover, HCQ is known to decrease type I IFN expression.[35] Accordingly, the observed trend in decreasing IFN-α and IFN-ϒ concentrations with increasing HCQ and DHQ concentrations is consistent with the known pathogenesis of SLE and mechanisms by which HCQ may improve SLE disease activity.

To our knowledge, we are the first to report the PK of DHCQ in children with SLE. In general, we found that HCQ concentrations are approximately twice those of DHCQ. Moreover, there were changes in the ratio of HCQ:DHCQ across time for most individuals, suggesting that metabolism may be influenced by concomitant medications or other environmental factors that cause CYP induction or inhibition that may change over time.[36] As expected, we observed the ratio of HCQ to DHCQ was higher in the one patient who was a *CYP2D6* poor metaboliser. We also found that the relationship between inflammatory cytokines and DHCQ concentrations was similar to HCQ, supporting reports that DHCQ is an active metabolite. Nevertheless, due to collinearity between HCQ and DHCQ concentrations, DHCQ would need to be directly administered to patients apart from HCQ to fully understand its PD effects.

There are several limitations to our analysis. First, there were several technological difficulties with the electronic pill bottle cap, including erroneous alarms and rarely, devices that failed to record all openings. Accordingly, there was a small number of either duplicative dosing records (requiring grouping of multiple events that occurred within a short period), missing dosing records or imprecise timing of administration, all of which may have increased residual error. Despite this limitation, due to the rich dosing records, sparse sampling and long half-life of HCQ, dosing inaccuracies are unlikely to have significantly influenced the results unless they occurred very close to a sampling time. Nevertheless, given the imprecision of V/F and some of the estimates for the exponential effect of weight crossing zero during the bootstrap analysis, it is likely that dosing imprecision or the small sample size contributed to high variability in the model fit between subjects. Second, we used a plasma matrix to measure HCQ and DHCQ concentrations,

which is known to have higher imprecision compared with whole blood (%CV 39 vs 3),[37] possibly due to the lysing of red blood cells that may occur during centrifugation or sample handling. Nevertheless, plasma HCQ samples have been used to develop other published PK models,[29 31] and has been used by the Food and Drug Administration (FDA) to support the drug labelling for chloroquine; underscoring that the use of plasma samples does not preclude its use for PK modelling. Additionally, although our assay had good accuracy and precision, six samples were analysed outside of the tested stability window.

There are also important limitations to the metabolite analysis. There is a lack of quantitative information in the literature regarding HCQ metabolism, the precise hepatic enzymes, the fraction of the dose biotransformed to each metabolite and hepatic clearance estimates are often extrapolated from data for chloroquine. Accordingly, we used the available data for chloroquine biotransformation to desethylchloroquine to fix the fraction of HCQ clearance to DHCQ and estimate DHCQ CL/F and V/F. Despite this potential limitation, we expect drug disposition to be similar because HCQ is the hydroxylated analogue of chloroquine. Additionally, we were unable to measure other HCQ metabolites, including bidesethylhydroxychloroquine and desethylchloroquine; therefore, HCQ clearance in our model represents both renal and non-renal (eg, hepatic) clearance pathways. To optimise PK model development, future studies could measure concentrations of all HCQ metabolites in both whole blood and urine to better characterise drug disposition, or directly administer the metabolites to estimate PK parameters.

CONCLUSIONS

In summary, we report the first population PK model and parameter estimates for HCQ and DHCQ in children using data from a novel direct-to-family clinical trial. We observed that the data needed for PK studies, including biological samples, dosing information and clinical covariates, can be obtained entirely outside of a brick-and-mortar research facility by leveraging a disease registry. Additionally, this pilot study confirmed the high inter-individual variability in HCQ PK and that weight-based dosing for HCQ correlates poorly with drug concentrations, indicating the need for alternative dosing strategies such as therapeutic drug monitoring. Furthermore, our results suggest that the current weight-based dosing paradigm for HCQ may result in suboptimal drug exposures, particularly for children with obesity. Accordingly, additional studies of HCQ are needed in pSLE to determine the optimal drug concentration and dosing to reduce disease activity and improve outcomes.

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