ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2010, p. 2611–2617 Vol. 54, No. 6
0066-4804/10/$12.00 doi:10.1128/AAC.01496-09

Population Pharmacokinetics and Pharmacodynamic Considerations of Amodiaquine and Desethylamodiaquine in Kenyan Adults with Uncomplicated Malaria Receiving Artesunate-Amodiaquine Combination Therapy▽

Vincent Jullien,[1] Bernhards Ogutu,[2] Elizabeth Juma,[3] Gwenaelle Carn,[4] Charles Obonyo,[3] and Jean-René Kiechel[4]*

Université Paris Descartes, Assistance Publique-Hôpitaux de Paris, Service de Pharmacologie Clinique, Groupe Hospitalier Cochin—Saint-Vincent de Paul, Inserm U663, WWARN, Paris, France[1]; Centre for Clinical Research, Kenya Medical Research Institute, Kisumu, Kenya[2]; Centre for Global Health Research, Kenya Medical Institute, Kisumu, Kenya[3]; and Drugs for Neglected Diseases initiative, Geneva, Switzerland[4]

Received 22 October 2009/Returned for modification 19 February 2010/Accepted 28 March 2010

Amodiaquine (AQ) is an antimalarial drug that was frequently combined with artesunate (AS) for the treatment of uncomplicated malaria due to *Plasmodium falciparum* and is now available as a fixed-dose combination. Despite its widespread use, the simultaneous pharmacokinetics in patients of AQ and its active metabolite, desethylamodiaquine (DAQ), were not characterized to date. The pharmacokinetics of AQ and DAQ in 54 adult patients receiving the AS/AQ combination were therefore investigated by the use of a population approach. AQ followed a 1-compartment model with first-order absorption and elimination, as well as a first-order and irreversible transformation into DAQ, which in turn followed a 2-compartment model with first-order elimination from its central compartment. The mean AQ apparent clearance and distribution volume were 3,410 liters/h and 39,200 liters, respectively. The mean terminal elimination half-life of DAQ was 211 h. Body weight was found to explain the interindividual variability of the apparent volume of distribution of AQ and the elimination rate constant of DAQ. A new dosage form consisting of a fixed-dose combination of AS and AQ was found to have no effect on the pharmacokinetic parameters of AQ and DAQ. All patients achieved parasite clearance within 4 days following the initiation of the treatment, which prevented investigation of the possible relationship between DAQ exposure and treatment outcome. This study provided the first simultaneous pharmacokinetic model for AQ and DAQ.

Artemisinin-based combination therapy (ACT) is currently the optimal treatment for uncomplicated malaria due to *Plasmodium falciparum* (8). These combinations comprise an artemisinin derivative and a slowly eliminated partner drug. The former, which is fast acting and has a short half-life, provides a rapid reduction of the parasite biomass, while the latter provides effective concentrations over a prolonged period, allowing the clearance of the residual parasites (15, 23). The high efficacy of these combinations has been documented in several trials (2, 7, 10, 13, 14, 17), and thus, ACTs are now the WHO recommended treatment for uncomplicated *P. falciparum* malaria.

Artesunate (AS) plus amodiaquine (AQ) is one of these recommended ACTs. Amodiaquine is a 4-aminoquinoline which inhibits the glutathione-mediated degradation of ferriprotoporphyrin IX (6). Despite its own efficacy, amodiaquine is considered a prodrug of its active metabolite, desethylamodiaquine (DAQ), since DAQ is eliminated much more slowly than AQ and is therefore the main agent responsible for treatment efficacy (8).

The PK parameters of the drug combined with the artemisinin derivative are of interest since they determine the length of treatment efficacy, as well as the beginning and duration of the selective window, i.e., the period corresponding to concentrations that are not high enough to be efficient against a new infection but are nevertheless sufficient to select resistant parasites (15, 22).

The pharmacokinetics (PK) of AQ/DAQ have been investigated in several studies, including population pharmacokinetics studies (1, 9, 16, 24, 25). However, to date, the PK parameters of DAQ were directly related to the AQ dose, which does not reflect the metabolic transformation of AQ into DAQ. Thus, the possible factors influencing this transformation could not be evaluated. Furthermore, such simplified models cannot be used when applying stringent conditions to predict DAQ concentrations after AQ intake, precluding the evaluation of different dosing regimens via PK simulations.

Therefore, the main objectives of the present study were to describe the pharmacokinetics of AQ and DAQ, taking into account the transformation of AQ into DAQ, and to investigate the possible influence of possibly relevant covariates on the different PK parameters. Among the covariates studied, a new fixed-dose combination (FDC) of AS/AQ, which is expected to improve adherence to the treatment, was evaluated (12). The secondary objective was to investigate the possible

* Corresponding author. Mailing address: Drugs for Neglected Diseases initiative, 15 Chemin Louis-Dunant, 1202 Geneva, Switzerland. Phone and fax: 33 1 64 92 20 65. E-mail: jean-rene.kiechel@wanadoo.fr.
▽ Published ahead of print on 5 April 2010.

TABLE 1. Characteristics of the study population

Parameter	Median	Minimum	Maximum
Age (yrs)	24	17	59
Body wt (kg)	59	39	90
ALAT (unit/liter)	24	2.1	123.1
Hemoglobin (g/100 ml)	12.4	8.7	18.3
Plasmodium count at beginning of treatment	17,180	1,127	109,360
Plasmodium count at end of follow-up[a]	0	0	40,365

[a] *Plasmodium* parasites were detectable in 2 patients.

relationships between AQ/DAQ PK parameters and treatment outcome.

MATERIALS AND METHODS

Patients and treatment. This was an open-label, randomized controlled clinical trial in two parallel groups, a fixed-dose AS/AQ combination therapy group (group A) versus a group receiving AS and AQ administered separately (group B). Subjects aged between 18 and 60 years with acute uncomplicated falciparum malaria defined by a *Plasmodium falciparum* monoinfection of more than 1,000 parasites/μl and either a history of fever in the last 24 h or a measured fever of ≥37.5°C were enrolled. The patients received 3 doses over 3 days with a time interval between 2 consecutive doses of 24 h. The doses consisted of two tablets of AS/AQ (100/270 mg) for the FDC group and 4 tablets of AS (50 mg) plus 4 tablets of AQ (153 mg) for the copackaged group. Possible differences between baseline characteristics of the FDC and the non-FDC group were investigated by a Mann-Whitney test. Blood sampling was performed before the 1st dose, between 15 min and 4 h after the 1st dose, between 15 min and 4 h after the 2nd dose, just before the 3rd dose, between 15 min and 4 h after the 3rd dose, and at days 7, 14, 21, and 28 after the beginning of treatment. This sampling design was determined based on previous knowledge of AQ/DAQ and AS pharmacokinetics in order to characterize the elimination phase of DAQ for at least 3 terminal half-lives and to simultaneously investigate AS and AQ with a minimal number of drawn samples, taking into account the pharmacokinetic differences between these 2 compounds (elimination half-lives of 20 min for AS, 1 h for its active metabolite dihydroartemisinin, and 4 h for AQ). The pharmacokinetic results for AS and its metabolite will be presented in another article.

Parasite density. Parasite density was determined on days 0, 1, 2, 3, 7, 14, 21, and 28 and was calculated by counting the number of asexual parasites against a set number of white blood cells (WBCs), typically 200, in the thick blood film using a hand tally counter. The final result, expressed as the number of asexual parasites per microliter (μl) of blood, was obtained by dividing the number of asexual parasites by the number of WBCs counted and then multiplying by either the measured total white cell count or an assumed WBC density (typically 8,000 WBCs/μl). This quantification of parasites was performed for each malaria blood slide by 2 qualified microscopists, and the average of the 2 results was accepted if the counts were concordant according to the standard operating procedure. In case of a discrepancy of >50% between the counts of the 2 primary slide readers, the quantification was performed by a 3rd microscopist and the final result was obtained by averaging the 2 most concordant counts.

Analytical method. AQ and its metabolite DAQ were separated from human plasma using an Oasis HLB solid-phase cartridge (Waters) under light-protected conditions. They were analyzed by reversed-phase liquid chromatography (XTerra MS C_{18} column, 3.5-μm particle size, 50-mm length by 3-mm inner diameter) and tandem mass spectrometry (Sciex API3000) detection in the turbo ion spray-positive mode. The assay was carried out using a 200-μl sampling volume of human plasma. Both AQ and DAQ were validated for a concentration range from 1 ng · ml^{-1} to 250 ng · ml^{-1} using a $1/X^2$-weighted least squares linear regression (where X is the theoretical concentration). The limit of quantification was 1 ng/ml for both compounds. The squared correlation coefficient determined on 9 batches was >0.99 for the 2 molecules. The assay bias and precision were <6 and 10%, respectively, over the calibration range.

Population pharmacokinetics modeling. Concentration-time data were analyzed by use of the First-Order method with Interaction of the nonlinear mixed effects modeling program NONMEM (version VI 2.0, double precision) (5). A pharmacokinetics model implemented using differential equations (ADVAN 6) was used to fit amodiaquine and desethylamodiaquine data simultaneously. Several structural pharmacokinetic models were investigated. Classical one- and two-compartment models with first-order absorption were evaluated for amodiaquine and desethylamodiaquine. The transformation of amodiaquine into desethylamodiaquine was assumed to be a linear process. This assumption was made based on the fact that the maximal plasma concentrations of AQ are usually 10 times lower than the value of the Michaelis-Menten constant associated to the transformation of AQ to DAQ via cytochrome P450 2C8 (i.e., around 0.290 mg/liter) (18). Since it could not be estimated, the apparent distribution volume of the central compartment of desethylamodiaquine was fixed to the value of 1 liter. Several error models (i.e., proportional, exponential, and additive random effects models) were also investigated as means of describing interpatient and residual variability. Systematic testing for the influence of continuous covariates on the pharmacokinetic parameters was performed by using a generalized model according to the following equation, by using, for example, the apparent clearance (CL/*F*) and body weight (BW): $CL/F = TV(CL/R) \times (BW/\text{median } BW)^{\theta}$, where TV(CL/*F*) was the typical value of the apparent clearance for a patient with the median covariate value and θ was the influential factor for body weight.

The influence of binary covariates (dosage form, sex, and alanine aminotransferase [ALAT]) was investigated as follows: $CL/F = TV(CL/F) \times \theta$, where θ was equal to the influential factor for fixed-dose combination, male, or ALAT >30 U/liter and was otherwise fixed to 1.

The significance of a relationship between a pharmacokinetic parameter and a covariate was assessed by use of the chi-square test of the difference between the objective functions of the basic model (without the covariate) and the model with the covariate. A covariate was retained in the model if it produced a minimum decrease in the objective function of 3.64 units ($P = 0.05$, 1 degree of freedom), if it decreased the interindividual variability of the corresponding pharmacokinetic parameter, and if its effect was biologically plausible. An intermediate multivariate model that included all selected covariates was then obtained. A covariate was retained in the final multivariate model if its deletion from the intermediate model led to a 6.63-point increase in the objective function ($P = 0.01$, 1 degree of freedom). At each step, the goodness of fit was evaluated by using a graph of the weighted residuals versus time after administration of the dose (time) or versus the predicted concentrations.

Model validation. The accuracy and robustness of the final population model were assessed by a visual predictive check. The final population model parameters were used to perform 300 simulations of the database. The 2.5th and the 97.5th percentiles, as well as the 50th percentile (median), of simulated concentrations were plotted against observed concentrations.

Evaluation of the results. Individual estimates of the pharmacokinetic parameters were used to calculate the terminal half-life of desethylamodiaquine ($t_{1/2\beta}$) as follows: $t_{1/2\beta} = \ln 2/\beta$ with $\beta = 0.5 \times \{k_{34} + k_{43} + k_{el} - [(k_{34} + k_{43} + k_{el})^2 - 4 \times k_{43} \times k_{el}]^{1/2}\}$, where β is the slope of the terminal elimination phase, k_{34} and k_{43} are the distribution micro constants of desethylamodiaquine between its central and

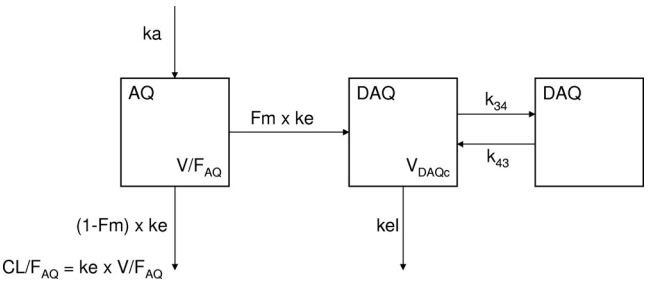

FIG. 1. Representation of the structural pharmacokinetics model. AQ, amodiaquine; DAQ, desethylamodiaquine; ka, absorption rate constant; F, amodiaquine bioavailability; V/F_{AQ}, apparent distribution volume of amodiaquine; CL/F_{AQ}, apparent clearance of amodiaquine; ke, elimination rate constant of amodiaquine; Fm, fraction of amodiaquine converted to desethylamodiaquine; V_{DAQc}, central distribution volume of desethylamodiaquine; kel, elimination rate constant of desethylamodiaquine; k_{34} and k_{43}, distribution rate constants between central and peripheral distribution volumes of desethylamodiaquine.

TABLE 2. Population PK estimates of the base and the final model

Parameter[a]	Base Model		Final Model	
	Mean	SE	Mean	SE
k_{aAQ} (h^{-1})	1.47	0.57	1.41	0.49
CL/F_{AQ} (liter/h)	3,620	659	3,410	540
V/F_{AQ} (liters)	37,500	7,310	39,200	6,350
$\theta BW_{V/F}$	NA	NA	1.72	0.59
FM (%)	0.389	0.121	0.411	0.115
k_{34} (h^{-1})	0.72	0.25	0.66	0.20
k_{43} (h^{-1})	0.0155	0.0033	0.0138	0.0025
k_{el} (h^{-1})	0.200	0.064	0.209	0.060
θBW_{kel}	NA	NA	0.74	0.27
$\omega^2 V/F_{AQ}$	0.311	0.092	0.296	0.089
$\omega^2 k_{el}$	0.0399	0.021	0.029	0.013
σ^2_{AQ}	0.476	0.164	0.444	0.129
σ^2_{DAQ}	0.213	0.034	0.203	0.033

[a] k_{aAQ}, absorption rate constant of AQ; CL/F_{AQ}, apparent clearance of AQ; V/F_{AQ}, apparent distribution volume of AQ; $\theta BW_{V/F}$, factor of influence of BW on AQ V/F; FM, fraction of AQ metabolized into DAQ; k_{34} and k_{43}, distribution rate constants of DAQ; k_{el}, elimination rate constant of DAQ; θBW_{kel}, factor of influence of BW on DAQ k_{el}; $\omega^2 V/F_{AQ}$, interindividual variability of V/F_{AQ}; $\omega^2 k_{el}$, interindividual variability of k_{el}; σ^2_{AQ}, proportional residual error for AQ; σ^2_{DAQ}, proportional residual error for DAQ.

peripheral compartments, and k_{el} is the elimination rate constant of desethylamodiaquine from its central compartment.

The cumulative area under the curve of DAQ (AUC_{DAQ}) between the first intake and the last blood sample (i.e., at day 28) was calculated by integrating DAQ concentration in the central compartment by the use of a dummy compartment.

Possible differences in $t_{1/2\beta}$ and AUC_{DAQ} values between the 2 dosage forms were investigated by a Mann-Whitney test. AUC_{AQ} and AUC_{DAQ} normalized by the dose in mg were also compared by the use of a Mann-Whitney test.

Monte Carlo simulations were performed, using the final model, to simulate the DAQ concentrations that would be achieved at days 3, 7, and 28 after the first dose for each dosage form in 1,000 patients with demographic characteristics similar to those of the patients in the study. The probability of achieving the DAQ concentration of 0.135 mg/liter at day 3 that was previously found to be an efficacy threshold (4) was calculated for the 2 dosage forms.

Relationship between PK and outcome. The chosen efficacy outcome was the parasite reduction ratio (PRR), defined as the ratio between the day 2 and day 0 parasitemias (23). It was anticipated to search for a possible relationship between PRR and AUC_{DAQ} by the use of linear and nonlinear regressions. Parasitemia was also investigated until day 28. In case of recurrence of parasitemia by the end of the follow-up, differentiation between recrudescence (same parasite) and a newly acquired infection (different parasite) was performed via PCR analysis of parasite genotype (21).

RESULTS

Patients and treatments. The population comprised 54 adult patients, 25 men and 29 women. Among them, 26 received the FDC (supplying 540 mg of amodiaquine) and the remaining 28 received amodiaquine (612 mg) and artesunate in 2 separate tablets. The characteristics of the population are displayed in Table 1. No significant difference regarding the baseline characteristics was observed between the FDC and non-FDC groups (not shown).

Population pharmacokinetics modeling. The final model was a 1-compartment model with first-order absorption for amodiaquine with an irreversible transformation into desethylamodiaquine, the disposition of which was characterized by a 2-compartment model with first-order elimination from the central compartment (Fig. 1). A proportional interindividual variability could be estimated only for the apparent distribution volume (V/F) and CL/F of amodiaquine and for the k_{el} of desethylamodiaquine, since including an interindividual variability for the other PK parameters led to unsuccessful convergence. However, the interindividual variability in the CL/F of amodiaquine was estimated with an important uncertainty (i.e., with a standard error of the estimate greater than 50% of the estimate value: 0.068 ± 0.070), so this parameter was also deleted from the model, which did not result in a significant change in the value of the objective function. Body weight was the only significant covariate and was found to explain the interindividual variability of the V/F of AQ and the elimination rate constant of DAQ.

The final covariate submodel, then, was $V/F_{AQ}(\text{liter}) = 39{,}200 \times (BW/59)^{1.72}$, k_{el} (h^{-1}) $= 0.209 \times (BW/59)^{0.74}$. The

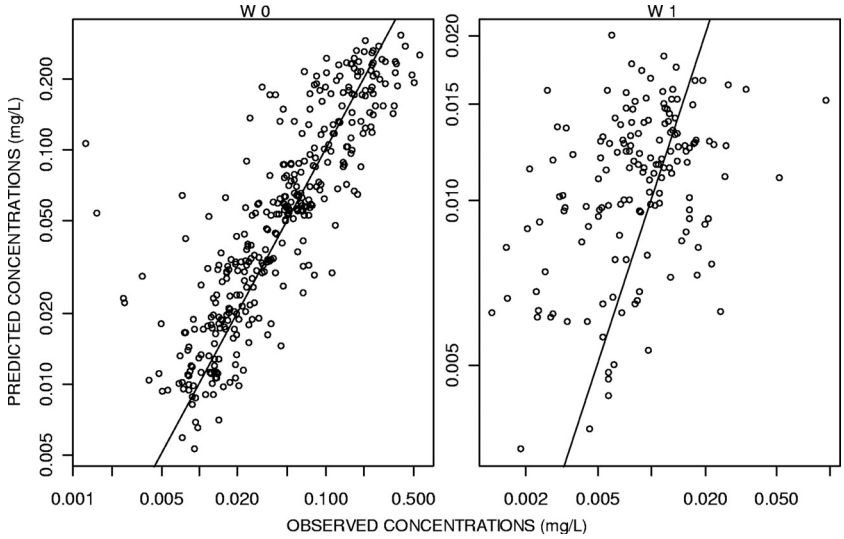

FIG. 2. Observed versus population-predicted concentrations (ng/ml) of amodiaquine (W1) and desethylamodiaquine (W0). The black lines represent the identity ($y = x$) lines. L, liter.

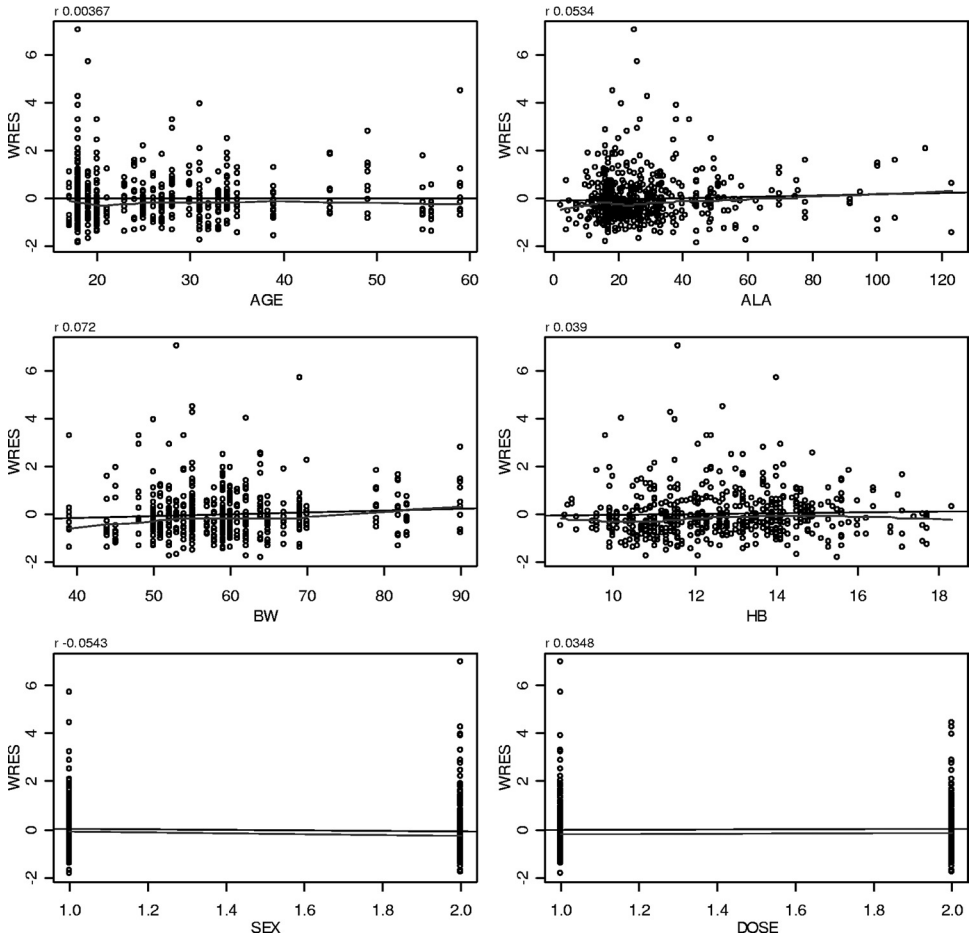

FIG. 3. Lack of residual trend on the weighted residuals (WRES) versus the different covariates: age (years); BW, body weight (kg); HB, hemoglobin (g/dl); ALA, alanine transaminase (unit/ml); sex (1 for men and 2 for women); and dose, dosage form (1 for fixed-dose combination and 2 for non-fixed-dose combination). Solid lines represent the Y = 0 line.

values of all the pharmacokinetic parameters are reported in Table 2.

Goodness of fit with the final model was evidenced by the lack of bias or residual trend on the graphs displaying the observed versus population-predicted concentrations (Fig. 2), the weighted residuals versus the different covariates (Fig. 3), and the weighted residuals versus time after dose and population-predicted concentrations (Fig. 4).

Validation of the model. The nonparametric 95% confidence intervals of the simulated concentrations and the observed concentrations are shown in Fig. 5. The observed AQ and DAQ concentrations were symmetrically distributed around the median, and 1.94% of the AQ concentrations and 4.55% of the DAQ concentrations were outside the confidence interval (Fig. 5).

Evaluation of the results. The mean values ± standard deviations for the terminal half-life of DAQ were 204 ± 22 h in the FDC group and 214 ± 37 h in the non-FDC group (P = 0.58). The mean values ± standard deviations of AUC_{DAQ} were 27.6 ± 3.2 mg · h/liter for the FDC and 32.7 ± 5.5 mg · h/liter for the non-FDC group (P = 0.0005). However, when AUC_{DAQ} was normalized by the dose, no significant difference was observed between the FDC and non-FDC groups (P = 0.73). The mean (standard deviation) simulated DAQ concentrations at days 3, 7, and 28 were 0.115 (0.057), 0.0607 (0.030), and 0.0127 (0.008) mg/liter for the non-FDC form and 0.101 (0.050), 0.054 (0.027), and 0.011 (0.007) for the FDC form. The probability of achieving a DAQ concentration of 0.135 mg/liter at day 3 was 35% for the non-FDC form and 23% for the FDC form. The evolution of AUC_{DAQ} or DAQ $t_{1/2\beta}$ with respect to BW for the FDC and non-FDC groups is displayed in Fig. 6.

Treatment outcome. All but 2 patients achieved complete parasite clearance within 2 days of the beginning of treatment. The 2 patients with PRR values of >0 on day 2 had cumulative AUC_{DAQ}s of 28.4 and 27.2 mg · h/liter. Complete parasite clearance was obtained for all patients at day 4. Nevertheless, a recurrence of parasitemia had occurred in two patients at day 28 which in both cases was secondary to a newly acquired infection. These 2 patients had cumulative AUC_{DAQ}s of 28.4 and 27.2 mg · h/liter, DAQ $t_{1/2\beta}$s of 212 and 214 h, and measured day 28 DAQ concentrations of 0.0119 and 0.0093 mg/liter. All of these values were in the ranges of the values observed in the rest of the study population of 20.5 to 45.2 mg · h/liter for AUC_{DAQ}, 159 to 319 h for DAQ $t_{1/2\beta}$, and 0.0040 to 0.045 mg/liter for day 7 DAQ concentration.

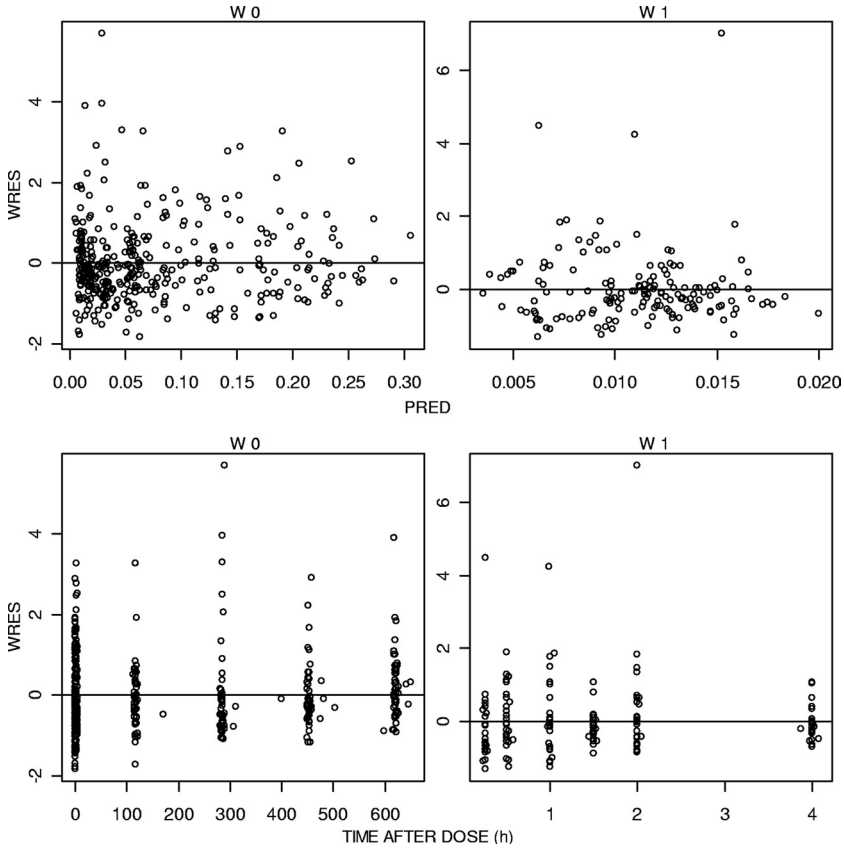

FIG. 4. Weighted residuals (WRES) versus time after dose (h) or population-predicted concentrations (PRED, mg/liter) for amodiaquine (W1) and desethylamodiaquine (W0).

DISCUSSION

This is the first pharmacokinetic analysis which simultaneously fitted AQ and DAQ concentrations and modeled the transformation of AQ into DAQ, as previously published population PK studies directly related the AQ dose to the DAQ concentration without considering the transformation of AQ into DAQ (1, 9). Although DAQ PK were accurately described in these studies, the model we developed is of interest because, by taking into account the metabolic transformation of AQ into DAQ, it allows for investigation of the influence of the AQ dose on the DAQ concentration. The PK parameters estimated are in the range of previously published results (Table 3), although the elimination half-life of AQ tended to be longer than the previously reported values. This might be a consequence of our study design, since no concentration was available more than 4 h after drug intake, whereas previous studies were based on rich sampling up to 12 h after the administration of AQ (12, 16, 24, 25).

An important residual variability was obtained for AQ, which can be explained by 2 measured concentrations that were much higher than the concentrations obtained from similar samples from other subjects. When these concentrations were discarded from the database, the corresponding σ^2 indeed decreased to 0.261, while the values of the other parameters were not substantially modified (not shown). These concentrations were nevertheless kept in the database as they could not be explained by a protocol violation or an analytical error. However, AQ concentrations were satisfyingly fitted, so the model appears reliable for the prediction of AQ concentrations, at least during the 4 hours following drug intake.

Fever, a surrogate marker of disease severity, was previously found to be related to the variability of the distribution volume of some antimalarial drugs, like mefloquine (3, 19). This covariate could not be investigated in the present study since self-medication with acetaminophen was not controlled. However, the lack of a residual trend between baseline hemoglobin and the weighted residuals (Fig. 3) suggests that disease severity does not explain the interindividual variation in AQ/DAQ PK in the present population.

On the other hand, body weight was found to significantly explain this interindividual variability, which suggests the use of a weight-based regimen, as DAQ exposure is inversely correlated to BW (Fig. 6). However, the excellent efficacy of the AS/AQ combination observed in the present study does not support such an approach. It is likely that the administered AQ dose was large enough to balance the PK variability due to BW in the population of the study and to provide to each subject a DAQ exposure corresponding to a maximal efficacy. This assumption should nevertheless be confirmed in a larger panel of patients. This very satisfactory efficacy precluded the investigation of the possible relationship between DAQ exposure and treatment outcome, and it is worthy of note that the 2 patients with slightly lower PRRs

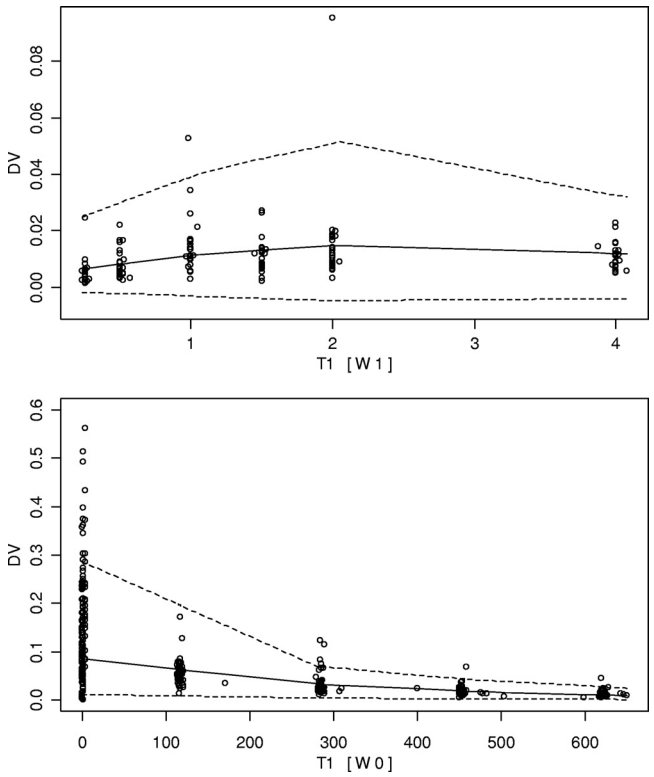

FIG. 5. Visual predictive checks for amodiaquine (W1) and desethylamodiaquine (W0). The solid lines show the median values, and the dotted lines show the 95% confidence intervals. DV, observed concentrations (mg/liter); T1, time after dose (h).

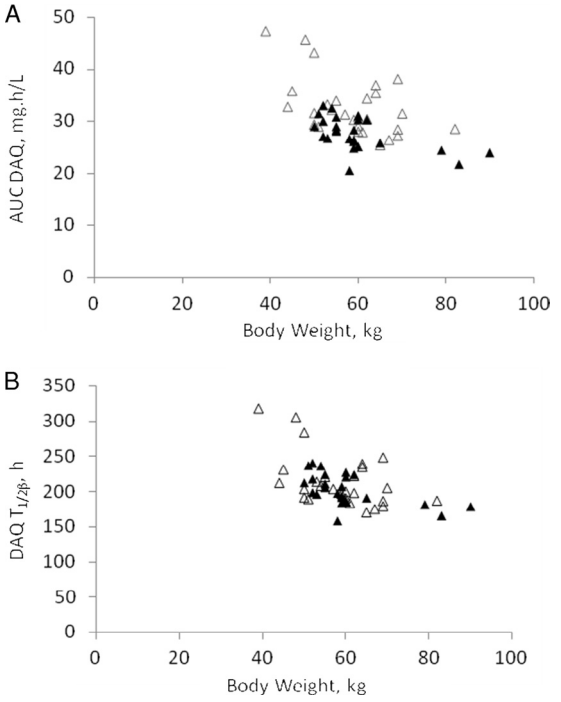

FIG. 6. Cumulative AUC of DAQ (A) and terminal half-life of DAQ (B) with respect to BW for both the non-FDC (open triangles) and the FDC (closed triangles) forms of administration.

and the 2 patients who experienced a new infection at the end of the 28-day follow-up were not characterized by a noticeable low exposure or short elimination half-life of DAQ. It therefore seems unlikely that these events were explained by an unusual PK profile of AQ/DAQ.

The occurrence of reinfection is a marker of high transmission rates in studies in western Kenya, and this has been seen with other antimalarials as well. However, the delayed parasite clearance may be explained by the fact that AQ has been used extensively in the study area; therefore, there may be parasites with high tolerance to AQ (11).

The PK parameters were not influenced by the form of dosage, indicating that AQ bioavailability is not modified by FDC, a result that was recently evidenced in healthy volunteers (12). The slightly lower DAQ exposure (i.e., −15%) obtained with the FDC, which is simply explained by the difference in the amount of AQ administered in the two forms (i.e., 12%), does not seem to be clinically relevant, based on the good observed therapeutic efficacy. The present study confirms in this specific patient population of adults the potential value of the AS/AQ fixed-dose combination since it seems to provide an efficacy similar to that of the non-FDC form and should, in addition, based on the simplicity of the administration schedule, improve adherence to treatment in a real-life context. Similar very good efficacy results for the FDC in children of various ages and in adult patients in other regions have been reported recently (13, 20). Our results also showed that a low percentage of patients would achieve the DAQ efficacy threshold previously determined for amodiaquine monotherapy in uncomplicated *Plasmodium falciparum* malaria with the current doses. This discrepancy confirms that the relationship between DAQ concentration and efficacy is significantly modified by artesunate, which was strongly suggested by the synergistic clinical efficacy of ACTs.

In conclusion, the present study provides the first simultaneous pharmacokinetic model for amodiaquine and its main active metabolite, desethylamodiaquine, in patients with uncomplicated malaria due to *Plasmodium falciparum*. The pharmacokinetic/pharmacodynamic profile of amodiaquine when combined with artesunate should be investigated in a larger cohort, as well as in the target population of young children.

TABLE 3. Comparison with previous results[a]

Source of results	$t_{1/2AQ}$ (h)	CL/F_{AQ} (liter/h)	$t_{1/2\alpha DAQ}$ (h)	$t_{1/2\beta DAQ}$ (h)
Present study	7.9	3,410	0.79	211
Navaratnam et al. (12)	2.3 ± 1.4	2,504 ± 2,000[b]	NA	201 ± 119
Orrell et al. (16)	3.9 ± 1.2	5,160 ± 1,560	NA	136.9 ± 83.8
Winstanley et al. (25)	3.7 ± 1.3	NA	NA	60
Winstanley et al. (24)	5.2 ± 1.7	6,060 ± 1,212[b]	NA	NA

[a] Values are means ± standard errors. $t_{1/2AQ}$, elimination half-life of amodiaquine; CL/F_{AQ}, apparent clearance of amodiaquine; $t_{1/2\alpha DAQ}$, distribution half-life of desethylamodiaquine; $t_{1/2\beta DAQ}$, terminal half-life of desethylamodiaquine; NA, not applicable.

[b] Values are derived from reported doses and AUCs.

ACKNOWLEDGMENTS

The present study was supported by DNDi (Drugs For Neglected Diseases initiative).

The contribution of the whole study team at Kenya Medical Research Institute, Kisumu, Kenya, is gratefully acknowledged. We thank the team for their dedication to the study and for the careful management of the patients. We thank Synexel Laboratories for performing the bioanalytical determination and Sanofi-Aventis which supported the bioanalytical development of Synexel for providing stable-isotope-labeled internal standards for bioanalytical work, as well as the study drugs. We thank also Julie A. Simpson for her contribution to the protocol design and the members of the FACT team for their contributions to the study design discussion. Patient listings were prepared by Cardinal Systems, Paris, France, which is in charge of the clinical study report.

The study has been published with the permission of the Director of the Kenya Medical Research Institute.

REFERENCES

1. **Adjei, G. O., K. Kristensen, B. Q. Goka, L. C. Hoegberg, M. Alifrangis, O. P. Rodrigues, and J. A. Kurtzhals.** 2008. Effect of concomitant artesunate administration and cytochrome P4502C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria. Antimicrob. Agents Chemother. **52:**4400–4406.
2. **Adjei, G. O., J. A. Kurtzhals, O. P. Rodrigues, M. Alifrangis, L. C. Hoegberg, E. D. Kitcher, E. V. Badoe, R. Lamptey, and B. Q. Goka.** 2008. Amodiaquine-artesunate vs artemether-lumefantrine for uncomplicated malaria in Ghanaian children: a randomized efficacy and safety trial with one year follow-up. Malar. J. **7:**127.
3. **Ashley, E. A., K. Stepniewska, N. Lindegardh, R. McGready, R. Hutagalung, R. Hae, P. Singhasivanon, N. J. White, and F. Nosten.** 2006. Population pharmacokinetic assessment of a new regimen of mefloquine used in combination treatment of uncomplicated falciparum malaria. Antimicrob. Agents Chemother. **50:**2281–2285.
4. **Aubouy, A., M. Bakary, A. Keundjian, B. Mbomat, J. R. Makita, F. Migot-Nabias, M. Cot, J. Le Bras, and P. Deloron.** 2003. Combination of drug level measurement and parasite genotyping data for improved assessment of amodiaquine and sulfadoxine-pyrimethamine efficacies in treating Plasmodium falciparum malaria in Gabonese children. Antimicrob. Agents Chemother. **47:**231–237.
5. **Beal, S. L., and L. B. Sheiner.** 1991. NONMEM user's guide. NONMEM Project Group, University of California at San Francisco, San Francisco, CA.
6. **Famin, O., and H. Ginsburg.** 2002. Differential effects of 4-aminoquinoline-containing antimalarial drugs on hemoglobin digestion in Plasmodium falciparum-infected erythrocytes. Biochem. Pharmacol. **63:**393–398.
7. **Faucher, J. F., A. Aubouy, A. Adeothy, G. Cottrell, J. Doritchamou, B. Gourmel, P. Houze, H. Kossou, H. Amedome, A. Massougbodji, M. Cot, and P. Deloron.** 2009. Comparison of sulfadoxine-pyrimethamine, unsupervised artemether-lumefantrine, and unsupervised artesunate-amodiaquine fixed-dose formulation for uncomplicated plasmodium falciparum malaria in Benin: a randomized effectiveness noninferiority trial. J. Infect. Dis. **200:**57–65.
8. **German, P. I., and F. T. Aweeka.** 2008. Clinical pharmacology of artemisinin-based combination therapies. Clin. Pharmacokinet. **47:**91–102.
9. **Hietala, S. F., A. Bhattarai, M. Msellem, D. Roshammar, A. S. Ali, J. Stromberg, F. W. Hombhanje, A. Kaneko, A. Bjorkman, and M. Ashton.** 2007. Population pharmacokinetics of amodiaquine and desethylamodiaquine in pediatric patients with uncomplicated falciparum malaria. J. Pharmacokinet. Pharmacodyn. **34:**669–686.
10. **Kobbe, R., P. Klein, S. Adjei, S. Amemasor, W. N. Thompson, H. Heidemann, M. V. Nielsen, J. Vohwinkel, B. Hogan, B. Kreuels, M. Buhrlen, W. Loag, D. Ansong, and J. May.** 2008. A randomized trial on effectiveness of artemether-lumefantrine versus artesunate plus amodiaquine for unsupervised treatment of uncomplicated Plasmodium falciparum malaria in Ghanaian children. Malar. J. **7:**261.
11. **Mbaisi, A., P. Liyala, F. Eyase, R. Achilla, H. Akala, J. Wangui, J. Mwangi, F. Osuna, U. Alam, B. L. Smoak, J. M. Davis, D. E. Kyle, R. L. Coldren, C. Mason, and N. C. Waters.** 2004. Drug susceptibility and genetic evaluation of Plasmodium falciparum isolates obtained in four distinct geographical regions of Kenya. Antimicrob. Agents Chemother. **48:**3598–3601.
12. **Navaratnam, V., S. Ramanathan, M. S. Wahab, G. Siew Hua, S. M. Mansor, J. R. Kiechel, M. Vaillant, W. R. Taylor, and P. Olliaro.** 2009. Tolerability and pharmacokinetics of non-fixed and fixed combinations of artesunate and amodiaquine in Malaysian healthy normal volunteers. Eur. J. Clin. Pharmacol. **65:**809–821.
13. **Ndiaye, J. L., M. Randrianarivelojosia, I. Sagara, P. Brasseur, I. Ndiaye, B. Faye, L. Randrianasolo, A. Ratsimbasoa, D. Forlemu, V. A. Moor, A. Traore, Y. Dicko, N. Dara, V. Lameyre, M. Diallo, A. Djimde, A. Same-Ekobo, and O. Gaye.** 2009. Randomized, multicentre assessment of the efficacy and safety of ASAQ—a fixed-dose artesunate-amodiaquine combination therapy in the treatment of uncomplicated Plasmodium falciparum malaria. Malar, J. **8:**125.
14. **Nosten, F., M. van Vugt, R. Price, C. Luxemburger, K. L. Thway, A. Brockman, R. McGready, F. ter Kuile, S. Looareesuwan, and N. J. White.** 2000. Effects of artesunate-mefloquine combination on incidence of Plasmodium falciparum malaria and mefloquine resistance in western Thailand: a prospective study. Lancet **356:**297–302.
15. **Nyunt, M. M., and C. V. Plowe.** 2007. Pharmacologic advances in the global control and treatment of malaria: combination therapy and resistance. Clin. Pharmacol. Ther. **82:**601–605.
16. **Orrell, C., F. Little, P. Smith, P. Folb, W. Taylor, P. Olliaro, and K. I. Barnes.** 2008. Pharmacokinetics and tolerability of artesunate and amodiaquine alone and in combination in healthy volunteers. Eur. J. Clin. Pharmacol. **64:**683–690.
17. **Owusu-Agyei, S., K. P. Asante, R. Owusu, M. Adjuik, S. Amenga-Etego, D. K. Dosoo, J. Gyapong, B. Greenwood, and D. Chandramohan.** 2008. An open label, randomised trial of artesunate+amodiaquine, artesunate+chlorproguanil-dapsone and artemether-lumefantrine for the treatment of uncomplicated malaria. PLoS One **3:**e2530.
18. **Parikh, S., J. B. Ouedraogo, J. A. Goldstein, P. J. Rosenthal, and D. L. Kroetz.** 2007. Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. Clin. Pharmacol. Ther. **82:**197–203.
19. **Simpson, J. A., R. Price, F. ter Kuile, P. Teja-Isavatharm, F. Nosten, T. Chongsuphajaisiddhi, S. Looareesuwan, L. Aarons, and N. J. White.** 1999. Population pharmacokinetics of mefloquine in patients with acute falciparum malaria. Clin. Pharmacol. Ther. **66:**472–484.
20. **Sirima, S. B., A. B. Tiono, A. Gansane, A. Diarra, A. Ouedraogo, A. T. Konate, J. R. Kiechel, C. C. Morgan, P. L. Olliaro, and W. R. Taylor.** 2009. The efficacy and safety of a new fixed-dose combination of amodiaquine and artesunate in young African children with acute uncomplicated Plasmodium falciparum. Malar. J. **8:**48.
21. **Snounou, G., and H. P. Beck.** 1998. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. Parasitol. Today **14:**462–467.
22. **Stepniewska, K., and N. J. White.** 2008. Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. Antimicrob. Agents Chemother. **52:**1589–1596.
23. **White, N. J.** 1997. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. Antimicrob. Agents Chemother. **41:**1413–1422.
24. **Winstanley, P., G. Edwards, M. Orme, and A. Breckenridge.** 1987. The disposition of amodiaquine in man after oral administration. Br. J. Clin. Pharmacol. **23:**1–7.
25. **Winstanley, P. A., O. Simooya, J. M. Kofi-Ekue, O. Walker, L. A. Salako, G. Edwards, M. L. Orme, and A. M. Breckenridge.** 1990. The disposition of amodiaquine in Zambians and Nigerians with malaria. Br. J. Clin. Pharmacol. **29:**695–701.