ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2008, p. 1052–1061 Vol. 52, No. 3
0066-4804/08/$08.00+0 doi:10.1128/AAC.00955-07

Population Pharmacokinetics of Piperaquine after Two Different Treatment Regimens with Dihydroartemisinin-Piperaquine in Patients with *Plasmodium falciparum* Malaria in Thailand▽

J. Tarning,[1,3] E. A. Ashley,[2,3,4,5] N. Lindegardh,[3,4] K. Stepniewska,[3,4] L. Phaiphun,[2] N. P. J. Day,[3,4] R. McGready,[2,3,4] M. Ashton,[1] F. Nosten,[2,3,4] and N. J. White[3,4]*

Department of Pharmacology, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden[1]; Shoklo Malaria Research Unit, Mae Sot, Thailand[2]; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand[3]; Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, United Kingdom[4]; and Epicentre, 8 rue Saint Sabin, 75011 Paris, France[5]

Received 25 July 2007/Returned for modification 30 October 2007/Accepted 28 December 2007

The population pharmacokinetics of piperaquine in adults and children with uncomplicated *Plasmodium falciparum* malaria treated with two different dosage regimens of dihydroartemisinin-piperaquine were characterized. Piperaquine pharmacokinetics in 98 Burmese and Karen patients aged 3 to 55 years were described by a two-compartment disposition model with first-order absorption and interindividual random variability on all parameters and were similar with the three- and four-dose regimens. Children had a lower body weight-normalized oral clearance than adults, resulting in longer terminal elimination half-lives and higher total exposure to piperaquine (area under the concentration-time curve from 0 to 63 days [$AUC_{day\ 0-63}$]). However, children had lower plasma concentrations in the therapeutically relevant posttreatment prophylactic period ($AUC_{day\ 3-20}$) because of smaller body weight-normalized central volumes of distribution and shorter distribution half-lives. Our data lend further support to a simplified once-daily treatment regimen to improve treatment adherence and efficacy and indicate that weight-adjusted piperaquine doses in children may need to be higher than in adults.

Malaria is the most important parasitic disease in the world, with between 300 to 500 million clinical episodes each year. The 4-aminoquinoline piperaquine, 1,3-bis-[4-(7-chloroquinolyl-4)-piperazinyl-1]-propane, is effective against multidrug-resistant *Plasmodium falciparum*. In 1978, it replaced chloroquine as the first-line treatment for malaria in China and was also used extensively as a mass prophylaxis in the 1970s until the emergence of resistance in the 1990s. Piperaquine has recently been the object of renewed interest as a partner drug in artemisinin-based combination therapy (ACT). ACTs are now recommended first-line treatments for *P. falciparum* malaria throughout the world.

In several studies, a fixed oral combination of dihydroartemisinin and piperaquine phosphate (Artekin; each tablet contains 320 mg piperaquine phosphate and 40 mg dihydroartemisinin) has given high cure rates with excellent tolerability in the treatment of multidrug-resistant *P. falciparum* malaria (1, 2, 8, 16, 25, 29). The dihydroartemisinin-piperaquine combination is being increasingly deployed in Southeast Asia and is already part of national treatment recommendations in Cambodia and Vietnam. It is considered a highly promising antimalarial drug for future global deployment and has great potential for intermittent presumptive treatment. The standard treatment regimen (DP4) comprises four treatment doses given over 3 days (i.e., an adult dose of two tablets given orally at 0, 8, 24, and 48 h). Ashley et al. (2) recently showed that the simpler regimen of an approximately equivalent total dose divided equally for once-daily treatment (DP3) given over the same period (i.e., three tablets given orally at 0, 24, and 48 h) is also a highly efficacious and safe treatment. In patients studied on the northwest border of Thailand, both regimens were well tolerated and there were no differences in PCR genotype-adjusted cure rates assessed at day 63.

Few studies have assessed the clinical pharmacokinetics of piperaquine despite its extensive use. In general, it has disposition kinetics similar to those of chloroquine. In eight healthy Caucasian subjects, exposure to piperaquine increased by twofold when piperaquine was administered with a high-fat meal compared to that for the fasting state (24). The oral bioavailability of piperaquine and other lipid-soluble antimalarial drugs, including mefloquine, atovaquone, and halofantrine, is limited by low water solubility and is therefore increased by administration with fats (7, 19, 22).

Population pharmacokinetic modeling was employed to characterize piperaquine kinetics in Cambodian patients (13) and healthy Vietnamese subjects (23). In both studies, oral piperaquine exhibited biphasic disposition kinetics with a large steady-state volume of distribution and low clearance, resulting in a long terminal half-life of about 2 to 3 weeks. Children had a twofold-higher body weight-normalized oral clearance than that for adults (13). Absorption in fasting subjects was erratic, resulting in multiple peaks (23). Neither study identified any covariates influencing piperaquine kinetics. Concerns that the terminal half-life was underestimated because of inadequate duration of sample collection and assay insensitivity have been raised (27).

The present study investigated the pharmacokinetic properties of piperaquine with a population-based modeling ap-

* Corresponding author. Mailing address: Mahidol - Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand. Phone: 662 354 9172. Fax: 662 354 9169. E-mail: nickw@tropmedres.ac.

▽ Published ahead of print on 7 January 2008.

proach and a sensitive assay in a larger series of adults and children with uncomplicated *P. falciparum* malaria treated with the ACT combination dihydroartemisinin-piperaquine once daily for three days (DP3) or with the previously standard four-dose regimen (DP4), who were monitored for 63 days following treatment.

MATERIALS AND METHODS

Study site and ethical approval. This pharmacokinetic study was nested into a larger randomized clinical trial of the efficacy and safety of dihydroartemisinin-piperaquine reported in detail elsewhere (2). The study was conducted in accordance with the Helsinki Declaration at two clinics run by the Shoklo Malaria Research Unit along the Thai-Myanmar border. This is an area of unstable low and seasonal transmission of multidrug-resistant *P. falciparum* malaria (18). The study was explained to patients in their own language, and written consent was obtained (by thumbprint in the cases of patients unable to read or write). Approval for the study was granted by the Faculty of Tropical Medicine Mahidol University Ethical Committee, Bangkok, Thailand, and the Oxford Tropical Research Ethics Committee, United Kingdom.

Study design. Full clinical details of this study have been reported previously (2). Upon enrollment of the patients, malaria parasitemia was counted on Giemsa-stained thick and thin blood films (9), the hematocrit was measured, and blood was stored for PCR genotyping. The exclusion criteria were pregnancy, lactation, having ≥4% parasitized red blood cells, age of less than 1 year or more than 65 years, having signs or symptoms of severe malaria, and/or having been treated with mefloquine during the previous 60 days.

Study drug. Patients were randomly allocated into one of the two treatment arms (DP3 or DP4) from a randomization list generated in Stata, version 7. Dihydroartemisinin-piperaquine (Artekin; Holleykin Pharmaceutical Co. Ltd., Guangzhou, China) was administered to achieve a total dose of 7 mg/kg of body weight of dihydroartemisinin and 55 mg/kg of body weight of piperaquine phosphate rounded up to the nearest half tablet. The dose was divided into four (DP4) and administered at 0, 8, 24, and 48 h or three (DP3) and administered at 0, 24, and 48 h. Each treatment was supervised. No restrictions regarding food intake before or after drug administration were specified. The treatment and study codes were both concealed in sealed envelopes. Laboratory staff reading the malaria smears or performing the drug quantification assay were blind to the treatment received.

Clinical and parasitological assessments. Blood smears to test for malaria were taken and tympanic temperature (Braun Thermoscan LF40 thermometer) was measured daily until clearance of the parasite and fever. The patients had weekly follow-up visits at the clinic during which clinical examinations, malaria smears, symptom inquiries, and hematocrit determinations were performed. Recrudescence was distinguished from reinfection by using PCR genotyping (6).

Plasma sample collection. All patients provided a pretreatment plasma sample and two to four additional samples drawn randomly from the following time windows after administration of the drug: 0 to 4, 8 to 12, 24 to 28, and 48 to 52 h (DP3) or 4 to 8, 12 to 24, 28 to 48, and 52 to 72 h (DP4) plus one additional sample on either day 7, 14, 21, 28, 35, 42, 49, 56, or 63. Blood samples (3 ml) were drawn into heparinized tubes which were inverted by hand and centrifuged for 10 min at 1500 × *g*. Aliquots of plasma were transferred to plastic cryotubes and frozen in liquid nitrogen. They were transferred at regular intervals to the freezers in the local laboratory, where they were stored at −80°C, and were later transported on dry ice to the pharmacology laboratory at the Faculty of Tropical Medicine in Bangkok. All samples were stored at −80°C until drug analysis was performed, within 12 months after collection.

Piperaquine assay. Plasma samples were analyzed for piperaquine by a high-throughput method utilizing solid-phase extraction and liquid chromatography (LC) with UV detection as described previously (17). The LC system used was a LaChrom Elite system consisting of an L2130 LC pump, an L2200 injector, an L2300 column oven set at 25°C, and an L2400 UV detector (Hitachi, Tokyo, Japan). Data acquisition was performed using LaChrom Elite software (VWR, Darmstadt, Germany). Triplicates of 20-, 100-, and 612.5-ng/ml quality control samples were used to ensure precision and accuracy during quantification. The lower limit of quantification was set to 5 ng/ml or 2.5 ng/ml, depending on whether 0.5 ml or 1.0 ml, respectively, of plasma was used.

Data analysis and pharmacokinetic modeling. Piperaquine plasma concentrations were transformed into their natural logarithms, and the concentration-time profiles were modeled with nonlinear mixed-effects population modeling, using NONMEM, version V, level 1.1, software (Icon Development Solutions, Maryland). Pharmacokinetic compartment models were fitted to the concentration-time profile for all patients, using the first-order estimation (FO) and first-order conditional estimation (FOCE) methods (5, 30, 31). The FO estimation method was used to produce a preliminary model, as it provides advantages in convergence and analysis time. The final model was constructed using the FOCE method. Census, version 0.998r5a (33); S-PLUS, version 7.0 for Windows (Insightful Corp., Seattle, WA); and the S-PLUS-based program Xpose, version 3.1 (15), were used to evaluate the goodness of fit during the model-building process and to produce graphs.

The data sets for DP4 and DP3 were combined, and potential treatment regimen differences were modeled as covariate inclusions for each parameter. Piperaquine is already known to exhibit multiphasic disposition kinetics. Two- and three-compartment pharmacokinetic models with elimination from the central compartment and with first-order absorption, with and without absorption lag times, were evaluated. The models were parameterized as oral clearance (CL/*F*), central volume of distribution, absorption rate constant, intercompartmental clearance(s) (*Q*/*F*), and peripheral volume of distribution(s), where *F* is oral bioavailability. Interindividual random variability in all parameters was modeled exponentially as illustrated for clearance: $(CL/F)_i = TV(CL/F) \times \exp(\eta_{i,CL/F})$, where $(CL/F)_i$ is the individually estimated oral clearance value for the *i*th patient, $TV(CL/F)$ is the typical clearance value for the modeled population, and $\eta_{i,CL/F}$ is between-patient random variability, assumed to be normally distributed (zero mean, variance ω^2). Additive, proportional, and slope-intercept error models were applied to explain the residual random variability, which originates from intraindividual variability, measurement errors, and model misspecification.

The possible influence of continuous covariates (age, weight, height, initial hematocrit, and parasitemia) and categorical covariates (gender and treatment group) were investigated using the stepwise general additive method (GAM) as implemented in Xpose. Covariates identified by the GAM to be potentially influential were included in the modeling. Those continuous covariates were evaluated by including them in the model as linear, allometric, or hyperbolic maximum effect functions centered on the median value. The treatment group (i.e., DP4 [DOSE = 0] or DP3 [DOSE = 1]) was incorporated as a dichotomous covariate on all pharmacokinetic parameters to evaluate potential differences based on the different treatment regimens as described for intercompartment clearance, as follows: $(Q/F)_i = [(TV(Q/F) \times (1 + \text{DOSE} \times \theta_{DP3})] \times \exp(\eta_i^{Q/F})$.

Model discrimination was assessed by a likelihood ratio test using the objective function values (OFVs) computed by NONMEM. The OFV is essentially equal to −2 log likelihood, and the difference in OFVs between models is assumed to be χ^2 distributed (4). A difference in OFVs of 3.84 was considered to be significant when *P* was < 0.05, with one degree of freedom (i.e., a difference of one parameter), in comparisons of two competing hierarchical models.

Standard diagnostic plots were used to evaluate the overall goodness of fit by measured, log-transformed piperaquine concentrations versus population-fitted and individually fitted log-transformed piperaquine concentrations and by plotting weighted residuals versus time and population-fitted, log-transformed piperaquine concentrations. Individually predicted pharmacokinetic parameter estimates were used to simulate full profiles of randomly selected male and female patients of low and median body weights, using WinNonlin, version 5 (Pharsight Corporation, CA).

The final model and estimated parameters with the original data set were used as the simulation input. A visual predictive check was performed by simulating 500 concentrations at each of the individual sampling times up to 63 days after the initiation of treatment (12). Median (50th percentile), 5th percentile, and 95th percentile population concentrations were calculated at each of the 374 sampling times and plotted together with the observed concentrations against time.

RESULTS

Safety and efficacy. Ninety-eight Burmese or Karen patients aged 3 to 55 years with symptomatic uncomplicated *P. falciparum* infections were enrolled in the nested population pharmacokinetic study (demographic characteristics are given in Table 1). Both treatment regimens were well tolerated, and no patient had a recrudescence. Four were reinfected with *P. falciparum* at 28, 35, 36, or 50 days after starting treatment, and 17 patients had presumed *P. vivax* relapses between days 32 and 63 (Fig. 1).

TABLE 1. Patient demographics and treatment regimen for the nested piperaquine pharmacokinetic study

Characteristic	Treatment regimen group[c]			
	DP4		DP3	
	Median (±SD)	Min-max	Median (±SD)	Min-max
Total no. of patients	50		48	
No. of males	27		32	
No. of females	23		16	
Total PQ[a] dose (mg/kg)	31 (±4.5)	23–43	31 (±5.0)	23–43
Age (yr)	25 (±12)	6–52	25 (±13)	3–55
Height (cm)	153 (±13)	110–170	157 (±18)	92–169
Body wt (kg)	47 (±11)	14–74	51 (±12)	12–59
Initial hematocrit (%)	39 (±5.4)	23–48	39 (±6.0)	20–48
Initial parasitemia (parasites, $10^3/\mu l$)[b]	13.4 (±54.4)	0.133–221	8.16 (±37.6)	0.083–153

[a] Piperaquine.
[b] Geometric mean.
[c] DP4, dihydroartemisinin-piperaquine four-dose regimen at 0, 8, 24, and 48 h; DP3, dihydroartemisinin-piperaquine three-dose regimen at 0, 24, and 48 h; SD, standard deviation; Min-max, minimum to maximum.

Pharmacokinetic modeling of piperaquine. There were no major differences in patient demographics between the two treatment groups (Table 1). A total of 480 venous blood samples were taken from 98 patients over a period of 63 days following treatment. Piperaquine concentrations could be determined in 469 plasma samples. The coefficients of variation during the piperaquine analysis (n = 18) were 7%, 4%, and 4% at 20 ng/ml, 100 ng/ml, and 612.5 ng/ml, respectively. Measured concentrations below the limit of quantification (n = 11, <2.3% of total samples) were coded as missing data (3).

A first-order absorption, two-compartment disposition model without interindividual random variability and an additive error provided the base model. Inclusion of interindividual random variability in pharmacokinetic parameters provided a significant improvement, as evidenced by the changes in the OFVs and superior precision of parameter estimates. A three-compartment disposition model or a two-compartment model with absorption lag time was not supported by the data, as evidenced by a poor level of precision in parameter estimates or inability to converge the models.

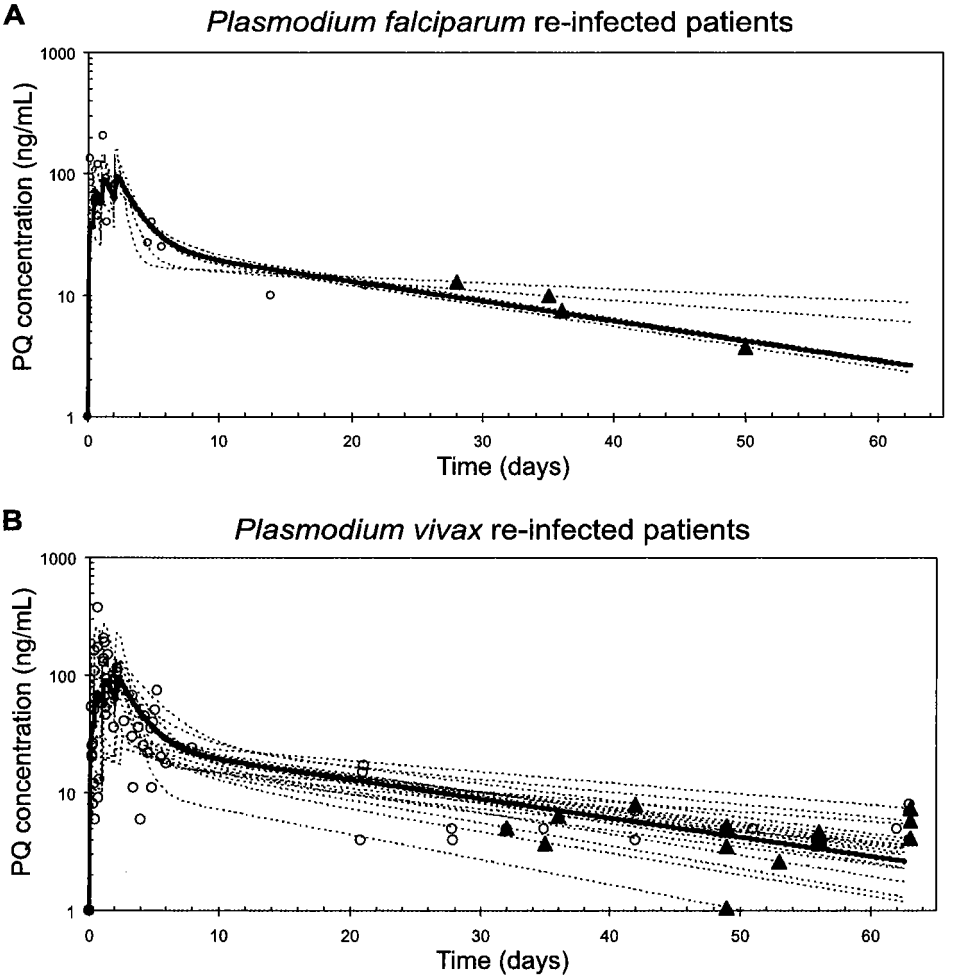

FIG. 1. Plasma concentration-time profiles of piperaquine (PQ) for *P. falciparum* (A)- and *P. vivax* (B)-reinfected patients. The solid lines (—) represent the population concentration-time profile, and the dashed lines (---) represent the concentration-time profiles for reinfected patients. The days of reinfection are marked by solid triangles (▲) and measured piperaquine concentrations by open circles (○). All y axes are on the logarithmic scale.

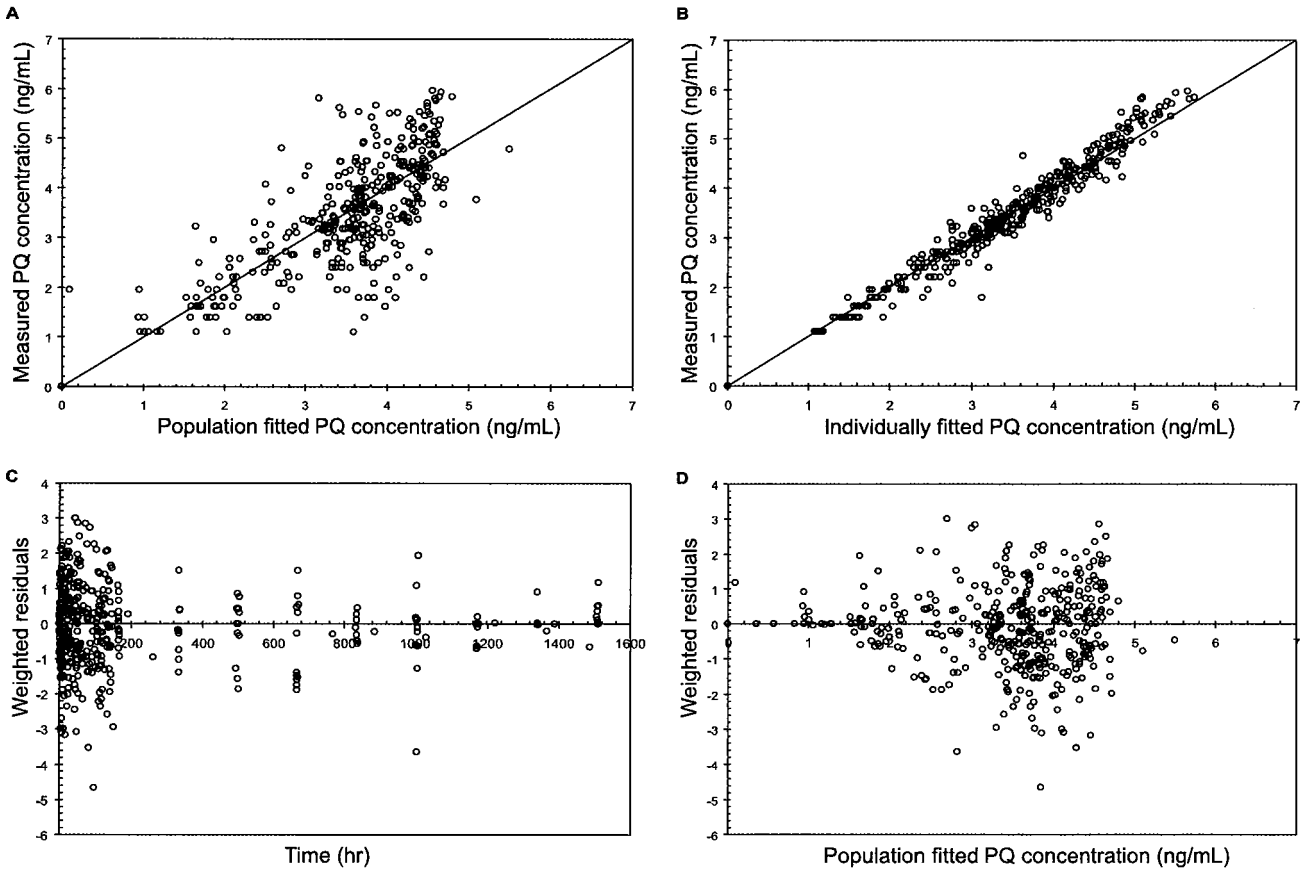

FIG. 2. Basic goodness-of-fit from the final two-compartment covariate model with first-order absorption rate and elimination from the central compartment. Measured piperaquine (PQ) concentrations are plotted against the population-fitted piperaquine concentration (A) and the individually fitted piperaquine concentration (B). The solid line (−) represents the line of identity. Weighted residuals are plotted against time (C) and the population-fitted piperaquine concentration (D). All axes except those for weighted residuals and time are on the logarithmic scale.

GAM analysis of the base model with interindividual random variability on all parameters, except on the absorption rate constant, indicated a linear correlation between covariates and pharmacokinetic parameters. Age and body weight were the covariates that produced the strongest correlation with pharmacokinetic parameters and were independently evaluated on each separate parameter and by forward stepwise inclusion during modeling.

A linear relationship between body weight and clearance and body weight and central volume of distribution gave the best fit to the data (2.6% and 2.7% increase in oral clearance and central volume of distribution, respectively, per kg of body weight increase from median weight). Use of an allometric or a hyperbolic maximum effect model for body weight on clearance and/or intercompartment clearance did not converge with the FOCE method. The same covariate models produced inferior parameter precision compared to a linear covariate model with the FO method. Variations in both clearance and central volume of distribution needed to be explained by body weight in order for NONMEM to converge. The additional inclusion of age to explain the residual variability of the peripheral volume of distribution in the final model was favored statistically (ΔOVF, −14.9, 1 degree of freedom) but provided inferior precision (relative standard error [RSE]) in the estimation of all pharmacokinetic parameters except for the central volume of distribution, which showed a minor improvement (RSE, 12% versus 14%). The inclusion of age resulted in decreased interindividual random variability for the peripheral volume of distribution (coefficient of variation, 50% versus 35%), but the precision of this variability was not justified (RSE, 76% versus 269%).

There was a linear correlation for children between body weight and age. Body weight as a covariate produced lower OFVs as evaluated on each separate parameter compared with the inclusion of age on the same pharmacokinetic parameter using the FO method. The addition of body weight as a covariate for pharmacokinetic parameters other than clearance and central volume of distribution or other combinations of covariates (i.e., age and/or body weight) was not supported during the inclusion and withdrawal of covariates. This finding was evidenced by a nonsignificant drop in OFV or the inability to converge NONMEM. No differences in the pharmacokinetics of the two treatment regimens were evident. The two dose regimens resulted in similar mean drug exposures (i.e., area under the concentration-time curve from 0 to 63 days [$AUC_{day\ 0-63}$] for DP3, 19.4 h × μg/ml, and for

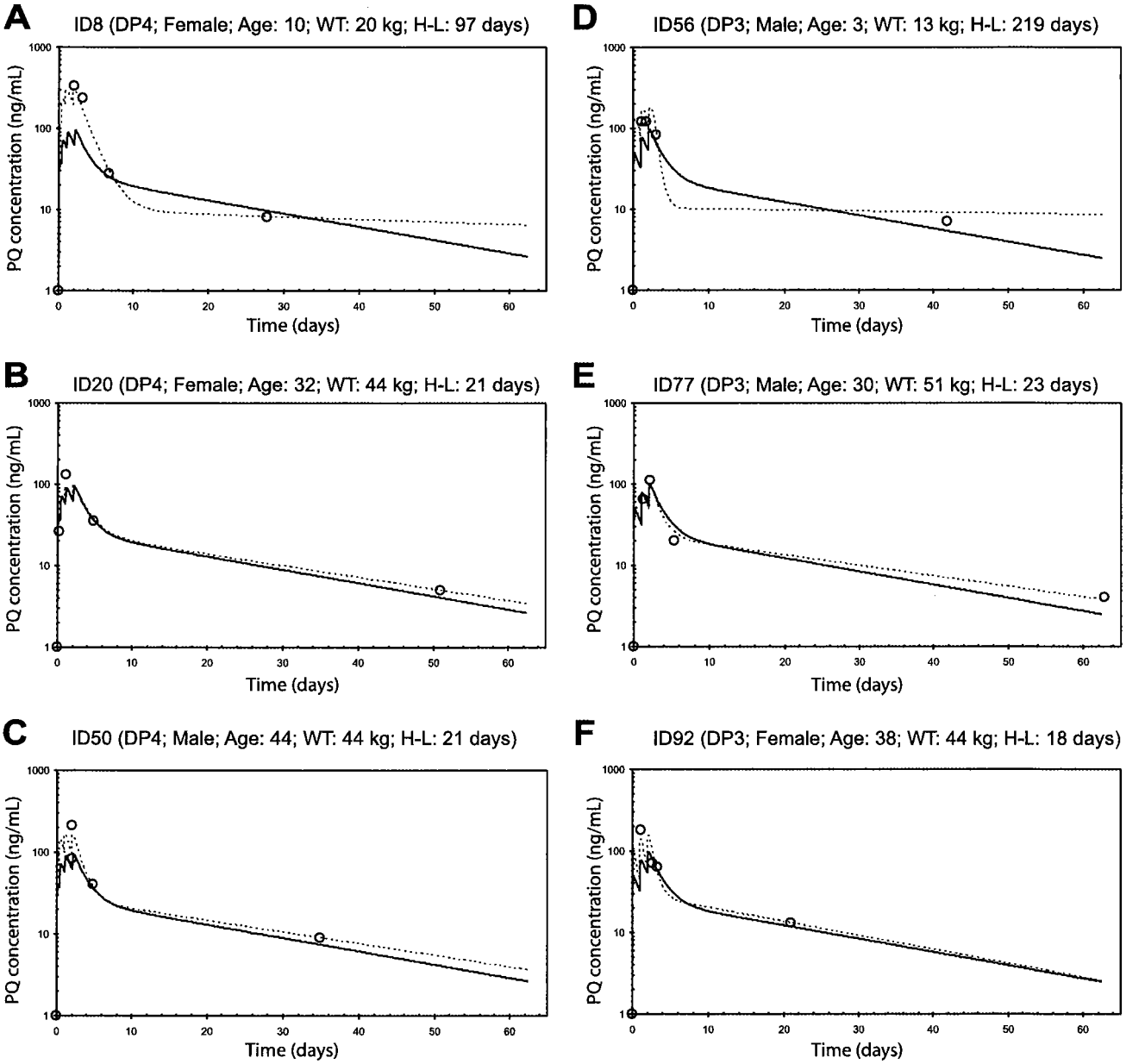

FIG. 3. Individual pharmacokinetic profiles simulated from individually estimated pharmacokinetic parameters for six randomly selected male and female patients of low and median body weight, displaying at least four measured concentrations. The patients represented in panels A, B, and C (A, ID8; B, ID20; C, ID50) received the standard four-dose treatment regimen (DP4; Artekin at 0, 8, 24, and 48 h), and the patients represented in panels D, E, and F (D, ID56; E, ID77; F, ID92) received the once-daily dose regimen (DP3; Artekin at 0, 24, and 48 h). The dashed lines (---) represent the simulated pharmacokinetic profiles, the solid lines (—) represent the population pharmacokinetic profile, and the open circles (O) represent the measured piperaquine (PQ) concentrations. Demographic data (i.e., gender, age, and body weight [WT]) and the terminal half-life (H-L) are stated for each patient above the relevant plot. All *y* axes are on the logarithmic scale.

DP4, 20.7 h × μg/ml; $AUC_{day\ 3-20}$ for DP3, 8.65 h × μg/ml, and for DP4, 9.07 h × μg/ml).

Basic goodness-of-fit plots are shown in Fig. 2, and individually simulated patient pharmacokinetic profiles for six randomly selected patients of different ages can be seen in Fig. 3. The population-derived estimates of the final model, interindividual random variability, and residual random variability are shown in Table 2 and compared with previously published reports (Table 3).

The mean terminal elimination half-life, calculated from individually obtained estimates, was approximately 28 days but was longer for children than for adults. A trend toward lower body weight-normalized oral clearance and a slightly higher body weight-normalized oral steady-state volume of distribu-

TABLE 2. Parameter estimates of the final two-compartment model describing piperaquine population pharmacokinetics in patients with uncomplicated *P. falciparum* malaria

Parameter[a]	Population estimate (% RSE)	% CV for interindividual variability (% RSE)
CL/*F* (liter/h)	66.0 (6.9) × {1 + 0.0262 (2.9) × [WT − 48]}	42 (44)
V_C/F (liter)	8,660 (14) × {1 + 0.0273 (11) × [WT − 48]}	101 (17)
Q/*F* (liter/h)	131 (13)	85 (18)
V_P/F (liter)	24,000 (13)	50 (76)
V_{ss}/F (liter)[b]	38,100[c]	
k_a (h^{-1})	0.717 (25)	168 (38)
σ (% CV)	31.4 (29)	
$t_{1/2\ \beta}$ (days)[b]	27.8[d]	

[a] *F*, oral bioavailability; CL, clearance; V_C, central volume of distribution; *Q*, intercompartment clearance; V_P, peripheral volume of distribution; V_{ss}, steady-state volume of distribution; k_a, first-order absorption rate constant; σ, additive residual error; $t_{1/2\ \beta}$, terminal half-life; RSE, relative standard error [(standard error/mean) × 100%]; WT, body weight with a median value of 48 kg.
[b] Mean population value.
[c] Minimum, 9980; maximum, 115,500.
[d] Minimum, 10.2; maximum, 216.

tion were observed in children (Fig. 4). The children in this study (11 patients ≤12 years of age and below 30 kg of body weight) had a higher total mean exposure for piperaquine ($AUC_{day\ 0–60}$, 25.9 h × μg/ml) than the population mean value ($AUC_{day\ 0–60}$, 19.4 h × μg/ml), but importantly, they also had a decreased mean exposure from days 3 to 20 ($AUC_{day\ 3–20}$ for children, 7.58 h × μg/ml; population mean value, 8.65 h × μg/ml).

The recently proposed method of internal evaluation by the posterior visual predictive check was used as a diagnostic tool for both the fixed and random effects in the model (12). Measured piperaquine concentrations were superimposed on simulations, and the former were symmetrically distributed on the 50th percentile profile, with approximately 9% of the data distributed outside the 5th- to 95th-percentile boundaries, reflecting that expected clinical variability is representatively described (Fig. 5). The log-normal distribution of simulated piperaquine plasma concentrations can be seen in the inserted panels at the common follow-up on days 28 and 42 (Fig. 5).

DISCUSSION

Dihydroartemisinin-piperaquine is an important artemisinin-based antimalarial treatment. This is to date the largest reported pharmacokinetic study of piperaquine conducted in patients with malaria. In the study, a population pharmacokinetic model for piperaquine was established by using NONMEM. The pharmacokinetic properties of piperaquine were characterized by a two-compartment disposition model with first-order absorption. The effective random residual error model should be considered multiplicative, since the modeled data was log transformed. This population pharmacokinetic study shows that there are no significant pharmacokinetic differences for piperaquine between the older, standard four-dose regimen and the newer, simplified three-dose regimen and therefore provides further support for this once-daily treatment regimen to improve treatment adherence and efficacy.

This study concentrated more on characterizing piperaquine

TABLE 3. Pharmacokinetics of piperaquine in present and previous studies

Subjects	Age (mean ± SD or range [yr])	No. of patients	Mean total piperaquine administered (mg/kg)	Total no. of samples	Duration of sampling (days)	Food intake during drug administration	Pharmacokinetic modeling	CL/*F* (liter/h/kg)[j]	V_{ss}/F (liter/kg)[k]	$t_{1/2\ z}$ (days)[l]	Reference
Patients	3–55	98	31	469	63	NC[i]	Mixed effects[d]	1.4[g]	874[g]	28	Present study
Patients	30 ± 13	38	32	213[b]	35	No food	Mixed effects[e]	0.90	574	23	13
Patients	2–10	47	35	132[b]	35	No food	Mixed effects[e]	1.85	614	14	13
Healthy volunteers	31 ± 3.5	12	25[a]	468	29	No food	Mixed effects[d]	1.0[h]	103[h]	12	23
Healthy volunteers	19–42	8	4.2	152[c]	42	No food	NCA[f]	1.14	716	20	24
Healthy volunteers	19–42	8	4.2	152[c]	42	High-fat food	NCA[f]	0.60	365	21	24

[a] These subjects received CV8; all other subjects received Artekin.
[b] Total number of samples above the lower limit of quantification; included in analysis according to graph in Hung et al. (13), Fig. 3, page 260.
[c] Based on duration of sampling, since number of samples included in analysis was not reported.
[d] Mixed effects, nonlinear mixed effects modeling using the modeling software NONMEM, version V, level 1.1.
[e] Mixed effects, nonlinear mixed effects modeling using the modeling software Kinetica, version 4.1.
[f] NCA, noncompartmental analysis using the modeling software Kinetica, version 4.3.
[g] Population estimate for a patient with a median body weight of 48 kg.
[h] Parameter estimates are weight normalized based on published population mean values divided by the mean weight of subjects.
[i] NC, not controlled in study design.
[j] CL/*F*, mean oral clearance.
[k] V_{ss}/F, mean oral steady-state volume of distribution.
[l] $t_{1/2\ z}$, mean terminal elimination half-life.

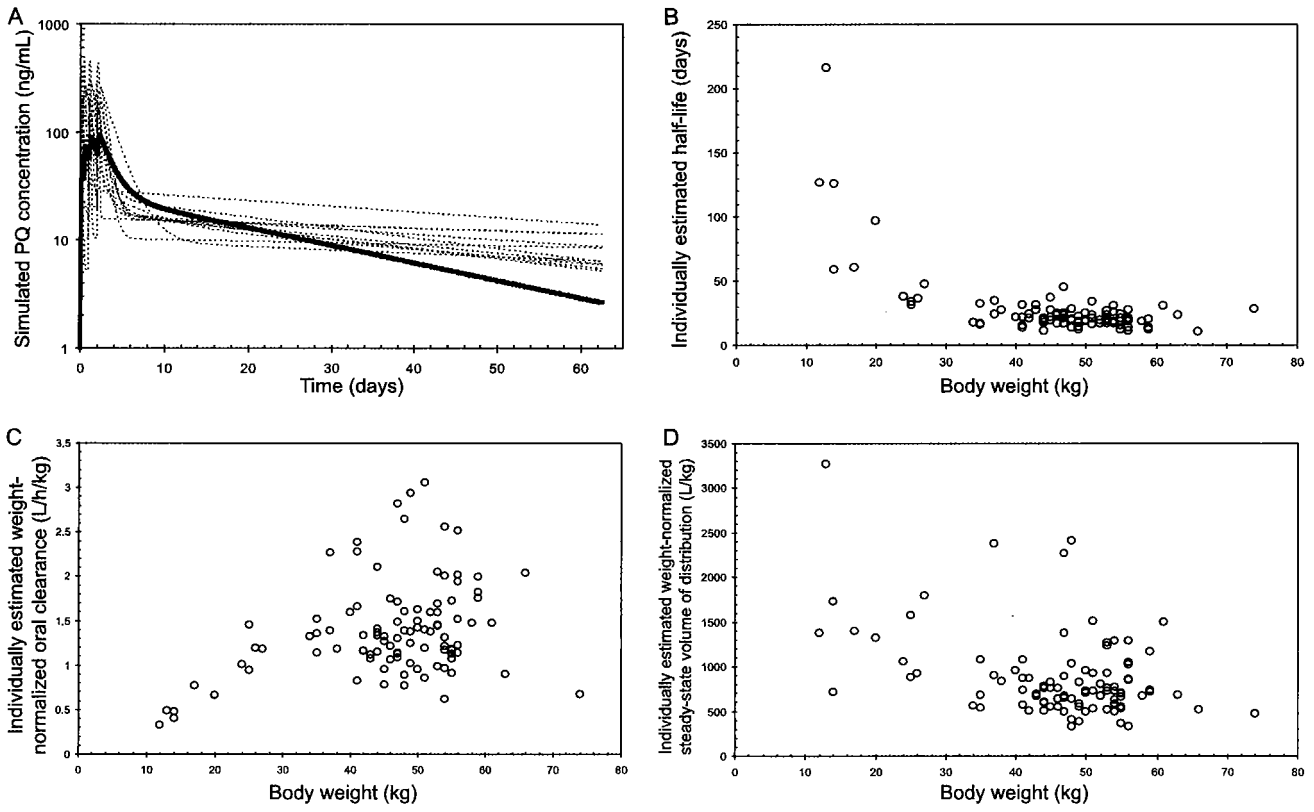

FIG. 4. (A) Simulated piperaquine (PQ) concentrations plotted on a semilogarithmic scale against time for the population mean (—) and patients below 30 kg of body weight (---). Individually estimated terminal half-lives (B), the individually estimated body weight-normalized oral clearance (C), and the individually estimated body weight-normalized oral steady-state volume of distribution (D) are plotted versus body weight for all patients.

disposition, so insufficient data were collected during the absorption phase, which partly explains the uncertainty in the absorption rate constant. The derived population estimates of pharmacokinetic parameters are otherwise generally similar to those previously reported, with a very large steady-state volume of distribution (V/F = 874 liter/kg) and a long estimated terminal half-life ($t_{1/2\beta}$) of 28 days (Tables 2 and 3). The half-life might still be underestimated, since the sparsely collected data were distributed over a long sampling period with no more than one measured concentration beyond 7 days after starting treatment. Even though the two-compartment model employed under-predicted the highest concentrations as well as those at the last sampling time point, the available data did not support a three-compartment disposition model (Fig. 2). The presence of an even longer terminal elimination phase has been suggested (27). In these respects, piperaquine is similar to chloroquine, which also has an extremely large total apparent volume of distribution and a very long terminal half-life. The terminal half-life is an important determinant of the temporal distribution of recrudescences (and thus the duration of follow-up required in clinical trials), as it determines the post-treatment prophylactic effect and affects the propensity to select for resistance (32).

Previous studies have shown a large interindividual random variability of piperaquine kinetics, which was also found in this study (13, 23). Body weight, centered on the median value, was included in the final model to explain a portion of the inter-individual random variability in clearance and in the central volume of distribution. There was an apparent increase in body weight-normalized oral clearance and a minor decrease in body weight-normalized oral steady-state volume of distribution with increasing body weight (Fig. 4). Body weight-normalized oral clearance was lower in children than in adults, and the resulting derived terminal half-life showed a marked prolongation in small children. However, the covariate function provided by these data should not be extrapolated beyond the studied population demographics, since for children below 10 kg of body weight parameter estimates will be unreasonable. The pharmacokinetic differences between children and adults observed in this study will need to be confirmed, since a relatively small number of children were studied and there could be model misspecification. However, if these differences are confirmed in larger studies, they might provide some indication of the drug-metabolizing enzymes involved. For example, it has been suggested that the cytochrome P450 (CYP) enzymes CYP1A2 and CYP2B6 are not fully matured until 10 years and 20 years of age, respectively (14). A retrospective study of 45 drugs also showed that glucuronidation substrates displayed significantly longer half-lives for children (2 to 12 years) and adolescents (12 to 18 years) than for adults (10). The trend in body weight-normalized clearance versus body weight is similar to that seen for caffeine (i.e., CYP1A2) and diclofenac (i.e.,

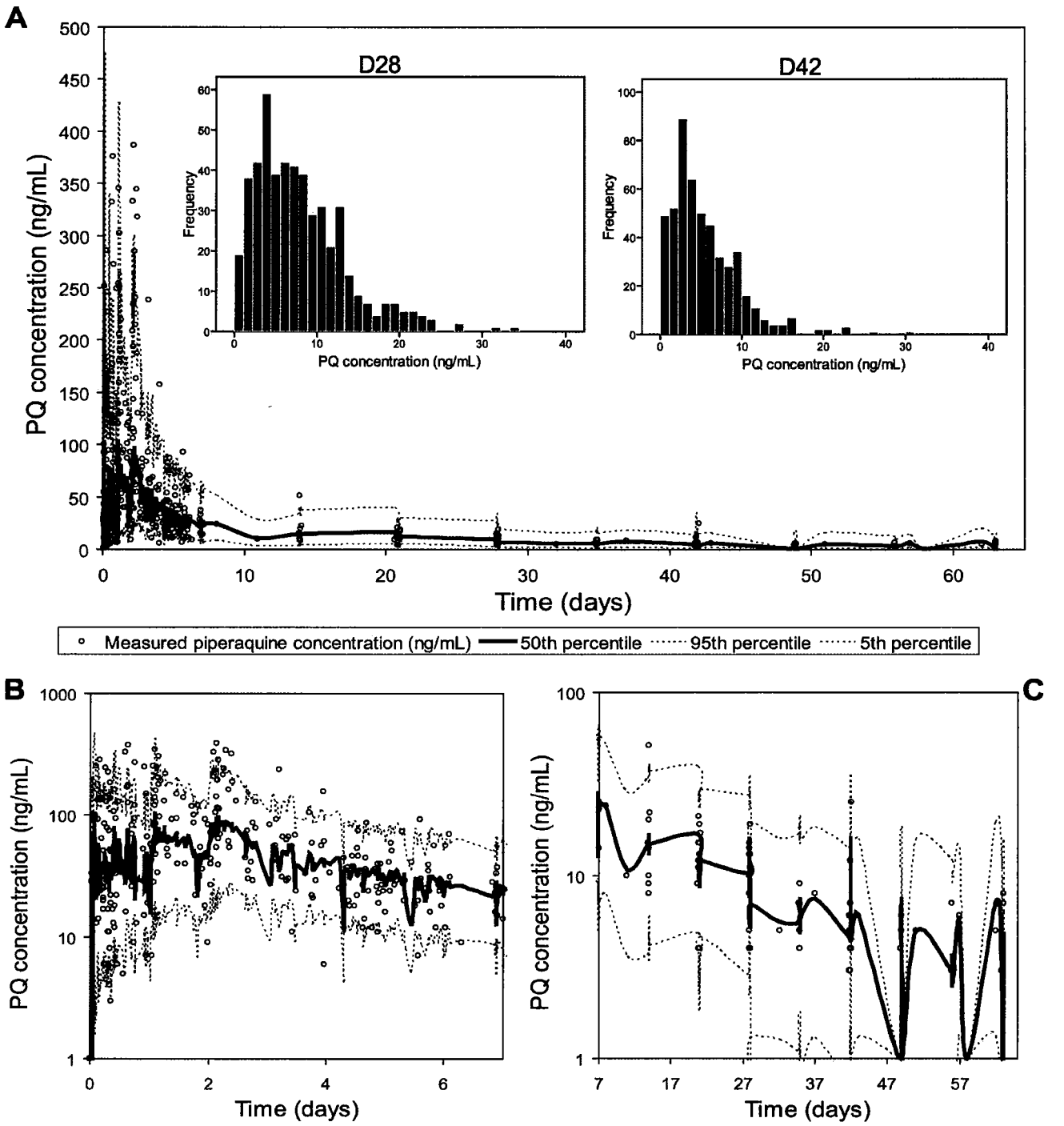

FIG. 5. Measured piperaquine (PQ) concentrations are superimposed over the population median concentration (50th percentile), the 5th percentile, and the 95th percentile simulated from the final model at each of the individual sampling times after treatment initiation (A). The inserted figures indicate the simulated distribution of piperaquine concentrations at the common follow-up days 28 and 42. Panels B and C show the data on a semilogarithmic scale for days 1 to 7 and days 7 to 63, respectively.

glucuronidation) and would also be reasonable for drugs that are eliminated predominantly by renal filtration (14). Piperaquine has previously been shown to be eliminated by both metabolism (26) and renal excretion (28), but the extent to which each elimination pathway contributes requires further evaluation.

Pharmacokinetics did not explain the reinfections in the study. The plasma concentrations were not different in rein-

fected patients, and there were no significant differences in the pharmacokinetic parameters compared to the population mean estimates (Fig. 1). The reinfected patients showed simulated piperaquine plasma concentrations of between 4 to 13 ng/ml and 1 to 8 ng/ml at the time of presentation with reinfections of *P. falciparum* and *P. vivax*, respectively. This is a low transmission setting, so these values must represent concentrations below the in vivo MIC for prevalent parasites. Considering a blood stage incubation period of not less than 7 days (reflecting multiplication rates of ≤10-fold per asexual cycle), the concentrations 1 week before reinfection of *P. falciparum* emerged were between 5 to 14 ng/ml, which suggests that this is a lower limit for the in vivo MIC in this region. Population mean plasma concentrations of 12 and 13 ng/ml could be seen at day 20 after the initiation of the DP3 and DP4 treatments, respectively. This suggests a mean posttreatment prophylactic effect of approximately 20 days with the current dosage. A simulated distribution of concentrations indicates most patients to be below 10 ng/ml at day 28 (Fig. 5, inserts). Estimating the MIC for *P. vivax* is more complex as, unlike *P. falciparum* reinfections, which may be assumed to be random, the emergence of relapses occurs at approximately 3-week intervals.

Although there were only 11 children in the study, they tended to have a smaller central volume of distribution, a shorter distribution half-life ($t_{1/2\alpha}$), and a more rapid fall in early piperaquine plasma concentrations compared to the population mean profile. This finding has potentially important therapeutic consequences for the use of the dihydroartemisinin-piperaquine combination. Thus, even though the initial concentrations of piperaquine were higher and the terminal elimination half-life was longer in children, they had lower plasma concentrations during the putative critical period between 3 and 20 days after starting treatment. The initial therapeutic response is determined almost entirely by the artemisinin derivative, so high early piperaquine levels offer no immediate benefit. However, after the second asexual cycle (>4 days), all the dihydroartemisinin has been eliminated, and parasite clearance depends entirely on the piperaquine partner drug. It is evident that the plasma concentration profiles in these children (Fig. 3 and 4) fall close to the putative in vivo MICs in approximately 10 days or less, so children would be expected to be at higher risk of recrudescence and earlier reinfection than the mean population. An increased risk of failure in children has been suggested in recent studies in West Papua (11, 21). Price et al. (27) showed plasma concentrations of piperaquine on day 7 to be a major determinant of the therapeutic response to dihydroartemisinin-piperaquine. Plasma piperaquine concentrations below 30 ng/ml on day 7 were associated with a higher failure risk (adjusted hazard ratio = 6.6 [95% confidence interval, 1.9 to 23]; $P = 0.003$) and were observed in 38% (21/56) of children less than 15 years of age and in 22% (31/140) of adults (20). The lower day 7 piperaquine plasma concentrations and the higher failure rates in children are in agreement with the present finding of an altered pharmacokinetic profile. This indicates that the time above MIC or AUC/MIC is important as a pharmacokinetic-pharmacodynamic determinant for slowly eliminated antimalarial drugs and that the total AUC can be a poor predictor of the therapeutic response for drugs with multiphasic disposition kinetics (Fig. 4). The piperaquine dose in children might need to be increased, although their tolerance for higher doses of piperaquine needs to be established.

These results are somewhat different from those in a previous study with 47 Cambodian children (2 to 10 years) with malaria, which described a shorter 14-day elimination half-life for children (13). The differences could result from the relatively small number of children studied here ($n = 11$), which could be nonrepresentative, as this compound displays large interindividual variability. Differences between studies might also result from differences in the duration of sampling and/or drug quantification sensitivity. Individual simulations of patients below 30 kg of body weight showed a good agreement between observed and predicted piperaquine concentrations, indicating a reasonably good pharmacokinetic characterization in these children. The lower $AUC_{day\ 3\text{-}20}$ values in this study are supported by studies in West Papua where day 7 piperaquine concentrations in children were lower than in adults when the subjects were dosed according to kilograms of body weight (11, 20, 21).

This study confirms that piperaquine exhibits considerable interindividual pharmacokinetic variability and has a very large apparent volume of distribution and a very slow elimination phase. It suggests that despite having a smaller central volume of distribution and a slower elimination rate than adults, children might have lower piperaquine concentrations in the therapeutically important period immediately following treatment. If this finding is confirmed in other malaria-affected regions, then consideration should be given to increasing the weight-adjusted dosage for children.

ACKNOWLEDGMENTS

This study is part of the Wellcome Trust-Mahidol University, Oxford Tropical Medicine Research Programme, funded by the Wellcome Trust. It was supported in part by the Swedish International Development Cooperation Agency (SIDA) and by the Medicines for Malaria Venture.

The authors gratefully thank all the patients who participated in the studies and all the staff involved in taking and processing the samples, as well as Daniel Röshammar for helpful suggestions on using the NONMEM software.

REFERENCES

1. **Ashley, E. A., S. Krudsood, L. Phaiphun, S. Srivilairit, R. McGready, W. Leowattana, R. Hutagalung, P. Wilairatana, A. Brockman, S. Looareesuwan, F. Nosten, and N. J. White.** 2004. Randomized, controlled dose-optimization studies of dihydroartemisinin-piperaquine for the treatment of uncomplicated multidrug-resistant falciparum malaria in Thailand. J. Infect. Dis. **190**:1773–1782.
2. **Ashley, E. A., R. McGready, R. Hutagalung, L. Phaiphun, T. Slight, S. Proux, K. L. Thwai, M. Barends, S. Looareesuwan, N. J. White, and F. Nosten.** 2005. A randomized, controlled study of a simple, once-daily regimen of dihydroartemisinin-piperaquine for the treatment of uncomplicated, multidrug-resistant falciparum malaria. Clin. Infect. Dis. **41**:425–432.
3. **Beal, S. L.** 2001. Ways to fit a PK model with some data below the quantification limit. J. Pharmacokinet. Pharmacodyn. **28**:481–504.
4. **Beal, S. L., and L. B. Sheiner.** 1982. Estimating population kinetics. Crit. Rev. Biomed. Eng. **8**:195–222.
5. **Beal, S. L., A. J. Boeckman, and L. B. Sheiner.** 1992. NONMEM users guides. NONMEM Project Group, University of California at San Fransisco, San Fransisco, CA.
6. **Brockman, A., R. E. Paul, T. J. Anderson, I. Hackford, L. Phaiphun, S. Looareesuwan, F. Nosten, and K. P. Day.** 1999. Application of genetic markers to the identification of recrudescent *Plasmodium falciparum* infections on the northwestern border of Thailand. Am. J. Trop. Med. Hyg. **60**:14–21.
7. **Crevoisier, C., J. Handschin, J. Barre, D. Roumenov, and C. Kleinbloesem.** 1997. Food increases the bioavailability of mefloquine. Eur. J. Clin. Pharmacol. **53**:135–139.

8. **Denis, M. B., T. M. Davis, S. Hewitt, S. Incardona, K. Nimol, T. Fandeur, Y. Poravuth, C. Lim, and D. Socheat.** 2002. Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. Clin. Infect. Dis. **35:**1469–1476.
9. **Feldman, C., and G. Sarosi.** 2005. Tropical and parasitic infections in the intensive care unit, vol. 9. Springer US, Boston, MA.
10. **Ginsberg, G., D. Hattis, B. Sonawane, A. Russ, P. Banati, M. Kozlak, S. Smolenski, and R. Goble.** 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol. Sci. **66:**185–200.
11. **Hasugian, A. R., H. L. Purba, E. Kenangalem, R. M. Wuwung, E. P. Ebsworth, R. Maristela, P. M. Penttinen, F. Laihad, N. M. Anstey, E. Tjitra, and R. N. Price.** 2007. Dihydroartemisinin-piperaquine versus artesunate-amodiaquine: superior efficacy and posttreatment prophylaxis against multidrug-resistant *Plasmodium falciparum* and *Plasmodium vivax* malaria. Clin. Infect. Dis. **44:**1067–1074.
12. **Holford, N.** 2005. A degenerative predictive check, abstr. 738, p. 14. Abstr. 14th Annu. Meet. Pop. Appr. Group Eur., Pop. Appr. Group Eur., Pamplona, Spain. www.page-meeting.org/?abstract=738.
13. **Hung, T. Y., T. M. Davis, K. F. Ilett, H. Karunajeewa, S. Hewitt, M. B. Denis, C. Lim, and D. Socheat.** 2004. Population pharmacokinetics of piperaquine in adults and children with uncomplicated falciparum or vivax malaria. Br. J. Clin. Pharmacol. **57:**253–262.
14. **Johnson, T. N., A. Rostami-Hodjegan, and G. T. Tucker.** 2006. Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. Clin. Pharmacokinet. **45:**931–956.
15. **Jonsson, E. N., and M. O. Karlsson.** 1999. Xpose—an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput. Methods Prog. Biomed. **58:**51–64.
16. **Karunajeewa, H., C. Lim, T. Y. Hung, K. F. Ilett, M. B. Denis, D. Socheat, and T. M. Davis.** 2004. Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin) in Cambodian children and adults with malaria. Br. J. Clin. Pharmacol. **57:**93–99.
17. **Lindegårdh, N., N. J. White, and N. P. Day.** 2005. High throughput assay for the determination of piperaquine in plasma. J. Pharm. Biomed Anal. **39:**601–605.
18. **Luxemburger, C., K. L. Thwai, N. J. White, H. K. Webster, D. E. Kyle, L. Maelankirri, T. Chongsuphajaisiddhi, and F. Nosten.** 1996. The epidemiology of malaria in a Karen population on the western border of Thailand. Trans. R. Soc. Trop. Med. Hyg. **90:**105–111.
19. **Milton, K. A., G. Edwards, S. A. Ward, M. L. Orme, and A. M. Breckenridge.** 1989. Pharmacokinetics of halofantrine in man: effects of food and dose size. Br. J. Clin. Pharmacol. **28:**71–77.
20. **Price, R. N., A. R. Hasugian, A. Ratcliff, H. Siswantoro, H. L. Purba, E. Kenangalem, N. Lindegardh, P. Penttinen, F. Laihad, E. P. Ebsworth, N. M. Anstey, and E. Tjitra.** 2007. Clinical and pharmacological determinants of the therapeutic response to dihydroartemisinin-piperaquine for drug-resistant malaria. Antimicrob. Agents Chemother. **51:**4090–4097.
21. **Ratcliff, A., H. Siswantoro, E. Kenangalem, R. Maristela, R. M. Wuwung, F. Laihad, E. P. Ebsworth, N. M. Anstey, E. Tjitra, and R. N. Price.** 2007. Two fixed-dose artemisinin combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. Lancet **369:**757–765.
22. **Rolan, P. E., A. J. Mercer, B. C. Weatherley, T. Holdich, H. Meire, R. W. Peck, G. Ridout, and J. Posner.** 1994. Examination of some factors responsible for a food-induced increase in absorption of atovaquone. Br. J. Clin. Pharmacol. **37:**13–20.
23. **Roshammar, D., T. N. Hai, S. Friberg Hietala, N. Van Huong, and M. Ashton.** 2006. Pharmacokinetics of piperaquine after repeated oral administration of the antimalarial combination CV8 in 12 healthy male subjects. Eur. J. Clin. Pharmacol.
24. **Sim, I. K., T. M. Davis, and K. F. Ilett.** 2005. Effects of a high-fat meal on the relative oral bioavailability of piperaquine. Antimicrob. Agents Chemother. **49:**2407–2411.
25. **Tangpukdee, N., S. Krudsood, W. Thanachartwet, K. Chalermrut, C. Pengruksa, S. Srivilairit, U. Silachamroon, P. Wilairatana, S. Phongtananant, S. Kano, and S. Looareesuwan.** 2005. An open randomized clinical trial of Artekin vs artesunate-mefloquine in the treatment of acute uncomplicated falciparum malaria. Southeast Asian J. Trop. Med. Public Health **36:**1085–1091.
26. **Tarning, J., Y. Bergqvist, N. P. Day, J. Bergquist, B. Arvidsson, N. J. White, M. Ashton, and N. Lindegardh.** 2006. Characterization of human urinary metabolites of the antimalarial piperaquine. Drug Metab. Dispos. **34:**2011–2019.
27. **Tarning, J., N. Lindegardh, A. Annerberg, T. Singtoroj, N. P. Day, M. Ashton, and N. J. White.** 2005. Pitfalls in estimating piperaquine elimination. Antimicrob. Agents Chemother. **49:**5127–5128.
28. **Tarning, J., T. Singtoroj, A. Annerberg, M. Ashton, Y. Bergqvist, N. J. White, N. P. Day, and N. Lindegardh.** 2006. Development and validation of an automated solid phase extraction and liquid chromatographic method for the determination of piperaquine in urine. J. Pharm. Biomed. Anal. **41:**213–218.
29. **Tran, T. H., C. Dolecek, P. M. Pham, T. D. Nguyen, T. T. Nguyen, H. T. Le, T. H. Dong, T. T. Tran, K. Stepniewska, N. J. White, and J. Farrar.** 2004. Dihydroartemisinin-piperaquine against multidrug-resistant Plasmodium falciparum malaria in Vietnam: randomised clinical trial. Lancet **363:**18–22.
30. **Wählby, U., M. R. Bouw, E. N. Jonsson, and M. O. Karlsson.** 2002. Assessment of type I error rates for the statistical sub-model in NONMEM. J. Pharmacokinet. Pharmacodyn. **29:**251–269.
31. **Wählby, U., E. N. Jonsson, and M. O. Karlsson.** 2001. Assessment of actual significance levels for covariate effects in NONMEM. J. Pharmacokinet. Pharmacodyn. **28:**231–252.
32. **White, N. J.** 1999. Delaying antimalarial drug resistance with combination chemotherapy. Parassitologia **41:**301–308.
33. **Wilkins, J. J.** 2005. NONMEMory: a run management tool for NONMEM. Comput. Methods Prog. Biomed. **78:**259–267.