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Population Pharmacokinetic and Pharmacodynamic Modeling of Amodiaquine and Desethylamodiaquine in Women with *Plasmodium vivax* Malaria during and after Pregnancy

Joel Tarning,[a,b] Palang Chotsiri,[a] Vincent Jullien,[c] Marcus J. Rijken,[d] Martin Bergstrand,[e] Mireille Cammas,[c] Rose McGready,[a,b,d] Pratap Singhasivanon,[f] Nicholas P. J. Day,[a,b] Nicholas J. White,[a,b] Francois Nosten,[a,b,d] and Niklas Lindegardh[a,b†]

Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand[a]; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, United Kingdom[b]; Université Paris Descartes, INSERM U663, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Vincent de Paul, Paris, France[c]; Shoklo Malaria Research Unit, Mae Sot, Thailand[d]; Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden[e]; and Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand[f]

Amodiaquine is effective for the treatment of *Plasmodium vivax* malaria, but there is little information on the pharmacokinetic and pharmacodynamic properties of amodiaquine in pregnant women with malaria. This study evaluated the population pharmacokinetic and pharmacodynamic properties of amodiaquine and its biologically active metabolite, desethylamodiaquine, in pregnant women with *P. vivax* infection and again after delivery. Twenty-seven pregnant women infected with *P. vivax* malaria on the Thai-Myanmar border were treated with amodiaquine monotherapy (10 mg/kg/day) once daily for 3 days. Nineteen women, with and without *P. vivax* infections, returned to receive the same amodiaquine dose postpartum. Nonlinear mixed-effects modeling was used to evaluate the population pharmacokinetic and pharmacodynamic properties of amodiaquine and desethylamodiaquine. Amodiaquine plasma concentrations were described accurately by lagged first-order absorption with a two-compartment disposition model followed by a three-compartment disposition of desethylamodiaquine under the assumption of complete *in vivo* conversion. Body weight was implemented as an allometric function on all clearance and volume parameters. Amodiaquine clearance decreased linearly with age, and absorption lag time was reduced in pregnant patients. Recurrent malaria infections in pregnant women were modeled with a time-to-event model consisting of a constant-hazard function with an inhibitory effect of desethylamodiaquine. Amodiaquine treatment reduced the risk of recurrent infections from 22.2% to 7.4% at day 35. In conclusion, pregnancy did not have a clinically relevant impact on the pharmacokinetic properties of amodiaquine or desethylamodiaquine. No dose adjustments are required in pregnancy.

The total global annual burden of *P. vivax* infections has been estimated at between 80 and 400 million cases, and about 3 billion people are at risk, mainly in Central and Southeast Asia (91%) (7, 16). Approximately 93 million pregnancies occur in areas where *P. vivax* is endemic, of which 40 million are in temperate regions with *P. vivax* transmission only (i.e., no *P. falciparum* transmission) (12). Vivax malaria rarely causes mortality but is associated with multiple relapses, anemia, abortion, and a reduction in birth weight in pregnant women with malaria (5, 32, 39, 47). Pregnant women with *P. vivax* infections are also more likely to experience relapses than nonpregnant women (39). Low birth weight increases the risk of infant mortality and may also have adverse consequences in the longer term (13).

Relapses of *P. vivax* arise from dormant liver stages (i.e., hypnozoites). Primaquine is the only generally available antimalarial drug with a parasiticidal effect on this dormant liver stage of the pathogen (61). However, primaquine may cause hemolysis and is not considered safe in the treatment of *P. vivax* malaria during pregnancy (42). Chloroquine has traditionally been used as prophylaxis and treatment of *P. vivax* malaria during pregnancy, but the increasing prevalence of resistance to chloroquine emphasizes the need for alternative safe and effective antimalarial drugs, especially during pregnancy (6, 46). Amodiaquine, like chloroquine, is a 4-aminoquinoline, but it is more effective in treating chloroquine-resistant strains of *P. vivax* (10). A study of 900 pregnant women with *P. falciparum* malaria receiving repeated amodiaquine treatments (600 mg for the first 2 days followed by 300 mg on the third day) alone or in combination with sulfadoxine-pyrimethamine (1,500 mg sulfadoxine and 75 mg pyrimethamine on the first day), sulfadoxine-pyrimethamine alone, or chloroquine alone (600 mg for the first 2 days followed by 300 mg on the third day) showed no liver toxicity or bone marrow depression (55). Spontaneous miscarriages, stillbirths, perinatal deaths, and neonatal deaths were similar among the four treatment groups. However, the proportion of women that were parasitemic at day 28 after drug administration was 5% in the amodiaquine group compared to 10% in the chloroquine group (55). Therefore, amodiaquine has been suggested as a safe and effective regimen for intermittent preventive treatment during pregnancy (54, 56).

Amodiaquine is rapidly absorbed after oral administration and extensively metabolized by the cytochrome P450 isoenzyme 2C8 (CYP2C8) to its active metabolite desethylamodiaquine (28, 41). Only a small fraction of amodiaquine (approximately 0.06% over

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Address correspondence to Joel Tarning, joel@tropmedres.ac.
† Deceased 18 January 2012.

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4 days) and desethylamodiaquine (approximately 0.02% over 8 days) is eliminated unchanged in the urine (64). Desethylamodiaquine is metabolized *in vivo* into its inactive metabolite bis-desethylamodiaquine by an unknown route (26). It has been suggested that the extrahepatic CYP1A1 is responsible for the *in vitro* metabolism of desethylamodiaquine to unknown other metabolites (14).

Amodiaquine and desethylamodiaquine are extensively bound (90 to 95%) to plasma proteins (43). Desethylamodiaquine, but not amodiaquine, is accumulated in erythrocytes, with an erythrocyte-to-plasma ratio of 3:1 in healthy subjects. The accumulation of desethylamodiaquine in erythrocytes is lower in patients with malaria (erythrocyte-to-plasma ratio of 0.8:1), with a gradual increase to healthy levels during recovery (65).

The pharmacokinetic properties of amodiaquine and desethylamodiaquine have been investigated in healthy volunteers, patients with *P. falciparum* malaria, and children (2, 17, 20, 23, 37, 38, 40, 45, 52, 53, 60, 64, 65). A multiphasic exponential decline has been suggested for both amodiaquine and desethylamodiaquine, with terminal elimination half-lives of approximately 10 h and 10 days, respectively (25). Thus, desethylamodiaquine provides approximately 100-fold-higher total drug exposure than amodiaquine and therefore contributes most of the antimalarial effect in amodiaquine treatment (45).

As pregnancy progresses, there are reduced gastrointestinal motility, increased blood volume, increased water content, decreased concentration of plasma protein, changed hormone content, and increased renal blood flow (11). The activities of the cytochrome P450 isoenzymes CYP2A6, CYP2C9, and CYP2D6 and the UDP glucuronosyltransferase (UGT) isoenzymes UGTA4 and UGT2B7 are reported to increase during pregnancy (4). In contrast, CYP1A2 and CYP2C19 activities are reduced in pregnant women (4). These physiological and enzymatic alterations might have important effects on the pharmacokinetic properties of several antimalarial drugs and consequently the clinical outcome. However, CYP2C8 and CYP1A1 activities have not been reported to be altered during pregnancy (14). A noncompartmental analysis of amodiaquine and desethylamodiaquine reported no significant pharmacokinetic difference in pregnant women and postpartum women (48). Studies in pregnant women with malaria reported relatively unchanged pharmacokinetic properties of chloroquine and quinine (1, 27). However, a recent publication reported significantly lower exposure of both chloroquine and its active metabolite, desethylchloroquine, when administered as intermittent presumptive treatment in pregnancy for malaria (24). Altered pharmacokinetic properties of artesunate, artemether, dihydroartemisinin, sulfadoxine, atovaquone, proguanil, cycloguanil, lumefantrine, and piperaquine have been observed in pregnant women compared to nonpregnant women (15, 31, 33–35, 49, 57–58).

Pharmacokinetic results from a noncompartmental analysis of this study have been reported elsewhere (48). Here, the pharmacokinetic and pharmacodynamic properties of amodiaquine and desethylamodiaquine were addressed by using nonlinear mixed-effects modeling. The aim of this study was to investigate the effect of pregnancy on the population pharmacokinetic and pharmacodynamic properties of amodiaquine and desethylamodiaquine in the treatment of *P. vivax* infection in the second and third trimesters of pregnancy and again after delivery.

MATERIALS AND METHODS

Study design. This study was conducted in an area of low seasonal malaria transmission along the northwestern border of Thailand. Clinical details and noncompartmental analysis results are reported in full elsewhere (48). The study was carried out at weekly antenatal clinics at the Shoklo Malaria Research Unit (SMRU). Study approval was obtained by the ethics committee of the Faculty of Tropical Medicine, Bangkok (MUTM 2007-112), and the Oxford Tropical Research Ethics Committee (OxTREC 024-06). Women in the second or third trimester of pregnancy with a *P. vivax* monoinfection were invited to participate in the study. The study was explained to patients in their own language, and written consent was obtained. Full medical history, physical examination, baseline drug levels, complete blood count, blood glucose, blood group, parasite count, and parasite culture were measured at admission. Thick and thin blood films were stained with Giemsa, and parasite density was expressed per 500 white blood cells.

Patients stayed at the inpatient departments in Wang Pha and Mawker Thai, Thailand, for 5 days and were monitored weekly in the antenatal clinics until delivery. All patients were scheduled to visit again 3 months postpartum.

Drug regimen. Women enrolled in this study received a daily dose of oral amodiaquine (Flavoquine; 10 mg/kg/day; Sanofi-Aventis, France) for 3 days at 0, 24, and 48 h. Amodiaquine was given with water, and drug administration was directly observed. The exact dose and dosing times were recorded and used in the modeling. Patients who vomited a dose within 60 min after administration were re-treated but excluded from pharmacokinetic sampling.

Blood samples. Venous blood samples (2 ml) were drawn into lithium heparin tubes from a catheter during the first 3 days and thereafter by venous puncture. Blood samples were taken at 0, 4, 24, 28, 48, 48.5, 49, 50, 51, 52, 54, 56, 58, and 72 h after initial drug administration. Additional blood samples were drawn at 4, 5, 7, 14, 21, 28, 35, and 42 days after initial drug administration. The exact sample times were recorded and used in the modeling. Blood samples were centrifuged at 1,500 to 2,000 $\times$ *g* at room temperature for 10 min and the plasma stored at −20°C. The samples were transferred within 2 months to a −80°C freezer and thereafter shipped to the Service de Pharmacologie Clinique, Hôpital St Vincent de Paul, Paris, France, on dry ice for drug analysis.

Amodiaquine and desethylamodiaquine quantification. Amodiaquine and desethylamodiaquine plasma samples were analyzed by using protein precipitation with acetonitrile and quantification by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (18). Hydroxychloroquine was used as the internal standard. Amodiaquine and desethylamodiaquine were quantified using a TSQ Discovery Max triple-quadrupole mass spectrometer (Thermo Finnigan, West Palm Beach, FL) operated in the positive-ion mode. Quantification was performed using selected reaction monitoring (SRM) for the transitions *m/z* 356–283 and 328–283 for amodiaquine and desethylamodiaquine, respectively, and 336–247 for the internal standard hydroxychloroquine. Total assay coefficients of variation (CV) for amodiaquine and desethylamodiaquine during analysis were less than 15% at all quality control levels (1, 5, 8, 25, and 80 ng/ml for amodiaquine and 2, 10, 16, 50, and 400 ng/ml for desethylamodiaquine). The lower limit of quantification (LLOQ) was set to 1 ng/ml and 2 ng/ml for amodiaquine and desethylamodiaquine, respectively.

Pharmacokinetic analysis. Amodiaquine and desethylamodiaquine plasma concentrations were transformed into their natural logarithms. The concentration-time profiles were characterized using nonlinear mixed-effects modeling and the first-order conditional estimation method with interaction in NONMEM version VI (Icon Development Solutions, Ellicott City, MD). Census version 1.1 (62), Perl-speaks-NONMEM (PsN; version 3.2.4), and Xpose version 4.0 (22) were used for automation and to evaluate the goodness of fit during the model-building process. Concentrations below the LLOQ were coded as missing data. Imputing the first concentration below the LLOQ with half of the

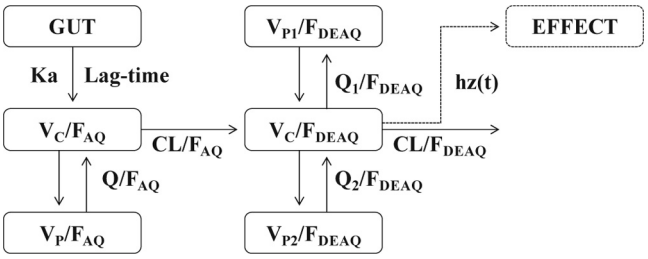

FIG 1 Final structural population pharmacokinetic model of amodiaquine and desethylamodiaquine. Amodiaquine is absorbed from the gut compartment via lagged first-order absorption (K_a) to the central amodiaquine compartment (V_C/F_{AQ}) and distributed into the peripheral compartment (V_{P1}/F_{AQ}). Amodiaquine is eliminated (CL/F_{AQ}) from the central compartment and forms desethylamodiaquine in the central desethylamodiaquine compartment (V_C/F_{DEAQ}). Desethylamodiaquine is distributed into two peripheral compartments (V_{P1}/F_{DEAQ} and V_{P2}/F_{DEAQ}) and then eliminated from the body (CL/F_{DEAQ}). Desethylamodiaquine concentrations were used to modify the hazard function [hz(t)] of the effect compartment. Parameter abbreviations are defined in Table 2.

LLOQ was investigated to determine the influence of the censored data. An approach of coding censored data as categorical data and maximizing the likelihood to predict these censored data below the LLOQ (i.e., M3 method) was also evaluated (3, 8). The objective function value (OFV), computed by the NONMEM program, was used to compare two hierarchical models ($P < 0.05$; ΔOFV > 3.84; 1 degree of freedom difference).

Elimination of amodiaquine and desethylamodiaquine was assumed to take place from the central compartments and amodiaquine was assumed to be metabolized completely into desethylamodiaquine. All possible combinations of one-, two- and three-compartment disposition models for amodiaquine and desethylamodiaquine were investigated in a simultaneously fitted drug-metabolite model using a first-order absorption model. The structural disposition model were parameterized with oral amodiaquine clearance (CL/F_{AQ}), central amodiaquine volume of distribution (V_C/F_{AQ}), amodiaquine intercompartmental clearance(s) (Q/F_{AQ}), peripheral amodiaquine volume of distribution(s) (V_P/F_{AQ}), oral desethylamodiaquine clearance (CL/F_{DEAQ}), central desethylamodiaquine volume of distribution (V_C/F_{DEAQ}), desethylamodiaquine intercompartmental clearance(s) (Q/F_{DEAQ}), and peripheral desethylamodiaquine volume of distribution(s) (V_P/F_{DEAQ}) (Fig. 1). The optimal combination of distribution compartments was selected and zero- and first-order absorption models with and without absorption lag time were evaluated. A transit compartment and a sequential zero- and first-order absorption model were also tried (50).

Interindividual random variability in all parameters was modeled exponentially: $\theta_i = \theta \times \exp(\eta_{i,\theta})$, where θ_i is the individually estimated parameter value for the ith patient, θ is the typical parameter value for the modeled population, and $\eta_{i,\theta}$ is the interindividual random variability assumed to be normally distributed with a zero mean and variance ω^2. Correlations between variability components were evaluated. Interindividual and interoccasion variability were investigated on the relative bioavailability (F): $F_{ij} = F \times \exp(\eta_{i,F} + \kappa_{j,F})$, where F_{ij} is the individual relative bioavailability for the ith patient on the jth dosing occasion, F is a fixed (100%) typical bioavailability, and $\kappa_{j,F}$ is the interoccasion variability in bioavailability between dose occasions. The residual unexplained variability was modeled with an additive error on the log-transformed drug and metabolite concentrations, which is essentially equivalent to an exponential residual error on an arithmetic scale.

Covariates. Continuous covariates (initial parasitemia, weight, height, age, hematocrit, bilirubin, platelet count, and estimated gestational age) and categorical covariates (smoking, pregnancy, and disease status) were investigated by using a stepwise forward inclusion (P values of <0.05 to be included in the model) followed by a stepwise backward elimination (P values of <0.01 to be retained in the model) (30). Body weight was also tried in the model as a simultaneous incorporation of an allometric function on all clearance and volume parameters (i.e., clearance parameters were defined by $\theta_i = \theta \times \exp(\eta_{i,\theta}) \times (BW_i/BW_{median})^{0.75}$ and volume parameters were defined by $\theta_i = \theta \times \exp(\eta_{i,\theta}) \times (BW_i/BW_{median})$, where BW_i is the individual body weight and BW_{median} is the median body weight of the modeled population) (19). Pregnancy as a categorical covariate was also evaluated simultaneously on all pharmacokinetic parameters for a full covariate approach.

Model evaluation. Basic goodness-of-fit diagnostics were used to evaluate systematic errors and model misspecification. Individual objective function values (iOFV) were computed and case deletion diagnostics were performed in order to determine the contribution of a single subject in the final model. Parameter estimate shrinkage (eta shrinkage) and epsilon shrinkage were calculated to evaluate the reliability of empirical Bayes estimates and the power to detect model misspecification in the goodness-of-fit diagnostics (51). Bootstrapping (n = 1,000) was performed to evaluate model robustness and to get nonparametric confidence intervals of pharmacokinetic parameter estimates. The final model were evaluated by visual and numerical predictive checks (n = 2,000). The 5th, 50th, and 95th percentiles of the observed data were overlaid with the 95% confidence intervals of simulations at the same percentiles to detect overall predictive performance.

Pharmacodynamic analysis. The final pharmacokinetic model and parameter estimates were fixed and used to produce individual concentration-time curves in the pharmacodynamic model. Times to recurrent *P. vivax* malaria were coded as an event, and patients with no recurrent *P. vivax* malaria were censored at the time of drop-out, *P. falciparum* infection, or first day of drug treatment in the postpartum period. The time-to-event model was performed by using the numerical Laplace method with interaction in NONMEM. A constant-hazard model and Weibull distribution hazard model were evaluated. The inhibitory effect of desethylamodiaquine was implemented as a sigmoid maximum effect (E_{max}) function on the baseline hazard: $hz(t) = BASE \times [1 - CP_{DEAQ}^{\gamma}/CP_{DEAQ}^{\gamma} - PC_{50DEAQ}^{\gamma}]$, where hz($t$) is the instantaneous hazard at time t, BASE is the constant baseline hazard function, CP_{DEAQ} is a predicted desethylamodiaquine concentration at time t, $PC_{50\,DEAQ}$ is the protective concentration for a 50% hazard reduction from the baseline hazard level, and γ is a shape parameter. This hazard function will be reduced to an E_{max} model when the shape parameter is fixed to 1. The survival was calculated by $S(t) = \exp(-H(t))$, where $S(t)$ is the survival at time t and $H(t)$ is the cumulative hazard to time t.

The pharmacodynamic models were compared by using the difference in objective function value as described previously. The final pharmacodynamic model was evaluated by a Kaplan-Meier visual predictive check (n = 1,000), where the observed time to recurrent infections was overlaid with the 95% prediction interval of the simulated time to recurrent infections. Bootstrapping (n = 500) was also performed for nonparametric confidence intervals of the final pharmacodynamic parameters as described above.

RESULTS

Twenty-seven pregnant women, 16 to 39 years of age, who were in the second or third trimester of pregnancy and had *P. vivax* infections were enrolled in this study (full demographic characteristics are given in Table 1). Nineteen women returned postpartum; 12 of these postpartum women were *P. vivax* negative, and 7 were *P. vivax* positive. Three pregnant women and one postpartum woman were lost to follow-up but were included in both the pharmacokinetic and pharmacodynamic models (i.e., censored at the time of dropout).

Pharmacokinetics of amodiaquine and desethylamodiaquine. Plasma amodiaquine and desethylamodiaquine concentration-

TABLE 1 Patient demographics and covariates in the clinical study of amodiaquine and desethylamodiaquine in pregnant and postpartum women

Parameter	Pregnant women	Postpartum women
Total no. of patients	27	19
No. of patients with *P. vivax*	27	7
Total dose of amodiaquine (mg/kg) [median (range)]	9.89 (9.32–10.7)	10.0 (9.32–10.7)
Continuous covariates [median (range)]		
Age (yr)	23 (16–39)	23 (18–35)
Body wt (kg)	49 (37–68)	44 (38–57)
Height (cm)	152 (137–164)	150 (137–164)
BMI (kg/m^2)	21.6 (17.3–25.2)	19.6 (16.8–22.6)
Pulse (min^{-1})	84 (62–120)	80 (68–92)
Temperature (°C)	36.5 (35.8–40.2)	36.8 (36.0–37.6)
Estimated gestational age (wk)	27.6 (13.0–37.4)	NA[a]
Parasitemia (no. of parasite/μl)	624 (96.0–50,400)	200 (0–7,810)
Hematocrit (%)	33.2 (22.8–39.8)	35.9 (22.3–42.1)
Bilirubin (mg/dl)	0.450 (0.150–1.46)	0.330 (0.190–0.330)
Platelet (10^3/μl)	172 (84.0–322)	262 (90.0–334)
Categorical covariates		
Smokers (%)	8 (29.6)	6 (31.5)
Outcome [no. (%)]		
Patients lost during follow-up	3 (11.1)	1 (5.26)
Patients with no recurrent malaria infection	10 (37.0)	18 (94.7)
Patients with recurrent *P. vivax* infection	10 (37.0)	0 (0)
Patients with novel *P. falciparum* infection	4 (14.8)	0 (0)

[a] NA, not applicable.

time profiles were best described by a first-order amodiaquine absorption with lag time followed by two amodiaquine disposition compartments and three desethylamodiaquine disposition compartments (Fig. 1). Other combinations of one, two, and three distribution compartments of drug and metabolite showed a systematic model misspecification or no additional improvement compared to the final model. An additional elimination pathway of amodiaquine to an unknown metabolite is not identifiable without data on additional metabolites and/or observations following intravenous administration. The assumption of complete *in vivo* conversion of amodiaquine into desethylamodiaquine results in disposition parameter estimates; CL/F_{DEAQ}, V_C/F_{DEAQ}, Q_1/F_{DEAQ}, Q_2/F_{DEAQ}, V_{P1}/F_{DEAQ} and V_{P1}/F_{DEAQ}, where F_{DEAQ} is the bioavailability of amodiaquine (F_{AQ}) multiplied with the fraction of amodiaquine that is metabolized into desethylamodiaquine.

Lag time of the first-order absorption process improved the model fit ($\Delta OFV = -37.7$), and this model was significantly better than all other absorption models. Separated error models for amodiaquine and desethylamodiaquine resulted in a statistically better model than a common error model. Interindividual variability in Q_1/F_{DEAQ}, Q_2/F_{DEAQ}, V_{P1}/F_{DEAQ}, and K_a was estimated with unacceptably low precision (relative standard error [RSE] > 60%) and therefore fixed to zero in the final model. Interoccasion random variability on the relative bioavailability resulted in a better model fit ($\Delta OFV = -45.5$) with no additional benefit of interindividual variability on the same parameter.

Incorporation of body weight as an allometric function on all clearance and volume parameters produced a significantly better model fit than the base model ($\Delta OFV = -11.3$). The power values were also estimated to support further the applied allometric model, which resulted in estimates close to the fixed values of 0.75 and 1.0 for clearance and volume parameters, respectively. Accuracy of parameter estimates was also comparable between the two models. Incorporation of pregnancy status on lag time and age on CL/F_{AQ} resulted in a significant improvement of the model when added in a stepwise manner and could also be retained in the backward elimination step with a higher significance level. The final parameter estimates are summarized in Table 2.

The full covariate approach (Fig. 2) resulted in minor effects of pregnancy on key pharmacokinetic parameters (pharmacokinetic parameters changed less than 25% as a result of pregnancy). Absorption lag time in pregnant patients was significantly shorter in the full covariate approach which was congruent with the previous stepwise covariate approach. Furthermore, the central volume of amodiaquine and desethylamodiaquine showed a relatively large but uncertain (nonsignificant) decrease and increase, respectively, in pregnant patients.

A total of 919 amodiaquine and 921 desethylamodiaquine venous plasma samples were quantified, resulting in 300 (33%) amodiaquine concentrations and 3 (<1%) desethylamodiaquine concentrations below the LLOQ. Coding these as missing data resulted in the same proportion of predicted and observed data below the LLOQ and suggests that the model was not biased on account of a high proportion of samples quantified below the LLOQ. Imputing the first concentration below the LLOQ in individual concentration-time profiles with half of the LLOQ or implementing the M3 method did not alter the model fit. Concentrations below the LLOQ were therefore coded as missing data.

Basic goodness-of-fit plots are shown in Fig. 3. Moderate epsilon shrinkage (12.2%) was present in the final model, but a relatively high eta shrinkage could be seen for certain parameters: CL/F_{AQ} = 5.38%, V_C/F_{AQ} = 16.6%, V_P/F_{AQ} = 33.8%, Q_P/F_{AQ} = 24.5%, CL/F_{DEAQ} = 7.64%, V_C/F_{DEAQ} = 34.6%, V_{P2}/F_{DEAQ} = 35.9%, lag time = 21.6%, relative bioavailability (first dose) = 13.6%, relative bioavailability (second dose) = 18.6%, and relative bioavailability (third dose) = 30.1%. Visual and numerical predictive checks were used to assess the predictive behavior of the final model (Fig. 4) (9). The numerical predictive checks resulted in 3.39% (95% confidence interval [CI], 2.58% to 8.07%) and 2.90% (95% CI, 2.58% to 8.07%) of observations below the 5th and above the 95th simulated percentile of amodiaquine, respectively, and 6.33% (95% CI, 2.72% to 7.86%) and 4.69% (95% CI, 2.73% to 7.75%) of observations below the 5th and above the 95th simulated percentile of desethylamodiaquine, respectively.

Pharmacodynamics of amodiaquine and desethylamodiaquine. Ten pregnant women presented with a recurrence of *P. vivax* malaria at a median of 63 (35 to 84) days, and four pregnant women showed *P. falciparum* infections at day 21, 41, 42, and 63. Seven women had recurrent *P. vivax* infections at the time of retreatment in the postpartum group (4 with a second episode of *P. vivax*, 2 after a previous *P. falciparum* infection during follow-up, and 1 with a first recurrent *P. vivax* episode). None of the treated postpartum women had recurrent malaria infections during the 42 days of follow-up. The constant-hazard model resulted in an adequate description of the time to recurrent infections with no additional benefit of a Weibull distribution of the hazard function ($\Delta OFV = -2.36$, 1 degree of freedom difference). The implementation of an inhibitory effect of desethylamodiaquine concentrations on the hazard function resulted in a significantly better model ($\Delta OFV = -4.85$, 1 degree of freedom difference), with no additional benefit of a shape parameter for a sigmoid dose-response shape ($\Delta OFV = -1.603$, 1 degree of freedom difference).

TABLE 2 Parameter estimates of the final amodiaquine and desethylamodiaquine population pharmacokinetic model in pregnant and postpartum women

Parameter[a]	Population estimate[b]	95% CI[c]	% RSE[d]
Primary			
Amodiaquine			
K_a (h^{-1})	0.515	0.439–0.587	6.93
Lag time (h)	0.395	0.359–0.456	7.48
CL/F_{AQ} (liters/h)	2,530	2,360–2,760	3.82
V_C/F_{AQ} (liters)	4,850	3,750–5,810	10.2
Q_1/F_{AQ} (liters/h)	2,750	2,450–3,090	5.53
V_{P1}/F_{AQ} (liters)	29,000	25,600–34,100	6.90
σ_{AQ}	0.0479	0.0372–0.0671	6.92
Desethylamodiaquine			
CL/F_{DEAQ} (liters/h)	34.3	31.4–37.7	4.43
V_C/F_{DEAQ} (liters)	197	167–233	8.13
Q_1/F_{DEAQ} (liters/h)	161	141–180	5.98
V_{P1}/F_{DEAQ} (liters)	2,670	2,370–2,960	5.41
Q_2/F_{DEAQ} (liters/h)	26.0	22.9–29.4	5.88
V_{P2}/F_{DEAQ} (liters)	5,700	5,200–6,410	5.16
σ_{DEAQ}	0.0416	0.0279–0.0548	8.57
Covariate effects			
Age effect on CL/F_{AQ} (% reduction per year)	1.36	0.321–2.30	34.6
Pregnancy effect on Lag-time (% decrease)	41.6	32.2–57.3	16.5
Interindividual variability (% CV)			
CL/F_{AQ} (liters/h)	0.0455 (21.6)	0.0250–0.700	23.3
V_C/F_{AQ} (liters)	0.349 (64.6)	0.198–0.523	22.4
Q_1/F_{AQ} (liters/h)	0.0639 (25.7)	0.0259–0.126	35.3
V_{P1}/F_{AQ} (liters)	0.0652 (26.0)	0.0180–0.103	31.8
CL/F_{DEAQ} (liters/h)	0.0535 (23.4)	0.0301–0.0833	25.0
V_C/F_{DEAQ} (liters)	0.210 (48.3)	0.0000212–0.369	44.9
V_{P2}/F_{DEAQ} (liters)	0.0321 (18.1)	0.00000534–0.134	67.3
Lag time (h)	0.325 (62.0)	0.141–0.474	27.4
Interoccasion variability (% CV)			
F	0.0575 (24.3)	0.0435–0.0748	13.1
Pharmacodynamics			
BASE (infections per year)	2.72	1.24–6.14	38.7
$PC_{50\ DEAQ}$ (ng/ml)	7.08	2.13–22.1	68.7
Secondary [median (range)][e]	Total (n = 46)	Pregnant (n = 27)	Postpartum (n = 19)
$C_{max\ AQ}$ (ng/ml)	30.2 (16.1–54.5)	30.3 (17.9–54.5)	29.4 (16.1–50.7)
$T_{max\ AQ}$ (h)	1.69 (0.938–2.55)	1.47 (0.938–2.54)	1.78 (1.12–2.55)
$t_{1/2\ AQ}$ (h)	15.6 (11.1–27.1)	18.4 (11.1–27.1)	14.6 (11.4–22.8)
$AUC_{5\ AQ}$ (h · ng/ml)	586 (265–890)	608 (350–890)	558 (265–811)
$C_{max\ DEAQ}$ (ng/ml)	350 (221–544)	349 (221–478)	362 (253–544)
$T_{max\ DEAQ}$ (h)	2.97 (1.66–4.72)	2.71 (1.66–4.72)	3.12 (2.13–4.13)
$t_{1/2\ DEAQ}$ (days)	12.4 (8.63–23.3)	12.3 (9.33–23.3)	12.6 (8.46–15.8)
$AUC_{60\ DEAQ}$ (h · μg/ml)	36.8 (19.0–69.9)	37.1 (19.0–69.9)	36.6 (22.1–59.8)

[a] AQ, amodiaquine; DEAQ, desethylamodiaquine; K_a, absorption rate constant; Lag time, absorption lag time; CL/F, oral clearance; V_C/F, central volume of distribution; Q/F, intercompartmental clearance; V_P/F, peripheral volume of distribution; F, relative bioavailability; σ, residual error variance; BASE, baseline hazard; $PC_{50\ DEAQ}$, 50% protective concentration; C_{max}, maximum concentration; T_{max}, time to maximum concentration; $t_{1/2}$, terminal elimination half-life; AUC_x, area under the concentration-time curve from time zero to x days.

[b] Computed population mean parameter estimates from NONMEM are calculated for a typical patient with a body weight of 48 kg and age of 23 years. The coefficient of variation (% CV) for interindividual variability is calculated as $[\exp(\text{estimate}) - 1]^{1/2} \times 100$.

[c] Computed from the nonparametric bootstrap method of the final pharmacokinetic model (n = 1,000) and pharmacodynamic model (n = 500).

[d] Calculated as 100 × (standard deviation/mean value).

[e] Secondary-parameter estimates are calculated from the Bayesian *post hoc* estimates.

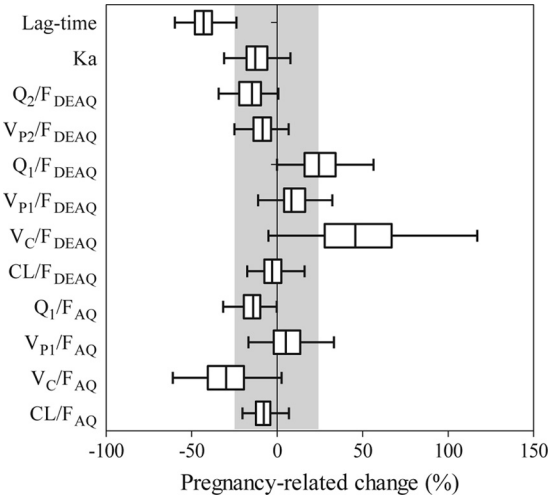

FIG 2 Pregnancy effects on pharmacokinetic parameters. Boxes represent the median value and interquartile range, and the whiskers represent the 95% confidence intervals, which were calculated from 200 bootstrap estimates. The shaded area represents a percentage parameter change in pregnant women between −25% and 25%. Parameter abbreviations are defined in Table 2.

Baseline hazard was estimated to 2.72 infections per year (38.7% RSE) and the $PC_{50\ DEAQ}$ was estimated at 7.08 ng/ml (68.7% RSE). The Kaplan-Meier plot of the observed time to recurrent *P. vivax* infections overlaid with the simulated 95% interval from the final model is shown in Fig. 5.

DISCUSSION

The pharmacokinetic properties of amodiaquine and desethylamodiaquine in adults and children have been described mainly using a noncompartmental approach (20, 38, 40, 53, 63–65). Three previous studies have described the pharmacokinetic properties of amodiaquine and desethylamodiaquine in nonpregnant adults and children using nonlinear mixed-effects modeling (2, 17, 23). A nonlinear mixed-effects approach, in contrast to results from a noncompartmental analysis, provides a mechanistic understanding of the pharmacokinetic properties of the drug with the ability to characterize potential covariate relationships with higher statistical power. A developed mixed-effects model can also be linked to pharmacodynamic outcome measurements to understand the clinical value of the studied drug and further used for dose optimizations and clinical trial simulations if necessary. Thus, the nonlinear mixed-effects approach utilized here offers a mechanistic understanding of the studied drug and population by a simultaneous drug-metabolite pharmacokinetic model compared to the previously published noncompartmental analysis (48). The present study also expands the knowledge of the studied treatment by the incorporation of a pharmacokinetic-pharmacodynamic model, which was used to describe the protective effect of the treatment.

Pharmacokinetic properties of amodiaquine and desethylamodiaquine. Amodiaquine absorption was characterized adequately by a first-order absorption rate (K_a = 0.515 h^{-1}) with lag time (0.395 h) in this study, and the more physiological transit-compartment absorption model did not improve the model fit.

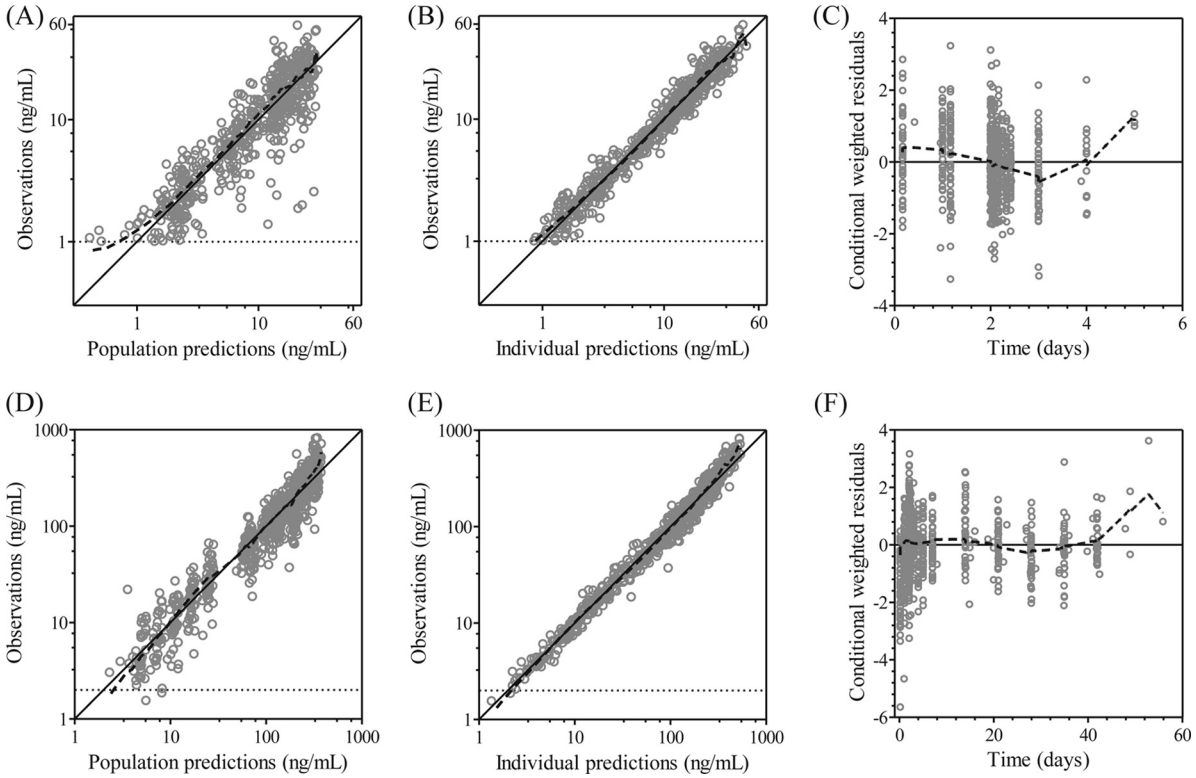

FIG 3 Basic goodness-of-fit plots of the final population pharmacokinetic model describing amodiaquine (A, B, and C) and desethylamodiaquine (D, E, and F). Observations versus population predictions (A and D), observations versus individual predictions (B and E), and conditional weighted residuals versus time (C and F) are shown. The horizontal dashed lines represent the lower limits of quantification of amodiaquine (1 ng/ml) and desethylamodiaquine (2 ng/ml).

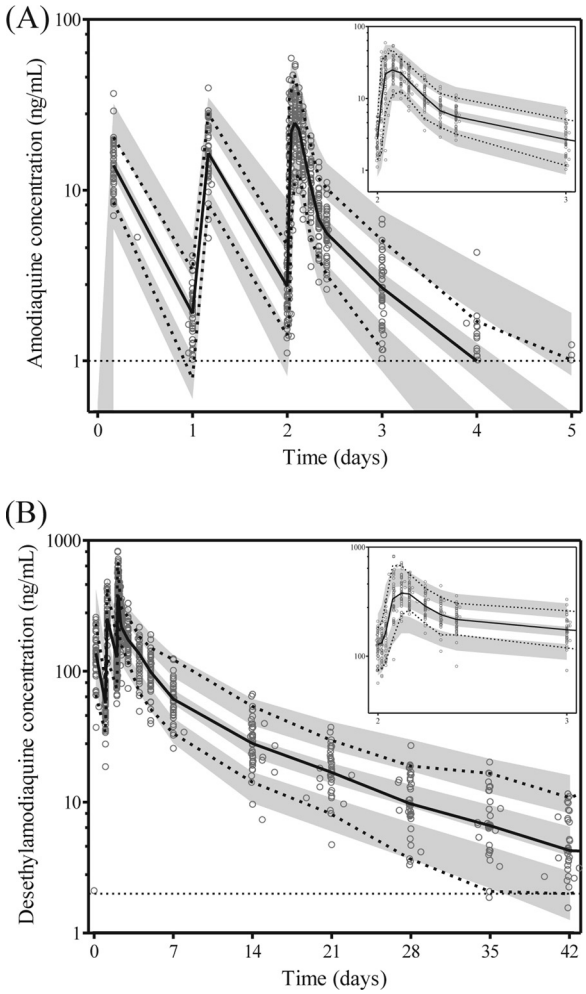

FIG 4 Visual predictive check of the final population pharmacokinetic model describing amodiaquine (A) and desethylamodiaquine (B) in pregnant and postpartum women. Insets show a visual predictive check for the last day of treatment. Open circles represent observed amodiaquine and desethylamodiaquine concentrations. Solid black lines represent the 50th percentiles of the observations, and dotted lines represent the 5th and 95th percentiles of the observations. Gray areas represent the 95% confidence intervals of the 5th, 50th, and 95th percentiles of the simulations. The horizontal dashed lines represent the lower limit of quantification of amodiaquine (1 ng/ml) and desethylamodiaquine (2 ng/ml).

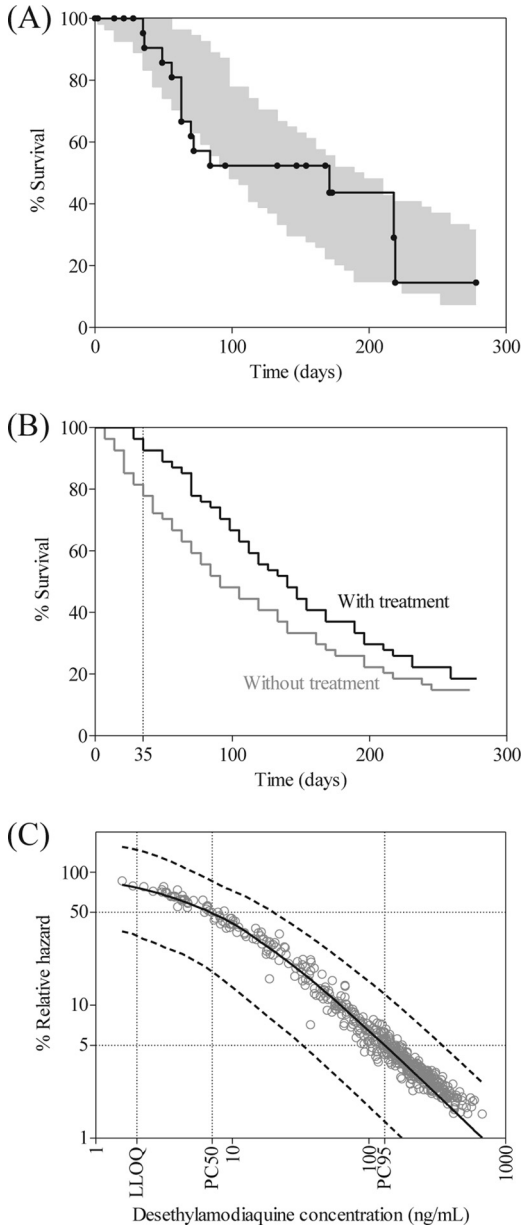

FIG 5 Pharmacodynamic model performance. (A) Visual predictive check of the final time-to-event model with an inhibitory effect of desethylamodiaquine on the hazard function. The solid black line is an observed Kaplan-Meier plot of recurrent *P. vivax* malaria during the follow-up period, and the gray area is the 95% prediction interval of the simulated time to recurrent infections. (B) Simulated median survival with (black line) and without (gray line) treatment. (C) Desethylamodiaquine concentrations plotted versus relative hazard of recurrent *P. vivax* malaria compared to baseline hazard. Open circles represent the observed desethylamodiaquine concentrations with the predicted hazard. Solid black line and dashed lines represent the median hazard and the 95% confidence interval of the estimated hazard function from 500 bootstrap results.

The absorption rate is within the range of the previously published estimates of 0.13 h^{-1} in children and 1.41 h^{-1} in nonpregnant adult patients (17). Parameterization of between-occasion variability of the relative bioavailability allowed a highly flexible absorption model. The increased relative absorption after the second ($F = 1.09$) and third ($F = 1.08$) doses compared to the first dose ($F = 0.899$) might result from recovery from malaria but could also be a result of different sampling after different doses. Two samples per subject were collected after the first and second dose, whereas dense samples were collected after the third dose.

The disposition of amodiaquine and desethylamodiaquine was best described by a simultaneous two- and three-compartment drug metabolite model. Multiphasic disposition has been observed previously in adults and children with malaria, and differences in compartment structure (i.e., three- versus two-compartment disposition for desethylamodiaquine) might be a result of modeling dense and sparse data in the different studies (17, 23, 60). The estimated amodiaquine and desethylamodiaquine population parameters are similar to those from the noncompartmental analysis and those reported in previous studies (2, 17, 23, 40, 44, 48, 64). Polymorphisms of CYP2C8, which is the main

enzyme for amodiaquine metabolism, might contribute to interindividual variability of amodiaquine elimination and formation of desethylamodiaquine. A CYP2C8*2 polymorphism resulted in a 6-fold-lower amodiaquine clearance in an African population (allele frequency = 0.115) (41). However, the Karen population in this study has a reported low frequency of CYP2C8 polymorphisms (CYP2C8*2, allele frequency = 0.008; CYP28*3, allele frequency = 0.12), which suggests that there are other factors contributing to the interindividual variability (36).

One previously published study of adult patients with falciparum malaria attempted to model amodiaquine and desethylamodiaquine simultaneously using nonlinear mixed-effects modeling (23). Jullien and colleagues suggested a supplementary unknown route of amodiaquine elimination in their model (28, 45, 63). However, desethylamodiaquine is the main *in vivo* metabolite of amodiaquine, and only low concentrations of other amodiaquine metabolites such as bis-desethylamodiaquine and 2-hydroxylamodaquine have been detected in plasma (45, 63). The present study therefore assumed that amodiaquine was metabolized completely into desethylamodiaquine.

Amodiaquine and desethylamodiaquine had terminal elimination half-lives of 16.8 h and 12.4 days, respectively, which are somewhat longer than those reported previously (5 to 10 h and 8 to 10 days) (23, 38, 40, 60). This finding might therefore reflect longer follow-up than that in previous studies. The long follow-up resulted in a large percentage of amodiaquine concentrations below the LLOQ (33%), while only a small fraction of desethylamodiaquine concentrations were below the LLOQ (<1%). However, the sensitivity of the bioanalytical method was high, and it is unlikely that a high percentage of amodiaquine LLOQ samples in this study would generate a model misspecification. Indeed, the fraction of observed amodiaquine samples below the LLOQ was in good agreement with the predicted fraction of amodiaquine samples below the LLOQ (data not shown).

Body weight was incorporated as a covariate into the pharmacokinetic model, which is in agreement with previous studies (2, 23). Age has been reported as a significant covariate of elimination clearance of desethylamodiaquine in pediatric patients (17). Here, age was incorporated as a covariate of amodiaquine clearance but could not be supported in desethylamodiaquine clearance. However, age had a relatively small effect on amodiaquine clearance (1.36% reduction per year), and this covariate relationship should not be extrapolated beyond the age range of the study. Furthermore, age did not explain much of the interindividual variability in amodiaquine clearance, and interindividual variability in this parameter decreased from 24.0% to 21.6% after covariate inclusion. An increase in amodiaquine clearance might be related to an increased immunity in older patients and reflect reduced severity of the disease. However, parasitemia was not identified as a covariate of any pharmacokinetic parameters, and there was no difference between *P. vivax*-infected and noninfected postpartum women. Pregnant patients had a reduced absorption lag time, which is contradictory to a reduced gastrointestinal motility (11) but might be a consequence of an increased blood flow to the stomach and small intestine, resulting from an increased cardiac output (30 to 50%) (29). The full covariate approach did not rule out the presence of a moderate pregnancy effect on the central volumes of distribution. However, neither a change in absorption lag time nor a change in central distribution volume of this kind is likely to have significant clinical implications in the treatment of uncomplicated malaria, and therefore these variations do not warrant a dose adjustment in pregnant women.

Pharmacodynamics of amodiaquine and desethylamodiaquine. Amodiaquine is not a radical cure for *P. vivax*, and in these regions (India, Myanmar, and Thailand), multiple short-interval relapses are common (21). Amodiaquine has a relatively short half-life of 16 h, and the posttreatment prophylactic effect is negligible compared to that of desethylamodiaquine. In this study, 39.2% (11/24) of the pregnant women had recurrent *P. vivax* infections during follow-up until the postpartum period and 16.7% (4/24) had a novel *P. falciparum* infection. Recurrent *P. vivax* infections were successfully modeled using a time-to-event approach where desethylamodiaquine concentrations affect the time to the recurrent episode (posttreatment prophylactic effect). This has not been described previously in the malaria literature. The baseline hazard in this population was calculated at 2.72 infections per year, which translates to approximately 22.2% of patients having a recurrent infection within 35 days without amodiaquine treatment. Desethylamodiaquine has a long terminal elimination half-life, which decreases the risk of a recurrent malaria infection for a sustained period (Fig. 5B). The pharmacodynamic model estimated that approximately 7.4% of patients would have a recurrent malaria infection within 35 days after treatment. The risk of a recurrent infection was reduced by 50% with a desethylamodiaquine concentration above 7.08 ng/ml, and the model predicted a 95% reduction in risk of recurrent infections above 131 ng/ml (Fig. 5). Using the $PC_{50\ DEAQ}$ to determine the protective effect of desethylamodiaquine indicated that patients in this study had a mean posttreatment prophylactic effect for 35 days (range from 17 to 75 days), which is in good agreement with the observed data. Monthly amodiaquine combination therapy might therefore be a suitable candidate for intermittent preventive treatment. A constant-hazard model might not mimic the real situation with periodicity of *P. vivax* relapses perfectly (59), and the pharmacodynamic parameters are uncertain as a natural consequence of the small sample size. However, this pharmacodynamic approach could be expanded and be useful in future studies incorporating more data.

Conclusion. The population pharmacokinetic properties of amodiaquine and desethylamodiaquine were successfully described by a simultaneous nonlinear mixed-effects model with complete *in vivo* conversion of amodiaquine into desethylamodiaquine. Pregnancy status and age had an effect on absorption lag time and amodiaquine clearance, respectively. However, neither pregnancy status nor estimated gestational age resulted in a clinically relevant impact on other pharmacokinetic parameters. The data presented here indicate that a dose adjustment in this vulnerable group of patients is not warranted. A concentration-effect relationship was established for the posttreatment prophylactic effect of desethylamodiaquine, with a 50% reduction in risk of recurrent infection for a concentration of 7.08 ng/ml.

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