Population Pharmacokinetic Estimates Suggest Elevated Clearance and Distribution Volume of Desethylamodiaquine in Pediatric Patients with Sickle Cell Disease Treated with Artesunate-Amodiaquine

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A B S T R A C T

Background: There is limited information on the safety or efficacy of currently recommended antimalarial drugs in patients with sickle cell disease (SCD), a population predisposed to worse outcomes if affected by acute malaria. Artesunate-amodiaquine (ASAQ) is the first-line treatment for uncomplicated malaria (UM) in many malaria-endemic countries and is also used for treatment of UM in SCD patients. There is, however, no information to date, on the pharmacokinetics (PK) of amodiaquine or artesunate or the metabolites of these drugs in SCD patients.

Objectives: This study sought to determine the PK of desethylamodiaquine (DEAQ), the main active metabolite of amodiaquine, among paediatric SCD patients with UM treated with artesunate-amodiaquine (ASAQ).

Methods: Plasma concentration-time data (median DEAQ levels) of SCD children (n = 16) was initially compared with those of concurrently recruited non-SCD paediatric patients with acute UM (n = 13). A population PK modelling approach was then used to analyze plasma DEAQ concentrations obtained between 64 and 169 hours after oral administration of ASAQ in paediatric SCD patients with acute UM (n = 16). To improve PK modeling, DEAQ concentration-time data (n = 21) from SCD was merged with DEAQ concentration-time data (n = 169) of a historical paediatric population treated with ASAQ (n = 103) from the same study setting.

Results: The median DEAQ concentrations on days 3 and 7 were comparatively lower in the SCD patients compared to the non-SCD patients. A two-compartment model best described the plasma DEAQ concentration-time data of the merged data (current SCD data and historical data). The estimated population clearance of DEAQ was higher in the SCD patients (67 L/h, 21% relative standard error (RSE) compared with the non-SCD population (15.5 L/h, 32% RSE). The central volume of distribution was larger in the SCD patients compared with the non-SCD patients (4400 L, 43% RSE vs. 368 L, 34% RSE).

Conclusions: The data shows a tendency towards lower DEAQ concentration in SCD patients and the exploratory population PK estimates suggest altered DEAQ disposition in SCD patients with acute UM. These findings, which if confirmed, may reflect pathophysiological changes associated with SCD on DEAQ dispo-

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sition, have implications for therapeutic response to amodiaquine in SCD patients. The limited number of recruited SCD patients and sparse sampling approach however, limits extrapolation of the data, and calls for further studies in a larger population.

Introduction

Sickle cell disease (SCD) is a common autosomal recessive genetic disorder caused by a single nucleotide substitution (T>A) in the sixth codon of the β-globin gene. The single point mutation resulting in a Glu to Val substitution promotes polymerization and precipitation of haemoglobin S (HbS) during deoxygenation or dehydration, resulting in sickling of red blood cells. The associated pathophysiological changes result in vaso-occlusive crises and other clinical manifestations of SCD, and ultimately, organ damage.[1] The highest frequencies of homozygous SCD are found in sub-Saharan Africa, and although a linkage exists between the presence of sickle haemoglobin (HbS) and protection from malaria in the heterozygous state,[2,3] malaria is a frequent cause of hospitalization and poor outcome among children with homozygous SCD in endemic areas[4,5] and malaria is associated with a higher mortality in hospitalized SCD patients.[6–8] These imply the need to ensure efficacious antimalarial therapy in SCD patients who develop acute malaria but information on the efficacy or safety of recommended antimalarial drugs in SCD patients is limited or non-existent.

Amodiaquine (AQ) is currently used in combination with artesunate (AS) as first-line treatment for uncomplicated *falciparum* malaria (UM) in many countries. AQ is a 4-aminoquinoline metabolized by hepatic cytochrome P450 2C8 (CYP2C8) to desethylamodiaquine (DEAQ) its active metabolite, which is subsequently eliminated via extra-hepatic biotransformation by CYP1A1 and CYP1B1.[9] DEAQ is primarily responsible for the pharmacological activity of AQ (parent drug), due to its long elimination half-life.[10] The pharmacokinetics (PK) of AQ when used in combination with artesunate (AS), has been described in healthy volunteers,[11,12] children,[13–15] and adults,[16] with UM. Estimates of DEAQ clearance have been reported to range between 6–17 L/h,[13,14] and distribution volume between 350–500 L,[13,14] in non-SCD children. There is the possibility that the disposition of AQ could be altered in patients with SCD given anticipated pathophysiological changes in SCD patients. It is known that both white blood cells and platelets accumulate large amounts of chloroquine,[17] therefore, excessive thrombocytosis or leukocytosis, both of which are features of SCD, could alter the distribution of this 4-aminoquinoline in blood. Indeed, plasma concentrations of chloroquine and desethylchloroquine, its major metabolite have been shown to differ between SCD and non-SCD erythrocytes *in vivo and in vitro*.[18] There are no reports on the PK of AQ or DEAQ in SCD patients. In this study, the PK of DEAQ in paediatric SCD patients with acute UM who participated in a clinical trial and received treatment with ASAQ is described.

Methods

Study site

The study was a sub-component of a clinical trial (IS-RCTN96891086) that was conducted to evaluate the efficacy and safety of ASAQ and artemether-lumefantrine (AR-L) among SCD and non-SCD children with acute UM. The study was conducted at the Outpatients Department (OPD) and Paediatric Sickle Cell Clinic of the Department of Child Health, Korle Bu Teaching Hospital,

Accra, Ghana, between January 2010 and December 2011. The full details of the clinical trial have been previously reported.[19] Briefly, children of known SCD status registered at the Paediatric Sickle Cell Clinic who presented to the Paediatric Emergency Department or the Paediatric Sickle Cell Clinic with an acute febrile illness confirmed parasitologically to be UM were recruited, if they fulfilled study criteria and if written informed consent was obtained from an accompanying parent or guardian. Recruited children were treated with ASAQ or AR-L and followed up on days 1, 2, 3, 7, 14, 28, 35, and 42, according to a pre-defined schedule. Ethical approval was obtained from the Ethical and Protocol Review Committee, University of Ghana Medical School. Inclusion criteria for the study were, child of confirmed SCD status aged six months to 12 years with an acute febrile illness (history of fever within the previous 72 hours, or an axillary temperature $\geq$37.5°C at presentation; *Plasmodium falciparum* infection of parasite density <200,000/μL; and, willingness of the accompanying parent/guardian to comply with the study procedures and follow-up schedule). Exclusion criteria were, symptoms or signs of severe malaria; known intolerance or allergy to study medications; and, reported treatment with any of the study drugs one-month preceding enrolment.

Drug dosing

ASAQ (Coarsucam; Sanofi Aventis, France; 25/50/100 mg artesunate and 67.5/135/270 mg amodiaquine) single daily dose, was administered for three days according to body weight: $\geq$4.5 - <9 kg (25 mg/67.7 mg), one tablet/dose; $\geq$9 - <18 kg (50 mg/135 mg), one tablet/dose; $\geq$18 - <36 kg (100 mg/270 mg), one tablet/dose; $\geq$36 kg (100 mg/270 mg) two tablets/dose.

Blood sampling

A venous blood sample (2–5 ml) was collected into heparinized polypropylene tubes before treatment and on days 3 or 7 in children who were haemodynamically stable and/or in whom a blood sample was required for laboratory analysis for clinical management. The heparinized blood was immediately centrifuged, and plasma was transferred into polypropylene tubes and stored at −20°C until analysis. The exact time of drug administration was recorded for each participant on days 0, 1, and 2, and the exact time of blood sampling was recorded on days 3 or 7.

High Performance Liquid Chromatographic (HPLC)

The concentrations of DEAQ were measured in plasma by means of a reverse-phase HPLC method. Heparinized plasma samples (100 µl) were pipetted into 10 ml glass tubes and 90 µl milli-Q water, 200 µl sodium carbonate buffer (0.2 M, pH 9.5) and 2000 µl tert-butylmethylether were added. After reciprocal shaking for 20 min. and centrifugation (3000 rpm., 10 min.) the organic phase was transferred to new tubes and extracted for 20 min. with 120 µl of phosphate buffer (0.1 M, pH 4). After separation, the organic phase was discarded, and the aqueous phase was transferred into polypropylene HPLC vials. Aliquots of 90 µl were injected into

the chromatograph. The HPLC system consisted of an 1100 Quaternary pump with 1100 Degasser and an 1100 Autosampler (Agilent Technologies (Palo Alto, CA, USA) with a Zorbax SB-CN column, 5μm (4.6 × 250 mm) (Agilent), protected by a LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 μm) guard column (Merck, Darmstadt, Germany). The mobile phase was acetonitrile-phosphate buffer (0.1 M, pH 2.3)-sodium perchlorate (1 M) (14:85:1) operating at a flow rate of 1.2 ml/min. The wavelength was set at 237 nm (1100 Diode array detector, Agilent Technologies). The within-day, intra-assay variation was determined by replicate analysis of samples spiked with different concentrations. The between-day, inter-assay variation was performed by two determinations at each concentration on different days. Five calibrators were run in duplicate each day of analysis. Accuracy was determined by replicate analysis of spiked plasma samples. The recovery in plasma at three different concentrations were determined by comparing peak areas obtained after extraction of spiked human plasma with those of directly injected standards dissolved in the mobile phase. The limit of quantitation (100 μl) was 10 ng/ml, and coefficient of variation (CV) was <10%.

PK model building

The total AQ dose, number of administered (AQ) doses, sampling and dosing times, plasma DEAQ concentrations, and demographic information was available. The sampling time was calculated relative to the time of first drug administration, and drug concentrations below LOQ (3 time points) were excluded.

A nonlinear mixed-effects modeling approach was applied to estimate the PK parameters of DEAQ, using NONMEM (Version 7.3, ICON Development Solutions, Ireland), with Pirana® software (Version 2.8.2, Pirana Software and Consulting BV) and the First Order Conditional Estimation with Interaction (FOCEI) algorithm for the model building. Graphical analysis of model outputs and covariate analysis were explored, using R Software® (Version 3.0.2), and R Package Xpose® (Uppsala University, Sweden).

Due to a high eta-shrinkage and poor goodness of fit plots (due to the sparse data and limited sample size) from the 16 sickle cell disease (SCD) children (21 concentration-time points), and 13 non-sickle cell patients (14 concentrations-time points), the data from the SCD patients was merged with a previous dataset that described the PK of DEAQ in 103 non-SCD children (169 measurable DEAQ concentration-time points) with UM in the same study setting,[13] giving a total of 190 concentration time points. Of these 103 children, 15 (median age = 5.3 years; median body weight = 18 kg) received AQ only, whilst 88 (median age = 6.0 years; median body weight = 18 kg) received AS plus AQ. After merging the data, SCD was included in the model as categorical covariate.

For the structural PK model, one- and two-compartmental models with first order absorption were fitted to the plasma DEAQ concentration-time data. The two-compartment model appeared to describe data better, based on the objective function. Due to the lack of data on the absorptive phase of AQ disposition, the absorption rate constant (K_a) and lag time (T_{lag}) were fixed at 0.867 h^{-1} and 0.84 h, respectively, as described previously.[13] Furthermore, the fixed Ka was viewed as a hybrid parameter that includes the Ka of AQ, the volume of distribution (V_1) of AQ, and the formation clearance (CL1/f) of DEAQ, as described previously.[13] Therefore, the two-compartment model was parametrized as clearance (CL/f), central volume of distribution (V2/f), intercompartmental clearance (Q/f), and peripheral volume of distribution (V3/f). Between patient variability (BPV) was modeled exponentially assuming a log normal distribution. BPV was estimated assuming parameters had a mean of zero, and a variance of omega squared (ω^2). Additive, proportional and combined error models were

Table 1

Baseline demographic characteristics and plasma levels of desethylamodiaquine (DEAQ) on days 3 and 7 after administration of first amodiaquine dose among recruited children with sickle cell disease (SCD) and without SCD.

Parameter	SCD (n = 16)	No SCD (n = 13)	P value
	n (%)		
Male sex	6 (37.5)	7 (53.8)	
	median (range)		
Age (y)	9 (1.9–13.8)	6 (2–11)	0.15
Parasite density (/μL)	652 (48–63,360)	24240 (160–148,160)	0.001
Haemoglobin (g/dL)	6.3 (3.6–12.8)	10 (5.8–12.2)	0.004
White blood cells (10^9/L)	12.8 (6–73.4)	9.3 (6.1–19.3)	0.14
Platelet count (10^9/L)	201 (87–813)	152 (41–283)	0.03
Day 3 DEAQ (ng/mL)	72.0 (20–169.3)	117.6 (36–317)	0.27
Day 7 DEAQ (ng/mL)	19.0 (14–70)	32.2 (14.4–51.5)	0.12

tested in the model building process, and residual error was assumed to have a mean of zero and variance of sigma squared (σ^2). The effect of age, body weight and sex were explored as covariates on the PK parameters. These covariates were retained in the model if they improved fit (backward elimination and forward inclusion). Allometric scaling of all distribution parameters were done as reported by Holford.[20] Models were compared by the likelihood ratio test, represented in NONMEM as a difference in objective function value (ΔOFV) of 3.84, and corresponding to a level of significance of <0.05, and by using a decrease in the variance of random effects, eta-shrinkage and goodness of fit plots [observed (DV) vs. individual-predicted (IPRED) and population predicted (PRED), as well as conditional weighted residuals (CWRES) vs. PRED].

The model that best described the data was evaluated by means of visual predictive check (VPC).

Statistical analysis

Entire pharmacokinetic modeling process (software used, description of structural and statistical models, model comparison and evaluation) have been described earlier. Continuous variables were summarized as medians with accompanying ranges, and group medians compared with Wilcoxon signed-rank test. A p value <0.05 was considered statistically significant.

Results

Characteristics of patients and plasma DEAQ concentrations on days 3 and 7

All recruited participants demonstrated an adequate clinical and parasitological response. The median age of the SCD patients was higher, and admission parasitaemia was lower, compared to the non-SCD group. There was a total of 35 measurable plasma DEAQ concentrations above LOQ from 16 SCD (n = 21), and 13 non-SCD (n = 14) patients obtained between 64.3 and 169.2 hours. There were comparatively lower (though statistically non-significant) DEAQ concentrations amongst the SCD children compared to the non-SCD children on Days 3 (p = 0.27) and 7 (p = 0.12). The baseline demographic characteristics and median DEAQ concentrations of SCD and non-SCD children on day 3 (SCD, n = 10: non-SCD, n = 8) and day 7 (SCD, n = 11: non-SCD, n = 6) is shown (Table 1).

Population PK modelling of DEAQ plasma concentrations of merged data [SCD (n = 16) and non-SCD (n = 103) children]

The DEAQ plasma concentration from the merged data of SCD children (21 concentration-time points) and non-SCD (169
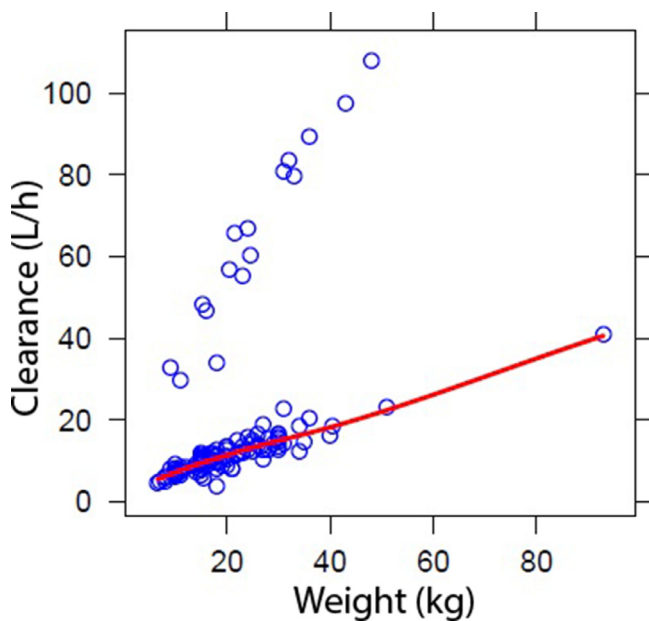
Figure 1. Clearance derived from the base model of merged data versus body weight. Red line shows line of best fit (or line of correlation)

concentration-time points) showed that a two-compartment best described the base model. The PK parameter versus covariate plot of the merged data showed that body weight would explain some of the variability in DEAQ clearance, as shown in Figure 1. Parametrization of the two-compartment model and application of allometric scaling yielded a much better fit, and diagnostic plots of the best model that described data are shown (Figures 2A and B). This model had a variance in BPV of 32.6% on CL (26% shrinkage) and 49.6% on V_2 (67% shrinkage). A set of simulated datasets generated, using the best model was compared to real observations, i.e., VPC, which showed that the sparse (plasma DEAQ) observations limited the creation of an appropriately robust model for the current dataset. The VPC of the historical dataset (Figure 3A), and the DEAQ data of the children with SCD (Figure 3B), are shown.

The parameter covariate plots of CL and central volume of distribution (V_2) of DEAQ among non-SCD and SCD children of the merged dataset, with inclusion of SCD as a categorical covariate in the model, are shown (Figures 4A and B), respectively. The population clearance and volume of distribution of DEAQ in SCD and non-SCD children is summarized in Table 2. Population CL was found to be approximately 5-fold greater in the SCD children compared to the non-SCD children. The population V_2 of DEAQ for the children with SCD children was more than 10-fold that of the non-SCD children.

Discussion

In this study, we used a population PK modelling approach to estimate selected primary PK parameters of DEAQ in a limited sample of SCD children versus non-SCD children treated with ASAQ for acute uncomplicated *falciparum* malaria. We are not aware of any previous report on the PK of antimalarial drugs in SCD patients with acute malaria. The data is consistent with previous studies that have showed wide inter-individual variation in DEAQ concentrations.[21] The relatively low day 3 and day 7 plasma DEAQ concentrations in the SCD patients are comparable to similarly low plasma DEAQ concentrations demonstrated in HIV-infected children treated with ASAQ,[22] but almost two-fold lower on average, than plasma DEAQ concentrations of non-SCD children.[13–15,23]

The low day 3 DEAQ concentrations in the SCD patients could be due to excessive thrombosis and leukocytosis in SCD patients,[19] and to associated pathophysiological changes in SCD including hyposthenuria and hyperfiltration,[24,25] which could be acting in concert with increased hepatic and renal blood flow from increased cardiac output or chronic haemolytic anaemia, to accelerate solute (drug) clearance.[26–28] Impaired hepatic and renal blood flow has been linked to elevated morphine clearance in SCD patients,[29] and to impaired lidocaine metabolism.[30] The large differences in clearance and volume of distribution is also consistent with the results of several studies that have shown differences in PK parameters between SCD and non-SCD populations for various drugs: the clearance of ciprofloxacin is reported to be 89% higher in SCD patients compared to non-SCD patients;[31] cefotaxime clearance has been shown to be higher in SCD patients, and higher still in SCD patients with acute chest syndrome;[32] and morphine clearance is reported to be 3-10 fold higher in SCD patients compared to published estimates in the non-SCD population.[29] The chronic inflammation in SCD may also induce drug metabolism, as demonstrated for codeine in SCD patients.[33] It is also plausible that other commonly occurring changes in SCD patients including malnutrition,[34,35] a condition reported in up to 63% of SCD children in our study setting,[36] and which is associated among others, with increase in total body water and hypoalbuminaemia, increases the unbound drug concentration and thus, alter the kinetics of drug disposition. However, due to the physicochemical characteristics of amodiaquine, the overall pharmacodynamic effect(s) of these PK parameter changes in SCD patients, on the therapeutic effect of amodiaquine, given the interplay of multiple factors, is difficult to anticipate.

The limitation of the study is the limited sample and sparse dataset and resulting high shrinkage on the PK parameter estimates. These places limitations on the robustness of the model and on interpretation or extrapolation of the findings. These limitations notwithstanding, we consider the data and findings to be potentially important because there is lack of data on the pharmacokinetics of amodiaquine, a widely used antimalarial drug, in SCD and non-SCD patients. Given the higher malaria-associated mortality in SCD, difficulty in recruiting sufficient number of parasitologically-

Table 2
Estimated population pharmacokinetic parameters of desethylamodiaquine in patients with pediatric sickle cell disease (n = 16) and without pediatric sickle cell disease (n = 103).

| Sickle cell disease (n = 16)				No sickle cell disease (n = 103)			
Parameter	Unit	Estimate	Relative SE (%)	Parameter	Unit	Estimate	Relative SE (%)
Absorption rate constant	h^{-1}	0.867 (fixed)		Absorption rate constant	h^{-1}	0.867 (fixed)	
Lag time	h	0.84 (fixed)		Lag time	h	0.84 (fixed)	
Clearance	L/h	67	21	Clearance	L/h	15.5	32
Central volume of distribution	L	4400	43	Central volume of distribution	L	368	34
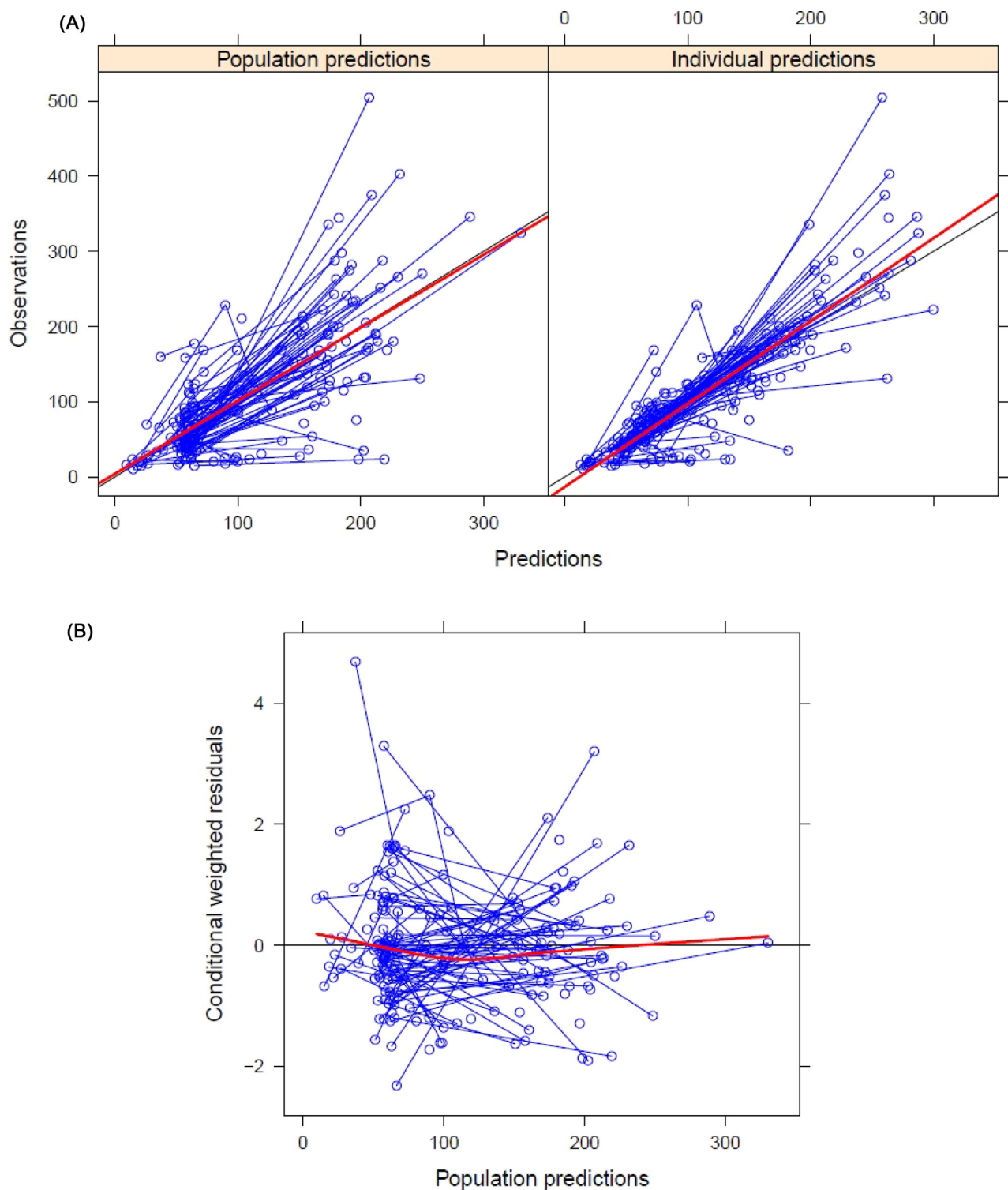
Figure 2. A: Merged observed dataset versus population and individual predictions from the most appropriate model. *Red line shows how best the final model is able to predict individual and population concentrations.* **B:** Conditional weighted residuals versus population predictions plot of merged dataset

confirmed malaria patients among SCD patients, the difficulties of taking multiple blood samples from such a chronic haemolytic anaemia prone paediatric SCD patients and the ethical implications of multiple blood sampling in such a vulnerable population, the findings from this limited dataset, obtained during routine clinical procedures, provides important preliminary information in a population in which such information is lacking and in which such a dataset is difficult to obtain.

In summary, we have employed a population modelling approach (merging sparse SCD data with historical data) to estimate primary PK parameters of DEAQ in paediatric SCD patients, a population in which such data is lacking. The findings suggest altered DEAQ disposition in SCD patients which calls for further studies and raise questions on potential therapeutic effects of these changes on amodiaquine use in SCD patients.
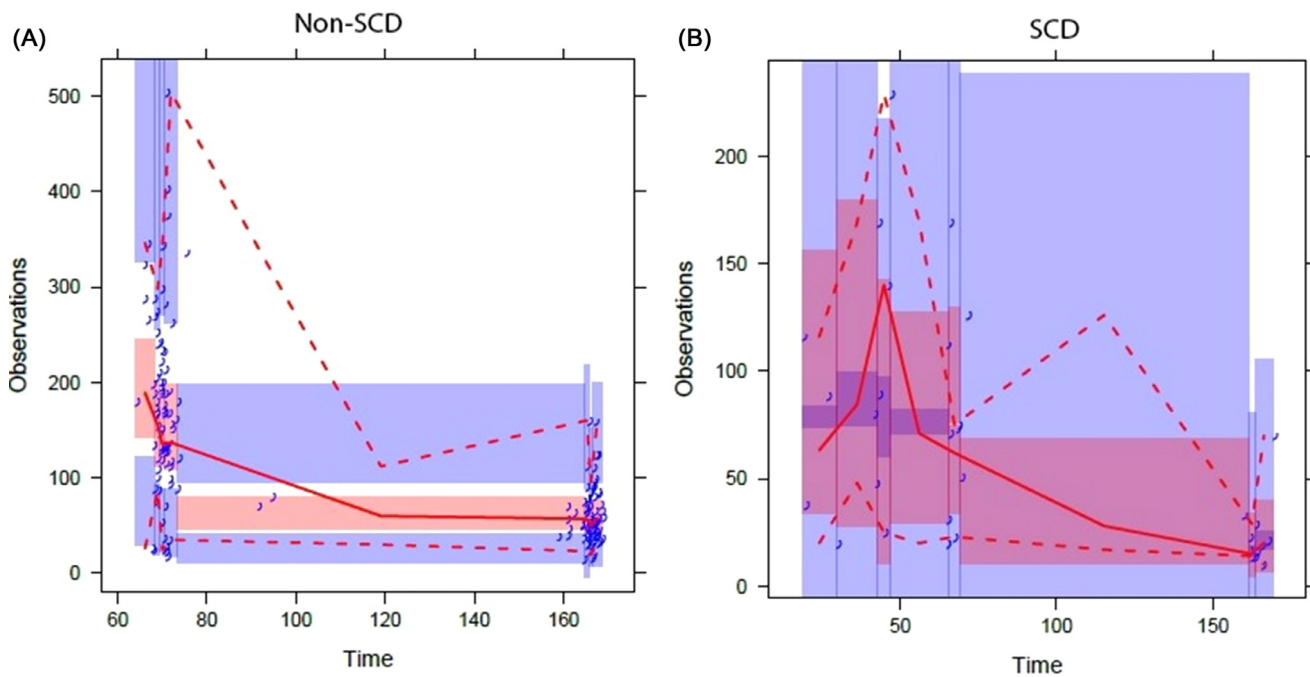
Figure 3. A: Visual Predictive Check (VPC) of DEAQ observations (ng/ml) versus time (h) of non-SCD (n = 103). **B:** Visual Predictive Check (VPC) of DEAQ observations (ng/ml) versus time (h) of SCD (n = 16). Blue half circles are individual observations, solid and doted red lines – observation percentile intervals (upper 5%, lower 5% and a median), the blue and orange areas are 95% confidence intervals around each of the prediction percentile intervals obtained by simulation.
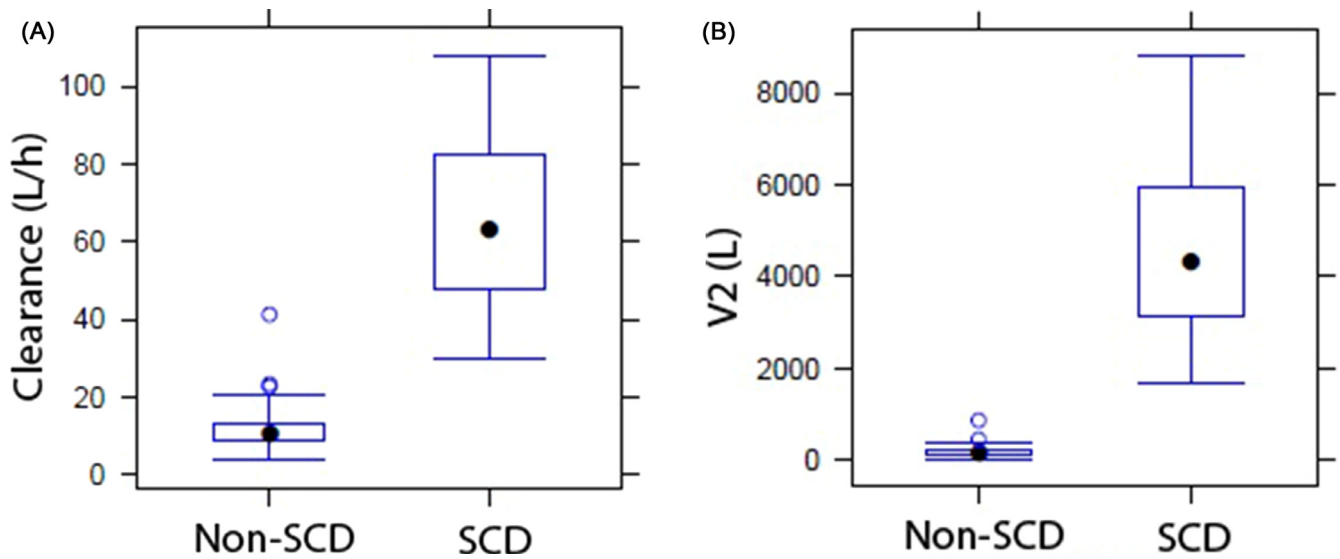
Figure 4. A: Population Clearance (L/h) of DEAQ derived from the most appropriate model after merged data among SCD and non-SCD children. **B:** Population V₂ (L) of DEAQ derived from the most appropriate model after merged data among SCD and non-SCD children. SCD: Sickle cell disease children. Non-SCD: Non-sickle cell disease children

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