Pharmacokinetic Properties of Sulfadoxine-Pyrimethamine in Pregnant Women[▽]

Harin A. Karunajeewa,[1,2] Sam Salman,[1] Ivo Mueller,[3] Francisca Baiwog,[3] Servina Gomorrai,[3]
Irwin Law,[1] Madhu Page-Sharp,[1] Stephen Rogerson,[4] Peter Siba,[3]
Kenneth F. Ilett,[1,5] and Timothy M. E. Davis[1]*

*School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, Australia[1]; Western Health,
Melbourne, Victoria, Australia[2]; Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea[3]; Faculty of
Medicine, University of Melbourne, Melbourne, Victoria, Australia[4]; and Clinical Pharmacology and
Toxicology Laboratory, Path West Laboratory Medicine, Nedlands, Western Australia, Australia[5]*

To determine the pharmacokinetic disposition of sulfadoxine (SDOX) and pyrimethamine (PYR) when administered as intermittent presumptive treatment during pregnancy (IPTp) for malaria, 30 Papua New Guinean women in the second or third trimester of pregnancy and 30 age-matched nonpregnant women were given a single dose of 1,500 mg of SDOX plus 75 mg of pyrimethamine PYR. Blood was taken at baseline and 1, 2, 4, 6, 12, 18, 24, 30, 48, and 72 h and at 7, 10, 14, 28, and 42 days posttreatment in all women. Plasma samples were assayed for SDOX, N-acetylsulfadoxine (NASDOX), and PYR by high-performance liquid chromatography. Population pharmacokinetic modeling was performed using NONMEM v6.2.0. Separate user-defined mamillary models were fitted to SDOX/NASDOX and PYR. When the covariate pregnancy was applied to clearance, there was a significant improvement in the base model for both treatments. Pregnancy was associated with a significantly lower area under the concentration-time curve from 0 to ∞ for SDOX (22,315 versus 33,284 mg · h/liter), NASDOX (801 versus 1,590 mg · h/liter), and PYR (72,115 versus 106,065 μg · h/liter; $P < 0.001$ in each case). Because lower plasma concentrations of SDOX and PYR could compromise both curative efficacy and posttreatment prophylaxis in pregnant patients, IPTp regimens incorporating higher mg/kg doses than those recommended for nonpregnant patients should be considered.

Treatment strategies for malaria during pregnancy include drug administration for symptomatic episodes and, because asymptomatic infections in areas of malaria endemicity can also be associated with adverse maternal and fetal outcomes, giving antimalarial therapy at prespecified intervals during pregnancy. This latter approach, known as intermittent preventive treatment in pregnancy (IPTp), clears maternal parasitemia and prevents or suppresses subsequent infections (37). Although conventional adult antimalarial treatment doses are recommended, the physiologic changes that take place during pregnancy may significantly alter drug disposition through a variety of mechanisms, including increases in plasma volume, increased clearance, and altered protein binding (2, 16). Despite these considerations, <150 pregnant women have been enrolled in published studies of antimalarial pharmacokinetics (14, 18, 33), and such data have been identified as an urgent priority for optimization of IPTp strategies (33).

The treatment with the best evidence for use as IPTp is sulfadoxine (SDOX) plus pyrimethamine (PYR) (SP) (22, 26, 31), but its effectiveness appears paradoxical given the component drugs have a relatively short elimination half-life ($t_{1/2\beta}$), specifically 6 to 11 days for SDOX (12, 19, 21, 24, 25, 32, 34, 35) and 3 to 5 days for PYR (3, 5, 9, 11, 12, 14, 19, 21, 25, 30, 32, 34, 35, 38). SDOX is acetylated by the enzyme N-acetyltransferase 2. Polymorphisms

in the N-acetyltransferase 2 gene are associated with rapid or slow acetylation (20), and there is some evidence that the extent of acetylation is linked to treatment failure in malaria (24). In Papua New Guinea (PNG), the national treatment policy is to give SP with chloroquine (CQ) as presumptive treatment at the first antenatal visit. We have investigated the pharmacokinetic properties of SDOX and PYR in pregnant PNG women and in nonpregnant female controls. Since PNG populations comprise predominantly rapid acetylators (8, 23), we measured N-acetylsulfadoxine (NASDOX), the primary metabolite of SDOX, to assess its potential clinical importance.

MATERIALS AND METHODS

Study site and sample. The study was conducted at the Alexishafen Health Centre, Madang Province, on the north coast of PNG between February and July 2006. The local population is almost exclusively Melanesian. Because *Plasmodium falciparum* transmission is hyperendemic (6), infections in women of childbearing age are usually asymptomatic. Approximately 29% of antenatal clinic attendees have peripheral blood P. falciparum parasitemia, with similar rates among nonpregnant women in the community (4).

First-time antenatal clinic attendees were recruited provided that (i) there was no history of SP use in the previous 14 days, (ii) the subjects displayed no features of severe malaria (40), (iii) there was no clinical evidence of other significant illness, (iv) follow-up was feasible, and (v) written informed consent was obtained. The nonpregnant subjects were recruited from villages where previously enrolled pregnant cases of similar age also resided. Women were eligible regardless of baseline parasitemia status. The study was approved by the Medical Research Advisory Committee of PNG.

Clinical procedures. Study procedures were identical for pregnant and nonpregnant subjects except that initial clinical assessment included estimation of gestational age by fundal height in the pregnant group. A 3-ml venous blood sample was taken for blood film and baseline tests including hemoglobin and blood glucose (HaemoCue, Angelholm, Sweden), and an aliquot of plasma was

* Corresponding author. Mailing address: School of Medicine and Pharmacology, Fremantle Hospital, P.O. Box 480, Fremantle 6959, Western Australia, Australia. Phone: (618) 9431 3229. Fax: (618) 9431 2977. E-mail: tdavis@cyllene.uwa.edu.au.

stored frozen for subsequent drug assay. All women received a single dose of SP (Fansidar; Roche, Basel, Switzerland; 1,500 mg of SDOX and 75 mg of PYR). In line with PNG standard treatment recommendations (13), all subjects were also given three tablets of CQ (Chloroquin [Astra, Sydney, Australia], 450 mg of CQ base) daily for 3 days. All drugs were administered as whole tablets swallowed with water under direct supervision. Subjects were not required to fast. In addition to the baseline sample, 3-ml venous blood samples were drawn at 1, 2, 4, 6, 12, 18, 24, 30, 48, and 72 h and then at 7, 10, 14, 28, and 42 days postdose. All patients were admitted for at least 48 h to enable blood sampling and hemodynamic monitoring and then reviewed as outpatients on days 7, 10, 14, 28, and 42. An axillary temperature and thick blood film were taken on each occasion. In patients with *P. falciparum* parasitemia at baseline, efficacy outcomes were assessed according to World Health Organization definitions (39).

Laboratory methods. Thick blood smears were examined independently by at least two skilled microscopists who were blinded with regard to pregnancy status. Each microscopist viewed >100 fields at ×1,000 magnification before a slide was considered negative. Any slide discrepant for positivity or negativity or speciation was referred to a third microscopist for final determination.

For drug assays, SDOX, PYR, and sulfamethazine were obtained from Sigma-Aldrich Australia (Castle Hill, Australia), and midazolam hydrochloride was obtained from Pfizer Australia (West Ryde, Australia). N_4-Acetysulfadoxine was synthesized according to the method of Whelpton et al. (36) and determined by high-pressure liquid chromatography to have a melting point of 230°C and >99.9% purity. Acetonitrile was obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical or high-pressure liquid chromatography grade. Extraction of SDOX and NASDOX from 50 μl of plasma and PYR from 500 μl of plasma was by previously published methods (10, 30). Separations were performed on a 5-μm, 250-mm-by-4-mm (internal diameter) Lichrospher RP Select B column (Merck) at 30°C. For SDOX and NASDOX, the mobile phase of 25% (vol/vol) acetonitrile in 0.01% (vol/vol) phosphoric acid and 0.01% (wt/vol) NaCl and was pumped at 1.5 ml/min. For PYR, the mobile phase contained 30% (vol/vol) acetonitrile in 0.01% (vol/vol) phosphoric acid and 0.01% (wt/vol) NaCl and was pumped at 1 ml/min. All analytes were detected by their UV absorbance at 270 nm, and analysis of chromatograms was undertaken using Chemstation Software (version 9; Agilent Technology, Waldbronn, Germany).

The standard curves were linear over 0.1 to 250 mg/liter for SDOX, 0.2 to 10 mg/liter for NASDOX and 2.5 to 1,000 μg/liter for PYR. At 1, 50, and 250 mg/liter the intraday relative standard deviations (RSDs) for SDOX were 6.2, 5.3, and 5.1%, respectively ($n = 5$), and the interday RSDs were 8.7, 7.2, and 5.4% ($n = 25$). For NASDOX at 0.1, 1, and 10 mg/liter the intraday RSDs were 7.7, 4.2, and 4.3%, respectively ($n = 5$), and the interday RSDs were 9.0, 7.7, and 5.7% ($n = 25$). For PYR at 5, 200, and 1,000 μg/liter the intraday RSDs were 5.5, 5.6, and 4.6%, respectively ($n = 5$), and the interday RSDs were 8.0, 6.9, and 4.6% ($n = 25$). The limits of quantitation (LOQs) for SDOX, NASDOX, and PYR were 0.1, 0.02, and 2.5 μg/liter, respectively. The limits of detection for SDOX, NASDOX, and PYR were 0.05 mg/liter, 0 0.01 mg/liter, and 1 μg/liter, respectively, with signal-to-noise ratios of 5.

Population pharmacokinetic analysis. Concentration-time datasets for SDOX, NASDOX, and PYR were analyzed by nonlinear mixed effect modeling using NONMEM (version 6.2.0; Icon Development Solutions, Ellicott City, MD) with a Intel Visual Fortran 10.0 compiler. Pirana (http://pirana.sourceforge.net/) was used as a graphical user interface for NONMEM.

For the SDOX data set, the subroutines within NONMEM were user-defined linear mamillary models (ADVAN5 in the PREDPP library) with provision for both SDOX and its metabolite NASDOX (one or two compartments) and first-order absorption (with or without a lag time) for the parent drug. For the PYR data set, we used ADVAN2/TRANS 2, ADVAN4/TRANS 4, and ADVAN12/TRANS 5 for one-, two-, and three-compartment models with first-order absorption (with or without a lag time), respectively. First-order conditional estimation with η-ε interaction was utilized for modeling. The minimum value of an objective function (OFV; a NONMEM-calculated global goodness-of-fit indicator equal to −2 log likelihood value of data) was used to choose suitable models during the model-building process. Unless otherwise specified, a difference in OFV of ≥6.63 (χ^2 with 1 degree of freedom [df], $P < 0.01$) was considered significant. For graphic model diagnosis the R (http://www.r-project.org/)-based model-building aid Xpose 6.0 was used (17). Derived pharmacokinetic parameters, including the volume of distribution at steady state ($V_{ss} = V_C + V_P$, where V_C and V_P are the central and peripheral volumes of distribution, respectively), were obtained from post hoc Bayesian prediction in NONMEM using the final model parameters. The significance of differences between the pregnant and nonpregnant groups was then compared by using the

rank-sum test. Drug concentration values below the LOQ (<0.5% of all values) were fixed at 1/2 × the LOQ.

A one-compartment model with first-order absorption of SDOX, conversion to NASDOX into a metabolite compartment, and first-order elimination of parent drug and metabolite from the respective compartments was fitted to the data. The initial model parameters were k_a (first-order absorption rate constant), t_{lag} (lag time), CL (clearance), V_C for SDOX; CL_M (metabolic conversion of SDOX to NASDOX); and CL and V for NASDOX. Subsequently, the addition of a peripheral compartment (Q = intercompartment clearance and a V_P) for SDOX was also assessed. Allometric scaling was used on all volume [*(weight/70)$^{1.0}$] and clearance [*(weight/70)$^{0.75}$] terms (1) after assessment of body weight as a covariate yielded similar model outcomes. Between-subject variability (BSV) was added to parameters for which it was able to be estimated reasonably from the available data. For residual unexplained variability (RUV), both exponential (proportional) and combined (exponential plus additive) error models were tested. Renal clearance is the predominant pathway for excretion of both SDOX and NASDOX. A previous study in which both compounds were administered separately to the same subjects has shown that CL_{NASDOX} is 10-fold greater than CL_{SDOX} (15). Therefore, CL_{NASDOX} was fixed at 10 times that of CL_{SDOX} to facilitate model identifiability. For the PYR data set, model building proceeded from one through to three compartments with allometric scaling on all volume and clearance terms, and investigation of t_{lag}, BSV, and RUV as described above for the SDOX data set. The initial model parameters were k_a, t_{lag}, CL, and V_C. Subsequently, a second compartment was added, necessitating the additional parameters V_P and Q. All V and CL parameters were relative to bioavailability (/F).

We assessed the influence of the covariates age, pregnancy, gestational age, hemoglobin, parasitemia, and glucose on model parameters. Xpose was used for exploration of covariate relationships by using the generalized additive modeling procedure function. Relationships between these covariates and individual pharmacokinetic parameters were also explored by inspection of correlation plots. Identified covariate relationships were evaluated within the NONMEM model using a stepwise, forward and backward covariate building process. A decrease of ≥6.63 in the OFV (χ^2 df = 1, $P < 0.01$) was required for inclusion of a covariate, and an increase of ≥10.82 (χ^2 df = 1, $P < 0.001$) was required to retain the covariate. Finally, the impact of the included covariates on BSV and the weighted residual (WRES) plots was assessed before they were incorporated into the final model.

To evaluate the model, a bootstrap procedure was performed by first using Perl speaks NONMEM (http://psn.sourceforge.net) to sample individuals from the original data set with replacement and generate 1,000 new datasets, which were subsequently analyzed by using NONMEM. The resulting parameters were then summarized as median and 2.5th and 97.5th percentiles (95% empirical confidence interval [CI]) to facilitate evaluation of the final model parameter estimates. In addition, a stratified visual predictive check (VPC) was also performed using Perl speaks NONMEM with 1,000 replicate datasets simulated from the original. The resulting 80% prediction intervals for SDOX, NASDOX, and PYR were plotted with the original data to assess the predictive performance of the model.

Statistical analysis. Sample size calculations assumed that (i) a 30% difference in the magnitude of a pharmacokinetic parameter between pregnant and nonpregnant groups would be of clinical importance, (ii) V/F and/or CL/F would increase with pregnancy, and (iii) the disposition of SDOX and PYR in the nonpregnant females would be the same as that in a previous study of SP in nonpregnant adults (12). At $\alpha = 0.05$ and $\beta = 0.1$, up to 21 subjects/group were required, depending on the pharmacokinetic parameter. To allow for a 15% attrition rate, a sample size of 30/group was chosen. It was expected that ca. 30% of women in both groups would have asexual parasitemia at recruitment (4) and that this variable and other covariates such as age, weight, parity, gestational age, and pregnancy status could be evaluated as covariates in the final model. Student's t test or the Mann-Whitney U test for non-normally distributed data was used to compare admission characteristics between the two groups. Categorical data were compared using either the Pearson chi-squared or the Fisher exact test where appropriate. A two-tailed level of significance of $P < 0.05$ was used for all tests (v9.0; SPSS, Chicago, IL).

RESULTS

Patient characteristics. The baseline features of the 30 pregnant and 30 nonpregnant subjects are summarized in Table 1. The groups were well matched for age, weight, and height. Although close to half of each group had detectable para-

TABLE 1. Baseline characteristics of pregnant subjects and nonpregnant controls

Characteristic	Mean ± SD, median [IQR], or no. (%)		P
	Pregnant (n = 30)	Nonpregnant (n = 30)	
Age (yr)	26.0 ± 50.9	25.5 ± 8.9	0.8
wt (kg)	54.0 ± 6.4	51.8 ± 5.5	0.16
ht (cm)	151 ± 5	149 ± 5	0.17
Axillary temp (°C)	36.3 ± 0.5	36.2 ± 0.7	0.6
P. falciparum parasitemia	13 (43)	7 (23)	0.1
P. vivax parasitemia	2 (7)	2 (7)	1.0
P. malariae parasitemia	2 (6)	0	0.5
Gestational age (wk)	22 [20–28]		
Gravidity	3 [1–5]	0 [0–2]	<0.001
Parity	2 [0–4]	0 [0–2]	0.03
Respiratory rate (/min)	23.3 ± 5.3	22.9 ± 4.1	0.7
Supine pulse rate (/min)	87.7 ± 11.7	74.9 ± 8.8	<0.001
Supine blood pressure (mm Hg)	98 ± 8/60 ± 8	100 ± 13/62 ± 9	0.5
Hemoglobin (g/liter)	80 ± 13	107 ± 19	<0.001
Blood glucose (mmol/liter)	4.8 ± 2.0	5.8 ± 1.5	0.02

sitemia (usually *P. falciparum*), no subject had an axillary temperature of >37.5°C. Twenty-three pregnant subjects were enrolled during the second trimester (range, 14 to 27 weeks), and seven were enrolled during the third trimester (28 to 31 weeks). The majority of nonpregnant controls were nulliparous. Pregnant subjects had a significantly higher pulse rate, and a lower hemoglobin and blood glucose, findings consistent with the physiologic changes of pregnancy (7).

Treatment tolerability and efficacy. No significant adverse events were reported. Of 13 pregnant and 7 nonpregnant subjects with *P. falciparum* at entry, 5 and 2 women, respectively, redeveloped parasitemia during the 28-day follow-up, representing an uncorrected adequate clinical and parasitological response rate of 65%. The four subjects with *P. vivax* infection and two subjects with *P. malariae* infection did not redevelop parasitemia.

Pharmacokinetics of SDOX and NASDOX. Median plasma SDOX concentrations were all significantly higher in nonpregnant versus pregnant subjects (Fig. 1), especially at later time points. Plasma NASDOX concentrations averaged between 1 and 7% those of the parent drug at each time point. The initial one-compartment model for SDOX and its metabolite NASDOX using first-order absorption and no lag time for SDOX yielded a valid description of the data and an OFV of 6,613.315. However, inspection of the WRES-time plots showed systematic positive bias in the SDOX concentrations, particularly at the 42-day time point (data not shown). We therefore added a peripheral compartment for SDOX, which resulted in a significant reduction in OFV to 6600.806 (χ^2 df = 2, $P < 0.01$) and an improvement in the residuals. BSV could be estimated on k_a, CL/F_{SDOX}, V/F_{SDOX}, and CL_M/F. For RUV, a proportional model was used for SDOX, while a combined model, both proportional and additive, was most suitable for NASDOX.

The structure of the final model is shown in Fig. 2 and the derived pharmacokinetic parameters, BSV and RUV terms for
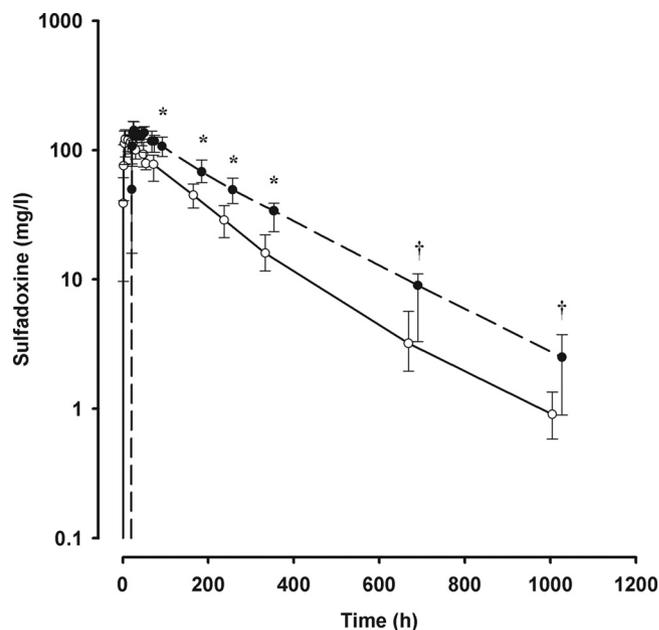

FIG. 1. Median and interquartile range (IQR) plasma SDOX concentrations in pregnant (solid line and open circles) and nonpregnant (dashed line and closed circles) groups. The symbols "†" and "∗" indicate P values of <0.01 or <0.001, respectively, for between-group differences.

both the base and the final models are summarized in Table 2. Of the available covariates, only the effect of pregnancy on CL/F_{SDOX} significantly improved the base model. The bootstrap results are also shown in Table 2 and demonstrate robust fixed and random parameter estimates for the final model, with biases of <5 and <6.1%, respectively. The derived pharmacokinetic parameters are summarized in Table 3. The median SDOX area under the concentration-time curve from 0 to ∞ ($AUC_{0-\infty}$) was 33% lower in the pregnant group. The WRES-time plots for NASDOX showed a modest positive bias at the first, second, and last data collection times (data not shown). VPC plots of actual drug concentrations stratified by pregnancy status together with the 10th and 90th percentile bound-
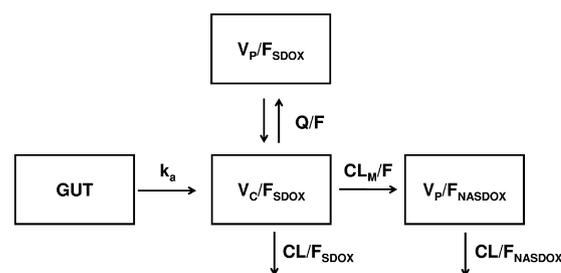

FIG. 2. Structural model used in the final pharmacokinetic analysis. F, bioavailability; k_a, first-order absorption rate constant; V_C/F_{SDOX}, central compartment volume of distribution for SDOX; V_P/F_{SDOX}, peripheral compartment volume of distribution for SDOX; Q/F, intercompartmental clearance for SDOX; CL/F_{SDOX}, clearance of SDOX; CL_M/F, metabolic clearance for SDOX; V/F_{NASDOX}, V for NASDOX; and CL/F_{NASDOX}, clearance of NASDOX (fixed at 10 × CL/F_{SDOX}).

TABLE 2. Model building, parameters, and bootstrap runs for SDOX and NASDOX disposition

Parameter	Base model	Final covariate model	Bootstrap replicates ($n = 1,000$; median [95% empirical CI])
OFV	5,856.304	5,826.383	5,805.270 [5,467.562–6,105.249]
Pharmacokinetic parameters (estimate [RSE%])			
CL/F_{SDOX} (liters/h/70 kg)	0.0476 [6.1]	0.0379 [7.4]	0.038 [0.034–0.043]
Pregnancy on CL/F_{SDOX} (liters/h/70 kg)		0.0181 [19.0]	0.0179 [0.0122–0.024]
V_C/F_{SDOX} (liters/70 kg)	15.8 [2.7]	15.8 [3.0]	15.7 [14.9–16.7]
V_P/F_{SDOX} (liters/70 kg)	1.11 [22.9]	1.11 [19.4]	1.165 [0.715–1.74]
k_a (/h)	0.769 [13.3]	0.769 [15.3]	0.763 [0.583–1.08]
CL_Q/F (liters/h/70 kg)	0.0051 [47.8]	0.0052 [44.5]	0.0051 [0.00251–0.0125]
CL_M/F (liters/h/70 kg)	0.0226 [5.0]	0.0227 [5.0]	0.023 [0.021–0.025]
V/F_{NASDOX} (liters/70 kg)	3.68 [4.8]	3.69 [5.3]	3.72 [3.36–4.10]
Random parameters (CV% [RSE%])			
BSV CL/F_{SDOX}	36.3 [11.6]	28.1 [10.1]	27.1 [21.6–33.2]
BSV V_C/F_{SDOX}	19.8 [16.7]	19.9 [9.1]	19.6 [15.1–23.7]
BSV k_a	99.3 [23]	99.6 [15.2]	97.1 [63.9–135]
BSV CL_M/F	27.3 [12.2]	27.3 [13.50]	26.8 [20.6–33.2]
Residual unexplained variability (RUV)			
Proportional error in SDOX (CV% [RSE%])	24.2 [5.7]	24.1 [5.8]	24.0 [21.4–26.7]
Proportional error in NASDOX (CV% [RSE%])	16.0 [7.8]	16.0 [7.6]	15.8 [11.5–18.3]
Additive error in NASDOX (mg/liter [RSE%])	0.202 [13.6]	0.2 [13.5]	0.202 [0.153–0.343]

aries from 1,000 simulations are shown in Fig. 3A and B for SDOX and Fig. 3C and D for NASDOX.

Pharmacokinetics of pyrimethamine. Median plasma PYR concentrations were higher in nonpregnant versus pregnant subjects at most time points (Fig. 4). The initial one-compartment model using first-order absorption and no lag time for PYR yielded a valid description of the data and an OFV of 9,417.018. However, inspection of the WRES-time plots showed systematic positive bias at later time points (data not shown). The addition of a peripheral compartment to the model reduced the OFV to 8,134.589 (χ^2 df = 2, $P < 0.01$) and improved the residuals. Adding a third compartment for PYR did not improve the model. For the final two-compartment model, BSV could be estimated on k_a, CL/F, V_C/F, V_P/F, and Q/F. The proportional error model was optimal for the RUV.

Derived pharmacokinetic parameters and the BSV (including correlations between pairs) and RUV terms for both the base and final models are summarized in Table 4. Of the available covariates, the effect of pregnancy on CL/F, V_C/F, and V_P/F produced a significant improvement in the base model. The bootstrap results are also shown in Table 4 and demonstrate robust fixed and random parameter estimates for the final model, with bias <3.1 and <10.6%, respectively. Derived pharmacokinetic parameters are summarized in Table 5. There was a 32% lower median $AUC_{0-\infty}$ in the pregnant group. VPC plots of actual drug concentration versus time stratified by pregnancy status including 10th and 90th percentile boundaries from 1,000 simulations are shown in Fig. 5.

DISCUSSION

We found that plasma concentrations of both SDOX and PYR were present in measurable concentrations for the full 42 days of follow-up. This could explain why SP appears to have

TABLE 3. Post hoc Bayesian predicted pharmacokinetic parameters for SDOX and NASDOX for PNG nonpregnant and pregnant women

Parameter	Median [IQR] Nonpregnant ($n = 30$)	Median [IQR] Pregnant ($n = 30$)	P^a
$t_{1/2abs\ SDOX}$ (h)	0.93 [0.55–1.23]	1.46 [0.65–2.15]	0.064
CL/F_{SDOX} (liters/h)	0.030 [0.026–0.035]	0.050 [0.043–0.053]	<0.001
V_C/F_{SDOX} (liters)	10.6 [9.9–11.9]	12.3 [11.1–13.9]	0.003
V_P/F_{SDOX} (liters)	0.81 [0.75–0.88]	0.86 [0.79–0.91]	0.136
V_{ss}/F_{SDOX} (liters)	11.5 [10.7–12.8]	13.1 [11.9–15.0]	0.002
$t_{1/2\alpha\ SDOX}$ (h)	110.8 [103.9–117.4]	103.5 [93.3–108.7]	0.013
$t_{1/2\beta\ SDOX}$ (h)	200.5 [176.8–222.4]	173.5 [162.3–182.6]	<0.001
$AUC_{0-\infty\ SDOX}$ (mg · h/liters)	33,284 [27,923–35,428]	22,315 [19,844–24,967]	<0.001
CL_M/F (liters/h)	0.017 [0.014–0.019]	0.019 [0.017–0.023]	0.011
CL/F_{NASDOX} (liters/h)	0.338 [0.295–0.397]	0.567 [0.483–0.603]	<0.001
V/F_{NASDOX} (liters)	3.1 [2.8–3.3]	3.3 [3.0–3.4]	0.136
$t_{1/2\ NASDOX}$ (h)	6.5 [5.0–7.7]	4.1 [3.5–4.5]	<0.001
$AUC_{0-\infty\ NASDOX}$ (mg · h/liters)	1,590 [1,227–1,933]	801 [610–989]	<0.001

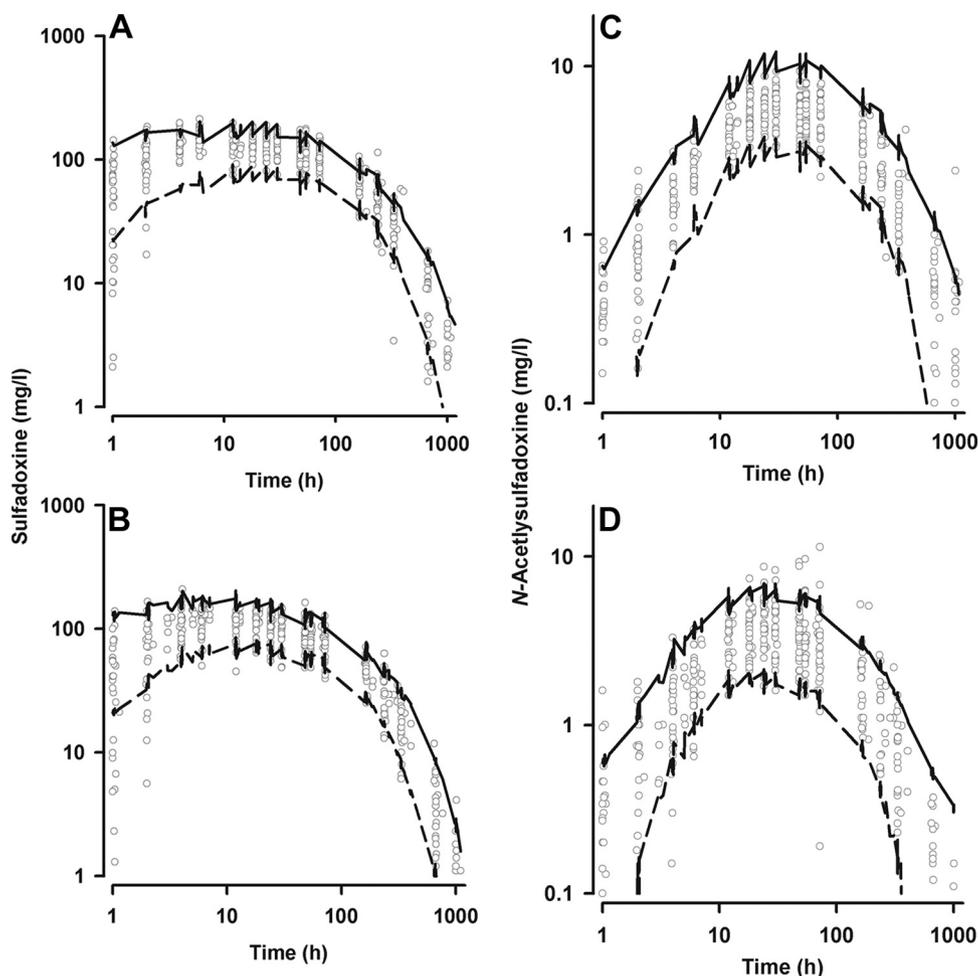^{a} As determined by the rank-sum test.

FIG. 3. Visual predicted check plots showing simulated 10th (dashed line) and 90th (solid line) percentile concentrations and observed concentration (log scale) data versus time (log scale) for SDOX (nonpregnant [A] and pregnant [B]) and NASDOX (nonpregnant [C] and pregnant [D]).

an extended posttreatment prophylactic effect in pregnancy (22, 26, 31). However, the plasma SDOX and PYR levels were significantly lower in our pregnant PNG women than in the nonpregnant group for the same mg/kg SP dose, suggesting that higher doses could be given safely in pregnancy. We found that the $t_{1/2}$ of PYR was longer than previously reported and that variability in SDOX acetylation has minimal impact on its disposition.

Most SDOX pharmacokinetic parameters in the nonpregnant control group were within ranges found previously in adults, including healthy volunteers (12, 19, 21, 24, 25, 32, 34, 35), asymptomatic parasitemic women (14), and patients with symptomatic malaria (3, 5). These include mean or median $t_{1/2abs}$ (0.5 to 0.8 h versus 0.93 h in the present study), $t_{1/2\beta}$ (5.6 to 10.9 days versus 8.4 days), V/F (4 to 20 liters versus combined V_{ss}/F of 11.5 liters), and CL/F (0.02 to 0.08 liters/h versus combined CL/F_{SDOX} + CL_M/F of 0.047 liters/h). Previous studies have shown monoexponential (3, 9, 14, 32) or biexponential (19, 21, 25, 34, 35) SDOX kinetics. We suggest that our 42-day sampling period, compared to ≤28 days in many previous studies (5, 12, 14, 21, 32), facilitated identification of a second compartment. Consideration of the α and β disposition exponentials for SDOX indicated that they accounted for ca.

40 and 60% of the $AUC_{0-\infty}$, respectively, in nonpregnant subjects and 62 and 38% in pregnant subjects. This reflects a more rapid distribution exponential in pregnant subjects, perhaps as a result of physiological changes that have become established in the first trimester (2, 7).

Our analysis shows that the polymorphic variability in SDOX acetylation anticipated from previous phenotypic studies in PNG populations (8, 23) has minimal impact on the disposition of SDOX in both nonpregnant and pregnant subjects. In the postdistribution phase, the plasma concentration-time profiles for SDOX and NASDOX were parallel in all patients. This is consistent with the finding that NASDOX elimination is formation rate limited, being largely controlled by renal excretion which, in humans, occurs at a rate ~10-fold greater than that for SDOX (15). Hence, in contrast to a previous report in Thai subjects (24), acetylation phenotype is unlikely to alter the pharmacokinetics of SDOX in PNG subjects.

The final model for the disposition of SDOX and its primary metabolite NASDOX incorporated first-order SDOX absorption with no lag time, two compartments for SDOX, and one compartment for NASDOX. The minor positive bias in WRES plots for NASDOX at 1 h, 2 h, and 42 days postdose was considered acceptable given the low concentrations at these
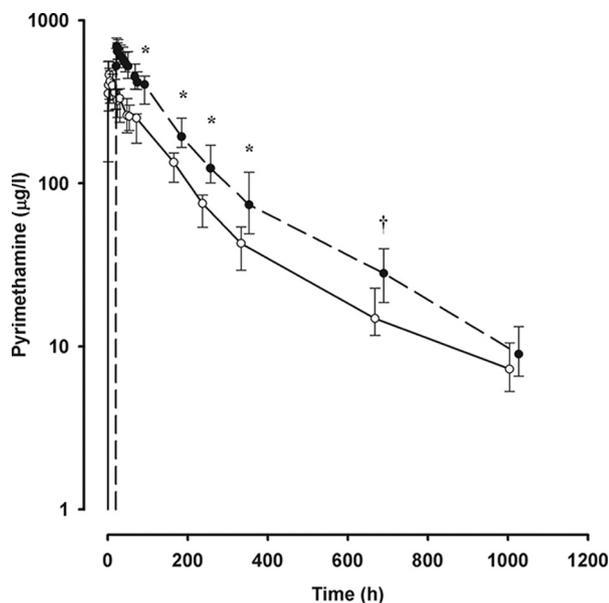
FIG. 4. Median and IQR plasma PYR concentrations in pregnant (solid line and open circles) and nonpregnant (dashed line and closed circles) groups. The symbols "†" and "∗" indicate P values of <0.01 and <0.001, respectively, for between-group differences.

F_{SDOX} (67%) and lower $AUC_{0-\infty}$ (33%) in pregnancy, together with an increase in V_{ss}/F (14%) and a decrease in $t_{1/2\beta}$ (13.5%). The large increase in glomerular filtration rate and the decreased plasma protein concentrations that accompany pregnancy (2) may underlie the higher CL/F observed in pregnant women in the present study. Although CL_M/F_{SDOX} was significantly increased in pregnancy, this increase was small and unlikely to influence overall SDOX disposition.

In contrast to SDOX, the PK parameters for PYR in both our patient groups differed markedly from those found in a wide variety of clinical and ethnogeographic settings (3, 5, 9, 11, 12, 14, 19, 21, 25, 30, 32, 34, 35, 38). In particular, the median $t_{1/2\beta}$s of 18.8 and 9.9 days for pregnant and nonpregnant women, respectively, are higher than previously reported values, which have ranged from 2.9 to 5.1 days in adults (3, 5, 12, 14, 19, 25, 32, 34, 35) and from 2.8 to 4.5 days in children (3, 9, 30, 38). Most other studies have utilized a single-compartment model (3, 9, 14, 30, 32) and/or sampling durations of ≤14 days (12, 14, 32, 38). In one study that attempted to measure PYR concentrations to 42 days, 38% of plasma concentration data was excluded from analysis because levels were either undetectable or below the 10-μg/liter LOQ of the LCMS assay (3). We suggest, therefore, that the second compartment revealed by our extended sampling time and high assay sensitivity (LOQ of 2.5 μg/liter) may account for our discrepant results. This situation appears analogous to the manner in which calculated $t_{1/2\beta}$ values for other antimalarial drugs, such as CQ and piperaquine, have become longer as longer sampling durations and more sensitive assay methodologies have been used (28, 29). The efficacy of IPTp may depend on a prolonged posttreatment prophylactic effect (37) and, although it is difficult to define an in vivo MIC for PYR, the longer $t_{1/2\beta}$

time points (data not shown). A bootstrap analysis of the data set demonstrated that the model was robust, as did the VPC plots for both parent drug and metabolite. Only pregnancy (on CL/F_{SDOX}) was a significant covariate in the final model, accounting for 32% of the total CL/F_{SDOX}. The post hoc Bayesian parameter estimates showed a significantly increased CL/

TABLE 4. Model building, parameters, and bootstrap runs for PYR disposition

Parameter	Base model	Final covariate model	Bootstrap replicates (n = 1,000; median [95% empirical CI])
OFV	8,134.589	8,088.822	8,071.221 [7,830.382–8,312.113]
Pharmacokinetic parameters (estimate [RSE%])			
CL/F (liters/h/70 kg)	1.22 [5.2]	0.87 [5.2]	0.869 [0.788–0.962]
Pregnancy on CL/F (liters/h/70 kg)		0.439 [19.3]	0.432 [0.302–0.569]
V_C/F (liters/70 kg)	189 [5.5]	146 [4.0]	145 [135–156]
Pregnancy on V_C/F (liters/70 kg)		76 [1.8]	76.7 [56–99.8]
V_P/F (L/70 kg)	123 [13.2]	76.8 [8.7]	77.1 [64.8–90.7]
Pregnancy on V_P/F (liters/70 kg)		98 [23.9]	101 [53.2–1205]
k_a (/h)	1.84 [22.8]	1.84 [19.4]	1.83 [1.32–2.7]
Q/F (liters/h/70 kg)	0.52 [16.3]	0.51 [12.9]	0.51 [0.4–0.66]
Random parameters (CV% [RSE%])			
BSV CL/F	36.5 [9.4]	27.6 [8.5]	7.3 [4.9–10.1]
BSV V_C/F	36.1 [9.8]	24.6 [10.7]	5.8 [3.6–8.6]
BSV V_P/F	65.3 [12.3]	42.5 [13.6]	16.3 [4–28.6]
BSV Q/F	55.9 [15.8]	57.5 [13.7]	29.6 [12.1–49.3]
BSV k_a	10.6 [14]	10.7 [13.3]	112 [67–187]
Correlations between BSV pairs			
R (CL/F, V_C/F)	0.889	0.797	0.802 [0.672–0.897]
R (CL/F, V_P/F)	0.832	0.756	0.787 [0.458–0.995]
R (V_C/F, V_P/F)	0.858	0.731	0.728 [0.427–0.995]
Residual unexplained variability (RUV)			
Proportional error in PYR (μg/liter [RSE%])	19.3 [5]	19.2 [4.9]	19.4 [17.3–21.3]

TABLE 5. Post hoc Bayesian predicted pharmacokinetic parameters for PYR for PNG nonpregnant and pregnant women

Parameter	Median [IQR]		P^a
	Nonpregnant ($n = 30$)	Pregnant ($n = 30$)	
$t_{1/2abs}$ (h)	0.41 [0.32–0.48]	0.37 [0.29–0.47]	0.234
CL/F (liters/h)	0.707 [0.571–0.875]	1.040 [0.901–1.383]	<0.001
V_C/F (liters)	108.0 [96.6–117.8]	174.2 [148.8–232.1]	<0.001
V_P/F (liters)	67.4 [53.9–82.3]	184.3 [154.7–222.6]	<0.001
V_{ss}/F (liters)	175.9 [150.9–200.1]	381.6 [301.5–434.9]	<0.001
$t_{1/2\alpha}$ (h)	51.9 [43.7–56.6]	75.1 [60.9–84.5]	<0.001
$t_{1/2\beta}$ (h)	237.3 [211.3–261.9]	450.1 [349.1–500.3]	<0.001
$AUC_{0-\infty}$ ($\mu g \cdot$ h/liters)	106,065 [85,693–131,350]	72,115 [54,250–83,229]	<0.001

[a] As determined by the rank-sum test.

of PYR in the present study, especially in pregnant patients, could be advantageous. The relatively long PYR $t_{1/2\beta}$ in the pregnant group may reflect the effect of the final sampling time point (day 42), the only one at which pregnant and nonpregnant patient had similar plasma PYR concentrations.

The α and β disposition exponentials accounted for ca. 30 and 70%, respectively, of the PYR $AUC_{0-\infty}$ in nonpregnant subjects and 45 and 55%, respectively, of the PYR $AUC_{0-\infty}$ in pregnant subjects. The β disposition reflected a CL/F estimate
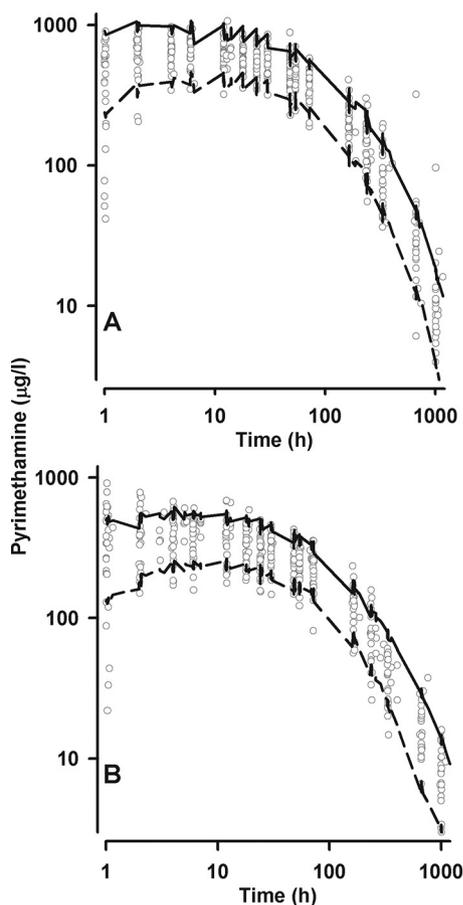

FIG. 5. Visual predicted check plots showing simulated 10th (dashed line) and 90th (solid line) percentile concentrations and observed concentration data (log scale) versus time (log scale) for PYR (nonpregnant [A] and pregnant [B]).

in our study (0.71 liters/h/70 kg) that was at the lower end of the range in previous studies of nonpregnant adults (0.7 to 2.0 liters/h) (3, 12, 14, 19, 34, 35), as well as the much higher median $AUC_{0-\infty}$ (106,065 $\mu g \cdot$ h/liters in nonpregnant subjects) than in other studies of adults administered comparable doses (34,700 to 42,000 $\mu g \cdot$ h/liters) (3, 14).

The final model for the disposition of PYR comprised two compartments with first-order absorption and no lag. A bootstrap analysis of the data set demonstrated that the model was robust, as did the VPC plots. Only pregnancy was a significant covariate in the final model, accounting for 33.5, 34, and 56%, respectively, of the total values for CL/F, V_C/F, and V_P/F. A 47% higher median CL/F in the pregnant group was also seen in the post hoc data and was associated with an increase in median V_{ss}/F (117%) in pregnancy. A substantial proportion of PYR clearance is probably renal, with urinary excretion accounting for 16 to 33% of a single oral dose (27). Therefore, as for SDOX, both the increased glomerular filtration rate and decreased protein binding in pregnancy (2) may underlie the higher CL/F observed in pregnant women in the present study.

The only other published study to have evaluated the pharmacokinetics of SP in pregnancy (14) produced similar findings to our own with respect to pregnancy-related differences in SDOX but not PYR disposition. Although these authors studied pregnant Kenyan women, many of whom (like our subjects) had asymptomatic malaria parasitemia, the subjects were mostly also human immunodeficiency virus infected (14). A crossover design was used such that 10 subjects were reevaluated 2 to 3 months postpartum in place of a nonpregnant comparator group. The $AUC_{0-\infty}$ for SDOX was 45% lower in pregnant versus postpartum subjects, a difference similar to the 33% lower value in our pregnant versus nonpregnant subjects. However, in contrast to our study, the authors of that study did not demonstrate significant between-group differences in any of the pharmacokinetic parameters evaluated using a one-compartment model for PYR (14). This may have reflected the short sampling duration (10 days) that would not have captured the late terminal elimination phase demonstrated in the present study, the crossover study design, and/or the relatively small size of the postpartum group ($n = 10$).

Our study was not designed to assess the efficacy of SP as an IPTp. However, the high rate of reemergent parasitemia in the pregnant women (38.5% of those with *P. falciparum* by day 28) may indicate a suboptimal response to SP. This observation and the pregnancy-related pharmacokinetic data suggest that

SP doses could be increased when administered as IPTp. For example, giving four SP tablets to pregnant women as a single dose would increase the $AUC_{0-\infty}$ for SDOX from 71 to 95% of the nonpregnant mean value after three tablets as simulated using our final model and that of PYR from 60 to 80%. The equivalent increases in peak plasma concentrations (C_{max}) would be from 95 to 127% for SDOX and from 57 to 76% for PYR. Giving three daily doses of 1.5 tablets (total of 4.5 tablets) would result in an $AUC_{0-\infty}$ for SDOX that was 107% of the nonpregnant mean value for 3 tablets stat and a C_{max} of 125%, with equivalent figures for PYR of 90 and 71%, respectively. Thus, the three-dose regimen would provide the closer equivalent to the $AUC_{0-\infty}$ of both drugs achieved with conventional dosing in nonpregnant women ($\geq$90%), with similar potential to a four-tablet stat regimen for increased toxicity associated with higher peak plasma SDOX concentrations. Nevertheless, compliance with a more complex regimen needs to be considered even if it could be linked to CQ dosing in countries such as PNG where both treatments are given together as IPTp.

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