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Population pharmacokinetics of amodiaquine and desethylamodiaquine in pediatric patients with uncomplicated falciparum malaria

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Abstract The study aimed to characterize the population pharmacokinetics of amodiaquine (AQ) and its major metabolite *N*-desethylamodiaquine (*N*-DEAQ), and to assess the correlation between exposure to *N*-DEAQ and treatment outcome. Blood samples from children in two studies in Zanzibar and one in Papua New Guinea were included in the pharmacokinetic analysis ($n = 86$). The children had been treated with AQ in combination with artesunate or sulphadoxine-pyrimethamine. The population pharmacokinetics of AQ and *N*-DEAQ were modeled using the non-linear mixed effects approach as implemented in NONMEM. Bayesian post-hoc estimates of individual pharmacokinetic parameters were used to generate individual profiles of *N*-DEAQ exposure. The correlation between *N*-DEAQ exposure and effect was studied in 212 patients and modeled with logistic regression in NONMEM.

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The pharmacokinetics of AQ and N-DEAQ were best described by two parallel two-compartment models with a central and a peripheral compartment for each compound. The systemic exposure to AQ was low in comparison to *N*-DEAQ. The $t_{1/2\lambda}$ of *N*-DEAQ ranged from 3 days to 12 days. There was a statistically significant, yet weak, association between *N*-DEAQ concentration on day 7 and treatment outcome. The age-based dosing schedule currently recommended in Zanzibar appeared to result in inadequate exposure to *N*-DEAQ in many patients.

Keywords Amodiaquine · Desethylamodiaquine · Malaria · Child · Population pharmacokinetic/pharmacodynamic modeling

Introduction

Amodiaquine (AQ) is an aminoquinoline structurally related to chloroquine. The increase in chloroquine and sulphadoxine-pyrimethamine resistant *Plasmodium falciparum* has resulted in a renewed interest in amodiaquine, particularly as part of combination treatments. Given the high incidence of malaria in the African pediatric population, estimated to be 1.6–5.4 malaria episodes per child year [1], pediatric patients must be considered as the primary recipients of antimalarial treatment. Though numerous recent studies address the efficacy of amodiaquine treatments in children little is known regarding its pharmacokinetics in this population.

From studies in adult patients and healthy volunteers, it is known that orally administered AQ is rapidly metabolized to the active metabolite *N*-desethylamodiaquine (*N*-DEAQ). The metabolism of AQ to *N*-DEAQ is catalyzed *in vitro* by the liver enzyme Cytochrome P450 2C8 (CYP2C8) [2]. Little AQ is detected in the systemic circulation following oral administration [3,4]. The half-life of AQ is approximately 4 h in adults [5]. Urine collection in four patients over three days of intravenous treatment with AQ (10 mg base/kg over 4 h) resulted in recovery of less than 2% of administered dose in the form of AQ in each 24 h interval [6]. *N*-DEAQ has a considerably longer terminal half-life ranging from 2.5 days to 18.2 days in adults [3,4]. The main route of elimination of *N*-DEAQ is unknown. Further metabolism of *N*-DEAQ to bis-DEAQ has been suggested although the plasma and urine concentrations of this metabolite were low in healthy volunteers receiving 300 mg AQ [7].

Both AQ and *N*-DEAQ have been shown to possess antimalarial activity in vitro [8,9] while there are no reports on the antimalarial activities of bis-*N*-DEAQ and the proposed *N*-hydroxyl-DEAQ [3]. Due to the rapid conversion of AQ to *N*-DEAQ the metabolite is assumed to be responsible for the main clinical effect, but in vitro studies also suggest a synergism between AQ and *N*-DEAQ [10]. A study of the correlation between *N*-DEAQ blood concentrations and treatment outcome in 118 Gabonese children showed that concentrations >135 ng/ml *N*-DEAQ on day four following a treatment regimen of 10 mg/kg for 3 days was associated with an adequate clinical response [11].

There is, to date, only one published study addressing the pharmacokinetics of amodiaquine in pediatric patients [12]. The findings of that study in Papua New Guinean children suggested that the blood concentrations of AQ and *N*-DEAQ may be higher than in earlier studied adult African populations.

The aim of the present study was to characterize the population pharmacokinetics of AQ and its major metabolite *N*-DEAQ in pediatric patients with uncomplicated malaria, and to assess the correlation between treatment outcome and pharmacokinetics of *N*-DEAQ.

Methods

Blood concentrations of AQ and *N*-DEAQ from three studies, two unpublished and one published, were included in the pharmacokinetic analysis. The pharmacodynamic variable, presence or absence of parasitemia on days 7, 14, and 28 after treatment initiation, was available in one of the studies.

The aim of study 1 was to compare the effectiveness of AQ-artesunate with that of sulphadoxine-pyrimethamine (SP) in uncomplicated malaria patients in Zanzibar, Tanzania. Samples for determination of drug concentration were obtained on days 7 and 14. This sampling schedule precluded description of AQ pharmacokinetics. Study 2 was conducted specifically to obtain information on the immediate post-dose pharmacokinetics of AQ and *N*-DEAQ in pediatric patients from the same area. Study 3 was a pharmacokinetic study on AQ and *N*-DEAQ in pediatric patients in Papua New Guinea [12].

Subjects and study designs

Study 1

Blood samples for drug concentration analyses were obtained from 212 pediatric patients. The study was conducted at the Primary Health Care Centre (PHCC) in Kivunge and Micheweni, Zanzibar in 2004 as part of a larger study on the effectiveness on AQ + artesunate compared to SP. Included in this study were children aged 3 months to 5 years presenting at the PHCC with a clinical episode of microscopically confirmed falciparum malaria. Informed consent from parent or guardian was a prerequisite for inclusion.

Doses of AQ and artesunate (supplied in the combination package Arsucam®, Creapharm, France, batch CLI3296) were determined on the basis of age, in accordance with the national treatment policy in Zanzibar. Patients younger than 12 months received 25 mg of artesunate and 50 mg of AQ-HCl (equivalent to 38.3 mg AQ) and patients aged 1–5 years received 50 mg of artesunate and 100 mg of AQ-HCl (equivalent to 76.5 mg AQ) once daily for three consecutive days. The drugs were supplied by the study personnel but treatment was unsupervised.

Thick and thin blood films were prepared to determine presence and density of malaria parasites on days 0, (and during follow-up on days) 7, 14,

and 28. Blood films were stained with 5% Giemsa's stain for 30 min and asexual parasite density was calculated against 200 white blood cells, assuming a WBC count of 8,000/μl. If less than 10 parasites were detected per 200 white blood cells, estimates were made against another 300 white blood cells. Slides were prepared and examined at the respective study sites.

Capillary blood samples for drug concentration analyses were obtained through finger prick on day 7 and day 14 following treatment initiation. The blood was collected into an eppendorf tube and 100 μl transferred by volumetric pipette to a filter paper (Whatman 31ETCHR). The samples were dried at room temperature and stored individually in zip-lock bags. The samples were stored at room temperature while in Zanzibar, and at −20°C in Sweden.

The study was approved by the Ministry of Health in Zanzibar and by the research ethics committee at the Karolinska Institute, Stockholm, Sweden.

Study 2

This study was performed in Kivunge, Zanzibar. Twelve children aged 3–12 years presenting at the PHCC, with uncomplicated malaria, i.e., fever, or a history of fever, and a parasitemia of 2000–250,000 parasites per μl blood were eligible for the study. Patients were asked to remain hospitalized for 8 h following treatment initiation and to return for supervised treatment on days 2 and 3. Doses of Arsucam® (Creapharm, France, batch number CLI3296) were determined on the basis of age as in study 1. Patients aged 3–6 years received 50 mg of artesunate and 100 mg AQ-HCl once daily for three consecutive days and patients aged 7–12 years received 100 mg of artesunate and 300 mg of AQ-HCl once daily for three consecutive days. Additional medication given was paracetamol (in two patients) and amoxicillin (one patient).

A total of eight samples were obtained from each subject. Venous blood samples were obtained through an indwelling catheter on day 1 and capillary blood by lancing a finger on days of follow up (days 7 and 14). Patients were subjected to one of two sampling schedules on the first day of treatment:
Schedule A: 0 (pre-dose), 0.25, 1, 3, 5, and 7 h following treatment initiation and Schedule B: 0 (pre-dose), 0.5, 2, 4, 6, and 8 h after start of treatment. Samples for drug concentration analyses were prepared and stored as in study 1.

The study was approved by the Ministry of Health in Zanzibar and the research ethics committee at Gothenburg University, Gothenburg, Sweden.

Study 3

Data from the 20 patients in a previously published study conducted in Papua New Guinea was included in the analysis [12]. Patients aged 1–10 years with uncomplicated falciparum malaria (fever >37.5°C, parasitemia) were included in the study. The children received a total oral dose AQ (infant Camoquin®, Prawll Laboratories Ltd., India, 100 mg tablet) of 30 mg kg^{-1} (10 mg kg^{-1} day 1 for 3 days) and a single dose of SP on day 7 (25 mg kg^{-1}, based on the sulphadoxine component) [9]. All doses were administered under supervision. AQ

and *N*-DEAQ concentrations were determined at 0 (pre-dose), 2, 4, 12, 24, 36, and 48 h following treatment initiation and on days 3, 5, 7, and 14.

The study was approved by the National Department of Health Medical Research Advisory Committee, Papua New Guinea, and Tokyo Women's Medical University Ethical Committee, Japan.

Chemical assay

Drug concentration determinations for studies 1 and 2 were conducted at Gothenburg University, Gothenburg, Sweden. Blood concentrations of AQ and *N*-DEAQ, were determined using a previously described method [13]. In brief; AQ and *N*-DEAQ were separated from blood components using solid phase extraction (PRS columns, Sorbent AB, Västra Frölunda, Sweden) followed by concentration determination using HPLC (column Zorbax SB-CN, Chromtech AB, Hägersten, Sweden) with UV detection at 242 nm. The interday coefficient of variation (CV) in quality control samples for AQ was 15%, 9%, and 11% at 183, 457, and 1,097 nM, respectively. The interday CV for *N*-DEAQ was 15%, 7%, 11% at 200, 500, and 1,200 nM respectively. The lower limit of quantification (LLOQ) for both AQ and *N*-DEAQ was set at 50 nM (intraday CV was 5% and 2% for AQ and *N*-DEAQ, respectively).

The drug concentrations in study 3 were determined by the same method but at a different laboratory as described by Hombhanje and colleagues [12].

Pharmacokinetic modeling

The population pharmacokinetics of AQ and *N*-DEAQ were modeled using the non-linear mixed effects approach as implemented in NONMEM version V level 1.1 (Icon Development Solutions, Maryland, USA). During the initial search for an appropriate structural model and initial parameter estimates the first order method was used. The final structural model, models of interindividual variability, error models as well as covariate effects were investigated using the first order conditional estimation method (FOCE). Models describing the formation of *N*-DEAQ from AQ as well as parallel models where AQ and *N*-DEAQ were introduced in the model from separate dosing compartments were fitted to log-transformed concentrations. One and two compartment models for the disposition of both compounds were investigated.

Homoscedastic and heteroscedastic error models were tested to explain residual error. Differences between populations (Papua New Guinean and Zanzibari patients) was assessed as random and fixed effects influencing interindividual variability, and as part of the residual error model.

Patients in studies 1 and 2 who had AQ and/or *N*-DEAQ in the pre-dose sample were excluded from the pharmacokinetic analysis ($n = 22$). Patients from study 1 who had no AQ or *N*-DEAQ on day 7 ($n = 94$), or AQ concentrations >500 nM in the 7 day sample ($n = 4$) were excluded as this indicated a lack of compliance with the treatment schedule. The first sample following

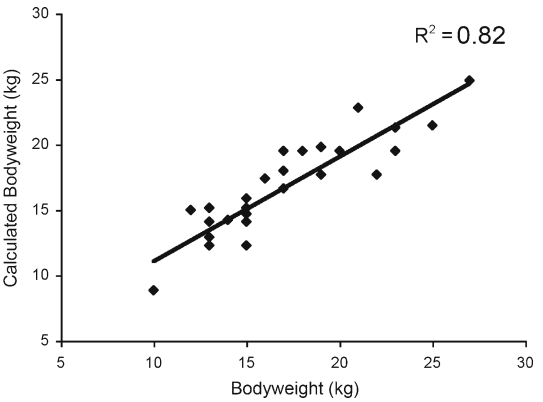

Fig. 1 Correlation between estimated bodyweights based on the linear relationship between age and weight in patients from studies 2 and 3 and actual bodyweights. The function (BW(kg) = 0.16* AGE(months)+6.8) was used to calculate bodyweights for patients in study 1

each dose that was below the limit of quantitation was set equal to half the LLOQ. Remaining data below the LLOQ were excluded. The total number of samples included in the analysis was 121 (41 and 79 from studies 2 and 3, respectively) and 374 (169, 71, and 134 from studies 1, 2, and 3, respectively) for AQ and DEAQ, respectively, obtained from a total of 117 patients. There were no records of body weight for patients in study 1. The linear relationship between body weight and age with slope (SE) 0.16 (0.01) and intercept (SE) 6.8 (0.9) identified from patients in studies 2 and 3 was used to estimate the body weight of these patients ($r = 0.82$). The correlation between the actual bodyweights and the calculated bodyweights for subjects in studies 1 and 2 is shown in Fig. 1.

Possible covariate effects on fixed parameters were identified using the general additive method (GAM) as implemented in Xpose Version 3.1 [14]. Covariates investigated in the model were age, gender, study # (study 1, 2, or 3) and study population (Zanzibari and Papua New Guinean). The value of the objective function (OFV) was used to discriminate between nested models. The OFV is essentially equal to $-2x$ log likelihood of the data and the difference in OFV is approximately χ^2 distributed [15]. Using the FOCE estimation method a decrease in the OFV exceeding 6.6 indicates a statistically significant better fit, however the actual significance level of a covariate depends on the number of individuals, the number of samples per individual as well as the residual error structure [16,17]. Forward inclusion of a covariate in the final model was based on the difference in OFV as well as a decrease in the interindividual variability.

To elucidate the possible influence of erratic dosing in study 1 (with unsupervised treatment) on the estimated typical values of the pharmacokinetic parameters the final model was applied to a dataset including only data from studies 2 and 3, with direct observed treatment.

The performance of the final model was assessed with a visual predictive check as described by Holford [18]. The model stability was investigated by calculating the condition number i.e., the ratio of the largest to the smallest eigenvalue of the covariance matrix. A condition number exceeding 1,000 indicates ill conditioning [19].

Generating complete pharmacokinetic profiles to determine individual exposure to *N*-DEAQ

Complete pharmacokinetic profiles for all individuals included in the pharmacokinetic analysis ($n = 117$) were simulated using the Bayesian post-hoc estimates of the individual pharmacokinetic parameters. A total of 40 sampling time points from 0.25 h to 500 h after treatment were simulated for each individual. The median extrapolated portion of the $AUC_{0-\infty}$ was 5.6% and ranged from 0.8 to 43%. Individual area under the concentration–time curve ($AUC_{0-\infty}$), the time to maximum concentration following the first dose (t_{max}), $t_{1/2\,\lambda}$, and the maximum concentration reached during treatment (C_{max}) were determined by non-compartmental analysis in WinNonLin Version 5 (Pharsight Corporation, California, USA). The area under the concentration–time curve was calculated using linear interpolation between increasing concentrations and logarithmic interpolation between declining concentrations.

Assessment of correlation between drug concentrations and pharmacokinetic parameters and parasitemia during follow-up

Parasitemia within one month of treatment initiation (i.e., on days 7, 14, and/or 28) was defined as a single outcome variable. The influence of *N*-DEAQ concentrations on days 7 and 14, pharmacokinetic parameters and patient factors on the defined outcome variable was assessed by binary logistic regression using the conditional Laplacian likelihood option in NONMEM. The relationship between the probability of parasitemia during follow up and the predictors were assessed with the model:

$$P\,(Parasitemia_i|\,\eta_i) = \frac{e^{a+b\times x+\eta_i}}{1+e^{a+b\times x+\eta_i}}$$

where x represents one of the following predictors: observed *N*-DEAQ concentrations on days 7 and 14, $AUC_{0-\infty}$, $t_{1/2\,\lambda}$, C_{max}, age and initial parasitemia, a is an intercept and b describes the magnitude of the change in probability associated with x. The $-2x$ log likelihood ratio was used to determine the statistical significance of the correlations. All patients in study 1 ($n = 212$) were included in the assessment of the influence of observed *N*-DEAQ concentrations on day 7 and 14, patient factors and parasitemia during follow up. The correlation between pharmacokinetic factors and treatment outcome could only be assessed in the patients in study 1 included in the pharmacokinetic analysis ($n = 86$).

A non-parametric bootstrap evaluation (1,000 bootstraps) of the logistic model was performed using Wings for NONMEM Version 600 [20]. The bootstrapped parameter values were used to determine the 90% confidence interval of the probability curve illustrated in Fig. 7.

Table 1 Patient characteristics

	Study 1 $n = 212$	Study 1[a] $n = 86$	Study 2 $n = 11$	Study 3 $n = 20$
Age (years) mean (range)	2 (0.3–5)	3 (0.3–5)	5 (3–10)	5 (1–9)
Body weight (kg) mean (range)	NA	12 (8–17)[b]	17(13–27)	16 (10–25)
Gender ratio (male/female)	107/105	43/43	4/7	12/8
Initial parasitemia (Parasites*$10^3/\mu$l)	7.51 (0.08–383.3)	8.27 (0.4–383.3)	3.08 (0.13–71.6)	>1
Recurrence of parasitemia within 1 month[c]	68	22	0[d]	0[d]

[a] Patients from study 1 included in the pharmacokinetic analysis
[b] Calculated using the formula: BW(kg) = 0.16*AGE(months) +6.8
[c] Recrudescence and reinfections not distinguished
[d] On days 7 and 14
[e] Geometric mean (range)

Table 2 Final parameter estimates of the pharmacokinetic model

Substance	Parameter	Estimate (RSE %)	IIV CV % (RSE%)	Definitions Coefficient of variation (residual standard error)
	ka (h^{-1})	0.13 (31)	100 (33)	Rate of presentation of AQ and *N*-DEAQ
AQ	CL/F_{AQ} ($l\,h^{-1}\,kg^{-1}$)	14 (8)		Oral clearance
	Vc/F_{AQ} ($l\,kg^{-1}$)	11.7 (91)		Volume, central compartment
	Q ($l\,h^{-1}\,kg^{-1}$)	17 (28)		Intercompartment CL
	Vp/F_{AQ} ($l\,kg^{-1}$)	311 (18)		Volume, peripheral compartment
	$\sigma 1$ (%)	41 (23)		Residual proportional error
	$\sigma 2$ (nM)	25 Fixed		Residual additive error
N-DEAQ	$CL/(F_{N-DEAQ})$ ($l\,h^{-1}\,kg^{-1}$)	0.67(10) $\exp^{-0.006(29)*AGE}$	36 (28)	Oral clearance
	$Vc/(F_{N-DEAQ})$ ($l\,kg^{-1}$)	12.8 (44)	139 (42)	Volume, central compartment
	Q ($l\,h^{-1}\,kg^{-1}$)	1.3 (23)		Intercompartment CL
	$Vp/(F_{N-DEAQ})$ ($l\,kg^{-1}$)	62.4 (9)		Volume, peripheral compartment
	$\sigma 1$ (%)	49 (9)		Residual proportional error
	$\sigma 2$ (nM)	25 Fixed		Residual additive error

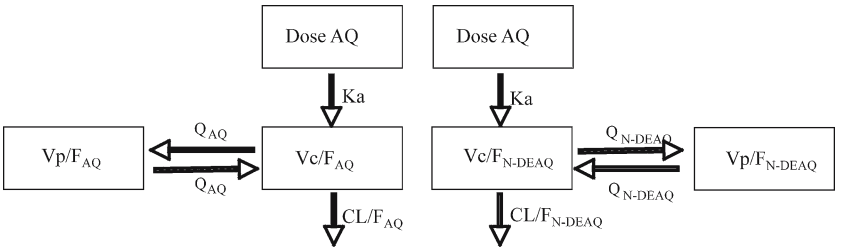

Fig. 2 The population pharmacokinetic model for AQ and *N*-DEAQ. AQ and *N*-DEAQ are introduced in the model through separate dosing compartments with identical molar doses. Both substances share the same rate of presentation, ka, while remaining parameters are estimated separately for AQ and *N*-DEAQ

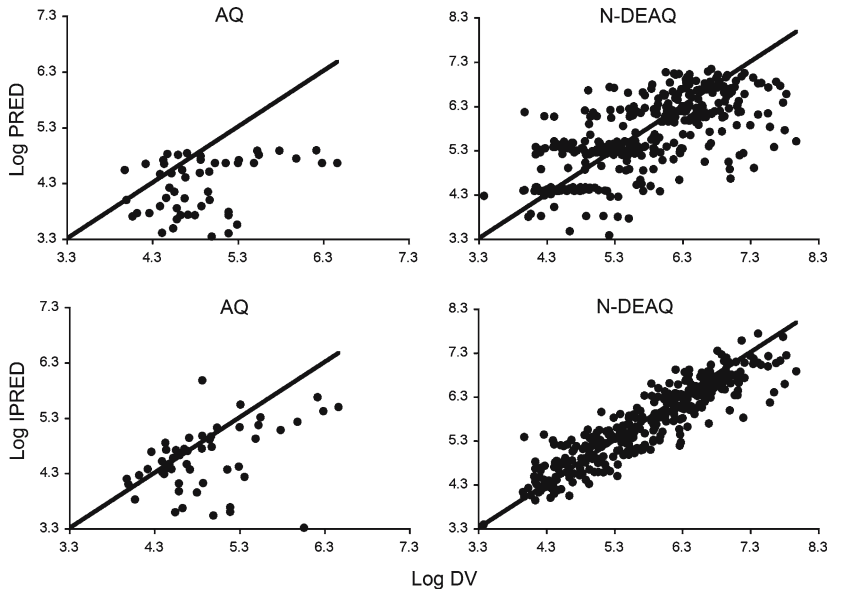

Fig. 3 Goodness of fit plots showing the overall fit of the AQ and *N*-DEAQ-pharmacokinetic model. Concentrations below LLOQ are excluded from the plot

Results

Population pharmacokinetics

Study population characteristics are summarized in Table 1. The pharmacokinetics of AQ and *N*-DEAQ were best described by two parallel two-compartment models with a central and a peripheral compartment for each compound (Fig. 2). Population estimates, inter-and intra-individual variability are presented in Table 2. Figure 3 illustrates the correlation between observed and model predicted concentrations.

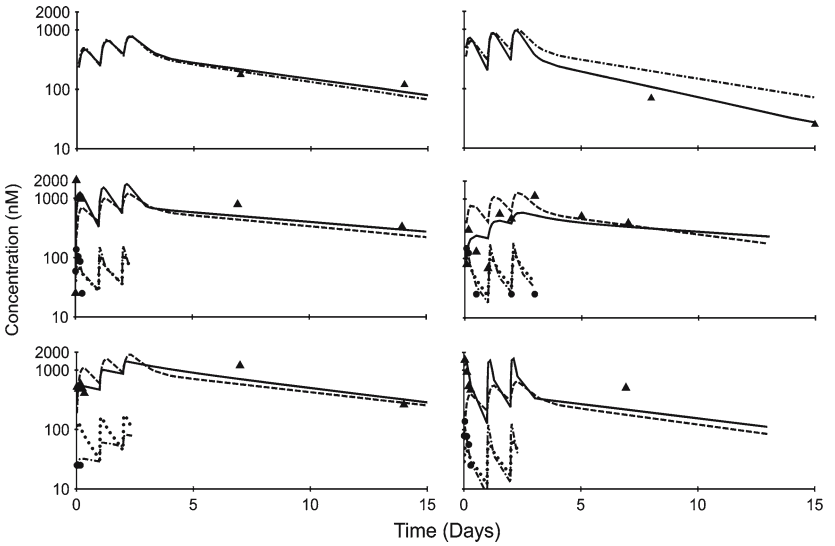

Fig. 4 Concentrations of AQ (*solid circles*) and *N*-DEAQ (*solid triangles*), individual predicted concentrations of AQ (●●●●) and N-DEAQ (*solid line*) and population predictions of AQ (●–●–) and *N*-DEAQ (- - - -) for two subjects from each study. The first concentration following each dose below the LLOQ (50 nM) was set to 1/2LLOQ

In the final model the pharmacokinetics of AQ and *N*-DEAQ were described separately, except for the shared estimate of ka. A model including a systemic conversion of AQ to *N*-DEAQ requires an estimate of the relative bioavailabilities of the two compounds. An attempt was made to estimate the fraction of dose absorbed as AQ (F) and the fraction metabolized, presystemically, to *N*-DEAQ ($1-F$). Given the lack of a priori information on the fraction of *N*-DEAQ formed, initial estimates of F ranged from 0.1 to 0.9. This model resulted in highly variable parameter estimates and the covariance step was not completed.

AQ and *N*-DEAQ both appeared rapidly in the systemic circulation following oral administration (Fig. 4). AQ was detectable within 0.5 h in 8/11 patients in study 2 (frequent sampling). In the remaining three patients no AQ was detected in any sample. *N*-DEAQ was detectable within 1 h in all patients in study 2. A model describing the absorption of AQ and the formation of *N*-DEAQ as consecutive processes did not converge. Simultaneous introduction of AQ and *N*-DEAQ from separate dosing compartments adequately described the data.

It was not possible to distinguish between rate of absorption, ka, and the rate of formation of *N*-DEAQ from AQ. Given the rapid conversion of AQ to *N*-DEAQ the absorption of AQ was assumed to be the rate limiting step thus *ka* was used to describe the rate of systemic presentation of both compounds. The data did not support a lag time in the formation of *N*-DEAQ.

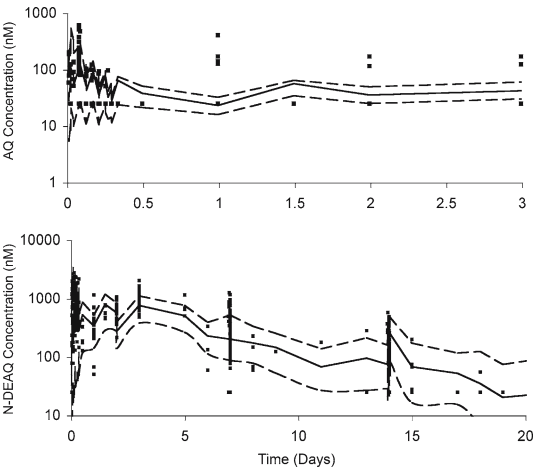

Fig. 5 The visual predictive check illustrating the distribution of observed AQ (first panel) and *N*-DEAQ (second panel) concentrations in relation to their respective simulated 90% prediction interval. Note the different time-scales for parent compound and metabolite

A correlation between study-number and *ka* was suggested by the GAM, however the limited number of samples obtained during the absorption phase precluded a further investigation of this covariate effect. No other influence of study-number or population (Zanzibari or Papua New Guinean) on parameter estimates was identified.

Residual error was best described by a combined additive and proportional error model.

Age was the only significant covariate identified by the GAM. The inclusion of age as a predictor on body weight normalized *N*-DEAQ clearance significantly reduced the OFV ($\Delta_{\mathrm{OFV}} = -12.3$). The relationship was modeled with the function:

$$TVCL_{N-DEAQ}/(F_{N-DEAQ}) = \theta_x \exp^{-\theta_y \times AGE}$$

Exclusion of data from study 1 (unobserved treatment) did not significantly alter the typical parameter estimates.

A visual predictive check is presented in Fig. 5. A total of 13% of the observed AQ concentrations were outside the 5–95 percentile range and the corresponding percentage for *N*-DEAQ was 9%. The condition number for the final model was 465, which indicates that the parameter estimates were not severely influenced by ill-conditioning.

Correlation between drug exposure and parasitemia during follow-up

The difference in patient characteristics and pharmacokinetic parameters between patients who had parasitemia during follow up and patients who had a successful treatment outcome are visualized in box plots (Fig. 6, Table 3).

An association between *N*-DEAQ concentration on day 7 and the risk of parasitemia within one month of treatment was identified in the PK/PD model.

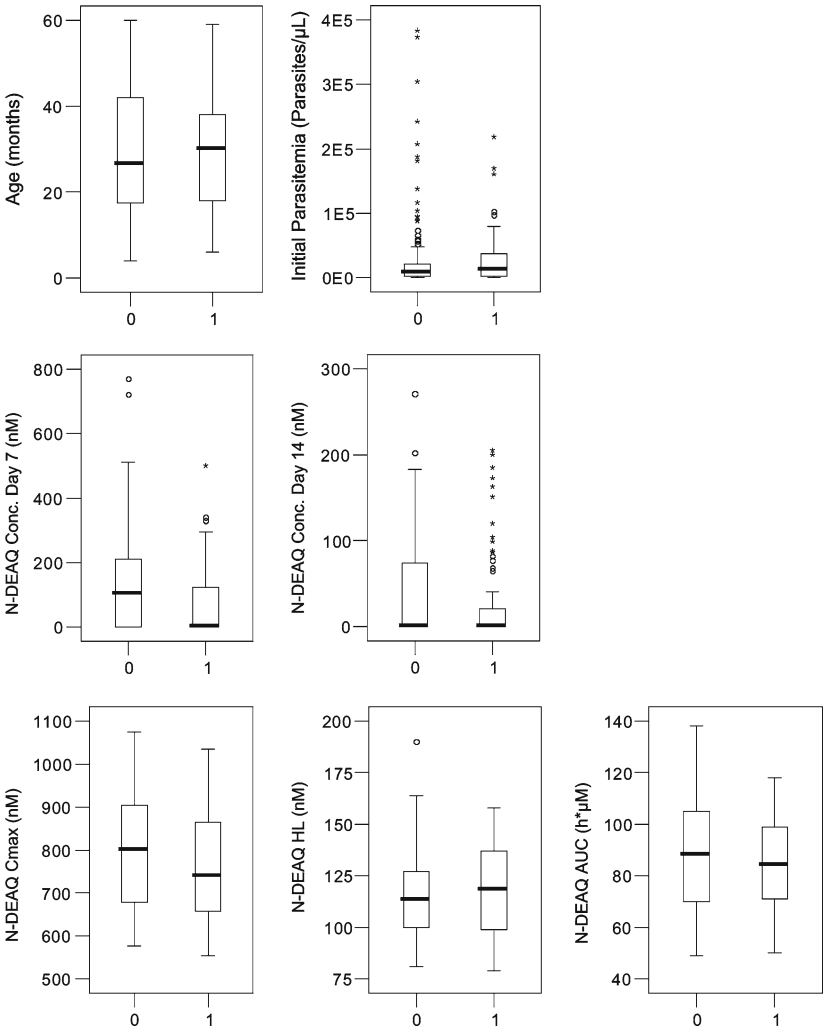

Fig. 6 Box plots illustrating variability in predictors between patients who had no parasitemia (0) and patients who had parasitemia (1), during the first month following treatment (on day 7, 14, and/or 28). Open circles and stars represent outliers and extreme values, respectively

Table 3 Summary of the pharmacokinetic parameters investigated in the pharmacodynamic model

Parameter	*N*	Mean	Range
Blood concentration of *N*-DEAQ on Day 7 (nM)	212	108	0–770
Blood concentration of *N*-DEAQ on Day 14 (nM)	212	37	0–271
Secondary pharmacokinetic parameters of N-DEAQ			
C_{max} (nM)	86	751	528–1,012
$t_{1/2}$ (h)	86	118	79–193
$AUC_{0-\infty}$ (h*μM)	86	88	49–139

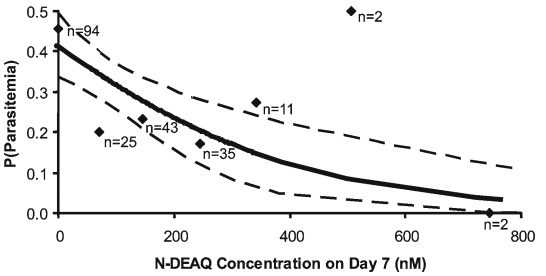

Fig. 7 The risk of recurring parasitemia against the mean *N*-DEAQ concentration on day 7. Observations are binned in increments of 100 nM. The solid line represents the model predicted probability to have parasitemia within one month of treatment initiation (assessed on days 7, 14, and 28) against *N*-DEAQ concentration on day 7. The broken lines represent the 90% prediction interval of the probability curve calculated from estimates obtained from 1,000 bootstrapped datasets

The slope and intercept of the model was −0.0041 (RSE 39%) and −0.36 (RSE 54%), respectively. The influence of observed *N*-DEAQ concentration on day 7 on the probability of having parasitemia within one month of treatment initiation is illustrated in Fig. 7. The model estimated risk of having parasitemia during follow up was 40% in patients with undetectable *N*-DEAQ concentration on day 7. This corresponds well with the actual figure of 45%. The bootstrapped mean (RSE) of the slope and intercept were −0.0044 (39%) and −0.35 (55%), respectively.

As indicated by the changes in OFV shown in Table 4, the inclusion of age, initial parasitemia, observed *N*-DEAQ concentration on day 14, $AUC_{0-\infty}$, C_{max} or $t_{1/2\lambda}$, did not improve the PK/PD model.

Parasite clearance and safety

Among the 212 children in study 1, 68 were found parasite positive during follow up (on days 7–28), 10 on day 7, 20 on day 14 and 47 on day 28. Nine patients were parasite positive on more than one occasion during follow up. No severe manifestations were encountered and all patients therefore received the

Table 4 Difference in OFV with the inclusion of predictors in the logistic PK/PD model

Model	OFV[a]
Age	−0.15
Initial Parasitemia	−0.07
N-DEAQ Concentration on Day 7	−10.09
N-DEAQ Concentration on Day 14	−2.30
$AUC_{0-\infty}$	−0.11
C_{max}	−0.98
$t_{1/2\lambda}$	−0.07

[a] Difference in OFV compared to the reduced intercept model

present second line treatment for complicated malaria in Zanzibar, i.e., artemether-lumefantrine (Coartem). No serious adverse events among the children in the study were reported and there were no treatment related adverse effects reported.

There were no parasitemias detected on days 7 or 14 in study 2. Two patients did not complete follow up on day 14. During follow-up two patients were treated for upper respiratory tract infections, two patients received treatment for urinary tract infection and two children found to have schistosomiasis were treated accordingly. One patient reported abdominal discomfort on day 14 which was assessed as a likely parasitic infection and treated accordingly.

The safety of the amodiaquine treatment in study 3 has been reported earlier by Hombhanje and colleagues [12].

Discussion

The objective of this study was to describe the population pharmacokinetics of AQ and *N*-DEAQ in pediatric patients with uncomplicated malaria. Further we aimed to assess the correlation between the pharmacokinetics of *N*-DEAQ, the active metabolite of AQ, and treatment outcome in these patients. Treatment outcome was described as a dichotomous variable determined by the presence or absence of parasitemia on days of follow up.

AQ and *N*-DEAQ exhibited two-compartment disposition kinetics in pediatric patients with uncomplicated malaria. Pharmacokinetic studies in adult patients have also shown multiexponential profiles for both AQ and *N*-DEAQ [4–6,21]. As indicated in Fig. 3 the individual model-predicted concentrations of *N*-DEAQ correlated well with observed concentrations. The precision in parameter estimates for *N*-DEAQ was adequate (RSE <30%) except pertaining to the volume of the central compartment and the rate of absorption. The pharmacokinetics of AQ was less well described by the model. It was not possible to account for interindividual variability in the AQ pharmacokinetics except in ka. Similar to earlier findings in adults the systemic exposure to AQ was low in comparison to *N*-DEAQ (Fig. 4) [4,5,7]. Even with the frequent sampling schedule used in study 2 no AQ could be detected in three out of 11 patients. Thus the parameter estimates for AQ are based on less data compared to those for *N*-DEAQ which partly explains the lower precision in AQ estimates.

There was some variability in the terminal elimination half-life of *N*-DEAQ in the study populations. The mean terminal elimination half-life was 125 ± 32 (mean $\pm$ sd) and 183 ± 57 h for the Zanzibari patients (Studies 1 and 2) and Papua New Guinean patients (study 3), respectively. Similarly, reported mean terminal elimination plasma half-life of *N*-DEAQ in healthy adults ranges from to 60 h to 311 h [3,4,22].

The pharmacokinetic model includes relatively high proportional residual errors, 41% and 49% for AQ and *N*-DEAQ, respectively. This unexplained intra-individual variability could be caused by model misspecification, noise due to sampling errors and/or interoccasion variability. A high heteroscedastic

residual error component may reduce the possibility to correctly detect and define covariate relationships [16]. The only covariate that could be identified was the effect of age on clearance.

According to our model, weight normalized clearance of *N*-DEAQ decreases with age. A negative correlation between age and weight normalized clearance in pediatric patients has been described for other drugs and is likely to be explained by the nonlinearity in the relationship between the function of the eliminating organs (liver and kidneys) and body weight [23]. In consequence doses normalized to body weight should rather be greater in children than in adults. In the studies conducted in Zanzibar, the dosing of AQ was based on age in accordance with the national treatment policy and as recommended in a recent WHO guideline [24]. Resulting mean dose per body weight was 7.4 mg kg^{-1} day^{-1} in study 2 (where actual body weights were recorded) and 6.2 mg kg^{-1} day^{-1} in study 1 (based on calculated body weights), i.e., lower than the recently described target dose of 7–15 mg kg^{-1} day^{-1}[25]. Further, only five patients in study 1 had estimated concentrations above 135 ng ml^{-1} on day 4, the cut-off concentration associated with a positive outcome of AQ monotherapy according to Aubouy and colleagues [11]. These findings indicate that the currently recommended age based dosing in Zanzibar may result in inadequate exposure to *N*-DEAQ in pediatric patients.

There was a statistically significant, albeit weak, association between *N*-DEAQ concentration on day 7 and clinical outcome within one month following treatment initiation. The inclusion of estimated individual pharmacokinetic parameters of *N*-DEAQ (AUC, C_{max} and $t_{1/2\lambda}$) did not improve the pharmacodynamic model. A possible explanation for the failure to establish a correlation between treatment outcome and pharmacokinetic parameters is the small number of patients in the analysis ($n = 86$).

The cure rate, defined as absence of parasitemia during the first month following treatment, was 68% in study 1. PCR was not performed to distinguish between true recrudescences and reinfections. In an efficacy study conducted at the same locations, and around the same time, the PCR unadjusted 28-day cure rate for the AQ-artesunate combination was 72% while the PCR-adjusted cure rate was 91% [26]. Similarly the unadjusted 28-day cure rate of the combination AQ-artesunate in a clinical trial in pediatric patients in Uganda was 42% while the PCR adjusted cure rate was 100% [27]. Thus frequent reinfections may have caused a lower response rate and obscured the concentration–effect correlation in this study.

Further, this study only addressed the pharmacokinetics and dynamics of *N*-DEAQ despite the fact that patients were treated with both AQ and artesunate. There is a considerable risk of recrudescence associated with short course artesunate monotherapy, thus the long term effect of combination treatments is likely to be attributable largely to the longer acting component [28]. A meta analysis of studies on artesunate combination treatments in Africa indicated that treatment outcomes were correlated to the degree of resistance to the partner drug (SP, AQ and chloroquine) [29]. In the present study, however, the 28-day cure rate in the 94 patients without detectable *N*-DEAQ

concentrations was 55%. A PCR adjusted 28-day cure rate of 72% has been reported in Gabonese children ($n = 50$) following a three day artesunate monotherapy (4 mg/kg) [30]. Artesunate kinetics and dynamics may have had a significant impact on the outcome in this study causing the correlation between *N*-DEAQ concentration and the clinical outcome to be weaker than previously described [11].

It is noteworthy that 44% of patients in study 1 did not have measurable *N*-DEAQ concentrations on day 7. This implies a remarkably low compliance with the current treatment. The relatively high cure rate in these patients (55%), suggests that these patients may have used AS monotherapy. It is possible that an improvement in compliance, and in clinical outcome, would be achieved through the use of a coformulation of AQ+AS.

The findings of this study suggest that the pharmacokinetics of AQ and *N*-DEAQ in pediatric patients are similar to those in adult patients in that AQ is rapidly eliminated from the systemic circulation while *N*-DEAQ has a long terminal half-life exhibiting interindividual variability. A correlation between observed *N*-DEAQ concentrations on day 7 and the risk of recurring parasitemia during the first month after treatment was found. The currently recommended age based dosing schedule in Zanzibar may result in inadequate dosing in pediatric patients and needs to be addressed in further studies.

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