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ORIGINAL ARTICLE

Population Pharmacokinetics of Lumefantrine in Pregnant and Nonpregnant Women With Uncomplicated *Plasmodium falciparum* Malaria in Uganda

F Kloprogge[1,2], P Piola[2,3,4], M Dhorda[3,5,6], S Muwanga[5], E Turyakira[4,5], S Apinan[1], N Lindegårdh[1,2,8], F Nosten[1,2,7], NPJ Day[1,2], NJ White[1,2], PJ Guerin[2,3] and J Tarning[1,2]

Pregnancy alters the pharmacokinetic properties of many antimalarial compounds. The objective of this study was to evaluate the pharmacokinetic properties of lumefantrine in pregnant and nonpregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda after a standard fixed oral artemether–lumefantrine treatment. Dense venous (*n* = 26) and sparse capillary (*n* = 90) lumefantrine samples were drawn from pregnant patients. A total of 17 nonpregnant women contributed with dense venous lumefantrine samples. Lumefantrine pharmacokinetics was best described by a flexible absorption model with multiphasic disposition. Pregnancy and body temperature had a significant impact on the pharmacokinetic properties of lumefantrine. Simulations from the final model indicated 27% lower day 7 concentrations in pregnant women compared with nonpregnant women and a decreased median time of 0.92 and 0.42 days above previously defined critical concentration cutoff values (280 and 175 ng/ml, respectively). The standard artemether–lumefantrine dose regimen in *P. falciparum* malaria may need reevaluation in nonimmune pregnant women.

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Approximately 660,000 patients died from malaria and an estimated 219 million malaria infections occurred in 2010.[1] In 2007, an estimated 85 million pregnancies took place in areas with *Plasmodium falciparum* transmission.[2] Artemisinin-based combination therapy is now recommended by World Health Organization as first-line treatment for uncomplicated *P. falciparum* malaria, including the second and third trimester of pregnancy. The fixed dose drug combination of artemether and lumefantrine is the artemisinin-based combination therapy most widely used for the treatment of uncomplicated *P. falciparum* malaria during pregnancy.[3]

Artemether is rapidly metabolized into dihydroartemisinin and has a very rapid and potent antimalarial effect, which results in a prompt resolution of symptoms. Lumefantrine is relatively slowly eliminated from the body and kills the residual parasites after the rapidly eliminated artemether and dihydroartemisinin have been cleared. This addition of a long-acting drug prevents recrudescent malaria and results in cure of *P. falciparum* malaria. Blood or plasma lumefantrine concentrations at day 7 are generally considered a useful pharmacokinetic predictor of treatment failure. Two different venous lumefantrine plasma target concentrations at day 7 have been reported in the literature; 280 and 175 ng/ml, respectively.[4,5] The relative risk of recrudescent malaria was reported to be substantially higher in patients with day 7 concentrations <175 ng/ml (adjusted hazard ratio of 17) compared with those above this reported threshold.[5] A day 7 concentration <280 ng/ml in Thai patients resulted in a 51% cure rate compared with 75% for patients with a day 7 concentration >280 ng/ml.[4]

Artemether–lumefantrine has been reported to provide high cure rates in nonpregnant patients in both Thailand and Africa.[6–12] Cure rates were high in pregnant women in Uganda (polymerase chain reaction–corrected cure rate of 98.2% (93.5–99.7%)) where the transmission of malaria is high[10], but lower cure rates have been reported in pregnant women in Thailand where the transmission is low (polymerase chain reaction–corrected cure rate of 82.0% (74.7–89.3%)).[13] A similar proportion of pregnant women in Thailand (35%) and in Uganda (32%) had day 7 lumefantrine concentrations below the previously set threshold of 280 ng/ml.[13] The difference in cure rates between the two groups suggests that background immunity makes a substantial contribution to drug efficacy. However, this should be interpreted with caution because the end points were defined differently, which can also be a source of variability and discrepancy.[10,13] Even though the low lumefantrine exposure in Uganda did not result in a low cure rate, subtherapeutic drug exposures may select for parasites with reduced drug susceptibility, encouraging the development and spread of drug resistance.[14]

Important changes in the pharmacokinetics of many antimalarial compounds in pregnant women have been observed, such as for artemether/dihydroartemisinin,[15] artesunate/dihydroartemisinin,[16] amodiaquine and desethylamodiaquine,[17,18] dihydroartemisinin,[19,20] lumefantrine,[21] atovaquone,[22] proguanil,[22] sulfadoxine,[23,24] and pyrimethamine.[25] To date,

[1]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [2]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK; [3]Epicentre, Paris, France; [4]Mbarara University of Science and Technology, Mbarara, Uganda; [5]Epicentre, Mbarara, Uganda; [6]Malaria Group, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, USA; [7]Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand. [8]Deceased. Correspondence: Joel Tarning (joel@tropmedres.ac)
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there are no available comparative studies using a nonpregnant control group to assess the effect of pregnancy on lumefantrine pharmacokinetics, although reported levels tend to be higher in nonpregnant patients compared with pregnant women.

The aim of this study was to investigate the population pharmacokinetics of lumefantrine in pregnant women (second and third trimester) compared with a nonpregnant control group after receiving a standard artemether–lumefantrine treatment for uncomplicated *P. falciparum* malaria in Uganda.

RESULTS

This study was nested into a larger efficacy study, and a total of 116 pregnant women in the second and third trimesters were enrolled in the pharmacokinetic cohort. In addition, 17 nonpregnant women with uncomplicated *P. falciparum* malaria in Uganda were enrolled in the pharmacokinetic cohort (**Table 1**). The standard oral fixed dose treatment of artemether and lumefantrine (twice daily for 3 days) was well tolerated and no cases of vomiting were reported. No patients presented with recurrent malaria infections during the follow-up (until delivery or day 42). Twenty-six pregnant and 17 nonpregnant women contributed with densely sampled plasma, and 90 pregnant women contributed with sparsely sampled capillary plasma (**Table 1**). One individual in the capillary arm was omitted from the data analysis because of an unexplainable high baseline plasma lumefantrine concentration of 7,717 ng/ml. Demographic values were generally similar between pregnant and nonpregnant women, except for a higher body weight in pregnant women as compared with that in nonpregnant women (**Table 1**). Furthermore, pregnant women in the group with venous plasma sampling had lower admission parasitemias as compared with both pregnant women with capillary sampling and nonpregnant women (**Table 1**).

Several absorption, distribution, covariate, and residual error models were fitted to the data to construct the best-performing model. The final nonlinear mixed-effects model consisted of a flexible transit absorption model (five transit compartments) followed by a two-compartment disposition model and described the lumefantrine data well (**Figure 1** and **Supplementary Data**). Residual variability was best described using an additive error model on the log-transformed data. Implementation of between-patient variability, between-dose occasion variability, and a Box–Cox transformation of the relative bioavailability of lumefantrine resulted in a significant improvement of the model (objective function value differences (ΔOFV) of –261, –539, and –21.5, respectively). Venous and capillary data were successfully modeled simultaneously using a proportional correction factor for the capillary samples on a population level because no patient contributed with both capillary and venous samples. Capillary concentration samples were estimated to be 11.9% (relative standard error of 9.32%) lower compared with venous concentration samples. However, this conversion factor did not improve the model fit significantly.

The following covariates were all selected in the forward covariate search ($P < 0.05$): estimated gestational age on bioavailability (exponential relationship), apparent distribution volume of the peripheral compartment (linear relationship), body temperature on mean transit time (linear relationship), and pregnancy on intercompartmental clearance (categorical). However, only body temperature on mean transit time (linear relationship) and pregnancy on intercompartmental clearance (categorical) could be retained as significant covariates ($P < 0.01$) in the backward elimination step. Mean absorption transit time increased with 16.5% with every degree centigrade increase of body temperature between 36.0 and 39.8 °C ($P < 0.01$) and intercompartmental

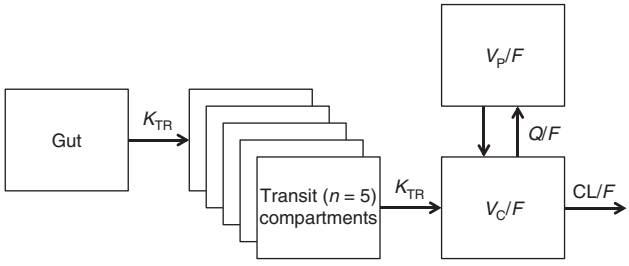

Figure 1 Visual representation of the population pharmacokinetic lumefantrine model. CL/F, elimination clearance; K_{TR}, transit rate constant; Q/F, intercompartmental clearance; V_C/F, apparent central volume of distribution; V_P/F, apparent peripheral volume of distribution.

Table 1 Demographic summary of the study population

	All	Venous, nonpregnant	Venous, pregnant	Capillary, pregnant
Number of patients	132	17	26	89
Total lumefantrine dose (mg/kg)	51.4 (34.7–72.0)	58.8 (45.7–72.0)	51.4 (38.9–65.5)	50.5 (34.7–65.5)
Total number of samples	1,580	440	620	517
Sample size (samples/patient)	6 (4–26)	26 (25–26)	25 (14–26)	6 (4–8)
Body weight (kg)	56.0 (40.0–83.0)	49.0 (40.0–63.0)	56.0 (44.0–74.0)	57.0 (44.0–83.0)
Age (years)	21.0 (15.0–38.0)	21.0 (18.0–29.0)	20.0 (18.0–38.0)	22.0 (15.0–38.0)
Parasitemia (μl^{-1})[a]	1,357 (24.0–194,000)	1,677 (48.0–152,000)	401 (32.0–11,800)	1,868 (24.0–194,000)
Gestational age (weeks)	21 (0–39.0)	–	22.5 (16.0–38.0)	22.0 (13.0–39.0)
Body temperature (°C)	36.9 (36.0–39.8)	36.7 (36.1–38.2)	36.7 (36.0–39.3)	37.0 (36.0–39.8)

Values are given as median (range).
[a]Reported as geometric mean (range).

clearance was 36.5% lower in pregnant women compared with that in nonpregnant women ($P < 0.01$). Similar trends of pregnancy-related effects were seen in the full-covariate model for pregnancy (**Figure 2**).

Basic goodness-of-fit diagnostics (**Figure 3**) of the final covariate model did not show any trends of model misspecification (20.6% ε-shrinkage). Additional simulation-based diagnostics (i.e., visual predictive checks) indicated high predictive performance of the final model (**Figure 4**). Similarly, the numerical predictive check computed 4.07% (95% confidence interval (CI): 2.56–7.87%) and 3.80% (95% CI: 2.35–8.29%) of the observed lumefantrine concentrations above and below the 90% prediction interval, respectively. Parameter estimates were robust with reasonably low relative standard errors (**Table 2**). η-Shrinkage estimates were relatively high for certain parameters (15.5–54.0%), which suggests that *post hoc* estimates should be interpreted with caution.

Monte Carlo simulations performed with the final population pharmacokinetic model indicated lower day 7 venous plasma concentrations in pregnant women (414 ng/ml) compared with those in nonpregnant women (566 ng/ml) in this study (**Figure 5**). This produced a decreased median time of 0.92 and 0.42 days over the 280 and 175 ng/ml targets, respectively, in pregnant women compared with nonpregnant women (**Figure 5b**). This translates into 7, 16, 20, 20, and 18% lower exposure for pregnant women compared with nonpregnant women when calculating the posttreatment exposures from day 3–14, day 4–14, day 5–14, day 6–14, and day 7–14, respectively. However, the total exposure was not affected by pregnancy, and lumefantrine plasma concentrations from day 12 onwards were slightly higher in pregnant women compared with those in nonpregnant women. Monte Carlo simulations were also performed to compare results from this study with those previously published for pregnant women in Thailand[21] (**Figure 5d**). This resulted in a similar median day 7 capillary concentration of 370 ng/ml in this study compared with 392 ng/ml in pregnant women in Thailand.

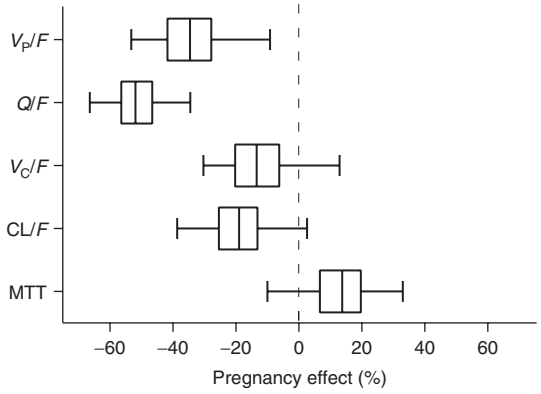

Figure 2 Box and whisker plot visualizing the effect of pregnancy on pharmacokinetic parameters from 200 bootstrap runs (boxes represent the 25–75 percentiles and whiskers represent the 2.5–97.5 percentiles of the bootstrap estimates). CL/*F*, elimination clearance; K_{TR}, transit rate constant; MTT, mean transit time; *Q*/*F*, intercompartmental clearance; V_C/F, apparent central volume of distribution; V_P/F, apparent peripheral volume of distribution.

DISCUSSION

No comparative pharmacokinetic analyses have been performed previously for lumefantrine in pregnant and nonpregnant women, despite intensive use of this antimalarial drug in the second and third trimester of pregnancy and poor cure rates reported in pregnant women in Thailand.[13] An analysis to assess potential effects of pregnancy on lumefantrine pharmacokinetics using a comparable nonpregnant control group was therefore needed.

A transit-compartment absorption model followed by a two-compartment disposition model described the data well in this study. This is in good agreement with the disposition model presented for sparsely sampled pregnant women in Thailand.[21] However, a three-compartment model was used to describe the disposition pharmacokinetics of lumefantrine in Papua New Guinean children.[26] The extended duration of sampling is likely to explain why a second peripheral compartment was supported by those data. The developed model allowed for a difference in biological matrices (e.g., capillary vs. venous plasma), but the 95% CI for the conversion factor ranged from below 1 to above 1 (**Table 2**). This indicated that there was no significant difference between venous and capillary plasma concentrations on a population level, which was also confirmed by backward deletion of the conversion factor parameter (ΔOFV = 2.369). Similar findings have been reported previously using a linear regression model to compare capillary and venous lumefantrine plasma concentrations.[27]

Shrinkage estimates were relatively high due to the sparse sampling in the capillary arm. For the purpose of this analysis, the shrinkage is acceptable, but potential future simulations on an individual level for dose optimization using this model should be treated with caution. The visual predictive check indicates adequate overall predictive power of the model (**Figure 4**). However, confidence intervals in the visual predictive checks stratified for pregnant women with capillary data, venous data, or nonpregnant women with venous data were wider compared with the combined prediction-corrected visual predictive check. This is a common phenomenon driven by the smaller sample size in the strata.

Body temperature was a significant covariate on mean absorption transit time. With every degree Celsius increase in body temperature, the mean absorption transit time increased by 16.5%. This resulted in a mean transit time of 3.48 and 6.05 hours for a patient with a 36.0 and 39.8 °C admission body temperature, respectively. Lumefantrine is poorly absorbed from the gastrointestinal tract into the systemic circulation with an absolute bioavailability of 5–12% in rats.[28] Patients with higher fever might have reduced gut motility and prolonged lumefantrine absorption into the systemic circulation. However, this covariate relationship did not affect the systemic lumefantrine exposure in the patients studied here.

Geometric mean values of parasitemia at admission differed between the three study arms (**Table 1**). However, parasitemia was tried in the model and did not significantly influence the pharmacokinetics of the drug. This may be explained by similar ranges for the pregnant and nonpregnant group. Furthermore, it has not been shown in previous

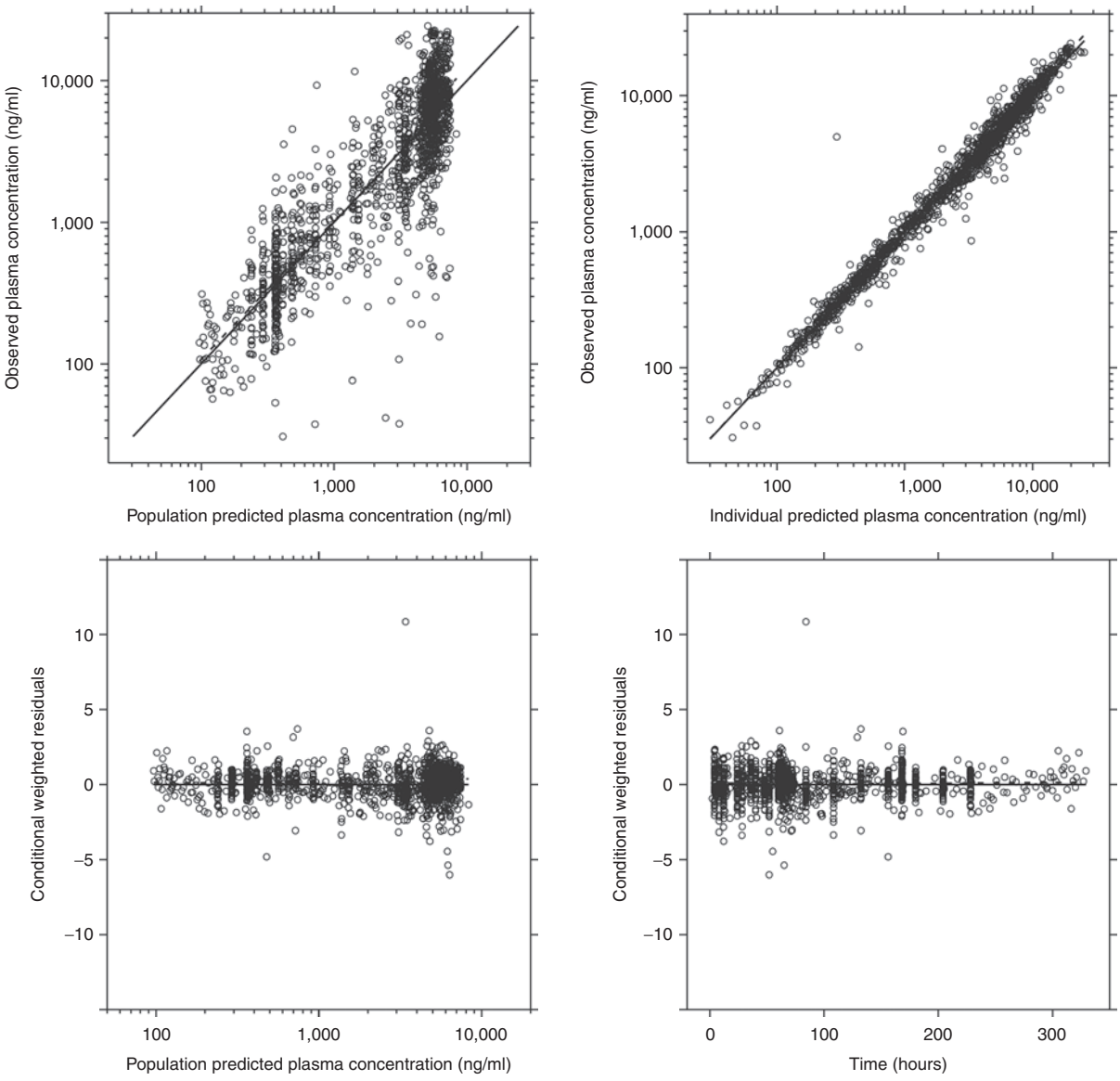

Figure 3 Basic goodness-of-fit plots from the final lumefantrine model. The lines of identity are represented by the black solid lines and the trend lines (local polynomial regression fitting using 50 evaluations) are represented by the black dashed line.

literature that parasitemia influences the pharmacokinetics of lumefantrine.

Lumefantrine plasma concentration at day 7 has previously been used as a readily measurable simple pharmacokinetic end point to predict treatment failure. Simulations using the final population pharmacokinetic model showed lower venous plasma lumefantrine concentrations at day 7 in pregnant women compared with those in nonpregnant women (**Figure 5**). Confidence intervals from the simulations should be interpreted with caution due to relatively large shrinkage values. The lower lumefantrine day 7 plasma concentrations during pregnancy were caused by a decrease of 36.5% in the intercompartmental clearance in pregnant women. Due to changes in body composition (increased plasma volume, water, and fat content) during pregnancy,[29] the distribution of the lipophilic lumefantrine through the body might be altered substantially, which would explain this finding. Total lumefantrine exposure was not affected by pregnancy in this study. However, exposures were higher in nonpregnant women compared with those in pregnant women in the post-treatment phase (area under the plasma concentration–time curve $(AUC)_{day\ 3–14}$, $AUC_{day\ 4–14}$, $AUC_{day\ 5–14}$, $AUC_{day\ 6–14}$, and $AUC_{day\ 7–14}$). This might have consequences in terms of efficacy because residual parasites will be eliminated during this phase, preventing recrudescent malaria. Pregnant women displayed higher plasma concentrations from day 12 onwards compared with nonpregnant patients, but this is not expected to have a major clinical impact as plasma concentrations were very low by this time. These findings should be interpreted with caution as the control group was not equally sized compared with the arm with pregnant women, and this covariate effect may be confounded by the high between-patient variability and between-dose occasion variability on bioavailability. A larger study with a control group

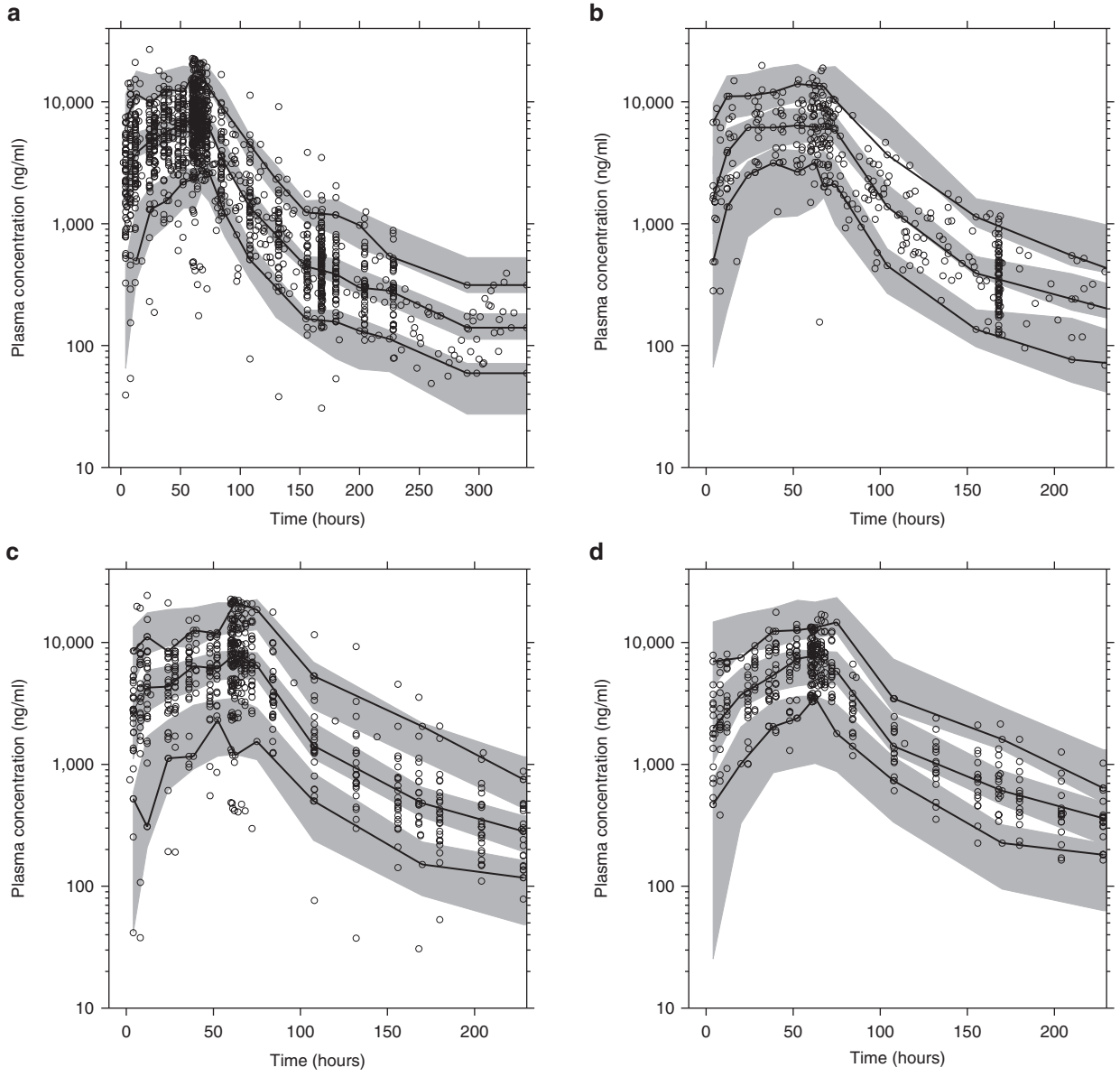

Figure 4 Prediction-corrected visual predictive check of the final lumefantrine model for (**a**) all available data and when stratified for (**b**) pregnant women with capillary data, (**c**) pregnant women with venous data, and (**d**) nonpregnant women with venous data. Open circles represent the observed lumefantrine plasma concentrations. The 5th, 50th, and 95th percentiles of the observed data are represented by the solid black lines. The 95% confidence intervals of the 5th, 50th, and 95th percentiles of the 2,000 simulations are represented by gray-shaded areas.

would, therefore, be necessary to confirm the current findings and the potential impact of different pharmacokinetic end points (i.e., exposure vs. day 7 levels). The bootstrap diagnostics of the full covariate model (**Figure 2**) showed a trend toward lower peripheral volume of distribution during pregnancy, but this covariate did not contribute to a significant improvement of the model. This might be because of the small nonpregnant control group studied here. Simulated capillary lumefantrine pharmacokinetic profiles indicated similar day 7 concentrations in Thailand compared with that in Uganda (**Figure 5d**). Both these efficacy studies also reported low observed day 7 lumefantrine concentrations: 35 and 32%, respectively, below the previously defined target concentration of 280 ng/ml.[10,13] However, low cure rates were reported only in Thailand.[13] The most likely explanation for this difference in efficacy is the substantially higher background immunity in Uganda compared with that in Thailand, which would compensate for the underexposure of lumefantrine. More speculative contributors might be altered lumefantrine susceptibility or emerging artemisinin resistance in Southeast Asia.[30] Patients infected with artemisinin-resistant parasites would have more residual parasites needing to be cleared by lumefantrine, which could lead to increased failure rates. Lower lumefantrine day 7 concentrations in pregnant patients compared with that in nonpregnant patients might also accelerate the development of resistance because

Table 2 Population pharmacokinetic estimates from the final lumefantrine model in pregnant and nonpregnant women with *P. falciparum* malaria in Uganda

	Fixed effect[a]	95% CI[b]	IIV/IOV† (%CV)[a]	95% CI[b]
Parameter	**(% RSE)[b]**		**(% RSE)[b]**	
CL/*F* (l/hour)	5.09 (7.90)	4.35–5.87	17.5 (20.6)	13.8–21.0
V_c/F (l)	123 (8.40)	104–145	–	–
Q/*F* (l/hour)	1.68 (10.2)	1.35–2.00	–	–
V_p/F (l)	110 (9.07)	91.7–131	21.5 (65.2)	0.213–40.0
MTT (hours)	4.09 (5.22)	3.70–4.55	36.6 (32.0)	22.9–47.5
F	One fixed	–	44.8 (31.2)/104 (12.2)†	29.0–57.8/87.74–125
Box–Cox shape parameter of *F*	–0.376 (21.7)	–0.516 to –0.227	–	–
Transit compartments (*n*)	Five fixed	–	–	–
Temperature on MTT	0.165 (44.1)	0.0328–0.329	–	–
Pregnancy on *Q*	–0.365 (14.3)	–0.455 to –0.259	–	–
Matrix conversion factor	0.881 (9.32)	0.733–1.05	–	–
σ venous	0.0595 (15.3)	0.0382–0.0914	–	–
σ capillary	0.0207 (11.8)	0.00957–0.0343	–	–
Post hoc estimates[c]	All women	Nonpregnant women	Pregnant women	
$AUC_{0-\infty}$ (hours × μg/ml)	594 (76.4–1850)	630 (285–1,240)	570 (76.4–1,850)	
C_{max} (μg/ml)	8.37 (0.722–25.6)	8.33 (4.36–15.0)	8.40 (0.722–25.6)	
$T_{1/2}$ (hours)	89.5 (54.3–121)	69.8 (54.3–78.3)	90.3 (64.3–121)	
Day 7 concentration (μg/ml)	0.454 (0.045–2.61)	0.592 (0.258–1.67)	0.423 (0.045–2.61)	

σ, residual variability for capillary and venous data; AUC, area under the plasma concentration–time curve; CI, confidence interval; CL/*F*, elimination clearance; C_{max}, maximum lumefantrine concentration; CV, coefficient of variation; *F*, relative bioavailability; IIV, interindividual variability; IOV, inter occassion variability; MTT, mean transit time; *Q*/*F*, intercompartmental clearance; RSE, relative standard error; $T_{1/2}$: elimination half-life; V_C/F, apparent volume of distribution of central compartment; V_p/F: apparent volume of distribution of peripheral compartment.
[a]Population mean values, IIV, and IOV estimated by NONMEM. IIV and IOV are presented as 100 × (EXP(mean estimate)–1)$^{1/2}$. [b]Relative standard errors are calculated as 100 × (standard error/mean value) from 1,000 iterations of a nonparametric bootstrap. The 95% CI is displayed as the 2.5–97.5 percentiles of the bootstrap estimates. [c]*Post hoc* estimates were calculated as the median and ranges of the empirical Bayes estimates.

subtherapeutic drug exposures may select for parasites with low drug susceptibility.[14]

The main metabolite of lumefantrine, desbutyl-lumefantrine, has been reported to have substantial antimalarial activity.[31,32] It has been suggested that future efficacy and pharmacokinetic studies should include measurement of both lumefantrine and desbutyl-lumefantrine to enable the assessment of a weighted lumefantrine/desbutyl-lumefantrine drug effect.[26] In this study, it was not possible to combine the pharmacokinetic model with a pharmacodynamic model as cure rates were high. Recently, model efforts have been made to describe the pharmacodynamics of malaria with a time-to-event approach.[33] This type of modeling would be particularly suitable for assessing the individual contributions of lumefantrine and desbutyl-lumefantrine on treatment outcome. Results presented in this article and model parameters could be used for power calculations and optimal design theory to design an appropriate follow-up study.

In conclusion, lumefantrine pharmacokinetics were well described using a transit compartment absorption model followed by two disposition compartments. The pharmacokinetic end point, day 7 lumefantrine plasma concentration, was 27% lower in pregnant women compared with that in nonpregnant women, and the median times above previously defined target concentrations of 280 and 175 ng/ml were decreased by 0.92 and 0.42 days, respectively. Simulations of capillary plasma concentrations showed similar exposures compared with a previous study in pregnant women in Thailand. However, a high cure rate was seen in this study, most likely because of higher background immunity or increased drug susceptibility to lumefantrine and/or artemether. To avoid the development of resistance and to improve the cure rates of artemether–lumefantrine in other geographical regions, more studies and modeling work have to be performed to support dose optimization, in combination with a confirmatory pharmacokinetic study using an equal-sized control group.

METHODS

This nested pharmacokinetic study was conducted in the Mbarara National Referral Hospital (MNRH) antenatal clinic in Uganda and pregnant patients were recruited from October 2006 to December 2008.[10] Inclusion criteria were pregnant women with *P. falciparum* mixed- or mono-infection (detected by microscopy), residence in the Mbarara municipality (radius 15 km from MNRH), and an estimated gestation age of at least 13 weeks. Exclusion criteria were *P. falciparum* parasitemia (>250,000 parasites/μl), severe anemia (Hb <7 g/dl), signs or symptoms of severe malaria requiring parental treatment, known allergy to artemisinin derivates, lumefantrine, or quinine, and previous participation in the efficacy study or inability to comply with the specified follow-up schedule. Patients in the nonpregnant venous

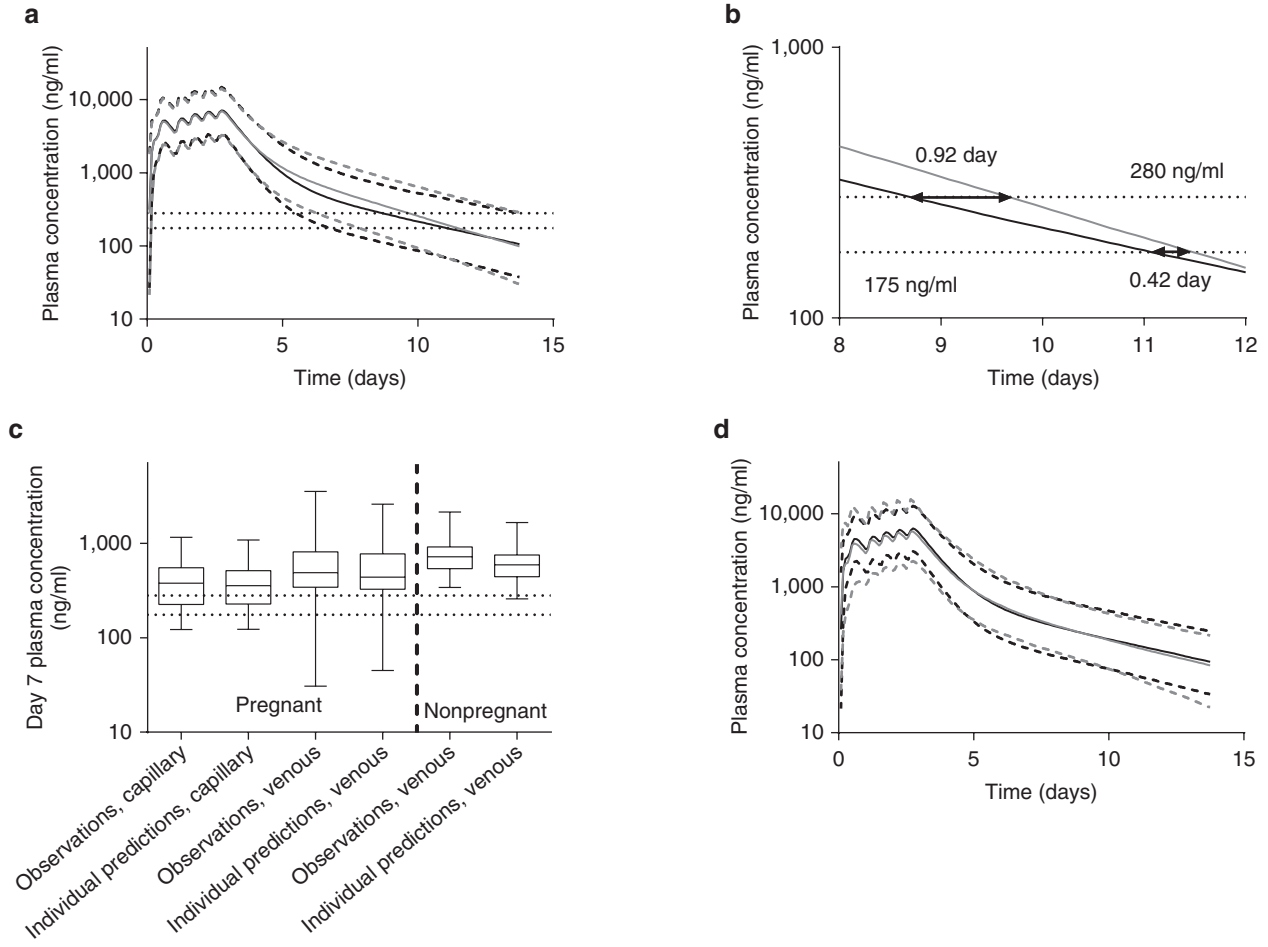

Figure 5 An overview of the pregnancy effect on lumefantrine pharmacokinetics. (**a**) Population predicted concentration–time profiles of lumefantrine venous plasma concentrations in pregnant (black) and nonpregnant (gray) women based on 2,000 simulations from time 0 to day 14 and (**b)** zoomed in from day 8 to day 12. (**c**) Shows observed and individually predicted day 7 lumefantrine concentrations using the final population pharmacokinetic model. (**d**) Displays the simulated profiles for lumefantrine capillary plasma concentrations in Uganda (black) and Thailand (gray). Solid lines represent population means and dashed lines represent the 95th and 5th percentiles. The upper and lower horizontal dashed lines in **a**, **b**, and **c** represent the 280 and 175 ng/ml day 7 plasma concentration thresholds, respectively.

control group were recruited (from February to August 2009) as postpartum women (>8 weeks) if a match (with respect to history of fever; axillary temperature >37.5 °C; smoking; and parasitemia: <1,000, 1,001–25,000, 25,001–250,000 parasites/μl) could be found in the densely sampled venous lumefantrine pregnant arm. Written informed consent was obtained before inclusion. Ethical approval was obtained from the Mbarara University Faculty of Medicine Research and Ethics Committee, the Mbarara University Institutional Ethics Committee, the Uganda National Council for Science and Technology (ethics committee), and the "Comité de Protection des Personnes" (Ile de France XI, France). The trial was registered at ClinicalTrials.gov (NCT00495508).

The combination of artemether and lumefantrine (Coartem; Novartis Pharma AG, Basel, Switzerland; each tablet contained 20 mg artemether and 120 mg lumefantrine) was administered with 200 ml of milk tea to optimize the oral bioavailability of lumefantrine.[34] Four tablets per dose were administered twice daily for 3 days (at 0, 8, 24, 36, 48, and 60 hours). If the dose was vomited within 30 minutes, a full replacement dose was given. If the dose was vomited between 30 minutes and 1 hour, a half replacement dose was given. If the replacement dose was vomited again within 30 minutes, the patient was withdrawn from the study and treated with rescue treatment.

Two sample matrices were used for collecting pharmacokinetic samples. Venous blood samples (2 ml) in the venous sampling group were drawn from each patient from a cannula into heparinized tubes at 0, 4, 8, 12, 24, 28, 36, 40, 48, 52, 60, 60.5, 61, 62, 64, 66, 68, 72, 84, 108, 132, 156, 180, 204, and 228 hours after the first dose. Two capillary blood samples in the capillary sampling group were taken from each patient at 0 hours (pretreatment) and at day 7 after treatment initiation. Additional capillary samples were taken from each patient at random within the following time windows after the first dose 0–71, 72–95, 96–143, and 144–336 hours.

Plasma was separated by centrifugation (1,400*g* for 5 minutes) and subsequently stored at −70 °C or in liquid nitrogen until analysis. The plasma samples were transported on dry ice to the Mahidol-Oxford Tropical Medicine Research Unit, Department of Clinical Pharmacology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Lumefantrine was quantified by high-performance liquid chromatography with ultraviolet detection according to a validated and published method.[35] Triplicates of quality control samples at three concentrations were analyzed within each batch to ensure precision and accuracy during quantification. The overall relative standard deviation was <10% and the lower limit of quantification was set to 26 ng/ml. The Department of Clinical Pharmacology is participating in the World Wide Antimalarial Resistance Network quality control and assurance proficiency testing program (http://www.wwarn.org/toolkit/qaqc).

The natural logarithms of venous and capillary lumefantrine plasma concentrations were modeled simultaneously. Estimations and simulations were performed on a Windows XP operating system (Microsoft Corporation, Seattle, WA) with a G95 Fortran compiler (Free Software Foundation, Boston, MA) using NONMEM v.7.1 (ICON Development Solutions, Ellicott City, MD). ADVAN5, TRANS1, and the first-order conditional estimation method with interaction were used during model building.[36] Postprocessing and automation was performed using Pearl-Speaks-NONMEM (PsN) v. 3.4.2,[37,38] Xpose v. 4[39], and R v. 2.13.1 (The R Foundation for Statistical Computing, Vienna, Austria). Competing models were evaluated during the model building process by the OFV (computed as minus twice the log likelihood of the data), physiological plausibility, and goodness-of-fit diagnostics. A significant (P = 0.05) improvement, after the introduction of one new parameter (one degree of freedom), was concluded if the OFV dropped with 3.84 points or more.

Several combinations of absorption models (first order, first order with lag-time, and transit absorption), distribution models (one-, two-, and three-compartment distribution), variability models (between-subject variability and between-occasion variability), and error models (additive, proportional, and combined additive and proportional error models) were assessed. Relative bioavailability was implemented as a fixed parameter (100% for the population), which allowed for estimation of the between-subject and between-occasion variability of the relative bioavailability. Covariate modeling was done using the best-performing structural model. Parameter-covariate relationships that showed a significant correlation (P < 0.05 in Pearson, Spearman, and/or Kendall test) and/or that were considered physiologically plausible were evaluated with forward addition and backward elimination covariate selection.[37,40] A P value of 0.05 was considered significant for retaining the covariate in the model and a P value of 0.01 (ΔOFV > 6.63) was considered as significant in the backward elimination. The impact of bodyweight, age, estimated age of gestation, parasitemia, body temperature, and pregnancy (categorical) were evaluated on all parameters (CL/F, V_c/F, Q/F, V_p/F, mean transit time, and F). Systolic blood pressure was evaluated on elimination clearance and intercompartmental clearance and liver status results (alanine transaminase) on elimination clearance. A linear (equation 1), exponential (equation 2), and power relationship (equation 3) were tested sequentially for continuous covariates. All covariates were centered on the median value of the population except estimated gestational age that was centered on nonpregnant woman. Categorical covariates were implemented as a relative difference (%) between groups.

$$P_i = P_{TV} \times \left(1 + \Theta_n \times (\text{covariate value} - \text{median value})\right) \times e^{\eta_n} \quad (1)$$

$$P_i = P_{TV} \times e^{\Theta_n \times (\text{covariate value} - \text{median value})} \times e^{\eta_n} \quad (2)$$

$$P_i = P_{TV} \times \left(\frac{\text{covariate value}}{\text{median value}}\right)^{\Theta_n} \times e^{\eta_n} \quad (3)$$

where P_i is the individual parameter estimate, P_{TV} is the typical parameter estimate for the study population, Θ_n is a fixed effect, and η_n is a random effect. Body weight was also evaluated as an allometric function on all clearance and volume parameters. The disposition and absorption model was reconsidered using the final covariate model. One model with pregnancy as a categorical covariate on all clearance parameters, volume parameters, and mean transit time was also developed and bootstrapped for a full-covariate model approach.[41] Parameter-covariate relations were considered potentially significant in the full-covariate model if the 95% CI did not include zero effect.

To assess the reliability of individual parameter estimates, η- and ε- shrinkages were calculated.[42] A stratified (for pregnancy, trimester, and biological matrix) nonparametric bootstrap of 1,000 data sets was performed to calculate reliable standard errors of parameter estimates and the nonparametric CIs around these estimates. Using 2,000 simulations of each individual plasma sample, a prediction-corrected visual predictive check and a numerical predictive check was performed to examine the predictive power of the model.[43] The 95% CIs of the simulated 5th, 50th, and 95th percentiles were overlaid with the 5th, 50th, and 95th percentiles of the observed data to visualize the predictive check. Monte Carlo simulations were performed using the population pharmacokinetic parameter estimates of the final model to assess potential differences in exposure between pregnant and nonpregnant women.

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Conflict of interest. The Wellcome Trust is a UK-based medical research charity and is independent of all drug companies. It has no financial links with the manufacturers of either the diagnostic tests or the drugs used in this study. The other authors declared no conflict of interest.

Author contributions. J.T., F.K., N.P.J.D., and N.J.W. wrote the manuscript. J.T., F.K., P.P., M.D., S.M., E.T., F.N., and

P.J.G. designed the research. J.T., F.K., P.P., M.D., S.M., E.T., F.N., and P.J.G. performed the research. J.T., F.K., S.A., and N.L. analyzed the data. M.D., S.A., and N.L. contributed new reagents/analytical tools.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ The day 7 blood or plasma lumefantrine concentration is commonly used as a measure of artemether–lumefantrine exposure in antimalarial drug trials. Reported day 7 lumefantrine concentrations in pregnant women with uncomplicated malaria tend to be lower compared with nonpregnant uncomplicated malaria patients. However, no comparative analysis using a nonpregnant control group has been performed.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ The aim of this study was to evaluate lumefantrine population pharmacokinetics in pregnant women and a nonpregnant control group.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ Results showed 27% lower day 7 lumefantrine concentrations in pregnant women compared with that in nonpregnant women and a decrease of 0.92 and 0.42 days in the median time above previously defined critical concentration cutoff values (280 and 175 ng/ml, respectively).

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ The standard dosing regimen in pregnant women resulted in substantially lower day 7 concentrations, and this may have an impact on cure rates.

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Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (http://www.nature.com/psp)