Artemisinin population pharmacokinetics in children and adults with uncomplicated falciparum malaria

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Aims To investigate the pharmacokinetics of the antimalarial artemisinin in the field setting using sparsely collected data.

Methods Artemisinin concentrations were determined by h.p.l.c. in a total of 107 capillary plasma samples collected on the first day and in 33 samples on the last day of a 5-day oral artemisinin regimen of 10 mg kg^{-1} day^{-1} in 23 paediatric (aged 2–12 years) and 31 adult (aged 16–45 years) Vietnamese patients with uncomplicated falciparum malaria. The population model was developed using NONMEM, incorporating interoccasion variability and accounting for a systematic change in artemisinin pharmacokinetics with time, modelled as a change in oral bioavailability.

Results Clinical efficacy, in terms of parasite clearance and fever subsidence times, was comparable between children and adults. A one-compartment model with separate pharmacokinetic estimates for children and adults was found best to describe the disposition of artemisinin after oral administration. The population estimates for artemisinin clearance and distribution volume, respectively, were 432 l h^{-1} and 1600 l for adults and 14.4 l h^{-1} kg^{-1} and 37.9 l kg^{-1} for children, with an intersubject variability (collectively for both age groups) of 45% and 104%, respectively. The oral bioavailability was estimated to decrease from Day 1 to Day 5 by a factor of 6.9, a value found to be similar for children and adults.

Conclusions Artemisinin pharmacokinetic data was successfully derived in both paediatric and adult patients using 2–3 capillary blood samples taken in conjunction with parasitaemia monitoring. This study's findings advocated the dosing of artemisinin to children according to bodyweight and to adults according to a standard dose.

Keywords: artemisinin, capillary blood, malaria, paediatrics, population pharmacokinetics

Introduction

With the spread of parasite resistance to chloroquine, Fansidar® and mefloquine, the control of malaria has deteriorated in recent years. In terms of numbers affected, the most severe morbidity and mortality occur in children in rural areas. It has been stated that globally, on average, two children die of malaria every minute [1]. In Vietnam, malaria is one of the most important infectious diseases with about 666 000 clinical cases reported in 1995 (data from Institute of Malariology, Parasitology and Entomology, Hanoi). The artemisinin group of compounds have become first-line drugs against falciparum malaria at the primary health care level in parts of Southeast Asia and are now emerging on the 'Essential Drugs Lists' of several African countries, being indicated for severe cases. The pharmacodynamics of these drugs are characterised by rapid parasitological and clinical responses [2] and a broad stage specificity of antimalarial action [3]. A notable problem with artemisinin and its derivatives is a high recrudescence rate associated with monotherapy indicating that present dosage strategies may not be optimal. Artemisinin elimination is presumed to occur by hepatic metabolism [2]. Data acquired in adult patients have demonstrated that the pharmacokinetics of artemisinin are characterised by a short plasma half-life of about 2 h and a marked time-dependency in pharmacokinetic behaviour resulting in plasma artemisinin concentrations following 5 to 6 days of repeated oral administration approximately 20 to 30% of those following the first drug dose [4, 5]. Similar kinetic knowledge in the paediatric population is presently lacking. This has primarily been due to limitations in sensitive analytical methodologies and to the problems associated with performing an intensive blood sampling typically required in pharmacokinetic analyses. As various differences in drug disposition may exist between children and adults [6], acquisition of paediatric pharmacokinetic data for artemisinin is important in the optimal design of dosage regimens in this group of patients.

We present an application of numerical analysis of sparse data in deriving pharmacokinetic information in patients receiving artemisinin in the field setting employing small volumes of capillary blood taken in conjunction with parasitaemia monitoring. The validity of employing capillary drug concentrations in pharmacokinetic studies with artemisinin was recently demonstrated in a pilot study conducted

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in healthy subjects [7]. In addition, we wished to determine whether the time-dependent changes in artemisinin kinetics described for adults is also a phenomenon in children.

Methods

Study design

Twenty-three children aged 2 to 12 years and 31 adults aged 16 to 45 years (Table 1) were investigated. The study, in lieu of a local research ethics committee, was reviewed by the Ministry of Health, Hanoi, and was separately approved by the ethics committee of the Medical Faculty of Uppsala University and by the Medical Products Agency, Uppsala, Sweden. Written, informed (patient, parental or guardian) consent was obtained for each subject prior to study inclusion. The study was conducted at various health stations of Phu Rieng rubber plantation, Song Be Province, Vietnam. Patient recruitment was performed in a stratified manner (2 to 7 years ($n=10$), 8 to 12 years ($n=13$), 16 to 30 years ($n=19$) and 31 to 45 years ($n=12$)) so as to avoid the inclusion of predominantly older children and younger adults into the study. Symptomatic and asymptomatic male and female patients suffering acute, uncomplicated *P. falciparum* malaria were entered into the study. The following were exclusion criteria: intolerance to oral medication, adult females returning a positive pregnancy test, intake of artemisinin or any of its derivatives within the preceding 2 days, persistence of vomiting or severe diarrhoea, symptoms of cerebral or severe malaria as defined by the WHO [8], children with a total body weight <5 kg, whole blood haemoglobin value <80 g1^{-1} and mixed infection with *P. vivax*. Subjects who had, in the opinion of the examining physician, a clinically significant cardiovascular, renal, hepatic, pulmonary or haematological disease state or an infectious condition resulting in fever were also excluded from the study. Patients were studied for 5 days and were only kept at the local health station (after verification of their complete parasite clearance; see *Clinical assessments*) if they required supervision on other medical grounds. Others returned to the station twice daily for study procedures (including supervised drug administration).

Necessary comedications were restricted to paracetamol (up to 500 mg day^{-1}), diazepam (5 mg day^{-1}) and thiamine (up to 100 mg day^{-1}). Eighteen out of 19 adult male patients and one male in the paediatric group were smokers, whereas no female subjects smoked. Body weight was measured upon study inclusion. Cigarette usage and alcohol consumption were recorded. Patients refrained from solid food 2 h prior to and 2 h after the first drug dose on the days of blood sampling (Days 1 and 5), and xanthine-containing beverages for the duration of these 2 days.

Clinical assessments

Clinical indices Whole blood haemoglobin (Hb) was measured (HemoCue® B-Hemoglobin Photometer, Ängelholm, Sweden) on a single occasion prior to patient inclusion in the study. Other clinical biochemical and haematological indices were not determined due to the difficulties associated with performing such tests in the field. Physical examination was performed for each patient prior to the study and was repeated on the last day of drug administration. Oral temperature was measured every 6 to 8 h until normalised and then daily for the remaining duration of drug administration. Giemsa-stained blood films were prepared for parasite identification and asexual parasites counted by light microscopy of thick smears prior to patient inclusion. Following the (first) dose on Day 1 parasitaemia measurements were timed to coincide with blood sampling for pharmacokinetic determinations. Thereafter, parasitaemia was monitored every 6 to 8 h until three consecutive negative smears were obtained. Following parasite clearance, smears were prepared once daily for the remainder of the study. The number of asexual parasites per 300 white blood cells (WBC) were determined. Parasite density, expressed as the number of parasites per μl blood, was calculated using a WBC correction factor of 8000. Patients (or their parents/guardians) were interviewed about adverse events on Days 1, 3 and 5, initially in an open fashion followed by specific questions according to a check list.

Pharmacodynamic parameters Parasite clearance time (PCT) was defined as the time from commencement of artemisinin dosing to the first of three consecutive negative blood smears. The time of parasitaemia reduction to 50% (PC_{50}) and 95% (PC_{95}) of the initial value was determined by linear interpolation of plots of parasite density versus time. Fever subsidence time (FST) was taken as the time required for oral temperature to fall below 37.5° C and remain so for

Table 1 Patient demographics and pharmacodynamic parameters in Vietnamese patients receiving artemisinin orally for 5 days. Values given as median (range).

	Adults	*Children*
Patients	31 (19 M/12 F)	23 (16 M/7 F)
Age (years)	31 (16–45)	9 (2–12)
Weight (kg)	46.5 (34–56)	20 (8–32)
Height (cm)	158 (148–168)	120 (55–144)
Hb (g1^{-1})	118 (32–137)[#]	102 (82–136)[#]
Initial parasitaemia (/μl blood)[a]	5638 (213–266 667)	8785 (773–800 000)
Initial body temp (°C)	38.0 (37.3–41.2)	38.3 (37.5–40.2)
PCT (h)	30 (14–53)	32 (8–69)
PC_{50} (h)	9 (3–21)	9 (2–22)
FST (h)	24 (3–60)	25 (3–52)

[a]Geometric mean.

[#]$P=0.004$ between children and adults. No statistical differences in any pharmacodynamic parameter.

three consecutive readings. The interpretation of FST was complicated and biased by the administration of paracetamol to 9 children and to 18 adults.

Drug administration

Artemisinin was administered orally as a single dose in the mornings of Days 1 and 5 (children: 10 mg kg^{-1}; adults: 2×250 mg) and twice daily (approximately 07.00 h and 19.00 h) on Days 2 to 4 (children: 5 mg kg^{-1}; adults: 1×250 mg) as hard gelatine capsules of 25 mg, 50 mg, 100 mg, 150 mg (Apoteksbolaget AB, Stockholm, Sweden) or 250 mg (Institute of Materia Medica, Hanoi, Vietnam). Dosing in children was individualised using the five capsule strengths available so as to achieve the weight-adjusted dosages mentioned. No more than two capsules were administered to any patient at each dosing event. All dosages were administered by a member of the medical team and capsule ingestion was followed by ingestion of 25 to 50 ml (children) or 100 ml (adults) of water.

Blood sampling

Capillary blood samples (0.5 ml each) were drawn by lancing (Microtainer® lancet, Becton-Dickinson, 2.2 mm depth) a fingertip which was first swabbed with 70% ethyl alcohol and allowed to dry. Squeezing of fingertips was minimised in order to avoid dilution of samples with interstitial tissue fluid. Patients were randomly allocated to have their blood sampled following administration of the first dose (Day 1) according to one of two schemes:

Children 2.5 h and 6 h ($n=12$) or 4 h and 8 h ($n=11$).

Adults 2.5 h and 6 h ($n=15$) or 4 h and 10 h ($n=16$).

Two blood sampling occasions per patient on Day 1 was expected to be the maximum in terms of local community acceptance and, thus, for facilitation of patient recruitment. The above times were selected to provide a characterisation of the elimination phase of the plasma concentration-time profile. As little value was placed on the modelling of the artemisinin's absorption and with maximal plasma concentrations occurring approximately 1 to 4 h postdose [4, 5] an initial sampling time of 2.5 h was selected to optimise population estimates of the remaining kinetic parameters. A time of no later than 8 h for collection of the second sample was chosen for children in order to avoid the possibility of undetectable artemisinin plasma concentrations due to potentially a higher clearance value in this population. Previous experience indicated that the majority of artemisinin concentrations determined later than 10 h following a 10 mg kg^{-1} dose in patients would be below the assay limit of quantitation.

In order to characterise any time-dependency in artemisinin pharmacokinetics, a single capillary sample was taken in 18 children and 15 adults on Day 5 at the earlier of their Day 1 blood sampling times (i.e., at 2.5 or 4 h).

Assessment of blood sampling schedules The sampling schemes in adults and children on Day 1 were assessed prior to the study by using data obtained from adult Vietnamese patients suffering from uncomplicated *P. falciparum* malaria in whom oral artemisinin pharmacokinetics were characterised from an intensive (12 samples from each of 15 subjects) blood sampling regimen [9]. Population pharmacokinetic parameter and variability estimates derived from data sets, with a reduced number of samples to mimic the stated sampling schemes, were found in statistical simulations to accord with those derived from the full data set (results not presented). Thus, the population pharmacokinetics of artemisinin were determined to be adequately described using the chosen sampling schedules.

Artemisinin assay

Following collection, blood samples were left standing at ambient temperature for 15 min, then centrifuged at 1000 *g* for 10 min and the plasma harvested and the samples kept in liquid nitrogen until transferred to Ho Chi Minh City for storage at -20° C. Plasma samples were then collectively transported on dry ice to Uppsala University for analysis. Within 3 months of collection, concentrations of artemisinin were determined in plasma samples using a reverse-phase h.p.l.c. method with post-column on-line alkali derivatisation and u.v. detection, as previously described [4]. The following assay modifications were undertaken to permit analysis of 0.1 ml plasma volumes: each plasma sample was mixed with 1.4 ml potassium phosphate buffer (pH 3.5) and the residue obtained following evaporation was reconstituted with 200 μl mobile phase, of which 100 μl was injected onto the column; with absorbance measured by a Shimadzu SPD-10 A u.v. detector. Within run imprecision for this modified assay was 16% ($n=10$) at 5 μg l^{-1} (limit of quantitation).

Statistical evaluation of pharmacodynamic parameters

Initial parasitaemia, PCT, PC_{50}, FST and Hb in children and adults were compared by the Mann–Whitney U test. Correlations were sought between initial parasitaemia and PC_{50}, PCT and FST, between PCT and FST and between initial body temperature and FST by Spearman's test. An α level of 0.05 was set in all statistical analyses.

Sparse data analysis

Population pharmacokinetic parameters of artemisinin were estimated using nonlinear mixed effects modelling, as implemented in the software package NONMEM (version V) [10]. A total of 140 artemisinin concentrations from 54 subjects were employed in the data analysis. The first-order estimation method was used to derive population pharmacokinetic parameters, the intersubject variability in these parameters, and residual variability between observed and predicted concentrations. This latter residual variability can arise from a host of factors, including variation introduced in drug assay, timing of blood collection and of dosing, and model misspecification. Description and explanation of the population method are provided elsewhere [11, 12].

The pharmacostatistical model was developed by comparing one-and two-compartment models with first-order absorption and elimination. The models were parameterised in terms of distribution volume (V/F) and clearance

(CL/F). Using CL/F as an example, intersubject variability was modelled as

$$(\mathrm{CL}/F)_i = (\tilde{\mathrm{CL}}/F)_p \cdot e^{\eta_i^{\mathrm{CL}/F}}$$

where $\eta_i^{\mathrm{CL}/F}$ denotes the (proportional) difference between the typical parameter value in the population $(\tilde{\mathrm{CL}}/F)_p$, and the parameter value for subject i, $(\tilde{\mathrm{CL}}/F)_i$. Intersubject variability was modelled the same way for all parameters. The ηs are zero mean, normally distributed, random variables with variance ω^2. The ω^2s are the diagonal elements of the interindividual variance-covariance matrix, Ω. The off-diagonal elements of this matrix were also considered to assess possible correlations between parameters.

As 'stationarity' in artemisinin pharmacokinetics may not be a valid assumption, time-variance in the pharmacokinetic parameters was modelled both as systematic and random variability. In the latter case, interoccasion variability (IOV) of a parameter P (CL/F and V/F only) for subject i during study occasion j was employed, defined as

$$P_{ij} = \tilde{P}_{ij} \cdot e^{\eta_i^P + \kappa_j^P}$$

where κ_{ij}^P is a random variable with variance π^2 [13].

Residual variability was modelled as essentially proportional, according to

$$\ln C_{ijk} = \ln \tilde{C}_{ijk} + \varepsilon_{ijk}$$

where C_{ijk} and $\tilde{C}_{ijk}$ are the kth measured and the model-predicted concentrations, respectively, and ε_{ijk} denotes the residual intrasubject random error, which is distributed with zero mean and variance σ^2. The variances ω^2, π^2 and σ^2 were estimated as components of the population model.

Model building and selection The construction of the pharmacokinetic models was performed along principles described by Ette & Ludden [14]. The initial step consisted of deriving the basic pharmacokinetic model and Bayesian individual parameter estimates, using the 'POSTHOC' option in NONMEM. The initial model consisted of no covariates and a commonality of parameter estimates for both children and adults. The distribution of the empirical Bayes estimates of individual kinetic parameters and their relationships with covariates were then examined. Covariates screened included demographic characteristics, patient group (adults or children), Hb, pharmacodynamic measures (initial body temperature and parasitaemia, PCT, PC_{50}, PC_{95} and FST) and categorical representation of alcohol intake and smoking. Covariate selection was primarily based on the generalised additive model (GAM) approach [15]. Covariates screened as influential by the graphic explorations were then introduced into the model and the significance of their explanatory power was judged by improvement of the objective function (OF), the latter being equal to twice the negative log likelihood of the data. A change in the OF (which approximates the χ^2 distribution) of 4 units was considered statistically significant ($P<0.05$) for addition of one parameter to a candidate model [16]. The goodness-of-fit of each NONMEM analysis was also assessed by residual analyses, the standard error of the parameters and changes to estimates of ω's and σ resulting from changes to the model [14].

Evaluation of population pharmacokinetic parameters In order to assess whether the final pharmacokinetic model was strongly dependent on or determined by only a small subgroup of individuals, case deletion diagnostics were employed as a measure of influence. Subjects were deleted one-at-a-time and the data reanalysed by NONMEM using the final model. The new parameter values obtained from the single case-deleted data set were compared qualitatively with those from all 54 individuals.

Results

A total of 107 and 33 plasma artemisinin concentrations were determined on Days 1 and 5, respectively (Figure 1). Absolute deviations of actual blood sampling times from those scheduled averaged 7 min (max 40 min). Artemisinin was found to be well tolerated and no adverse events were reported or observed. Patient demographic characteristics and indices of efficacy are presented in Table 1. Children were more anaemic compared with adults. Clinical efficacy, in terms of parasite clearance and fever subsidence times, were comparable in children and adults. Of the correlations between the pharmacodynamic parameters that were investigated, the only significant relationships found were between initial parasitaemia and PC_{50} ($r_s=0.34$, $P=0.01$) and PCT ($r_s=0.51$, $P<0.001$).

Model selection and development

A one-compartment model with first-order absorption (with K_a constrained to be greater than CL/V) was found to best describe the plasma artemisinin concentration data. The correlation between $\eta_i^{V/F}$ and $\eta_i^{\mathrm{CL}/F}$ was found to be near unity. The need for modelling of a change in artemisinin pharmacokinetics from Days 1 to 5 in both age groups was evident in the initial model building process. This change was modelled collectively on both CL/F and V/F (i.e., on the compound's bioavailability) and was included as a component of the basic model. Covariates assessed to ascribe the variability in artemisinin pharmacokinetics was restricted to those factors influencing CL/F and V/F. Following selection of the basic model, patient group, gender and smoking were identified in the GAM analyses as being the influential factors on the residuals of both oral clearance and distribution volume. The categorical term, age group, was found to better improve the basic model than total body weight. Compared with a model without its presence, the estimation of IOV, which was greater for V/F than for CL/F, resulted in a decrease in σ by 16% together with smaller reductions in both $\omega^{\mathrm{CL}/F}$ and ω^{Ka}.

From the basic model (OF = 152), modified to separately characterise artemisinin pharmacokinetics for children and adults, the inclusion of smoking and gender on both CL/F and V/F for both age populations was found to cause the greatest reduction in the OF (by 26 units) and in the random effects estimates. However, population estimates of these two covariates (increasing F by factors of 3.8 and 3.4 for smoking and females, respectively) were associated with relative standard errors in the order of 60–90% and produced essentially the same change in F for males and females. It

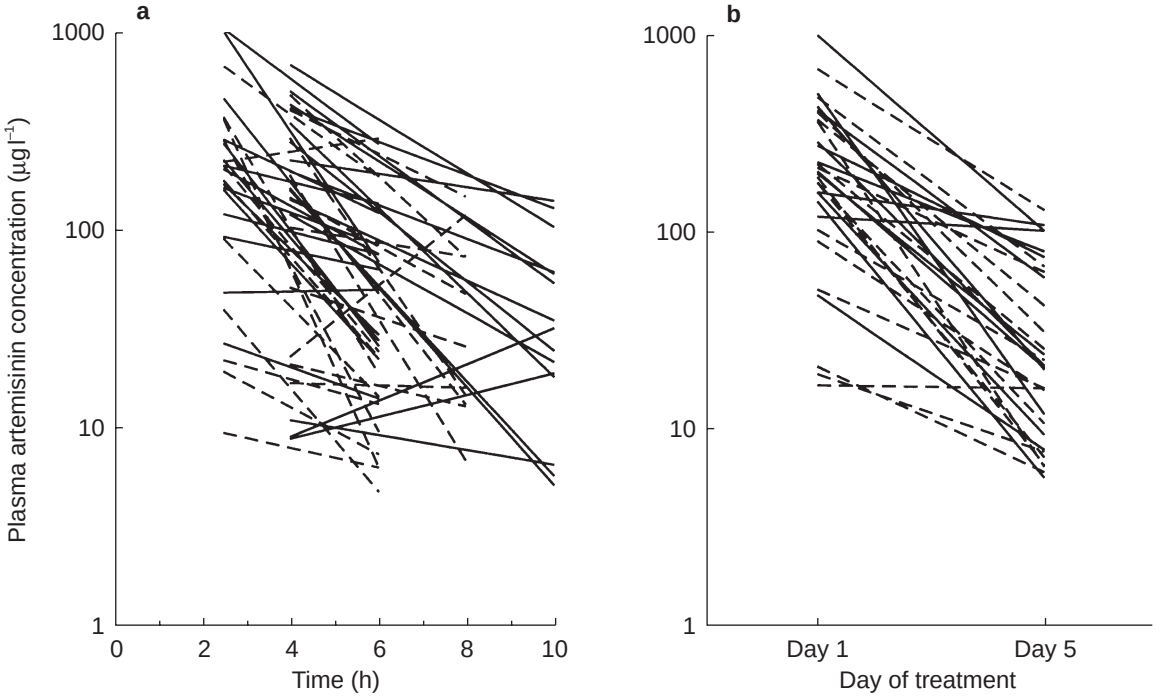

Figure 1 Individual artemisinin plasma concentrations ($\mu g\,l^{-1}$) in adults (—) and children (---) after oral artemisinin doses of 10 mg kg^{-1} (a). Plasma concentrations determined on two occasions, straddled with respect to time between patients, after the first dose are interconnected by a straight line. In a sub-group, time-dependent artemisinin pharmacokinetics was indicated by lower plasma drug levels at 2.5 or 4 h after the final dose of the 5-day oral regimen of 10 mg kg^{-1} day^{-1} compared with plasma concentrations determined at the same time-points after the first dose in the same adults (—) and children (---) (b).

was further determined that the presence of these covariates was biased by the data of two male subjects, an adult who was not a smoker and a child who was. In light of these influential behaviours and of the indiscriminate nature of smoking and gender in the adult population (nearly all males were smokers whilst no females smoked), these two covariates were subsequently excluded as potential descriptors of clearance and volume in adults. In children, however, gender alone was identified as being influential, resulting in a decrease in the OF value by 6 units from the base model and improvement in the goodness-of-fit plots, with female children being determined to have both a lower CL/F and V/F. The population estimate for this reduction in CL/F and V/F in the female, relative to the male, child was 0.32 (22% relative s.e.). However, in view of the small numbers of male and female children involved and of a slight inequality of both weight and age distributions between the subgroups, gender was not considered to be a reliable sole descriptor of paediatric artemisinin kinetics and was subsequently dropped. Weight was the only other covariate determined to improve pharmacokinetic estimates in the paediatric group (decrease OF by 2.5 and σ by 7%). Though the resultant change in the OF upon its inclusion was not statistically significant, weight was preferred and was subsequently retained as a covariate in structural models of CL/F and V/F in children over gender until this latter covariate's explanatory worth is verified in the paediatric population. There was no justification for the inclusion of weight in the modelling of clearance and/or volume for adults. Further, there was no advantage in the inclusion of body surface area over total body weight in the model development process.

Final model estimates

Results of the final structural and statistical models are summarised in Table 2. Goodness-of-fit plots for the final model, including those shown in Figure 2, were absent of strong trends and indicated a reasonable model fit to the observed data. Interindividual variability was largest for K_a and was approximately 2.5 times higher for V/F than CL/F. Fixing typical values of K_a or avoiding estimating its error

Table 2 Population parameter estimates for artemisinin pharmacokinetics. Estimates of CL/F, V/F and K_a, are those for Day 1.

Parameter	*Mean*	*Relative s.e. (%)*[a]
CL/F_{adults} ($l\,h^{-1}$)	432	19
$CL/F_{children}$ ($l\,h^{-1}\,kg^{-1}$)[b]	14.4	24
V/F_{adults} (l)	1600	28
$V/F_{children}$ ($l\,kg^{-1}$)[b]	37.9	33
$\Delta F_{Day1 \rightarrow Day5}$[c]	6.9	20
K_a (h^{-1})	1.7	25
$\omega^{CL/F}$	45%	44
$\omega^{V/F}$	104%	36
ω^{K_a}	576%	21
$\pi^{CL/F}$	53%	32
$\pi^{V/F}$	86%	36
σ	47%	41

[a]Relative standard error of estimate = (s.e./mean)*100%.
[b]Units adjusted for total body weight (WT) due to the presence of this covariate in the final model for population estimates in children.
[c]Factor change in both CL/F and V/F from Days 1 to 5, considered as a change in the bioavailability term (F_{Day1}/F_{Day5}).

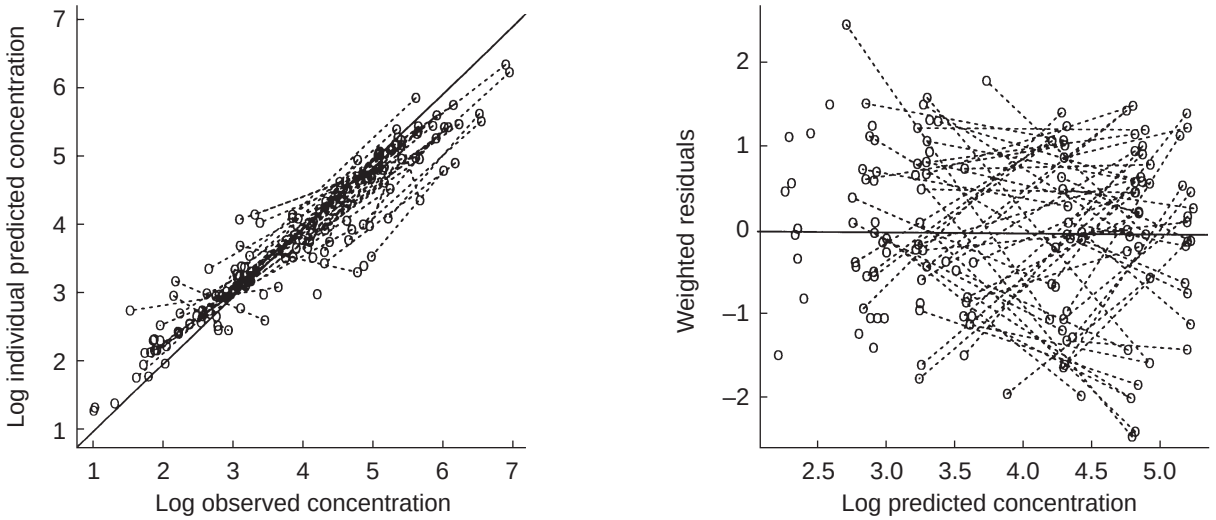

Figure 2 Goodness-of-fit plots for the final population model for artemisinin pharmacokinetics in children and adults. The left-hand graph shows how well the model can describe the data where, in a perfect fit of error-less data, all points would fall on the line of identity. Scatter of the observations around this line and over-/under- predictions at high/low concentrations is a result of unexplained variability in the data. The right-hand graph represents one of the goodness-of-fit plots used to assess the fit and it should, if the model is adequate, portray a random scatter around y = 0. In both graphs concentrations have been transformed to their natural logarithms.

had little effect on the estimates for the remaining kinetic parameters. Thus, population estimates for K_a were allowed to be derived. Population estimates of artemisinin half-life on Day 1 were 2.6 h (range of individual estimates: 1.0–11.8 h) in adults and 1.8 h (range: 0.8–7.9 h) in children. Regardless of gender, typical values of $CL/F = 432$ l h^{-1} and $V/F = 1600$ l would be expected for all adults in the study. For an adult weighing 46.5 kg (median for adults) this equates to CL/F and V/F values of 9.3 l h^{-1} kg^{-1} and 34.4 l kg^{-1}, respectively. The factor decrease in the oral bioavailability of artemisinin on Day 5 compared with Day 1 was determined to be 6.9. Separate population estimates of this change for children (6.7) and adults (7.3) and for males (6.7) and females (7.4) were found to be similar.

Model evaluation

Case deletion diagnostics for the 53 single case-deleted reanalyses of the final model are given in Table 3. Coefficients of variation for parameter estimates from each reanalysis were $<10\%$ for all parameters, except K_a.

Discussion

Children in the developing world are particularly at risk of suffering from malaria, yet they constitute a population in which the obtainment of pharmacokinetic information is inherently difficult, particularly in the field setting. The present study aimed to characterise the pharmacokinetics of artemisinin in both paediatric and adult patients by employing numerical analysis methodology of sparse data. A significant feature of the population approach in pharmacokinetic analysis is the ability to derive information from sparse or fragmented data whilst being able to consider and assess factors which may influence drug disposition. By tailoring the study design to derive pharmacokinetic data based on a minimum of two capillary blood samples taken in conjunction with parasitaemia monitoring, the need for collecting venous blood and of attaining sample intensive drug concentration data was avoided.

The population pharmacokinetic parameters determined in this study accorded well with parameter values in adults with uncomplicated malaria derived by noncompartmental methods used in studies involving intensive blood sampling schedules (range of mean reported CL/F, V/F and $t_{\frac{1}{2}}$ values: 299–318 l h^{-1}, 1578–1704 l and 2.0–2.5 h, respectively) [4, 5, 9, 17]. Compared with Day 1, the oral bioavailability of artemisinin was, on average, determined to be reduced by a factor of 6.9 on the fifth day of repeated oral drug administration ($F_{\text{Day 1}}/F_{\text{Day 5}}$). The degree of this change, representing a striking example of time-dependency in clinical pharmacokinetics, was remarkably consistent in the

Table 3 Case deletion diagnostics for evaluation of final model estimates.

Parameter	*Mean*	*CV (%)*[a]	*Min*[b]	*Max*[b]
CL/F_{adults} (l h^{-1})	402	5	361	437
$CL/F_{children}$ (l h^{-1} kg^{-1})	13.2	8	11.2	16.2
V/F_{adults} (l)	1504	6	1310	1740
$V/F_{children}$ (l kg^{-1})	36.7	8	27.8	44.6
$\Delta F_{Day1-Day5}$[c]	6.6	5	6.2	7.3
K_4 (h^{-1})	5.8	55	1.5	10.0

[a]Coefficient of variation and [b]minimum and maximum values of all 53 estimates (each from one case deletion re-run of final model; see text for explanation).

study population. Delayed absorption may have contributed to the apparent lack of change in three individuals, two of whom were sampled at 2.5 h after dose. A similar, three-to-six-fold, decrease in areas under the plasma artemisinin concentration-time curves following artemisinin therapy has been reported in adult Vietnamese [4] and Tanzanian patients [5]. These time-dependent changes are thought to result from autoinduction of drug elimination capacity causing a decrease in oral bioavailability [4]. In the present study, modelling the time-dependency on bioavailability was superior to it having an effect on clearance alone. Interestingly, the change in artemisinin's disposition was presently determined to occur to a similar extent in both children and adults and between males and females. It is conceivable that lower drug levels towards the end of treatment may in some patients contribute to the risk of recrudescence.

As expected for a rapidly absorbed compound such as artemisinin [4, 5], the absence of plasma concentrations prior to 2.5 h postdose resulted in a very high estimate of intersubject variability for K_a. Instability in this parameter's estimate was also borne out in the large variability in values from case deletion diagnosis analyses. However, fixing estimates for K_a caused little change in the estimates of the other parameters. This is consistent with the findings of Wade and colleagues [18] that in the absence of data in the absorption phase, misspecification or poor estimation of the absorption characteristics of a rapidly absorbed drug in population analyses has little consequence for the estimation of the remaining population parameters.

In comparison with adults, the oral clearance of artemisinin was found to be slightly greater in children. As a marked gender difference was identified in the paediatric group, with females having CL/F and V/F estimates approximately 0.3 times that of males, the apparent kinetic differences between children and adults were most likely due to overall higher clearances in the male children. However, these differences should be interpreted in light of the small subgroup sizes involved and the large interindividual pharmacokinetic variability. It is known that a number of physiological and metabolic processes in children differ qualitatively as well as quantitatively when compared with adults [19]. For instance, the clearance of methotrexate [20], antipyrine [21], theophylline [22] and mefloquine [23] has been reported to be greater in children than adults. Whether the apparent gender difference in artemisinin disposition in children presently reported demonstrates a similar physiological phenomenon should be confirmed in future paediatric pharmacokinetic studies with the compound.

The clinical efficacy of the artemisinin group of antimalarials has been widely demonstrated with the response to monotherapy being both rapid and associated with relatively few documented adverse effects [2]. In addition to being well tolerated, the administration of artemisinin to patients in this study resulted in similar resolution of the clinical indices of malarial infection (PCT and FST) between children and adults. Further, the NONMEM and regression analyses presently undertaken failed to identify any influence of pharmacodynamic outcomes on artemisinin pharmacokinetics or vice versa.

In conclusion, in having identified potential influential covariates on artemisinin's disposition in uncomplicated malaria, only weight (in children) was determined to be a suitable descriptor of the compound's kinetics with the data at hand. Others such as gender and possibly smoking served to identify potential investigative needs for the compound. Thus, at present we advocate the dosing of artemisinin to children according to bodyweight and to adults according to a standard dose. The marked time-dependent pharmacokinetics of artemisinin, may affect the risk for recrudescence and the choice of dosage strategy. The present application of population analysis of sparse data obtained in a field setting serves to illustrate the particular value of this approach in the clinical development of drugs for tropical diseases.

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