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Pharmacokinetic Predictors for Recurrent Malaria After Dihydroartemisinin-Piperaquine Treatment of Uncomplicated Malaria in Ugandan Infants

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Background. Although dihydroartemisinin-piperaquine (DP) is used primarily in children, pharmacokinetic/pharmacodynamic (PK/PD) data on DP use in young children are lacking.

Methods. We conducted a prospective PK/PD study of piperaquine in 107 young children in Uganda. Samples were collected up to 28 days after 218 episodes of malaria treatment, which occurred during follow-up periods of up to 5 months. Malaria follow-up was conducted actively to day 28 and passively to day 63.

Results. The median capillary piperaquine concentration on day 7 after treatment was 41.9 ng/mL. Low piperaquine concentrations were associated with an increased risk of recurrent malaria for up to 42 days, primarily in those receiving trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis. In children not receiving TMP-SMX, low piperaquine concentrations were only modestly associated with an increased risk of recurrent malaria. However, for children receiving TMP-SMX, associations were strong and evident for all sampling days, with PQ concentrations of ≤27.3 ng/mL on day 7 associated with a greatly increased risk of recurrent malaria. Notably, of 132 cases of recurrent malaria, 119 had detectable piperaquine concentrations at the time of presentation with recurrent malaria.

Conclusions. These piperaquine PK/PD data represent the first in children <2 years of age. Piperaquine exposure on day 7 correlated with an increased risk of recurrent malaria after DP treatment in children receiving TMP-SMX prophylaxis. Interestingly, despite strong associations, infants remained at risk for malaria, even if they had residual levels of piperaquine.

Keywords. malaria; pharmacokinetics; piperaquine; artemisinin combination therapy; antimalarial.

The burden of malaria is highest in sub-Saharan Africa, with individuals <5 years of age experiencing the greatest morbidity and mortality from the disease [1]. Artemisinin-based combination treatments (ACTs) are the currently recommended first-line treatments for uncomplicated *Plasmodium falciparum* malaria worldwide. Among the most promising newer ACT options is dihydroartemisinin-piperaquine (DP), which offers the advantage of convenient once-daily dosing, compared with twice-daily dosing for artemether-lumefantrine, the most widely adopted ACT [2–6]. The World Health Organization (WHO) recently added DP to the list of recommended ACT regimens for the treatment of uncomplicated malaria [7].

DP combines a highly active artemisinin-based antimalarial, dihydroartemisinin, with a long-acting quinoline-based antimalarial, piperaquine. Dihydroartemisinin exhibits rapid and potent activity against all

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erythrocytic stages of multidrug-resistant parasites, but the duration of in vivo efficacy is limited by its extremely short half-life (<1 hour) [8, 9]. The longer-acting piperaquine is believed to act primarily on residual parasites that persist following cessation of the 3-day treatment course [10, 11]. The prolonged terminal half-life of piperaquine has been estimated previously to be 2–4 weeks [12, 13], providing a significant period of post-treatment exposure and potential prophylaxis against new infections [2, 14–16]. Any benefit of the slow elimination of piperaquine following rapid elimination of artemisinin must be weighed against the risk for the selection for newly introduced drug-resistant strains [9, 11].

The pharmacokinetics and pharmacodynamics of DP in its current 3-dose regimen have only been investigated recently. Piperaquine exhibits complex multiphasic pharmacokinetics, and fat has a variable influence on its absorption [17–21]. Moreover, children are prone to reduced exposure presumably because of differences in metabolic maturation, with some studies reporting lower piperaquine exposure in children aged 2–5 years, compared with children aged 6–10 years and adults, and suggesting dosage adjustment for children [2, 9, 12, 22, 23]. To optimize the clinical use of DP, it is essential to understand the disposition of the drug in the most vulnerable populations and to relate exposure to clinical outcomes [23].

Pharmacodynamic studies carried out previously have focused on evaluating the predictive value of single drug levels, obtained on day 7, with treatment outcomes. The concentration on day 7 serves as a surrogate for the area under the plasma concentration versus time curve (AUC), as has been reported for drugs such as amodiaquine, lumefantrine, and sulfadoxine-pyrimethamine [10, 11, 24, 25]. Treatment outcomes after DP are also associated with the level of piperaquine exposure on day 7 [22, 26, 27]. However, there are no studies in children <2 years old and limited studies of children in Africa, where the burden of disease is greatest [22]. Furthermore, potential pharmacokinetic and pharmacodynamic interactions are largely unexplored, and trimethoprim-sulfamethoxazole (TMP-SMX), a drug with antimalarial properties, is routinely given to children born to human immunodeficiency virus (HIV)–infected mothers to prevent opportunistic infections prior to the cessation of breastfeeding and determination of HIV infection status [28].

The primary aim of this analysis was to define the pharmacokinetic and pharmacodynamic properties of piperaquine, when administered in combination with dihydroartemisinin for treatment of uncomplicated *P. falciparum* malaria, among infants in a Ugandan setting where malaria transmission is high and reinfections are common [28]. This report establishes the relationship between piperaquine levels and recurrent malaria over extended periods of clinical follow-up, in the presence or absence of TMP-SMX prophylaxis.

METHODS

Study Area and Enrollment

Participants in this pharmacokinetic/pharmacodynamic study were part of a larger clinical trial comparing the efficacy of artemether-lumefantrine, the first-line treatment for malaria in Uganda, to DP in very young children [15]. The study took place in Tororo, Uganda, an area with a high intensity of malaria transmission. The entomological inoculation rate has been estimated at 562 infective bites/person per year in this area [29]. The main study cohort was enrolled beginning in August 2007. Convenience sampling was used for infants presenting to antenatal clinics for routine care. Eligibility criteria included the following: (1) the child resided within 30 km of the study site, (2) the child and mother had a documented HIV infection status, (3) the child was currently breastfeeding if HIV exposed, and (4) the child's parent(s) or guardian(s) agreed to bring the child to the clinic for any illness and to avoid giving the child medications received outside of the study clinic. All participants were given an insecticide-treated bed net at enrollment. Daily TMP-SMX prophylaxis was given to HIV-infected participants for the duration of the study and to HIV-exposed participants until completion of breastfeeding. After breastfeeding, HIV-exposed children who remained HIV uninfected were randomized to continue TMP-SMX until 2 years of age or to discontinue prophylaxis. HIV-infected participants were provided antiretroviral therapy (ART) according to national guidelines. The ART regimen was triple therapy with nevirapine, lamivudine, and either stavudine or zidovudine. Supplementary Figure 1 depicts the breakdown of TMP-SMX use, HIV infection status, and ART use among the 107 participants in the study.

The study population for pharmacokinetic/pharmacodynamic analyses consisted of children within the cohort study, aged 6–24 months, who were randomized to receive DP for each episode of uncomplicated malaria occurring during study follow-up (Supplementary Figure 1). Enrollment in the pharmacokinetic study began on 5 June 2008 and continued until 24 October 2008. All parents or guardians provided informed consent. Ethical approval was obtained from the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, the University of California–San Francisco Committee on Human Research, and the Centers for Disease Control and Prevention Global AIDS Program.

Diagnosis and Treatment of Malaria

Uncomplicated falciparum malaria was diagnosed in patients with both a thick blood smear positive for malaria parasites (regardless of parasite density) and either a documented fever (tympanic temperature, ≥38.0°C) or history of fever in the

previous 24 hours, and the diagnosis excluded those with symptoms indicative of complicated malaria [15]. Active follow-up was performed by study clinicians on days 0 (diagnosis), 1, 2, 3, 7, 14, 21, and 28, and passive follow-up, in which patients only presented to the clinic if they were sick, was performed up to day 63 to monitor for treatment outcome and adverse events. Treatment outcomes were assessed using standardized polymerase chain reaction genotyping methods to confirm parasite species and determine recrudescent cases [30]. For this study, recurrent malaria was defined as parasitemia and a documented fever or a history of fever in the previous 24 hours, occurring within 42 or 63 days after treatment, regardless of parasite genotype.

DP (Duo-Cotecxin, Holley-Cotec Pharmaceuticals) was administered in 3 daily doses, according to body weight at the time of diagnosis, with total dose targets of 6.4 and 51.2 mg/kg of dihydroartemisinin and piperaquine, respectively [15]. DP was administered as 20 and 160 mg of dihydroartemisinin and piperaquine, respectively, per dose for subjects weighing 5.1–10.4 kg and as 30 and 240 mg, respectively, per dose for subjects weighing 10.5–14.5 kg. All 3 doses were administered by study nurses as crushed tablets dispersed in approximately 5 mL of water and were followed by 150 mL of reconstituted cow's milk (Nido, Nestle), which contained approximately 5 g of fat, to ensure optimal and consistent absorption of piperaquine [21]. Parents who were breastfeeding were asked to feed their child after dosing. Patients were monitored for 1 hour to ensure that doses were tolerated. Doses vomited within 30 minutes of ingestion were recorded, and the full dose was readministered. Caretakers were questioned regularly to ensure that no medical treatment was undertaken outside of the study clinic. No additional medications with antimalarial activity or known hepatic enzyme inhibitors or inducers were administered to patients during the study, with the exception of daily TMP-SMX prophylaxis and ART.

Sample Collection and Analysis

Capillary plasma samples for pharmacokinetic analysis were taken on days 0 (before the first dose), 3 (24 hours after the third dose), 7, 14, 21, and 28 (Figure 1). Sampling on days 0 and 28 began in July and August 2008, respectively. For all episodes of uncomplicated malaria occurring from the time of enrollment until October 2008, patients underwent sampling for pharmacokinetic analysis. After finger-stick specimen collection, 125–200 μL of whole capillary blood was collected into heparinized microtubes and centrifuged at $2000 \times g$ for 10 minutes, and plasma was transferred to cryovials. Plasma was stored in liquid nitrogen or at −80°C for a maximum of 12 months and shipped on dry ice to the Mahidol Oxford Clinical Research Unit for analysis by liquid chromatography–tandem mass spectrometry, as described previously [31]. This method provided a limit of detection of 0.375 ng/mL, with a lower limit of quantification set at 1.50 ng/mL.

Statistical Analysis

The risk of recurrent malaria was estimated using the Kaplan-Meier product limit formula, with censoring for patients with incomplete follow-up. Associations were explored between quartiles of piperaquine concentrations on each day samples were obtained for pharmacokinetic analysis and the risk of recurrent malaria by days 42 and 63 of follow-up. After exploration, cutoffs for piperaquine level were identified by breaking

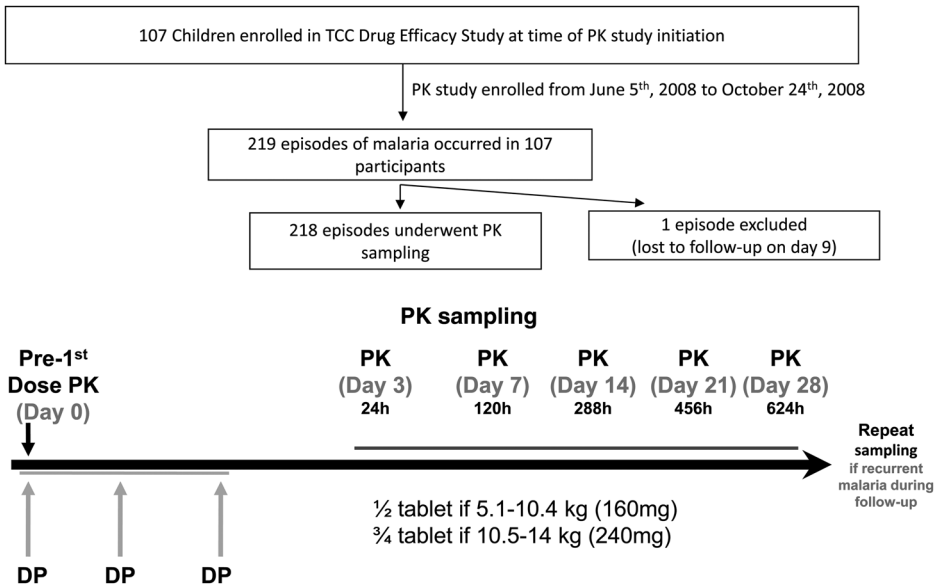

Figure 1. Trial profile and pharmacokinetic (PK) sampling scheme. Abbreviation: TCC, Tororo Child Cohort.

piperaquine levels into deciles. Measures of association between piperaquine level deciles and the risk of recurrent parasitemia were assessed using Cox proportional hazards models stratified by TMP-SMX use and controlled for age and place of residence (urban vs rural), with inference adjusted for repeated measures in the same patient. Optimal cutoffs were chosen on the basis of strength of statistical association [32]. Statistical analysis was performed using Stata, version 11.2 (Stata, College Station, TX). A 2-sided *P* value of < .05 was considered statistically significant.

RESULTS

Study Profile

Demographic characteristics of all subjects with episodes of uncomplicated malaria included in this DP pharmacokinetic study are provided in Table 1. Thirty-seven percent of the 107 children were receiving daily TMP-SMX prophylaxis, and 11% (12) were HIV infected (10 were receiving ART at the time of sampling for pharmacokinetic analysis). A total of 219 episodes of uncomplicated malaria were treated with DP. Sampling for pharmacokinetic analysis occurred during 218 episodes, with 1 child lost to follow-up. There was a median of 2 treatments/person (range, 1–4 treatments/person) during follow-up (Figure 1 and Supplementary Figure 1).

Table 1. Baseline Characteristics at Time of Malaria Diagnosis in Children Enrolled in the Tororo Child Cohort Study in Tororo District, Uganda, Between June and October 2008

Variable	Value
Episodes, no.	218
Children, no.	107
PK samples obtained, no.	1314
Malaria episodes/child, no., median (range)	2 (1–4)
Episodes in male children, %	62
Body weight, kg, median (range)	9.0 (5.1–12.5)
PQ dose/treatment course, mg/kg, median (range)	57.1 (46.2–94.1)
Age	
Overall, mo, median (range)	15.2 (6.8–22.8)
6 to <12 mo, no.	41
12–18 mo, no.	120
>18 to 24 mo, no.	57
Hemoglobin level <10 g/dL, %	45
Parasite density, parasites/μL, geometric mean (95% CI)	17 280 (13 371–22 332)
Episodes, by subject characteristic, %	
Breastfeeding	44
Urban residence	13
HIV infection	10
TMP-SMX prophylaxis	35
Recurrent malaria, by time point, % (no.)	
28 d	2 (5)
42 d	22 (48)
63 d	59 (129)
63 d due to recrudescence	4 (8)

Data are no. or % of children, unless otherwise indicated.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; PK, pharmacokinetic; PQ, piperaquine.

Piperaquine Levels

A total of 1096 capillary plasma samples were obtained for pharmacokinetic analysis, >99% of which were evaluable. Piperaquine was detected in all samples from days 3–28, with a median concentration of 41.9 ng/mL on day 7 and levels of 3.1–117 ng/mL on day 28 (Table 2). Supplementary Figure 1 displays the long terminal half-life of piperaquine. Because of the potential for drug-drug interactions between ART, TMP-SMX, and ACT regimens, the association between piperaquine levels and ART use was explored [33, 34]. Piperaquine levels were not associated with ART status or TMP-SMX use. Specifically, median levels on day 7 in those receiving TMP-SMX versus those not receiving TMP-SMX (39.3 vs 42.2 ng/mL) were not significantly different. In addition, differences were not seen in levels on day 7 in subjects receiving ART versus those not receiving ART (43.7 vs 41.6 ng/mL), although this comparison is limited because of the small numbers of individuals receiving ART in this study.

Pharmacodynamics of Piperaquine

A total of 129 of 218 treatments (59%) were followed by recurrent malaria within 63 days. Genotyping was successful in 128 of these episodes, revealing that only 6% of recurrent malaria episodes (8 of 128) were due to recrudescence and not to new infections.

Table 2. Capillary Plasma Levels of Piperaquine (PQ) on Each Measurement Day for Children Who Received Dihydroartemisinin-PQ for Uncomplicated Malaria

Sample	Time Since Last Dose, h	Samples, no.	PQ Concentration, ng/mL, median (IQR)
Day 0[a]	Before first dose	142	8.9 (2.9, 15)
Day 3	24	214	121 (86.7, 163)
Day 7	120	208	41.9 (30.2, 56.6)
Day 14	288	218	24.9 (17.4, 36)
Day 21	456	196	19.5 (12.6, 25.8)
Day 28[a]	624	118	14.5 (10.5, 19.6)

The dihydroartemisinin dose was 6.4 mg/kg, and the PQ dose was 51.2 mg/kg.

Abbreviation: IQR, interquartile range.

[a] Fewer samples were taken prior to the first dose and at day 28 because sampling at these time points began later in the course of the pharmacokinetic study.

Table 3. Relative Risk (RR) of Recurrent Malaria on Days 42 and 63, by Trimethoprim-Sulfamethoxazole (TMP-SMX) Use and Piperaquine (PQ) Capillary Plasma Levels at Specific Time Points

TMP-SMX Status, Time Point	Subjects, no.	PQ Level			HR[a] (95% CI)	*P*
		Cutoff, ng/mL	Below Cutoff, Subjects, % (no.)	Above Cutoff, Subjects, % (no.)		
No TMP-SMX use			RR on Day 42			
Day 7	136	≤56.4	29.4 (102)	23.5 (34)	0.83 (.36–1.94)	.67
Day 14	142	≤36.0	31.1 (106)	19.4 (36)	0.64 (.34–1.21)	.17
Day 21	127	≤25.8	34.0 (94)	12.1 (33)	0.32 (.11–.87)	.03
Day 28[b]	80	≤24.1	43.6 (62)	5.6 (18)	0.11 (.01–.74)	.02
			RR on Day 63			
Day 7	136	≤56.4	65.7 (102)	68.3 (34)	1.05 (.68–1.62)	.82
Day 14	142	≤36.0	66.0 (106)	67.4 (36)	1.01 (.67–1.52)	.95
Day 21	127	≤25.8	62.8 (94)	69.7 (33)	0.98 (.64–1.49)	.92
Day 28[b]	80	≤24.1	69.3 (62)	69.4 (18)	0.73 (.43–1.21)	.22
TMP-SMX use			RR on Day 42			
Day 7	72	≤27.3	31.3 (16)	5.4 (56)	0.13 (.03–.52)	.004
Day 14	76	≤16.0	35.7 (14)	5.9 (62)	0.10 (.03–.27)	<.001
Day 21	69	≤14.0	23.8 (21)	6.3 (48)	0.23 (.05–1.09)	.06
Day 28[b]	38	≤14.5	17.4 (23)	0 (15)	NA	
			RR on Day 63			
Day 7	72	≤27.3	72.5 (16)	39.3 (56)	0.28 (.13–.63)	.002
Day 14	76	≤16.0	57.9 (14)	43.9 (62)	0.43 (.23–.81)	.01
Day 21	69	≤14.0	54.3 (21)	45.8 (48)	0.58 (.26–1.30)	.19
Day 28[b]	38	≤14.5	52.2 (23)	20.0 (15)	0.28 (.08–.99)	.05

Abbreviations: CI, confidence interval; HR, hazard ratio; NA, not applicable.

[a] Controlled for age, household location, and repeated measures.

[b] Variability in capillary PK levels at later time points, combined with our smaller sample sizes, may affect association analyses at day 28 [40].

Examination of the association between the piperaquine concentration on specified days and the cumulative risk of recurrent malaria by days 42 and 63 was stratified by TMP-SMX use, as TMP-SMX was found to be an effect modifier. Levels on days 7, 14, 21, and 28 were divided into quartiles, revealing differences in the association between piperaquine levels and the risk of malaria between children receiving TMP-SMX and those not receiving TMP-SMX prophylaxis (Supplementary Table 1). In children who were not receiving TMP-SMX, levels in the highest quartiles on days 21 and 28 were associated with differences in the risk of malaria at 42 days but did not extend to outcomes at 63 days. However, in children receiving TMP-SMX prophylaxis, differences in the risk of recurrent malaria at 42 days were most apparent at the lowest quartiles of piperaquine, with trends extending to day 63.

To more precisely characterize the statistical associations between piperaquine levels and the risk of recurrent malaria, univariate and multivariate Cox proportional hazards analyses were performed after breaking piperaquine levels into deciles to define the optimal cutoffs on the basis of the strength of statistical associations (Table 3). The final multivariate model controlled for age, household location, and repeated measures in the same individual, with results stratified by TMP-SMX use. In children who were not receiving TMP-SMX, the associations between piperaquine levels and the risk of recurrent malaria were not significant until day 21 and only extended to day 42 outcomes. In particular, levels of <26 and <24 ng/mL on days 21 and 28, respectively, were associated with an increased risk of malaria by day 42.

In children receiving TMP-SMX, associations between piperaquine levels and the risk of malaria were much stronger and remained significant for longer periods of follow-up. In children receiving TMP-SMX, a level of 27.3 ng/mL on day 7 was most predictive and showed the strongest statistical association with the cumulative risk of recurrent malaria during the 63 days of follow-up (Figure 2). In addition, "threshold" piperaquine concentrations most predictive for the risk of malaria were notably lower in participants receiving TMP-SMX (Table 3).

Residual Piperaquine Levels at the Time of Reinfection

A total of 142 samples were collected on day 0, before the first dose. Ten of these samples were collected from participants who had not received DP for malaria since enrolling into the

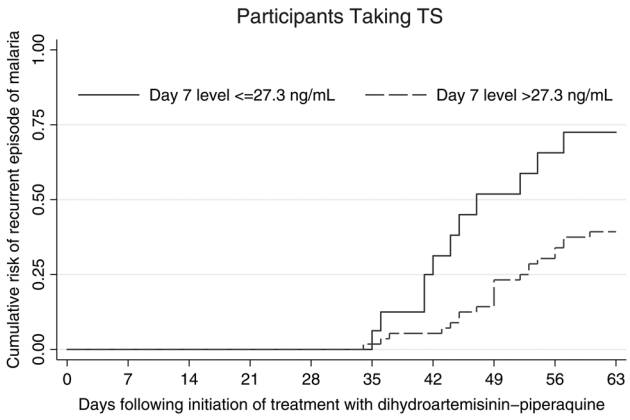

Figure 2. Cumulative risk of recurrent malaria at 63 days. The graph shows the risk of recurrence in participants receiving trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis, based on an optimal cutoff of 27.3 ng/mL at day 7.

drug efficacy study in 2007. Correspondingly, piperaquine was not detectable in these samples. For the remaining 132 samples, all participants had received a course of DP in the past for an episode of malaria. In 13 of these samples, piperaquine was undetectable on day 0. In those instances, the prior episode of malaria occurred a median of 124 days (range, 84–213 days) before the day 0 sample was collected. In the remaining 119 samples from day 0, piperaquine was detectable in the capillary plasma at the time of presentation (median level, 10 ng/mL; range, 0.5–44.1 ng/mL). In these cases, the prior episode of malaria occurred a median of 49 days earlier (range, 28–126 days; Figure 3). No difference in residual concentrations of piperaquine was observed between children receiving TMP-SMX and those not receiving TMP-SMX.

DISCUSSION

We report the first pharmacokinetic and pharmacodynamic data for piperaquine in children <2 years of age treated for malaria with DP. Children were followed for up to 5 months and underwent repeat sampling for all consecutive episodes of malaria that occurred during follow-up. Our data reveal that children <2 years of age have lower capillary levels of piperaquine on day 7, compared with previously evaluated older children (Table 4). This adds support to the findings that the disposition of piperaquine in children is altered and that drug exposure is reduced by a higher body weight–normalized rate of clearance [22]. Importantly, piperaquine exposure after treatment in these young children predicts their risk for recurrent malaria, with the magnitude of these associations influenced by the use of TMP-SMX prophylaxis. Since recurrent malaria after treatment was almost exclusively due to new infections, this study provides a detailed pharmacokinetic/pharmacodynamic

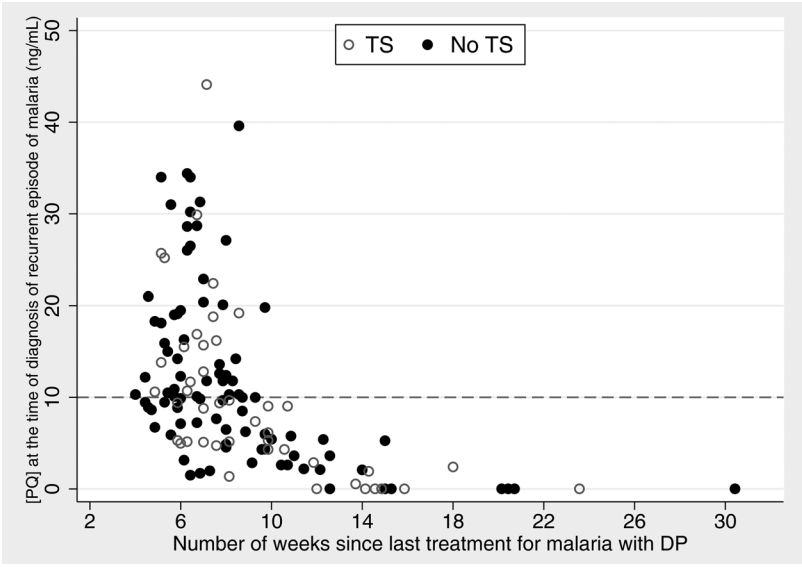

Figure 3. Scatterplot of levels of piperaquine in capillary plasma found at the time of diagnosis of recurrent malaria in patients with a previous episode of malaria who were treated with dihydroartemisinin-piperaquine (DP) during the study. A total of 132 episodes in the study had a prior episode of malaria treated with DP. In 13 of 132 episodes, piperaquine was undetectable, and the range of time since the prior episode of malaria was 84 to 213 days. In the remaining 119 episodes, piperaquine levels were still detectable (3 samples were below the lower limit of quantification), with a range of time since the prior episode of malaria of 28 to 126 days. The dotted line represents the median piperaquine level (10 ng/mL) in participants with a detectable level at the time of recurrent malaria. Closed circles represent episodes in children not receiving trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis, and open circles represent levels in children receiving TMP-SMX prophylaxis.

Table 4. Comparison of Published Day 7 Piperaquine (PQ) Levels

Study Location, Age	Day 7 Level, ng/mL		Reference
	Capillary	Venous	
Uganda			
6–23 mo	41.9 (median)	13.8 (median)[a]	Current study
Burkina Faso[b]			
2–5 y	58.7 (median)	36.6 (median)	[22]
5–10 y	67.5 (median)	44.1 (median)	
Papua New Guinea			
5–10 y	. . .	41.8 (median)[c]	[38]
Papua, Indonesia			
5–14 y	. . .	37.1 (mean)	[27]
15–60 y	. . .	50.4 (mean)	
Vietnam			
17–55 y	. . .	37–118 (range)[d]	[9]

All studies used a similar 3-dose regimen of dihydroartemisinin-PQ for the treatment of uncomplicated falciparum malaria.

[a] Converted from capillary plasma measurements, using published data comparing levels from these different sites [22]. Note that venous and capillary levels have been found to have a time dependant correlation [40].

[b] Venous and capillary measurements were taken simultaneously.

[c] Converted from a reported value of 78 nM.

[d] Median and/or mean data were not reported, but all patients had levels of >30 ng/mL.

analysis of the posttreatment prophylaxis of DP in a high-transmission setting.

Proper dosing of antimalarials in children is critical to ensure the efficacy and longevity of ACT regimens. For children, dosing has been largely deduced from adult data adjusted for body weight, and, as such, guidelines have largely ignored the impact of developmental changes on drug disposition [35, 36]. The relevance of developmental changes to antimalarial dosing can be seen in the case of sulfadoxine-pyrimethamine (SP) therapy. A pivotal study revealed that weight-based dosing in children aged 2–5 years led to significantly lower SP exposure, compared with that in adults and, importantly, that low SP levels were correlated with treatment failure [25]. Mounting evidence indicates that the problem associated with weight-based SP dosing is not an isolated example of inadequate dosing of antimalarials. The lumefantrine AUC was approximately 50% lower in children, compared with healthy adults [24, 33]. Piperaquine concentrations on day 7 were lower in children aged 2–5 years, compared with children aged 6–10 years, despite receipt of a higher body weight–normalized dose by the younger subjects, and these levels were associated with the risk of recurrent malaria [22]. In addition, a recent multicenter analysis at the individual patient level of the efficacy of DP revealed that children aged <5 years were at higher risk of recurrent malaria after treatment for both *P. falciparum* and *Plasmodium vivax* infection [37].

We aimed to extend these findings by assessing the relationship between piperaquine levels and recurrent malaria in young children, an age group at the highest risk for malaria. In our study, the median capillary level of piperaquine on day 7 was 29% and 38% lower than that in children aged 2–5 years and children aged 6–10 years, respectively, in Burkina Faso [22]. Prior studies that used venous plasma samples support these findings (Table 4) [2, 9, 27, 38, 39]. Venous plasma levels on day 7 in an adult population receiving similar DP dosing reported a mean piperaquine level of 50.4 ng/mL [27]. Notably, capillary plasma levels of piperaquine have been found to be higher than corresponding venous plasma levels [40]. Using a recently described relationship to convert capillary and venous piperaquine levels [22], we calculated that our median capillary level of 41.9 ng/mL on day 7 corresponds to a venous level of 13.8 ng/mL, which is 27%–37% of the mean venous levels reported for older individuals in the study by Price et al [27] (Table 4). In the same study, a venous level cutoff of 30 ng/mL on day 7 was found to be the best predictor of recurrent malaria. Levels in 75% of our children (157 of 208) were found to be below this "threshold" level on day 7, compared with 30%–43% of children aged 2–10 years in Burkina Faso [22].

This pharmacokinetic study was conducted in the context of a larger study that demonstrated the efficacy of TMP-SMX prophylaxis in the prevention of malaria [15, 28]. While our results showed significant associations independent of TMP-SMX use (data not shown), critical differences were seen in analyses stratifying for TMP-SMX use. Children who were not receiving TMP-SMX prophylaxis accounted for 65% of malaria episodes. In these children, only levels measured on days 21 and 28 were associated with the risk of malaria at 42 days, whereas for children receiving TMP-SMX, strong associations were observed for levels measured on days 7–28. Importantly, the threshold piperaquine concentration most predictive of risk of reinfection was approximately 50% lower for children receiving TMP-SMX (approximately 14 ng/mL) as compared to those not receiving TMP-SMX (approximately 25 ng/mL; Table 3). One can interpret these findings to suggest that TMP-SMX provided additional posttreatment prophylaxis for malaria, such that the risk of recurrence did not increase significantly until piperaquine levels were quite low. In addition, associations in subjects receiving TMP-SMX were maintained to day 63, likely because of the continued prophylaxis afforded by daily TMP-SMX throughout the follow-up period. In comparison, in subjects who were not receiving TMP-SMX prophylaxis, comparatively modest decreases in piperaquine levels were associated with an increased risk of recurrent malaria.

Our longitudinal follow-up of participants, sampled for up to 4 episodes of malaria, revealed an additional important concern in the use of DP for the treatment of malaria: 90% of

sampled children (119 of 132) who had received DP for a prior episode of malaria had detectable levels of piperaquine at the time of recurrence, occasionally remaining detectable out to 4 months. These levels were not insignificant, with 60% of levels (70 of 119) measured before receipt of the first dose exceeding the minimum levels seen on day 7 in this cohort. Thus, although piperaquine levels in these very young children are associated with the risk of recurrent malaria after treatment with DP, many children became symptomatic in the context of lingering piperaquine levels of up to 44.1 ng/mL (Figure 3).

While treatment with DP is effective at nearly eliminating the 28-day risk of malaria, piperaquine exposure can linger at potentially ineffective concentrations for up to 4 months. In our high-transmission setting, multilocus genotyping revealed an average complexity of infection of 3–4 different clones at the time of presentation and recurrence of malaria [41]. In such settings, piperaquine-tolerant parasites may preferentially outcompete strains that are more susceptible to low piperaquine levels, potentially promoting the spread of drug resistance. These findings need also be considered in studies evaluating the use of DP for intermittent preventive treatment. In addition, while recrudescences were rare in our study (8 of 132 recurrent infections), all 5 participants from whom samples collected before the first dose were obtained at the time of recrudescence had detectable levels of piperaquine on day 0 (range, 9.4–26.5 ng/mL), suggesting some degree of piperaquine "tolerance."

The link between the underdosing of SP in children and SP resistance has provided the malaria research community with a stark example of the potential implications of inappropriate dosing of antimalarials in young children. Recent articles call for refined dosing of DP in older children to reduce the risk for loss of this effective ACT [22, 25]. With the worldwide adoption of ACTs as first-line treatments for malaria, it is imperative that we conduct comparative pharmacokinetic/pharmacodynamic dosing studies in young children early in the course of drug deployment. Our longitudinal data point to a concerning finding that, because of the considerable "pharmacokinetic mismatch" between the short-acting dihydroartemisinin and long-acting piperaquine, the long unprotected piperaquine exposure after treatment raises the potential for selection of piperaquine-resistant strains upon exposure to new strains in high-transmission settings. However, one could argue that the risk of selection for resistant parasites is outweighed by the risks of undertreatment and the selection pressure on de novo resistance that are encountered through systematic underdosing of DP in young children [11].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflict of interest. All authors: No reported conflicts.

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