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Population Pharmacokinetics of Dihydroartemisinin and Piperaquine in Pregnant and Nonpregnant Women with Uncomplicated Malaria

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Pregnant women are particularly vulnerable to malaria. The pharmacokinetic properties of antimalarial drugs are often affected by pregnancy, resulting in lower drug concentrations and a consequently higher risk of treatment failure. The objective of this study was to evaluate the population pharmacokinetic properties of piperaquine and dihydroartemisinin in pregnant and nonpregnant women with uncomplicated malaria. Twenty-four pregnant and 24 matched nonpregnant women on the Thai-Myanmar boarder were treated with a standard fixed oral 3-day treatment, and venous plasma concentrations of both drugs were measured frequently for pharmacokinetic evaluation. Population pharmacokinetics were evaluated with nonlinear mixed-effects modeling. The main pharmacokinetic finding was an unaltered total exposure to piperaquine but reduced exposure to dihydroartemisinin in pregnant compared to nonpregnant women with uncomplicated malaria. Piperaquine was best described by a three-compartment disposition model with a 45% higher elimination clearance and a 47% increase in relative bioavailability in pregnant women compared to nonpregnant women. The resulting net effect of pregnancy was an unaltered total exposure to piperaquine but a shorter terminal elimination half-life. Dihydroartemisinin was best described by a one-compartment disposition model with a 38% lower relative bioavailability in pregnant women than nonpregnant women. The resulting net effect of pregnancy was a decreased total exposure to dihydroartemisinin. The shorter terminal elimination half-life of piperaquine and lower exposure to dihydroartemisinin will shorten the posttreatment prophylactic effect and might affect cure rates. The clinical impact of these pharmacokinetic findings in pregnant women with uncomplicated malaria needs to be evaluated in larger series.

Children under the age of 5 years and pregnant women are especially vulnerable to malaria. An estimated 85 million pregnancies occurred in areas with falciparum malaria transmission during 2007 (13). Malaria during pregnancy is responsible for maternal and fetal mortality (42, 43). Both falciparum malaria and vivax malaria during pregnancy are associated with low birth weight resulting from intrauterine growth restriction (8, 9, 48). Effective treatment and prophylaxis of malaria in pregnancy rely on the use of efficacious antimalarial drugs.

Pregnancy has substantial effects on the pharmacokinetic characteristics of many antimalarial drugs. Previous studies report artemesate, artemether, dihydroartemisinin, sulfadoxine, atovaquone, proguanil, cycloguanil, pyrimethamine, and lumefantrine concentrations to be reduced in pregnant women (25, 33, 34, 36, 44, 58). This may contribute to lower antimalarial cure rates in pregnant than nonpregnant adults (58). Other studies report no pharmacokinetic differences in pregnant women compared to nonpregnant women (e.g., pyrimethamine and amodiaquine/desethylamodiaquine) or even a higher drug exposure during pregnancy (pyrimethamine) (15, 25, 46).

Artemisinin-based combination therapy (ACT) is now recommended as first-line treatment for falciparum malaria in nearly all countries where malaria is endemic. ACTs are recommended by the World Health Organization in the second and third trimester of pregnancy (60). One of the most promising new ACTs is the fixed-dose oral dihydroartemisinin and piperaquine combination. Good tolerability (e.g., total incidence of early vomiting of 4.8% [95% confidence interval [CI], 3.7% to 5.9%]) and high efficacy (PCR-corrected cure rates of 98.7% [95% CI, 97.6% to 99.8%] at day 28) have been reported in 1,814 adult and pediatric patients with uncomplicated falciparum malaria from 12 different studies in 6 countries conducted between 2003 and 2006 (62). A recent case series reported the dihydroartemisinin and piperaquine combination to be an inexpensive, safe, and relatively effective treatment (PCR-adjusted cure rate, 92.2% [95% CI, 76.9% to 97.4%] at day 63) for pregnant women with multiple recurrences of Plasmodium falciparum malaria (45). The dihydroartemisinin and piperaquine combination is a potential first-line treatment for multidrug-resistant falciparum malaria during pregnancy and is a promising candidate for intermittent preventive treatment of pregnant women with malaria (59).

Piperaquine has a very large apparent volume of distribution (V) of approximately 600 to 900 liters/kg, an oral clearance (CL) of approximately 0.90 to 1.4 liters/h/kg, and a long terminal elimination half-life (t1/2) of approximately 20 to 30 days in adult patients (21, 55–56). Dihydroartemisinin pharmacokinetics are very different; it has a small apparent volume of distribution of approximately 1.5 to 3.8 liters/kg, an oral clearance of approximately 1.1 to 2.9 liters/h/kg, and a very short terminal elimination half-life of approximately 0.83 to 1.9 h in adult patients (27, 37, 39, 40). The population pharmacokinetic properties of the fixed oral combination of dihydroartemisinin and piperaquine have not been...
studied in pregnant women with malaria. Information for dose optimization in this vulnerable group is urgently needed. The aim of this study was to define the population pharmacokinetic properties of dihydroartemisinin and piperaquine in the treatment of uncomplicated malaria infections in nonpregnant and pregnant women living on the Thai-Myanmar border during the second and third trimesters of pregnancy.

**MATERIALS AND METHODS**

**Study design and ethical approval.** This pharmacokinetic study was conducted in the Wang Pha Clinic of the Shoklo Malaria Research Unit (SMRU), Mae Sot, Thailand. The clinical assessment with a preliminary noncompartmental analysis is reported in full elsewhere (47). Approval for the study was obtained from the ethics committee of the faculty of Tropical Medicine, Bangkok, Thailand (MUTM 2007-111) and the Oxford Tropical Research Ethic Committee (OxTREC 017-07). Pregnant women in the second and third trimesters of pregnancy and without signs of severe malaria were recruited. The purpose of the study was explained to the women in their own language, and written consent was obtained before study participation. Twenty-four pregnant women with malaria and 24 matched (by age, parasitemia, smoking, and history of fever) nonpregnant women were enrolled.

**Drug regimen and blood sampling.** Patients were treated with a total dose of 6.4 mg/kg of body weight dihydroartemisinin and 51 mg/kg piperaquine tetrathosphate (tablet contained 40 mg dihydroartemisinin and 320 mg piperaquine tetrathosphate [equivalent to 184 mg base]; Holleypharm, People’s Republic of China) once daily for 3 days (0, 24, and 48 h). Food intake was not controlled, and the daily dose was given with water, based on body weight, and divided to the nearest quarter tablet. Blood samples (2 ml) were drawn from an indwelling catheter into lithium heparin tubes for piperaquine quantification (1 ml) and into prechilled fluoride oxalate tubes for dihydroartemisinin quantification (1 ml) at baseline and at 0.5, 1.5, 4, 8, 24.5, 25.5, 28, 32, 48.5, 48.8, 50, 51, 52, 54, 56, 60, and 72 h after the first dose. Additional samples for piperaquine quantification were taken by venipuncture at days 5, 7, 14, 21, 28, 35, 42, 49, 56, 63, 77, and 84. Piperaquine blood samples were centrifuged at room temperature at 1,500 to 2,000 × g for 10 min and dihydroartemisinin blood samples were centrifuged for 4°C at 2,000 × g for 7 min to obtain plasma. Plasma samples were stored at −80°C and shipped on dry ice to the MORU Clinical Pharmacology Laboratory, Bangkok, Thailand, for drug quantification.

**Drug analysis.** Dihydroartemisinin and piperaquine plasma samples were quantified using solid-phase extraction, followed by liquid chromatography coupled to tandem mass spectrometry (17, 30). Quality control samples at low, middle, and high concentrations were analyzed in triplicate within each analytical batch to ensure accuracy and precision during the analysis. The coefficients of variation during piperaquine quantification (n = 68 at each concentration) were 3.06%, 2.39%, and 1.74% at 4.50 ng/ml, 20.0 ng/ml, and 400 ng/ml, respectively. The coefficients of variation during dihydroartemisinin quantification (n = 45 at each concentration) were 5.4%, 3.3%, and 4.2% at 5.87 ng/ml, 117 ng/ml, and 1,880 ng/ml, respectively. The lower limit of quantification was set to 1.50 ng/ml for piperaquine and to 2.0 ng/ml for dihydroartemisinin.

**Pharmacokinetic analysis.** Piperaquine and dihydroartemisinin venous concentration data were transformed into their natural logarithms.
FIG 1 Diagram of the final structural model for piperaquine (A) and dihydroartemisinin (B) population pharmacokinetics in pregnant and nonpregnant women with uncomplicated malaria. ktr, transit absorption rate constant \([ktr = (n + 1)/\text{mean transit time}];\) CL, elimination clearance; \(V_c\), apparent volume of distribution of the central compartment; \(Q_c\), apparent volume of distribution of the peripheral compartment; \(Q_{1/2}\), intercompartment clearance; \(F\), relative oral bioavailability.

and modeled separately using nonlinear mixed-effects modeling with NONMEM software (version VI; ICON Development Solutions, MD). Postprocessing and automation were performed using the Pearl-Speaks-NONMEM (PsN) (version 3.2.4) (28, 29), Census (version 1.1) (61), and Xpose (version 4.0) (23) programs in the programming language R (version 2.10.1; The R Foundation for Statistical Computing). The first-order conditional estimation method with interactions was used throughout the modeling. The objective function value (OFV; computed by NONMEM as minus twice the log likelihood of the data), goodness-of-fit graphical analysis, and physiological plausibility were used for model selection. Different \(P\) values were used as statistical criteria at different stages during the model-building process, as described below (changes in OFV \(\Delta\text{OFV}\) of 3.84, 6.63, and 10.8 are considered significant at \(P = 0.05, P = 0.01,\) and \(P = 0.001,\) respectively, with 1 degree of freedom). Observed data below the limit of quantification were coded as missing data or modeled as categorical data using the previously published M3 method (1, 4, 6).

The structural base models were parameterized as first-order absorption rate constant \(k_a\), apparent volume of distribution of the central compartment \((V_c/F)\), intercompartment clearance \((Q_c/F)\), apparent volume of distribution of the peripheral compartment \((V_p/F)\), elimination clearance \((CL/F)\), and relative oral bioavailability \((F)\). Interindividual random variability in all parameters was modeled exponentially, as illustrated for CL: \(CL/F_i = TV(CL/F) \times \exp(\eta_{CL/F_i})\), where \(CL/F_i\) is the individually estimated value of clearance for the \(i\)th patient, TV\((CL/F)\) is the typical clearance value for the population, and \(\eta_{CL/F_i}\) is the interindividual random variability for the described parameter. The interindividual random variability is assumed to be normally distributed with a zero mean and variance \(\sigma^2\). Intraindividual variability was explored between dose occasions 1, 2, and 3 (between-occasion variability). The residual random variability was assumed to be additive since data were transformed into their natural logarithms (i.e., essentially equivalent to an exponential error model on a linear scale).

### TABLE 2 Parameter estimates of the final model describing piperaquine population pharmacokinetics in pregnant and nonpregnant women with uncomplicated malaria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population estimate (% RSE)</th>
<th>Between-occasion variability (P% C.V.)</th>
<th>Population estimate (% RSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (liters/h)</td>
<td>161 (9.74)</td>
<td>25.0 (10.1)</td>
<td>60.2 (10.5)</td>
</tr>
<tr>
<td>(V_c) (liters)</td>
<td>3,210 (17.8)</td>
<td>42.7 (18.7)</td>
<td>3,070 (12.5)</td>
</tr>
<tr>
<td>Q_c (liters/h)</td>
<td>155 (14.9)</td>
<td>35.4 (19.6)</td>
<td>427 (14.9)</td>
</tr>
<tr>
<td>MTT (h)</td>
<td>21.8 (4.23)</td>
<td>4.1 (72.4)</td>
<td>24.9 (97.6)</td>
</tr>
<tr>
<td>Non-trait (a)</td>
<td>0.15 (0.04)</td>
<td>4.24 (19.4)</td>
<td>0.15 (0.04)</td>
</tr>
<tr>
<td>(Q_{1/2})</td>
<td>3,210 (17.8)</td>
<td>42.7 (18.7)</td>
<td>3,070 (12.5)</td>
</tr>
<tr>
<td>CL/F (liters/h)</td>
<td>161 (9.74)</td>
<td>25.0 (10.1)</td>
<td>60.2 (10.5)</td>
</tr>
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<tr>
<td>(V_p/F)</td>
<td>3,210 (17.8)</td>
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<td>3,070 (12.5)</td>
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</tr>
</tbody>
</table>

*Note: Values are for pregnant and nonpregnant women. CL, elimination clearance; \(V_c\), apparent volume of distribution of the central compartment; \(Q_c\), apparent volume of distribution of the peripheral compartment; \(Q_{1/2}\), intercompartment clearance; \(F\), relative oral bioavailability. Most pharmacokinetic parameters were assessed as proportional error (error assessing the percentage deviation from NONMEM-estimated values). The 95% confidence interval (CI) is displayed as the 2.5 to 97.5 percentile of bootstrap estimates. Coefficient of variation (% CV) for between-occasion variability was calculated as \(\sqrt{\text{exp(estimated variance)}} - 1\), where \(\sqrt{\text{RSE}}\) is the square root.
and first-order absorption models with and without an absorption lag time were evaluated. A stepwise addition of drug transit compartments in the absorption phase was also tried to find the optimal number of transit compartments for each of the two drugs (50, 51).

All available covariates at admission (i.e., age, initial parasitemia, body weight, blood pressure, hematocrit, smoking, estimated gestational age, and pregnancy) were evaluated with a stepwise addition ($P < 0.05$) and backward elimination ($P < 0.01$) approach. Linear, piecewise linear, and power relationships centered on the median value of the population were tried for continuous covariates. Smoking and pregnancy were modeled as categorical covariates. Body weight was also evaluated as a simultaneous incorporation of an allometric function on all clearance and volume parameters, where clearance values scale to a power of 0.75 and volume values scale to the first power [e.g., individual clearance value = typical clearance value $\times$ (individual body weight/median body weight in the population)]. The influence of estimated gestational age in the pregnant group was evaluated both as a continuous covariate at enrollment and as a time-dependent covariate.

Goodness-of-fit characteristics were evaluated by plotting observed drug concentrations against population predicted and individually predicted drug concentrations and by plotting conditional weighted residuals of the predicted time to peak concentration. The reliability of the goodness-of-fit diagnostics was evaluated by calculating eta and epsilon shrinkage (52). Numerical and visual predicted checks and nonparametric bootstrap diagnostics were obtained by the automated functionalities in PsN (5). The final model with estimated variability was used to simulate 2,000 concentrations at each sampling time point (binning was centered on protocol time points), and the 95% confidence intervals around the simulated 5th, 50th, and 95th percentiles were overlaid with the observed data to evaluate the predictive power of the model (visual predictive check). The nonparametric bootstrap diagnostics ($n = 1,000$) were stratified for pregnancy to maintain an equal distribution of pregnant and nonpregnant women in the resampled data.

Pharmacodynamics. Parasite smears were performed daily, and parasite clearance times were assessed. PCR blood spots were collected at admission and at recurrent malaria and evaluated by the PCR technique to distinguish between recrudescent and new malaria infections (10). The influence of piperaquine and dihydroartemisinin on the risk of the recurrent malaria infections was investigated using survival analysis (Cox regression analysis) in the STATA program (version 10; Stata Corp., TX). Group comparisons were performed with the nonparametric Mann-Whitney test in STATA.

RESULTS
The aim of this study was to define the population pharmacokinetic properties of dihydroartemisinin and piperaquine in the treatment of uncomplicated malaria infections in pregnant and nonpregnant women living on the Thai-Myanmar border during the second and third trimesters of pregnancy.

Pharmacokinetics of dihydroartemisinin and piperaquine. As reported previously, the pregnant and nonpregnant women were well matched (Table 1), and the study drug was efficacious, well tolerated, and with no serious adverse events (47). Patients in the nonpregnant group were followed for 63 days, and those in the pregnant group were followed for 63 days or to the time of delivery (whichever was latest). Parasite clearance times were very similar in pregnant and nonpregnant women in this study ($P = 0.499$; Table 1). Regression analysis of parasite clearance rate and maximal concentration and total exposure to dihydroartemisinin or piperaquine did not result in any significant pharmacokinetic-pharmacodynamic correlations ($P > 0.05$). Two women (one pregnant patient and one nonpregnant patient) had PCR-confirmed recrudescent *P. falciparum* infections, and three women (two pregnant patients and one nonpregnant patient) had a new *P. falciparum* infection during follow-up. The nonpregnant patient who had recrudescent falciparum malaria had low piperaquine concentrations (relative bioavailability of piperaquine, 27%), which is likely to explain the treatment failure. The other recurrent *P. falciparum* infection could not be explained by altered pharmacokinetic properties of dihydroartemisinin or piperaquine. Eighteen women (10 pregnant and 8 nonpregnant patients) had a *P. vivax* infection during follow-up. No pharmacokinetic parameters or demographic variables were significant predictors of the risk of developing vivax malaria in the time-to-event modeling, which indicates that other factors (such as immunity and concomitant illness) might be more important predictors of vivax malaria.

Pharmacokinetics of piperaquine. The disposition pharmacokinetics of piperaquine were best characterized by a 3-compartment model (for 3- versus 2-compartment model, $\Delta$OFV $= -79.5$) with no significant benefit of an additional peripheral compartment ($\Delta$OFV $= -2.89$) (Fig. 1). A transit compartment absorption model with a fixed number of transit compartments ($n = 5$) was superior to all other absorption models ($\Delta$OFV $> -62.0$). A model estimating both the absorption rate from the last transit compartment to the central compartment and the drug transit rate could not minimize successfully. Drug transit rate was therefore set identical to the absorption rate from the last transit compartment to the central compartment for robust and reliable parameter estimates. Allowing intrindividual variability between dose occasions in mean transit time ($\Delta$OFV $= -344$) and in relative bioavailability ($\Delta$OFV $= -165$) significantly improved the model fit. Observed data below the limit of quantification were coded as missing since only a total of 6.3% of the observed data and 3.8% of the observed data in the terminal phase were below the limit of quantification.

### TABLE 3 Post hoc parameter estimates of the final model describing piperaquine population pharmacokinetics in pregnant and nonpregnant women with uncomplicated malaria$^a$

<table>
<thead>
<tr>
<th>Group</th>
<th>CL/F (liters/h/kg)</th>
<th>$V_{p}$F (liters/kg)</th>
<th>$t_{1/2}$ (days)</th>
<th>$T_{max}$ (h)</th>
<th>$C_{max}$ (ng/ml)</th>
<th>AUC$_{0-92}$ (µg · h/ml)</th>
<th>Predicted concn (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1.30 (0.955–1.66)</td>
<td>664 (492–912)</td>
<td>20.2 (17.4–24.1)</td>
<td>3.06 (2.62–4.05)</td>
<td>244 (173–344)</td>
<td>25,300 (19,600–32,400)</td>
<td>28.8 (23.6–34.6)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>1.28 (0.955–1.59)</td>
<td>529 (464–708)</td>
<td>17.5 (16.2–19.4)</td>
<td>3.14 (2.84–3.84)</td>
<td>291 (194–362)</td>
<td>27,400 (21,000–32,400)</td>
<td>28.8 (23.6–34.6)</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>1.32 (0.987–1.85)</td>
<td>829 (655–1,110)</td>
<td>24.0 (22.0–26.1)</td>
<td>3.04 (2.36–4.13)</td>
<td>216 (139–276)</td>
<td>23,400 (17,400–35,100)</td>
<td>22.7 (17.6–32.8)</td>
</tr>
</tbody>
</table>

$^a$ Data are for 24 pregnant and 24 nonpregnant women. Post hoc estimates were calculated as median values (interquartile ranges) from empirical Bayes estimates, and statistical differences were estimated with a nonparametric Mann-Whitney test. CL, elimination clearance; $V_{p}$, apparent total volume of distribution ($V_{p}$F + $V_{p}$/F + $V_{p}$/H); $t_{1/2}$, terminal elimination half-life; $T_{max}$, predicted time to peak concentration; $C_{max}$, predicted peak concentration; AUC$_{0-92}$, area under the concentration-time curve from time point 0 to day 92.
A covariate model with body weight incorporated as an allometric function on volume and clearance parameters minimized successfully but with an R matrix algorithmically nonpositive-semidefinite but nonsingular warning and did not improve model fit compared to the base model ($\Delta$OFV = 4.62). Estimated gestational age did not explain parameter variability in the pregnant group and was not incorporated in the final covariate model. Pregnancy on elimination clearance ($\Delta$OFV = −14.1) and pregnancy on relative bioavailability ($\Delta$OFV = −7.30) were selected in the stepwise covariate selection. A physiologically plausible competing model with pregnancy as a covariate only on volume of distribution parameters resulted in a reduced fit ($\Delta$OFV = 144) compared to the above-described covariate model. This supports a true covariate relationship between clearance, bioavailability, and pregnancy, irrespective of covariate selection procedure. The final covariate model resulted in a 45.0% and 46.8% increase in piperaquine elimination clearance and relative bioavailability, respectively, in pregnant patients compared to nonpregnant patients. Both pregnant and nonpregnant patients displayed a time-dependent increase in relative bioavailability (101%, 130%, and 170% after doses 1, 2, and 3, respectively). Interindividual variability could be reliably estimated (relative standard error [RSE] = <50%) for elimination clearance, central volume of distribution, and intercompartment clearance. Final parameter estimates are summarized in Tables 2 and 3.

The final model showed satisfactory goodness of fit (Fig. 2) and...
predictive performance, as illustrated by the visual predictive check (Fig. 3). Shrinkage was estimated to be below 24% for all parameters. A numerical predictive check (2,000 Monte Carlo simulations) using the final model resulted in 5.1% (95% CI = 2.8 to 7.6%) and 4.0% (95% CI = 2.6 to 7.9%) of piperaquine observations being below and above the simulated 90% prediction interval, respectively (Fig. 3).

**Pharmacokinetics of dihydroartemisinin.** The disposition pharmacokinetics of dihydroartemisinin were best described by a one-compartment model with no benefit of an additional peripheral compartment ($\Delta$OFV = -0.096) when using a first-order absorption model. A transit compartment absorption model with a fixed number of transit compartments ($n$ = 7) was superior to all other absorption models ($\Delta$OFV > -140). Drug transit rate was set identical to the absorption rate from the last transit compartment to the central compartment since a model estimating both the absorption rate and the drug transit rate did not significantly improve the model fit ($\Delta$OFV = -1.16). Allowing interindividual variability in the relative bioavailability ($\Delta$OFV = -51.0) and intraindividual variability in mean transit time between dose occasions ($\Delta$OFV = -344) significantly improved model fit. There was no additional benefit of implementing intraindividual variability between doses in relative bioavailability or elimination clearance. The structural disposition model was reevaluated using the transit compartment absorption model with described variability and resulted in a significant improvement in model fit when adding a peripheral compartment compared to a one-compartment disposition model ($\Delta$OFV = -112). However, the two-compartment model displayed considerable model misspecification, and the improvement in model fit was a direct result of data censoring at later time points (77% of observations were below the limit of quantification at 12 h after dosing) (Fig. 4). Modeling data below the limit of quantification as categorical data (M3 method) improved the diagnostics for the two-compartment disposition model but did not change the diagnostic performance of the one-compartment disposition model (Fig. 4). A one-compartment model where data below the limit of quantification were censored as missing was therefore used in further model building. Furthermore, a two-compartment disposition model did not result in a significantly better model fit compared to that obtained with a one-compartment disposition model when incorporating censored data as categorical data in the final covariate model ($\Delta$OFV = -5.48). Body weight was implemented on clearance and volume parameters by an allometric function and resulted in a significant improvement in model fit ($\Delta$OFV = -9.08). Parasitemia on relative bioavailability ($\Delta$OFV = -22.1) and pregnancy on relative bioavailability ($\Delta$OFV = -17.8) were selected in the stepwise covariate selection. This resulted in an increase in relative bioavailability with increasing initial parasitemia (27.8% linear increase per unit logarithmic parasitemia) and a 37.5% lower relative bioavailability in pregnant women than nonpregnant women. No time-dependent differences in relative bioavailability or any other pharmacokinetic parameters could be seen. Interindividual variability on volume of distribution and relative bioavailability could be reliably estimated (RSE < 50%). Final parameter estimates are summarized in Tables 4 and 5.

The final model showed satisfactory goodness of fit (Fig. 2) and predictive performance, as illustrated by the visual predictive check (Fig. 3). Shrinkage was estimated to be below 22% for all parameters. A numerical predictive check (2,000 Monte Carlo simulations) using the final model resulted in 3.64% (95% CI = 3.08 to 7.14%) and 6.44% (95% CI = 3.08 to 7.28%) of dihydroartemisinin observations being below and above the simulated 90% prediction interval, respectively (Fig. 3).
DISCUSSION

Pharmacokinetics of piperaquine. Biphasic disposition has previously been described for piperaquine in nonpregnant adults and children with uncomplicated malaria (21, 24, 55). A three-compartment disposition model has been suggested in healthy adults and adults with malaria, but this has not been incorporated in a population model because of the poor precision of additional parameter estimates (49, 55). This frequently sampled population demonstrates that a three-compartment disposition model does provide a better description of piperaquine pharmacokinetics than a two-compartment disposition model when enough data are incorporated in the model. A transit compartment absorption model provided a clear advantage over the previously described first-order absorption models. Not enough data were collected in the absorption phase to support both an estimation of the drug transit rate and the absorption rate from the last transit compartment to the central compartment, and the two rate constants were therefore set to be identical. Piperaquine concentrations varied

FIG 4 Visual predictive check of dihydroartemisinin emphasizing the influence of censored data for a basic covariate-free one-compartment disposition model (A), a basic covariate-free two-compartment disposition model (B), a basic covariate-free one-compartment disposition model using the M3 method for censored data (C), and a basic covariate-free two-compartment disposition model using the M3 method for censored data (D). Binning was centered on protocol time points, and open circles represent the middle time point of each bin. Solid lines, observed fraction of data below the limit of quantification; shaded areas, 95% confidence interval of the simulated (\( n = 2,000 \)) fraction of data below the limit of quantification.
considerably between doses, which could be explained by variation in mean transit time (45.8%) and in relative bioavailability (56.3%). Piperaquine is a lipophilic compound with a low oral bioavailability (50% in the rat) (57) and variable food-dependent absorption and thus total exposure (3, 16, 41, 53). Food intake was not controlled in this study, which might explain part of the high intrapersonal variability observed in absorption parameters. The time-dependent increase in oral bioavailability might in part be explained by symptomatic recovery during treatment, with greater food intake in the later stage of treatment. Relative oral bioavailability was 47% higher in pregnant women than nonpregnant women, which might result from dietary differences, reduced small intestine motility, and prolonged gastric emptying in pregnant women because of elevated progesterone concentrations (Fig. 5) (12). Thus, the incomplete absorption of piperaquine is likely to be ameliorated by an increased mean residence time in the small intestine. An increased blood flow to the stomach and small intestine, resulting from an increase in cardiac output (30 to 50%) in pregnant women, might also explain the increased bioavailability of piperaquine in pregnant women compared to nonpregnant women (31). Elimination clearance of piperaquine was an estimated 45% higher in pregnant women than nonpregnant women in this study (Fig. 5). The activities of certain liver cytochrome (CYP) isoenzymes (such as CYP3A4 and CYP2D6) are increased during pregnancy, resulting in a higher rate of metabolism of drugs by these enzymes (31). In contrast, estrogens in pregnancy inhibit CYP2C19 (35). Piperaquine is suggested to be metabolized by CYP3A4, and increased CYP3A4 activity is likely to contribute to the increased elimination of piperaquine.

Renal blood flow and glomerular filtration rate rise during pregnancy, but these changes are a less likely explanation of the observed increase in elimination clearance since studies in the rat indicate negligible renal elimination of piperaquine (57). A non-compartmental analysis of these data suggested no difference in oral clearance and a significantly lower volume of distribution in pregnant women than nonpregnant women (47). This is in agreement with the results from the population modeling since a proportional increase in relative bioavailability and elimination clearance would explain the noncompartmental net result of a decreased apparent volume of distribution since changes in volume of distribution and relative bioavailability cannot reliably be separated in a noncompartmental analysis. The population pharmacokinetic properties of piperaquine have not previously been described in pregnant women, but results presented in this study for nonpregnant women with malaria are in agreement with previously published results for nonpregnant patients with uncomplicated malaria (55).

**Pharmacokinetics of dihydroartemisinin.** Population pharmacokinetic properties of dihydroartemisinin were best described by a one-compartment disposition model with a transit compartment absorption model. Not enough data were collected in the absorption phase to support both an estimation of the drug transit rate and the absorption rate from the last transit compartment to the central compartment, and the two rate constants were therefore set to be identical. Dihydroartemisinin has previously been described by one- and two-compartment models, when modeled as individual concentration-time data in nonpregnant healthy volunteers and patients with malaria, which supports the structural model result in this study (19, 26, 38, 39). Caution should be exerted when modeling artemisinin compounds since censoring of data (a high proportion of data below the limit of quantification at later time points) can lead to inappropriate structural models if...
model selection is based on statistical criterion alone (OFV) and not confirmed by simulation-based diagnostics (Fig. 4). Censored data can be incorporated correctly in the model by the use of the Laplacian estimation method, but this is often undesirable since the computational time increases with more complex estimation methods. A one-compartment disposition model did not result in any biased predictions of censored data and was therefore not improved by a more complex model incorporating censored data as categorical data (Fig. 4).

Dihydroartemisinin is almost insoluble in water and requires appropriate excipients to optimize oral absorption. This is likely to explain the large interindividual variability in relative bioavailability (30.3%) and mean transit time (50.9%). The final covariate model resulted in a 38% lower relative bioavailability in pregnant women than nonpregnant women (Fig. 5). Dihydroartemisinin is unstable in acidic pH, and a prolonged gastric retention in pregnant women might result in an increased presystemic decomposition of dihydroartemisinin and a reduction in relative bioavailability compared to nonpregnant women (12, 18). The metabolism of drugs catalyzed by UDP glucuronosyltransferase (UGT) isoenzymes (UGT1A4 and UGT2B7) is increased during pregnancy (2). Dihydroartemisinin is rapidly eliminated from the systemic circulation ($t_{1/2} \approx 1$ h) through hepatic glucuronidation by UGT1A9 and UGT2B7, and the induction of these enzymes during pregnancy could result in an increased first-pass metabolism of dihydroartemisinin and an accelerated clearance (22). These contributing factors are likely to explain why pregnancy had a larger effect on relative bioavailability than on elimination clearance of dihydroartemisinin. Relative bioavailability was an estimated 28% higher with each log-unit increase in initial parasitemia. A similar observation has previously been reported for dihydroartemisinin, with a proportional 5-fold reduction in both mean oral clearance and apparent volume of distribution detected in malaria patients compared to healthy volunteers when evaluated with noncompartmental analysis (36, 39, 40). This might be a result of a disease-related decrease in first-pass metabolism possibly compounded by reduced hepatic blood flow, as suggested for artesunate in acute malaria patients (32).

The pharmacokinetic properties of dihydroartemisinin in healthy volunteers and nonpregnant patients with malaria have been well characterized with noncompartmental analysis, but no data on the population pharmacokinetics of dihydroartemisinin when administered as the fixed dihydroartemisinin-piperaquine combination are available (7, 11, 19, 27, 40, 54). Results presented in this study for nonpregnant women with malaria are in agreement with previously published results for nonpregnant patients with uncomplicated malaria. Results from the noncompartmental analysis showed a nonsignificant trend of 30% higher elimination clearance in pregnant women than nonpregnant women (47). The difference did not reach statistical significance because of the large interindividual variability, which emphasizes the need for population pharmacokinetic modeling to estimate true population differences.

Pharmacodynamics of dihydroartemisinin and piperaquine. The altered pharmacokinetic properties of piperaquine in pregnant women compared to nonpregnant women are unlikely to have a clinical therapeutic impact since total drug exposure is unaltered (Tables 2 and 3). The effect of pregnancy on piperaquine elimination clearance and relative bioavailability resulted in an increased maximal concentration and a shorter terminal elimination half-life in pregnant women than nonpregnant women (Fig. 4). This will affect the period of posttreatment prophylaxis and would have an impact in intermittent preventive treatment if treatments are given too far apart, allowing piperaquine concentrations to fall below the MICs for prevalent parasites between doses. Simulated population mean profiles resulted in 8.7% and 45% higher piperaquine concentrations in nonpregnant women than pregnant women at 30 days and 60 days after treatment, respectively. This suggests that monthly administered intermittent preventive treatment should have similar clinical efficacy in pregnant and nonpregnant women but that treatment given further apart must be carefully evaluated before implementation. Pregnant women had a 38% lower dihydroartemisinin exposure resulting from reduced bioavailability. This could well have a clinical impact since piperaquine exposure is highly variable. A lower exposure to dihydroartemisinin results in larger numbers of residual parasites remaining to be killed by piperaquine, which might increase the risk of treatment failure and therefore fuel the emergence of resistance (14).

In conclusion, this parallel study in pregnant and nonpregnant women with uncomplicated malaria indicated a significant impact of pregnancy on the pharmacokinetic parameters of piperaquine and dihydroartemisinin. Pregnancy resulted in a 38% lower total dihydroartemisinin exposure, but there was no difference in piperaquine exposure in pregnant women compared to...
nonpregnant women. The efficacy of the fixed combination of dihydroartemisinin and piperaquine in pregnant women requires assessment in larger cohorts.

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