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Pharmacokinetic Properties of Conventional and Double-Dose Sulfadoxine-Pyrimethamine Given as Intermittent Preventive Treatment in Infancy▽

Sam Salman,[1] Susan Griffin,[2] Kay Kose,[2] Nolene Pitus,[2] Josephine Winmai,[2] Brioni Moore,[1] Peter Siba,[2] Kenneth F. Ilett,[1,3] Ivo Mueller,[2] and Timothy M. E. Davis[1]*

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

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Intermittent preventive treatment in infancy (IPTi) entails routine administration of antimalarial treatment doses at specified times in at-risk infants. Sulfadoxine-pyrimethamine (SDX/PYR) is a combination that has been used as first-line IPTi. Because of limited pharmacokinetic data and suggestions that higher milligram/kilogram pediatric doses than recommended should be considered, we assessed SDX/PYR disposition, randomized to conventional (25/1.25 mg/kg of body weight) or double (50/2.5 mg/kg) dose, in 70 Papua New Guinean children aged 2 to 13 months. Blood samples were drawn at baseline, 28 days, and three time points randomly selected for each infant at 4 to 8 h or 2, 5, 7, 14, or 21 days. Plasma SDX, PYR, and N_4-acetylsulfadoxine (NSX, the principal metabolite of SDX) were assayed by high-performance liquid chromatography (HPLC). Using population modeling incorporating hepatic maturation and cystatin C-based renal function, two-compartment models provided best fits for PYR and SDX/NSX plasma concentration profiles. The area under the plasma concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) was greater with the double dose versus the conventional dose of PYR (4,915 versus 2,844 μg/day/liter) and SDX (2,434 versus 1,460 mg/day/liter). There was a 32% reduction in SDX relative bioavailability with the double dose but no evidence of dose-dependent metabolism. Terminal elimination half-lives (15.6 days for PYR, 9.1 days for SDX) were longer than previously reported. Both doses were well tolerated without changes in hemoglobin or hepatorenal function. Five children in the conventional and three in the double-dose group developed malaria during follow-up. These data support the potential use of double-dose SDX/PYR in infancy, but further studies should examine the influence of hepatorenal maturation in very young infants.

Intermittent preventive treatment in infancy (IPTi) is a strategy in which infants in areas in which malaria is endemic are given treatment doses of antimalarial drugs at specified times, regardless of clinical and parasitologic status. Because of its availability, tolerability, and relatively low cost, sulfadoxine-pyrimethamine (SDX/PYR) has been used as a first-line treatment in IPTi programs, especially in Africa. A recent review of safety and efficacy data from six trials conducted from 1999 to 2007 revealed that, despite the emergence of molecular markers of parasite resistance, SDX/PYR IPTi reduced clinical malaria and malaria-related hospital admissions by about one-third and reduced anemia in the first year of life by 15% (23). The duration of effective antimalarial prophylaxis after a dose of SDX/PYR is 4 to 6 weeks (9, 17).

There is evidence that the efficacy of SDX/PYR IPTi is dose dependent. When given as a fixed dose (27), efficacy declines with age as lower doses (milligrams/kilogram of body weight) are taken (9). In addition, studies of older children aged 2 to 5 years with falciparum malaria have found higher clearance rates and larger apparent volumes of distribution for both SDX and PYR than those in adults (11). Consistent with these data, a population pharmacokinetic (PK) study in children with congenital toxoplasmosis showed that the elimination half-lives for both drugs were directly related to body weight, with the consequence that younger and thus lighter children had more rapid elimination (37). These studies suggest that the peak plasma concentration and area under the plasma concentration-time curve (AUC) will be reduced in younger children and that currently recommended doses of SDX/PYR of 25 mg/kg and 1.25 mg/kg, respectively, may be inadequate for full efficacy. Indeed, there is evidence that higher blood PYR concentrations enhance the ability of pediatric patients to clear resistant *Plasmodium falciparum* (19).

In view of these data and calls for doubling of the recommended treatment dose in children aged 2 to 5 years (11), we assessed the tolerability, safety, and pharmacokinetic properties of SDX/PYR given in recommended and double recommended doses to infants living an area of intense malaria transmission in Papua New Guinea (PNG).

MATERIALS AND METHODS

Study site, sample, and approvals. The present study was conducted at Alexishafen Health Centre, Madang Province, on the north coast of Papua New Guinea (PNG). Infants between the ages of 2 and 13 months from the surrounding area were eligible for recruitment provided that they (i) did not have features of severe malaria or significant nonmalarial illness, (ii) had not been treated with SDX or PYR in the previous 4 weeks, (iii) did not have a known allergy to either

* Corresponding author. Mailing address: Department of Medicine, 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.

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TABLE 1. Dosing guide for conventional and double-dose groups

Body wt	Dosage (mg SDX/PYR)	
	Conventional dose	Double dose
3–5.9 kg	¼ tablet (125/6.25)	½ tablet (250/12.5)
6–11.9 kg	½ tablet (250/12.5)	1 tablet (500/25)

SDX or PYR, and (iv) were available for assessment for the duration of follow-up. Written informed consent was obtained from the parents/guardians of all recruited infants. The study was approved by the Medical Research Advisory Committee of PNG and the Human Ethics Research Committee at the University of Western Australia.

Clinical procedures. At enrollment, a clinical assessment was performed that included a standard baseline symptom questionnaire completed by parents/guardians. A 500-μl finger prick capillary blood sample was taken for preparation of blood smears for microscopy, baseline drug assay, biochemical tests, and hemoglobin concentration (HemoCue, Angelholm, Sweden). Subjects were randomized to receive either the recommended dose of SDX/PYR (25/1.25 mg/kg Fansidar; Roche, Basel, Switzerland) or a double dose (50/2.5 mg/kg). Table 1 shows the dose administered based on body weight. All dosing was directly observed, with subsequent monitoring and readministration of the dose if the infant vomited within 30 min. Infants with a positive blood film were also given a 3-day course of amodiaquine according to PNG national treatment guidelines (33). All drugs were crushed and mixed with either water or breast milk before administration by mouth using a syringe.

All infants were reassessed on days 1, 2, 3, 5, 7, 14, 21, and 28. A hemoglobin concentration was determined on each occasion and a repeat symptom questionnaire administered at each visit up to day 7. Blood films were repeated on day 28 and/or when fever or a recent history of a fever was reported. For pharmacokinetic analysis, four additional 500-μl capillary blood samples were taken from each infant. The times for the first three of these were randomly selected for each infant from either 4 to 8 h or 2, 5, 7, 14, or 21 days postdose. A final sample was taken in all cases on day 28. The exact timing of each blood sample was recorded. All samples were centrifuged promptly, with red cells and separated plasma stored frozen at −80°C until assay.

Laboratory methods. Giemsa-stained thick blood smears were examined independently by at least two skilled microscopists who were blinded to dose group. Each microscopist viewed >100 fields at ×1,000 magnification before a slide was considered negative. Any slide discrepant for positivity/negativity or identification to the species level was referred to a third microscopist.

Cystatin C (CysC) concentrations were measured by particle-enhanced immunoturbidimetry (PETIA) using the Tina-quant cystatin C kit run on an Elecsys 2010 analyzer (Roche, Indianapolis, IN). Sodium, urea, creatinine, albumin, γ-glutamyl transferase, and bilirubin were measured using an Integra 800 analyzer (Roche) when sufficient plasma was available.

Sulfadoxine, sulfamethazine, and pyrimethamine were obtained from Sigma-Aldrich (Castle Hill, Australia), and midazolam hydrochloride was obtained from Pfizer (West Ryde, Australia). N_4-acetylsulfadoxine (NSX) was synthesized according to the method of Whelpton et al. (39) and found to have a melting point of 230°C and >99.9% purity by high-performance liquid chromatography (HPLC). Acetonitrile was obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical or HPLC grade.

For PYR, SDX, and NSX, extraction and separation were performed based on previously published HPLC-UV methods (26, 37). The internal standards were midazolam HCl for PYR and sulfamethazine for SDX and NSX. Analytes were assayed using UV detection at 270 nm. Chemstation software (version 9; Agilent Technology, Waldbronn, Germany) was used for analysis of chromatograms. Standard curves were linear from 5 to 1,000 μg/liter, 0.1 to 200 mg/liter, and 0.02 to 10 mg/liter for PYR, SDX, and NSX, respectively. Intra- and interday relative standard deviations (RSDs) were <15% for all analytes at all concentrations. The limits of quantification (LOQ) were 2.5 μg/liter, 0.1 mg/liter, and 0.02 mg/liter, and the limits of detection (LOD; determined as a signal-to-noise ratio of 5) were 1 μg/liter, 0.05 mg/liter, and 0.01 mg/liter for PYR, SDX, and NSX, respectively.

Population pharmacokinetic analysis. Log_e concentration-versus-time data sets for PYR, SDX, and NSX were analyzed by nonlinear mixed effect modeling using NONMEM (version 6.2.0; ICON Development Solutions, Ellicott City, MD) with an Intel Visual FORTRAN 10.0 compiler. Linear mammillary model subroutines within NONMEM (ADVAN2/TRANS2 and ADVAN4/TRANS4), first order conditional estimation (FOCE) with η-ε interaction, and the objective function value (OFV) were used to construct and compare plausible models. Unless otherwise specified, a difference in OFV of ≥6.63 (χ^2 distribution with 1 df, $P < 0.01$) was considered significant. Due to the small number of samples with low concentrations, those below the LOD were not included in the analysis, while levels between the LOD and LOQ were kept at their measured concentrations.

As the subjects were infants with a range of ages, it was important to incorporate maturation of clearance into the model. Therefore, total clearance (CL_T) was defined as the sum of hepatic clearance (CL_H) and renal clearance (CL_R), i.e., $CL_T = CL_H + CL_R$. The age-adjusted hepatic clearance, CL_H, was determined using a sigmoid maximum effect (E_{max}) model (7) as $TVCL_H \times [PMA^{HillCL}/(PMA^{HillCL} + MATCL_{50}^{HillCL})]$, where $TVCL_H$ is the population average value for hepatic clearance, PMA is the postmenstrual age (the age of the infant recorded from the last menstrual cycle of the mother during pregnancy rather than birth), HillCL is the Hill coefficient for hepatic clearance, and $MATCL_{50}$ is the PMA at which CL_H is 50% of the mature value. When an accurate PMA could not be obtained, it was estimated from the postnatal age (PNA) and average gestation in PNG (3, 15, 21). CL_R was adjusted to a standardized value for an estimated glomerular filtration rate (eGFR) of 120 ml/min/1.76 m^2, i.e., $TVCL_R \times (\text{eGFR}/120)$, where CL_R is the adjusted renal clearance, $TVCL_R$ is the population average value for renal clearance, and the eGFR was determined from the cystatin C concentration (CysC) as $91.62 \times (1/\text{CysC}^{1.123})$ (20).

Allometric scaling using weight (WT) was also used on all volume and clearance terms, which were multiplied by (WT/70) and $(\text{WT}/70)^{0.75}$, respectively. One- and two-compartment models with first order absorption without lag time were assessed for both SDX and PYR. As few data exist to describe the absorption phase of both drugs, the absorption rate constant (k_a) was fixed to the previously published value for infants (18). Between-subject variability (BSV) was added to parameters for which it could be estimated reasonably from available data. As $\log_e$ concentration data were used, an additive model (representing proportional error) was used for residual unexplained variability (RUV).

In the development of the final models, we investigated the influence of the covariates dosing group, relative dose (milligram/kilogram), PMA, malaria status, concomitant treatment with amodiaquine, and initial hemoglobin concentration using the generalized additive modeling procedure within Xpose (http://xpose.sourceforge.net) and by inspection of correlation plots. Covariate relationships identified by this procedure were evaluated within the NONMEM model, and inclusion of the covariate required a significant decrease in OFV accompanied by a decrease in the BSV of that parameter. Correlations among BSV terms and weighted residuals (WRES) plots were also used in model evaluation.

Once a final model for SDX was obtained, the parameter estimates were fixed and an additional compartment was added in order to model NSX concentrations. In order to allow identifiability in the model, the percentage conversion of SDX to NSX was fixed to 60% based on the product information (35). The elimination of NSX was assumed to be entirely renal (25).The influence of the covariates was assessed on new model parameters using the method described above.

A bootstrap procedure using Perl-speaks-NONMEM (PSN) (http://psn.sourceforge.net) and the resulting parameters were then summarized as median and 2.5th and 97.5th percentiles (95% empirical confidence interval [CI]) to facilitate validation of the final model parameter estimates. In addition, stratified visual predictive checks (VPCs) and numerical predictive checks (NPCs) were also performed using PSN with 1,000 replicate data sets simulated from the original data set. NPCs stratified according to PMA were assessed by comparing the actual with the expected number of data points within the 20, 40, 60, 80, 90, and 95% prediction intervals (PI). The resulting 80% PI for drug concentrations were plotted with the observed data to assess the predictive performance of the model.

Statistical analysis. As previously reported in a study of SDX/PYR pharmacokinetics in pregnant versus nonpregnant women (26), and using estimates of centrality and variance for pharmacokinetic parameters from previous pediatric studies (11, 19, 32, 37, 40) and an assumed 20% attrition rate, a sample size of 35 in each group in the present study would be expected to show a >30% increase in the magnitude of any pharmacokinetic parameter in the double-dose group at $\alpha = 0.05$ and $\beta = 0.1$. SPSS 17.0 (SPSS inc. Chicago, IL) was used for all statistical analysis unless otherwise specified. Data are summarized as mean ± standard deviation (SD) or median and interquartile range (IQR) as appropriate. Student's *t* test or the Mann-Whitney U test was used for two-sample comparisons. Categorical data were compared using either Pearson chi-squared or Fisher's exact test and multiple means by repeated measures analysis of variance (ANOVA). A two-tailed level of significance of 0.05 was used.

TABLE 2. Baseline characteristics of study participants

Parameter	Result for study group	
	Conventional dose (n = 35)	Double dose (n = 35)
Postmenstrual age, days [median (IQR)]	454 (383–513)	501 (428–532)
Sex [no. (%) male]	22 (63)	24 (69)
Weight (kg) (mean ± SD)	6.58 ± 1.31	6.98 ± 1.1
Height (cm) (mean ± SD)	61.8 ± 6.5	66.1 ± 7.8
Axillary temp (°C) (mean ± SD)	36.5 ± 0.6	36.4 ± 0.6
No. (%) with parasitemia[a]		
P. falciparum	1 (3)	0 (0)
P. vivax	3 (9)	3 (9)
Respiratory rate (per min) (mean ± SD)	40 ± 11	42 ± 11
Supine pulse rate (per min) (mean ± SD)	133 ± 14	133 ± 15
Mean upper arm circumference (cm) (mean ± SD)	13.2 ± 3.5	13.7 ± 2.6
Hemoglobin (g/liter) (mean ± SD)	9.5 ± 1.3	9.5 ± 1.2
eGFR (ml/min/1.73 m^2) (mean ± SD)	80 ± 20	84 ± 16
Sulfadoxine dose (mg/kg) (mean ± SD)[b]	35.6 ± 5.6	67.1 ± 12.6
Pyrimethamine dose (mg/kg) (mean ± SD)[b]	1.8 ± 0.3	3.4 ± 0.6

[a] One infant had a mixed *P. vivax/falciparum* infection.
[b] $P < 0.001$.

RESULTS

Patient characteristics. Seventy infants were enrolled between April 2008 and December 2008, with equal numbers in each dose group. Baseline subject characteristics are summarized in Table 2. The double-dose group received a significantly higher milligram/kilogram dose than the conventional dose group ($P < 0.001$) and was taller by a mean of 4.3 cm ($P = 0.015$). The double-dose group was also older (by a mean of 47 days) and heavier (by 0.4 kg) than the conventional dose group, but these differences were not statistically significant ($P > 0.05$).

Tolerability, safety, and efficacy. Both doses were well tolerated. There were no changes in symptoms in either group compared to predose profiles, including an absence of dermatological conditions. There were no significant changes in hemoglobin, or in plasma urea, creatinine, or CysC, over time. In the conventional dose group, there was a significant but transient mean fall in plasma albumin of 2 g/liter at day 2 (from 38 to 36 g/liter; $P < 0.01$), but there were no concomitant increases in plasma bilirubin or hepatic enzymes in either group.

Five infants with vivax malaria and one infant with a mixed *Plasmodium vivax/P. falciparum* infection at enrollment responded to treatment. Three other infants in the conventional dose group and two in the double-dose group were administered antimalarial drugs during follow-up at an external health care facility, and no blood smears were available for review. No other subjects became symptomatic during the study. Only two infants in the conventional dosing group and one in the double-dosing group who were aparasitemic at entry had a positive blood slide on day 28 (all for *P. vivax*). All were asymptomatic, and each was treated according to PNG national treatment guidelines.

Pharmacokinetic modeling. There were 248, 255, and 247 drug concentration measurements available for pharmacokinetic modeling for PYR, SDX, and NSX, respectively. There were four samples with PYR concentrations between the LOD and LOQ and a further four with concentrations below the LOD for PYR. In addition, seven samples were of insufficient volume for measurement of PYR after the SDX/NSX assay. There were no SDX or NSX concentrations below the LOQ, but NSX concentrations could not be determined in eight samples due to an unidentified interfering peak. For PYR, a two-compartment model was superior to a one-compartment model with a lower OFV (−87.081 versus −30.030) and a less-biased weighted residuals versus time (WRES) plot. The model parameters were k_a, CL_H/F, CL_R/F, central compartment volume of distribution (V_2/F), peripheral compartment volume of distribution (V_3/F), intercompartmental clearance (Q/F), HillCL, and $MATCL_{50}$. BSV was estimable on CL_T/F, V_2/F, and Q/F. As the correlation between the variability of V_2/F and Q/F was very close to 1, it was subsequently fixed to unity to assist with successful determination of the covariance matrix. None of the covariates tested improved the model significantly; therefore, the final model contained only the effects of PMA and WT anticipated from maturation and allometric scaling, respectively.

TABLE 3. Final population PK parameters and bootstrap results for PYR

Parameter[a]	Value	
	Final model	Bootstrap (n = 1,000) [median (95% CI)]
OFV	−97.384	−111.812 (−170.137 to −62.348)
PK parameters [estimate (% RSE)]		
k_a (per h)	0.779	Fixed
V_C/F (liters/70 kg)	222 (4)	221 (202–242)
V_P/F (liters/70 kg)	64.1 (24)	63.0 (41.8–128.5)
Q/F (liters/h/70 kg)	0.0735 (19)	0.0788 (0.0486–0.1470)
CL_R/F (liters/h/70 kg)	0.416 (64)	0.3820 (0.0621–0.9868)
CL_H/F (liters/h/70 kg)	0.854 (24)	0.878 (0.466–1.220)
$MATCL_{50}$ (days)	318 (8)	326 (286–367)
HillCL	7.39 (43)	7.80 (3.53–35.18)
Random parameters [% CV(% RSE)]		
BSV V_C/F	13.0 (36)	13.6 (3.6–24.7)
BSV CL_T/F	27.8 (13)	27.0 (18.2–35.0)
BSV Q/F	34.1 (32)	36.4 (17.4–53.4)
Correlations between BSV pairs		
R (V_C/F, CL_T/F)	0.533 (69)	0.563 (−0.059 to 0.826)
R (V_C/F, Q/F)	1	Fixed
R (CL_T/F, Q/F)	0.533 (69)	0.563 (−0.059 to 0.826)
Residual unexplained variability (RUV)		
Proportional error [% CV (% RSE)]	33.6 (23)	32.6 (26.9–37.4)

[a] % RSE, percent relative standard error.

The final parameter estimates and the results of the bootstrap procedure for PYR are shown in Table 3. All model parameters had a bias of <11%. Goodness-of-fit plots for PYR are shown in Fig. 1. NPCs of the data showed good predictive performance, as did VPC plots of the observed drug concentrations and their 80% PI (the 10th and 90th percentile boundaries) stratified by dosing group (Fig. 2A and B). *Post hoc* parameter estimates are shown in Table 4. There was no difference between the two groups for any of these parameters except for AUC from 0 h to infinity ($AUC_{0-\infty}$), which was significantly higher in the double-dose group (4,915 versus 2,844 μg/day/liter). Median steady-state volume (V_{SS}) for the

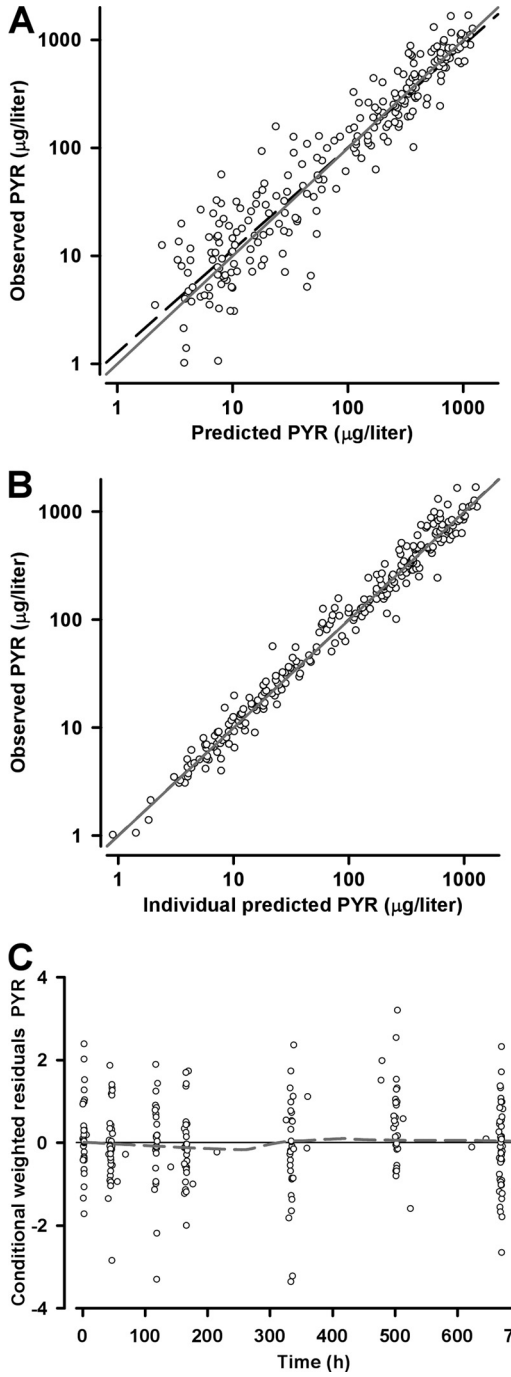

FIG. 1. Goodness-of-fit plots for PYR showing observed versus model predicted concentrations (A) and individual predicted concentrations (B) (both log scale) and conditional weighted residuals versus time (C). For panels A and B, the solid gray line represents the line of identity, while the dashed black line represents the linear regression line of best fit; in panel C, the solid gray line represents the locally weighted scatterplot smoothing (LOESS) smoothed fit.

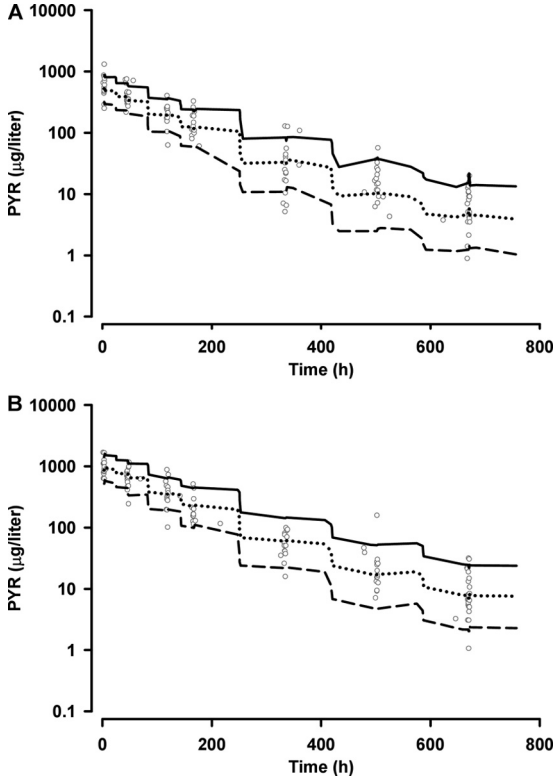

FIG. 2. Visual predicted check plots for PYR showing simulated 10th (short dashed line), 50th (dotted line), and 90th (solid line) percentile concentrations and observed concentration (log scale) data (gray open circles) versus time (log scale) for conventional dose (A) and double-dose (B) participants.

combined study sample was 27.8 liters, and the half-lives at α and β phases ($t_{1/2\alpha}$ and $t_{1/2\beta}$, respectively) were 72.7 and 374 h, respectively.

Initial modeling of SDX revealed that a one-compartment model was appropriate, as there was minimal bias in the WRES plot that was not improved when a two-compartment model was fitted. The model parameters were k_a, CL_H/F, CL_R/F, volume of distribution (V/F), HillCL, and $MATCL_{50}$. BSV was able to be estimated on CL_T/F and V/F. There was a significant relationship between relative dose (in milligrams/kilogram) and relative bioavailability which conformed to a power function; specifically, individual relative bioavailability = $1 \times$ ([individual relative dose]/[average relative dose]$^{\text{effect parameter}}$). The value of the power effect parameter was −0.56, indicating that, when the dose is doubled, the bioavailability falls by 32.2%. The final parameter estimates and the results of the bootstrap procedure are shown in Table 5. With the exception of CL_R, all parameter estimates had biases of <13%. The median bootstrap value for CL_R was almost double the initial estimate (195%), demonstrating the difficulty in delineating the difference between and estimating the hepatic and renal clearance using this methodology. Goodness-of-fit plots for SDX are shown in Fig. 3. NPCs of the data showed good predictive performance, as did VPC plots of the observed drug concentrations and their 80% PI stratified by dose group in Fig. 4.

An additional compartment was added to the final SDX PK model to incorporate the data for NSX. This resulted in three additional model parameters: volume of distribution of NSX (V_{NSX}/F), clearance of NSX (CL_{NSX}/F), and percentage of total SDX elimination representing conversion of SDX to NSX (%NSX). As these three parameters cannot be estimated simultaneously, %NSX was fixed to 60% based on published data (35). The estimates of V_{NSX}/F and CL_{NSX}/F are directly

TABLE 4. *Post hoc* Bayesian predicted PK parameters for PYR for PNG infants given conventional and double doses of SDX/PYR

Parameter	Median result (IQR) for study group		*P* value[a]
	Conventional dose (n = 35)	Double dose (n = 35)	
CL_T/F (liters/h)	0.183 (0.13–0.21)	0.199 (0.164–0.229)	NS
V_C/F (liters)	20.2 (17.8–24.1)	22.1 (19–25)	NS
V_P/F (liters)	6.18 (5.32–6.81)	6.49 (5.83–7.03)	NS
V_{SS}/F (liters)	25.781 (23.319–30.957)	28.8 (24.9–31.8)	NS
Q/F (liters/h)	0.0118 (0.0073–0.0165)	0.0135 (0.009–0.0196)	NS
$t_{1/2\alpha}$ (h)	73.7 (67.1–87.7)	70.7 (62.3–82.3)	NS
$t_{1/2\beta}$ (h)	391 (300–565)	361 (272–511)	NS
$AUC_{0-\infty\ PYR}$ (μg/day/liter)	2,844 (2,486–3,571)	4,915 (4,311–5,681)	<0.001

[a] Mann-Whitney test. NS, nonsignificant ($P > 0.05$).

related to %NSX; therefore, the value of these parameters should be interpreted with caution. However, AUC and $t_{1/2}$ for NSX remain unchanged for different values of %NSX. V_{NSX}/F and CL_{NSX}/F were not influenced by any of the available covariates. Final parameter estimates and results of the bootstrap procedure are shown in Table 5. Bias was <5% for all NSX parameters, and NPCs and VPCs were performed on the NSX data set and indicated good predictive performance of the model (data not shown).

There were some significant differences between conventional and double-dose groups in the *post hoc* parameter estimates for both SDX and NSX (Table 6). These included expected differences in the $AUC_{0-\infty}$ for both SDX and NSX, but also differences in the half-life and clearance for both drugs which were not revealed by the model covariate building stage. A higher clearance and lower half-life ($t_{1/2}$) in the double-dose group can be attributed to organ maturation, as these infants were older than those in the conventional dose group. The median $t_{1/2}$ of NSX for the combined study sample was shorter than that of SDX (8.9 versus 218 h). The percentage of the $AUC_{0-\infty}$ of NSX compared to that for SDX was the same for both dose groups (approximately 5%).

Sigmoid E_{max} curves of hepatic maturity for SDX and PYR by PMA are shown in Fig. 5. They are closely related to $MATCL_{50}$ values of 318 days and 271 days for PYR and SDX, respectively. Of the 70 infants, 48 (69%) and 38 (54%) had an estimated hepatic clearance that was >90% of adult values for PYR and SDX, respectively.

TABLE 5. Final population PK parameters and bootstrap results for SDX and NSX[a]

Parameter	Value	
	Final model	Bootstrap (n = 1,000) [median (95% CI)]
OFV	−521.177	−529.222 (−647.701 to −428.084)
PK parameters [estimate (% RSE)]		
k_a (per h)	1.23	Fixed
V/F (liters/70 kg)	24.2 (4)	24.2 (22.5–26.1)
CL_R/F (liters/h/70 kg)	0.0046 (113)	0.0086 (0.0005–0.0267)
CL_H/F (liters/h/70 kg)	0.0458 (16)	0.0427 (0.0290–0.0640)
$MATCL_{50}$ (days)	271 (8)	286 (248–360)
HillCL	4.07 (52)	4.61 (1.56–15.54)
Relative dose on relative bioavailability (power)	−0.56 (14)	−0.54 (−0.71 to −0.38)
% NSX (%)	**60**	**Fixed**
V_{NSX}/F (liters/70 kg)	**11.7 (10.7)**	**11.7 (9.4–14.4)**
CL_{NSX}/F (liters/h/70 kg)	**0.758 (5)**	**0.756 (0.690–0.838)**
Random parameters [CV% (% RSE)]		
BSV V/F	23.0 (11)	22.2 (17.1–26.5)
BSV CL_T/F	23.8 (11)	23.4 (17.9–28.3)
BSV V_{NSX}/F	**42.8 (19)**	**41.7 (21.0–56.3)**
BSV CL_{NSX}/F	**36.2 (26)**	**35.6 (25.5–45.1)**
Correlations between BSV pairs		
R (V/F, CL_T/F)	0.644 (26)	0.653 (0.439–0.814)
R (V_{NSX}/F, CL_{NSX}/F)	**0.218 (126)**	**0.226 (−0.474 to 0.729)**
Residual unexplained variability [CV% (% RSE)]		
Proportional error, SDX	16.5 (11)	16.4 (12.9–20.1)
Proportional error, NSX	**37.1 (9)**	**37.0 (30.2–43.1)**

[a] Parameters for NSX modeling obtained after fixing model parameters for SDX are highlighted in bold. % RSE, percent relative standard error.

DISCUSSION

The present study is the first to investigate the pharmacokinetics of SDX/PYR in infants living in a setting in which malaria is endemic and in which IPTi is appropriate. It is also the first to investigate the possibility that a higher dose than conventionally recommended should be given to achieve therapeutic plasma concentrations in this age group, as has been recommended for children aged 2 to 5 years (11). SDX/PYR was well tolerated by all infants, and there was no evidence of hepatorenal or bone marrow toxicity even at the higher dose. The $AUC_{0-\infty}$ of both SDX and PYR was significantly higher in the double-dose group. However, there was a 32% reduction in the relative bioavailability of SDX when the dose was doubled, possibly due to saturation of absorption. The percentage of NSX to SDX exposure (AUC) was the same in both groups, suggesting that a double dose does not affect the metabolic clearance of SDX. The pharmacokinetic properties of PYR were not dose dependent in the present study.

The pharmacokinetic parameters for PYR observed in our children are different from those observed in other pediatric studies (11, 18, 31, 37, 40). We found a longer $t_{1/2\beta}$ (15.6 versus 2.67 to 4.46 days) and a higher conventional dose AUC (2,844 versus 1,052 to 2,607 μg/day/liter). This may reflect the fact that most of our children were well. In addition, we employed a relatively long duration of sampling that facilitated identification of biexponential elimination, a profile reported previously in studies of adults (26, 28, 38) but not children. While one pediatric study sampled out to 42 days, the drug could not be quantified in 40% of the samples (11). Although the mean

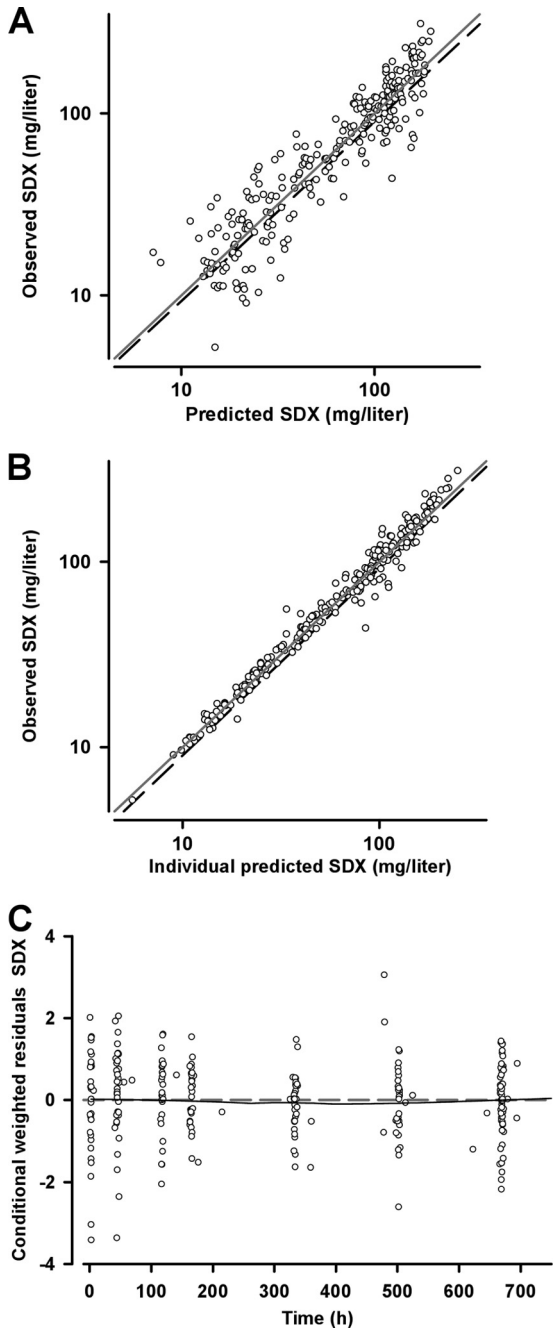

FIG. 3. Goodness-of-fit plots for SDX showing observed versus model predicted concentrations (A) and individual predicted concentrations (B) (both log scale) and conditional weighted residuals versus time (C). For panels A and B, the solid gray line represents the line of identity, while the dashed black line represents the linear regression line of best fit; in panel C, the solid gray line represents the LOESS smoothed fit.

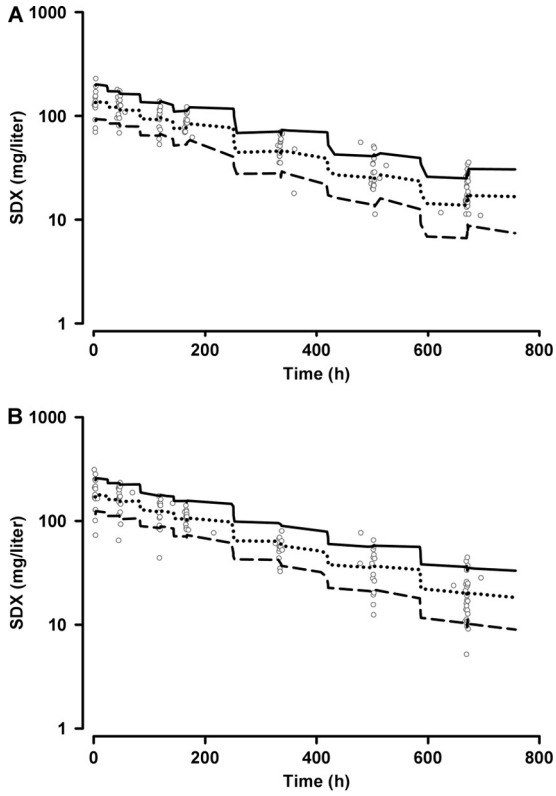

FIG. 4. Visual predicted check plots for SDX showing simulated 10th (short dashed line), 50th (dotted line), and 90th (solid line) percentile concentrations and observed concentration (log scale) data (gray open circles) versus time (log scale) for conventional dose (A) and double-dose (B) participants.

conventional dose PYR AUC in the present study was in the range of previously reported values in adults (1,602 to 3,166 μg/day/liter) (11, 16, 22, 28), the latter data may have been underestimates because of truncated sampling and/or use of a relatively insensitive assay. In a study of nonpregnant PNG women using a sampling profile, assay, and pharmacokinetic modeling techniques that were similar to those of the present study (26), the mean conventional dose PYR AUC (4,419 μg/day/liter) was similar to that in the present double-dose group. Together with the available tolerability and safety data from the present study, these considerations suggest that double-dose PYR is appropriate as part of SDX/PYR IPTi.

We found that SDX also had a longer mean elimination $t_{1/2}$ (9.1 versus 4.1 to 8.6 days) and a higher conventional dose mean AUC (1,460 versus 460 to 932 mg/day/liter) than those of children in other studies (11, 19, 32, 37, 40). However, the mean AUC was within the range found in adults (508 to 2,757 mg/day/liter) (11, 16, 22, 28), including nonpregnant women (1,386 mg/day/liter) from the same location as the present study (26). Although the difference in AUC compared to other pediatric populations may be explained, as with PYR, by our ability to detect drug concentrations for longer time postdose than in previous studies as well as by the relative health of our subjects, only a few studies have included infants aged <1 year, and these formed a minority of the patients recruited. As our sample includes only children <13 months of age, a limited maturation of elimination processes is likely to play a role in the longer $t_{1/2}$ and higher AUC observed for both drugs even in the conventional dose group. Indeed, we found evidence of a slower maturation of these processes for SDX than PYR.

In the present study, we used plasma CysC rather than creatinine to estimate GFR. The conventional Schwartz crea-

TABLE 6. *Post hoc* Bayesian predicted PK parameters for SDX and NSX in PNG infants given conventional and double dosing of SDX/PYR

Parameter	Result [median (IQR)] for study group		*P* value[a]
	Conventional dosing (n = 35)	Double dosing (n = 35)	
$CL_{T,SDX}/F$ (liters/h)	0.0068 (0.0057–0.0087)	0.0072 (0.0068–0.0105)	0.032
V_{SDX}/F (liters)	2.20 (1.95–2.53)	2.23 (1.97–2.64)	NS
$t_{1/2\ SDX}$ (h)	232 (203–252)	207 (179–232)	0.006
$AUC_{0-\infty\ SDX}$ (mg/day/liters)	1,460 (1,167–1,707)	2,434 (1,881–2,987)	<0.001
CL_{NSX}/F (liters/h)	0.081 (0.060–0.094)	0.101 (0.081–0.116)	0.012
V_{NSX}/F (liters)	1.14 (1.00–1.28)	1.17 (0.959–1.30)	NS
$t_{1/2\ NSX}$ (h)	10.3 (7.86–12.2)	8.69 (6.84–10.6)	0.027
$AUC_{0-\infty\ NSX}$ (mg/day/liter)	1,796 (1,397–2,154)	2,890 (2,482–3,609)	<0.001
$AUC_{0-\infty\ NSX}/AUC_{0-\infty\ SDX}$ (%)	5.0 (4.3–6.5)	5.0 (4.3–6.0)	NS

[a] Mann-Whitney test. NS, nonsignificant ($P > 0.05$).

tinine-based formula relies upon estimates of body composition (36), whereas CysC-based formulae do not (5), making the estimates more robust. We used the formula derived by Filler and Lepage (20), as it was derived from a large pediatric sample, and the same PETIA CysC assay used in the present study. CysC concentrations generated by other assays such as particle-enhanced immunonephelometry may differ from those from PETIA (5). The Filler and Lepage formula is comparable to others based on CysC derived from children (13, 14, 24, 41).

Since hepatic maturation would still be occurring within the age range of our subjects, it was appropriate to include this phenomenon in our model (1, 8, 12). We used a sigmoid E_{max} approach as this has been used previously with a number of other drugs (2, 4, 6, 8, 34) and our estimates of $MATCL_{50}$, namely, 315 and 271 days for PYR and SDX, respectively, fell in the range reported in these studies (270 to 380 days). The estimate of the Hill coefficient for SDX was also consistent (4.07 versus 2.78 to 4.6), but the Hill coefficient for PYR was higher than that previously reported (7.39). Although our study age range captured the process of maturation, most of our infants had clearances that were >90% of adult values and very few were <50% (Fig. 5). This limits our ability to characterize coefficients of maturation which are likely to be inappropriate outside this age range. For example, adult estimates of $t_{1/2}$ for SDX (333 h) and PYR ($t_{1/2\alpha}$, 113 h; $t_{1/2\beta}$, 647 h) based on the modeling presented here are higher than those previously reported (11, 16, 22, 26, 28, 38). Future studies of this type should include a larger range of ages so that the maturation process from birth to adult activity levels can be determined more accurately.

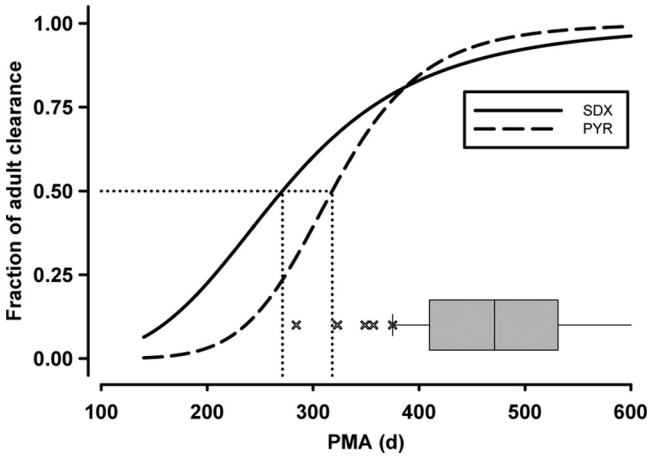

FIG. 5. Maturation as a fraction of adult clearance for PYR (dashed line) and SDX (solid line) predicted from the PK model plotted against PMA. A box plot of the PMA in the recruited subjects is included to show its distribution in relation to maturation of clearance. d, days.

Other studies have provided data relevant to the question of whether a higher SDX/PYR dose should be given to infants. A pharmacokinetic evaluation of SDX in children aged 6 months to 5 years with malaria found that those aged <24 months had a lower $AUC_{0\text{-}336\ h}$ than their older counterparts (12,500 versus 16,900 mg/h/liter) (32). However, all children <24 months of age received half the dose of older children regardless of body weight and no average dose by body weight was reported, thus complicating interpretation of the data. In a similar study (11), an age-stratified noncompartmental analysis of $AUC_{0-\infty}$ showed that 1- to 2-year-olds had sufficient drug exposure while children aged 2 to 5 years required a double dose. The study had only 11 children within the 1- to 2-year-old age range, and because only whole tablets were given, the mean dose in this group was almost twice that of ≥12-year-olds (50/2.5 versus 27.3/1.36 mg/kg). In a population-based pharmacokinetic analysis of SDX/PYR in children with congenital toxoplasmosis aged 1 week to 14 years (37), lighter-weight children had a shorter $t_{1/2}$ and therefore a lower drug exposure. This conclusion was based on the use of allometry, since age-based maturation contributed little to the model, perhaps because of the small numbers in the younger age groups. Interpreted within their limitations, these various studies also provide evidence that higher milligram/kilogram SDX/PYR doses are required in younger children, including those <1 year of age.

Relatively recent data from the study area indicate that amodiaquine-SDX/PYR treatment (until recently the recommended first-line antimalarial therapy for young PNG children) is associated with close to a 90% 28-day adequate clinical and parasitologic response for both falciparum (PCR-corrected) and vivax malaria (29). This is a suboptimal response but still suggests that either conventional or double-dose SDX/PYR treatment in the present study is likely to have contributed to the relatively small number of infections detected during follow-up. Although the present study was not designed to assess relative efficacy, especially since interpretation of emergent vivax infections remains problematic (10) and given that only one dose was administered rather than the several scheduled during IPTi, fewer children were treated for symptomatic malaria during follow-up or were

slide positive on day 28 in the double-dose group. Indeed, there is evidence from epidemiologic studies utilizing fixed-dose regimens (9, 27) that appropriate milligram/kilogram doses of SDX/PYR should be used in IPTi programs to ensure adequate levels of prevention, especially for symptomatic compared to asymptomatic falciparum malaria (30).

In light of this dose dependency, the fact that no study has shown >60% protective efficacy during the first year of life (9, 23), evidence that higher blood PYR concentrations facilitate parasite clearance in pediatric falciparum malaria (19), and the fact that double-dose SDX/PYR in our subjects was safe, well tolerated, and associated with higher exposure to both drug components (especially SDX), the present data argue for the potential use of double-dose SDX/PYR in infancy. As in recent adult studies of PYR disposition (26), we found that the mean elimination $t_{1/2}$ values of PYR and SDX were larger than previously reported, a factor that may contribute to the duration of effective prophylaxis. Although allometric considerations (shorter half-lives in smaller subjects) may justify higher SDX/PYR dosing in infants, we recommend that consideration must be given to the maturation of hepatorenal elimination processes and the possibility that increased doses may be inappropriate in very young infants.

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REFERENCES

1. **Alcorn, J., and P. J. McNamara.** 2002. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. Clin. Pharmacokinet. **41:**959–998.
2. **Allegaert, K., J. de Hoon, R. Verbesselt, G. Naulaers, and I. Murat.** 2007. Maturational pharmacokinetics of single intravenous bolus of propofol. Paediatr. Anaesth. **17:**1028–1034.
3. **Allen, S. J., A. Raiko, A. O'Donnell, N. D. Alexander, and J. B. Clegg.** 1998. Causes of preterm delivery and intrauterine growth retardation in a malaria endemic region of Papua New Guinea. Arch. Dis. Child. Fetal Neonatal Ed. **79:**F135–F140.
4. **Anand, K. J., et al.** 2008. Morphine pharmacokinetics and pharmacodynamics in preterm and term neonates: secondary results from the NEOPAIN trial. Br. J. Anaesth. **101:**680–689.
5. **Andersen, T. B., A. Eskild-Jensen, J. Frokiaer, and J. Brochner-Mortensen.** 2009. Measuring glomerular filtration rate in children; can cystatin C replace established methods? A review. Pediatr. Nephrol. **24:**929–941.
6. **Anderson, B. J., K. Allegaert, J. N. Van den Anker, V. Cossey, and N. H. Holford.** 2007. Vancomycin pharmacokinetics in preterm neonates and the prediction of adult clearance. Br. J. Clin. Pharmacol. **63:**75–84.
7. **Anderson, B. J., and N. H. Holford.** 2008. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu. Rev. Pharmacol. Toxicol. **48:**303–332.
8. **Anderson, B. J., and N. H. Holford.** 2009. Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab. Pharmacokinet. **24:**25–36.
9. **Aponte, J. J., et al.** 2009. Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. Lancet **374:**1533–1542.
10. **Baird, J. K.** 2008. Real-world therapies and the problem of vivax malaria. N. Engl. J. Med. **359:**2601–2603.
11. **Barnes, K. I., et al.** 2006. Sulfadoxine-pyrimethamine pharmacokinetics in malaria: pediatric dosing implications. Clin. Pharmacol. Ther. **80:**582–596.
12. **Bjorkman, S.** 2006. Prediction of cytochrome p450-mediated hepatic drug clearance in neonates, infants and children: how accurate are available scaling methods? Clin. Pharmacokinet. **45:**1–11.
13. **Bokenkamp, A., et al.** 1998. Cystatin C—a new marker of glomerular filtration rate in children independent of age and height. Pediatrics **101:**875–881.
14. **Bouvet, Y., et al.** 2006. GFR is better estimated by considering both serum cystatin C and creatinine levels. Pediatr. Nephrol. **21:**1299–1306.
15. **Brair, M. E., B. J. Brabin, P. Milligan, S. Maxwell, and C. A. Hart.** 1994. Reduced transfer of tetanus antibodies with placental malaria. Lancet **343:**208–209.
16. **Bustos, D. G., et al.** 2002. Pharmacokinetics of sequential and simultaneous treatment with the combination chloroquine and sulfadoxine-pyrimethamine in acute uncomplicated Plasmodium falciparum malaria in the Philippines. Trop. Med. Int. Health **7:**584–591.
17. **Cairns, M., et al.** 2008. Duration of protection against malaria and anaemia provided by intermittent preventive treatment in infants in Navrongo, Ghana. PLoS One **3:**e2227.
18. **Corvaisier, S., et al.** 2004. Population pharmacokinetics of pyrimethamine and sulfadoxine in children treated for congenital toxoplasmosis. Antimicrob. Agents Chemother. **48:**3794–3800.
19. **Dzinjalamala, F. K., et al.** 2005. Association between the pharmacokinetics and in vivo therapeutic efficacy of sulfadoxine-pyrimethamine in Malawian children. Antimicrob. Agents Chemother. **49:**3601–3606.
20. **Filler, G., and N. Lepage.** 2003. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? Pediatr. Nephrol. **18:**981–985.
21. **Garner, P., et al.** 1994. Birthweight and gestation of village deliveries in Papua New Guinea. J. Trop. Pediatr. **40:**37–40.
22. **Green, M. D., et al.** 2007. Pharmacokinetics of sulfadoxine-pyrimethamine in HIV-infected and uninfected pregnant women in Western Kenya. J. Infect. Dis. **196:**1403–1408.
23. **Grobusch, M. P., A. Egan, R. D. Gosling, and R. D. Newman.** 2007. Intermittent preventive therapy for malaria: progress and future directions. Curr. Opin. Infect. Dis. **20:**613–620.
24. **Grubb, A., et al.** 2005. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. Clin. Chem. **51:**1420–1431.
25. **Hekster, C. A., and T. B. Vree.** 1982. Clinical pharmacokinetics of sulphonamides and their N4-acetyl derivatives. Antibiot. Chemother. **31:**22–118.
26. **Karunajeewa, H. A., et al.** 2009. Pharmacokinetic properties of sulfadoxine-pyrimethamine in pregnant women. Antimicrob. Agents Chemother. **53:**4368–4376.
27. **Kobbe, R., et al.** 2007. A randomized controlled trial of extended intermittent preventive antimalarial treatment in infants. Clin. Infect. Dis. **45:**16–25.
28. **Mansor, S. M., et al.** 1989. Single dose kinetic study of the triple combination mefloquine/sulphadoxine/pyrimethamine (Fansimef) in healthy male volunteers. Br. J. Clin. Pharmacol. **27:**381–386.
29. **Marfurt, J., et al.** 2007. Low efficacy of amodiaquine or chloroquine plus sulfadoxine-pyrimethamine against Plasmodium falciparum and P. vivax malaria in Papua New Guinea. Am. J. Trop. Med. Hyg. **77:**947–954.
30. **May, J., et al.** 2008. Therapeutic and prophylactic effect of intermittent preventive anti-malarial treatment in infants (IPTi) from Ghana and Gabon. Malar. J. **7:**198.
31. **McLeod, R., et al.** 1992. Levels of pyrimethamine in sera and cerebrospinal and ventricular fluids from infants treated for congenital toxoplasmosis. Toxoplasmosis Study Group. Antimicrob. Agents Chemother. **36:**1040–1048.
32. **Obua, C., et al.** 2008. Population pharmacokinetics of chloroquine and sulfadoxine and treatment response in children with malaria: suggestions for an improved dose regimen. Br. J. Clin. Pharmacol. **65:**493–501.
33. **Papua New Guinea Department of Health.** 2005. Standard treatment of common illnesses of children in Papua New Guinea, 8th ed. Papua New Guinea Department of Health, Port Moresby, Papua New Guinea.
34. **Potts, A. L., G. R. Warman, and B. J. Anderson.** 2008. Dexmedetomidine disposition in children: a population analysis. Paediatr. Anaesth. **18:**722–730.
35. **Roche.** 2005. Fansidar product information. Roche Products Pty. Ltd., Dee Why, Australia.
36. **Schwartz, G. J., L. P. Brion, and A. Spitzer.** 1987. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr. Clin. N. Am. **34:**571–590.
37. **Trenque, T., et al.** 2004. Population pharmacokinetics of pyrimethamine and sulfadoxine in children with congenital toxoplasmosis. Br. J. Clin. Pharmacol. **57:**735–741.
38. **Weidekamm, E., H. Plozza-Nottebrock, I. Forgo, and U. C. Dubach.** 1982. Plasma concentrations in pyrimethamine and sulfadoxine and evaluation of pharmacokinetic data by computerized curve fitting. Bull. World Health Organ. **60:**115–122.
39. **Whelpton, R., G. Watkins, and S. H. Curry.** 1981. Bratton-Marshall and liquid-chromatographic methods compared for determination of sulfamethazine acetylator status. Clin. Chem. **27:**1911–1914.
40. **Winstanley, P. A., et al.** 1992. The disposition of oral and intramuscular pyrimethamine/sulphadoxine in Kenyan children with high parasitaemia but clinically non-severe falciparum malaria. Br. J. Clin. Pharmacol. **33:**143–148.
41. **Zappitelli, M., et al.** 2006. Derivation and validation of cystatin C-based prediction equations for GFR in children. Am. J. Kidney Dis. **48:**221–230.