Open Forum Infectious Diseases

MAJOR ARTICLE

Parasite Clearance and Artemether Pharmacokinetics Parameters Over the Course of Artemether-Lumefantrine Treatment for Malaria in Human Immunodeficiency Virus (HIV)-Infected and HIV-Uninfected Ugandan Children

Richard Kajubi,[1,a] Liusheng Huang,[2,a] Moses Were,[1] Sylvia Kiconco,[2] Fangyong Li,[3] Florence Marzan,[2] David Gingrich,[2] Myaing M. Nyunt,[4] Joshua Ssebuliba,[1] Norah Mwebaza,[1] Francesca T. Aweeka,[2] and Sunil Parikh,[3]

[1]Infectious Disease Research Collaboration, Kampala, Uganda; [2]University of California-San Francisco, San Francisco General Hospital; [3]Yale University School of Public Health and Medicine, New Haven, Connecticut; [4]Institute for Global Health, University of Maryland Baltimore School of Medicine

Background. Artemisinins are primarily responsible for initial parasite clearance. Antimalarial pharmacokinetics (PK), human immunodeficiency virus (HIV) infection, and antiretroviral therapy have been shown to impact treatment outcomes, although their impact on early parasite clearance in children has not been well characterized.

Methods. Parasite clearance parameters were generated from twice-daily blood smears in HIV-infected and HIV-uninfected Ugandan children treated with artemether-lumefantrine (AL). Artemether and dihydroartemisinin (DHA) area-under-the-curve from 0–8 hours ($AUC_{0\text{-}8hr}$) after the 1st AL dose was compared with $AUC_{0\text{-}8hr}$ after the last (6th) dose in a concurrently enrolled cohort. The association between post-1st dose artemisinin $AUC_{0\text{-}8hr}$ and parasite clearance was assessed.

Results. Parasite clearance was longer in HIV-infected versus HIV-uninfected children (median, 3.5 vs 2.8 hours; $P = .003$). Artemether $AUC_{0\text{-}8hr}$ was 3- to 4-fold lower after the 6th dose versus the 1st dose of AL in HIV-infected children on nevirapine- or lopinavir/ritionavir-based regimens and in HIV-uninfected children ($P \leq .002$, 1st vs 6th-dose comparisons). Children on efavirenz exhibited combined post-1st dose artemether/DHA exposure that was significantly lower than those on lopinavir/ritonavir and HIV-uninfected children. Multiple regression analysis supported that the effect of artemether/DHA exposure on parasite clearance was significantly moderated by HIV status.

Conclusions. Parasite clearance rates remain rapid in Uganda and were not found to associate with PK exposure. However, significant decreases in artemisinin PK with repeated dosing in nearly all children, coupled with small, but significant increase in parasite clearance half-life in those with HIV, may have important implications for AL efficacy, particularly because reports of artemisinin resistance are increasing.

Keywords. antimalarial; antiretroviral; artemisinin combination therapy; HIV; malaria.

Artemisinin compounds are extremely effective antimalarials, reducing parasite burden by up ~10 000-fold per asexual parasite cycle [1]. However, due to the risk of recrudescence and resistance when used as monotherapy, artemisinins are primarily used in combination with longer-acting partner drugs (artemisinin-based combination therapies [ACTs]), recommended as first-line antimalarial therapy since 2001 by the World Health Organization [2].

Unfortunately, resistance to artemisinins emerged on the Thai-Cambodia border, detected initially based on a phenotype of delayed clearance in peripheral blood parasitemia [3]. This is defined as a parasite clearance half-life of ≥5 hours after treatment with artesunate monotherapy or an ACT in a patient at a baseline parasite count of ≥10 000 parasites/mL [2, 4]. Independent emergence and spread of artemisinin resistance has since been seen in Southeast Asia, although resistance has not yet been established in Africa [5, 6]. The discovery of mutations in the Kelch 13 propeller region protein as a correlate to the delayed clearance phenotype has been a major advance; however, currently described mutations do not explain the delayed parasite clearance phenotype in all individuals, and, as such, it remains an important marker of artemisinin resistance [7, 8]. Moreover, our understanding of host, pharmacologic, and other parasite factors that can impact parasite clearance remains incomplete [4, 8–14].

The primary artemisinin derivatives in use are artemether and artesunate, with artemether-lumefantrine (AL) as the most

Received 1 September 2016; editorial decision 6 October 2016; accepted 11 October 2016.

[a]R. K. and L. H. contributed equally to this work.

Correspondence: S. Parikh, MD, MPH, Yale School of Public Health, 60 College Street, Room 724, New Haven, CT 06520 (sunil.parikh@yale.edu).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofw217

widely prescribed ACT worldwide. Artemisinin derivatives are converted into the active metabolite dihydroartemisinin (DHA), by cytochrome P450 (CYP) 3A4/5 and 2B6, and possibly CYP2A6 for artesunate [15–17], which in turn undergoes glucuronidation. Reductions in systemic exposure to artemisinins over the course of a standard twice-daily, 3-day malaria treatment regimen have been noted in the literature, findings ascribed to autoinduction of CYP3A4 metabolism or "disease effects" [18–20]. Artemisinin-based combination therapies are also subject to drug-drug interactions, particularly with HIV antiretroviral therapy (ART) [21]. After the last (6th) dose of AL, our group reported that efavirenz (EFV) and nevirapine (NVP), known CYP-inducers, and lopinavir/ritonavir (LPV/r), a potent CYP-inhibitor, dramatically decreases and increases artemether and lumefantrine exposure, respectively.

We hypothesized that post-1st dose artemisinin pharmacokinetic (PK) exposure may impact parasite clearance parameters. We further hypothesized that post-1st dose artemisinin PK and declines in artemisinin PK with repeated dosing will be modified in the setting of ART due to drug-drug interactions. To investigate this, we carried out intensive PK sampling after the initial dose of AL for uncomplicated malaria in HIV-uninfected and HIV-infected children on various ARTs, coupled with validated measures of parasite clearance.

METHODS

Study Area, Patients, and Clinical Management

This prospective PK and pharmacodynamics (PD) study of AL for the treatment of uncomplicated malaria in HIV-infected and HIV-uninfected children was conducted from August 2011 to November 2014 in the high transmission intensity district of Tororo, Uganda [21]. All parents or guardians provided informed consent. Children ages 0.5–8 years were enrolled if eligibility criteria were met (Supplementary Data). Children were enrolled into a "parasite clearance" cohort (providing 1st dose PK parameters and parasite clearance data) or a concurrent "intensive PK cohort" (providing 6th dose PK parameters only) [21]. Children had uncomplicated Plasmodium *falciparum* malaria confirmed by thick blood smear (regardless of parasite density) and documented or 24-hour fever history (≥38.0°C). Children received standard twice-daily, 6-dose, weight-based AL (Coartem Dispersible 20/120 mg; Novartis Pharma AG, Basel, Switzerland).

Parasite Clearance Estimation

In the parasite clearance cohort only, blood smears were collected via capillary finger prick every 12 hours until documented clearance. Parasite densities from thick blood smears were calculated by counting the number of asexual parasites per 500 leukocytes, assuming a leukocyte count of 8000/μL, providing a detection limit of 16 parasites/μL [22, 23]. A smear was declared negative (below the limit of detection) when no asexual parasites were seen after counting 500 white blood cells. Smears were read by trained technicians, with discrepancies resolved by a third reader.

The statistical models used to estimate the parasite clearance measures and lag phase duration were fitted using the Parasite Clearance Estimator (PCE) developed by WWARN [22]. The parameters were estimated: parasite clearance half-life, parasite clearance rate constant (K/hour), and the estimated time (in hours) to reduce parasitemia by 50% (PC50), 90% (PC90), 95% (PC95), and 99% (PC99). The parasite clearance half-life is based on the linear slope of decline in $\log_e$ parasitemia over time, and it is estimated as $\log_e(2)$/clearance rate, as detailed by WWARN PCE tool [22]. Parasite clearance half-life is defined as the estimated time in hours for parasitemia to decrease by one half. Additional detail on the PCE methodology is described in reference 23.

Pharmacokinetic Methods and Statistical Analysis

For children in the parasite clearance cohort, PK sampling was post-1st dose (7 venous samples on day 0 at time 0 [predose], 0.5, 1, 2, 3, 4, and 8 hours). For children in the concurrently enrolled intensive PK cohort, sampling was post-6th dose with 7 venous samples timed as described above [19]. Concentrations of artemether and DHA were determined using liquid chromatography tandem mass spectrometry, as previously described [24]. For both artemether and DHA, the calibration range was between 0.5 and 200 ng/mL, the lower limit of quantification was 0.5 ng/mL, and the coefficient of variation was <10% for each analyte.

Noncompartmental analysis of plasma drug concentrations was performed using WinNonlin (version 6.30; Certara L.P. Pharsight Corporation, Mountain View, CA). Primary outcomes were venous plasma PK parameters for artemether and DHA, including area-under-the-concentration versus time curve ($AUC_{0\text{-}8hr}$), maximal concentration (C_{max}), and time to C_{max} (T_{max}) (Supplementary Data). Data were analyzed using STATA version 14 (StataCorp, College Station, TX). The 1st dose PK (parasite clearance cohort) and post-last dose PK (intensive PK cohort) parameters were compared using a Wilcoxon rank-sum or χ^2 test, as appropriate. Because both artemether and DHA are clinically active, a "combined artemisinin AUC", defined as sum of artemether and DHA $AUC_{0\text{-}8hr}$ after a particular dose, was calculated. Linear regression on log-transformed parasite clearance half-life was performed and assessed the effects of covariates including HIV, parasite density at diagnosis, AUC for artemether or DHA or the combined artemisinin AUC, hemoglobin at diagnosis (g/dL), and age. Interaction of HIV and AUC was also included to assess effect modification by HIV status.

RESULTS

Study Profile

Participants were screened for the parasite clearance cohort over the course of 328 episodes of malaria (Supplementary Data). Of these, n = 110 met entry criteria and were included in the final PK/PD analysis (27 HIV-uninfected/83 HIV-infected; Table 1).

For the intensive PK cohort, n = 142 were included in the final artemether/DHA PK/PD analysis (51 HIV-uninfected and 91 HIV-infected), as previously described (Supplementary Data) [21]. Children were comparable between cohorts (Table 1), although children on NVP had a higher baseline parasite density and children on LPV/r had a lower baseline hemoglobin in the parasite clearance versus intensive cohort.

Parasite Clearance

Of the n = 110 participants in the parasite clearance cohort, n = 99 children had sufficient data to estimate parasite clearance parameters using the WWARN PCE [22]. Reasons for exclusion were insufficient blood smear data points (n = 6) and initial parasitemia below 1000 parasites/μL (n = 5). Data points were sufficient for calculation of lag phase in 13 of 110 participants, and no outliers were detected [22].

Parasite clearance parameters are summarized in Table 1, and parasite clearance slope half-life distribution by HIV status is depicted in Figure 1. Parasite clearance half-life was significantly longer in HIV-infected children on ART compared with HIV-uninfected children (median, 3.51 vs 2.80 hours; $P = .003$). Parasite clearance half-life and baseline parasite density were not

Table 1. Baseline Characteristics at Time of Malaria Diagnosis in Children Enrolled in the Parasite Clearance and Intensive Pharmacokinetics Cohort

Parasite Clearance Cohort (n = 110 Children)					
		HIV-Infected[a]			
Variable	HIV-Uninfected	HIV-Infected	Efavirenz-Based ART	Nevirapine-Based ART	Lopinavir/Ritonavir-Based ART
	n = 83	n = 27	n = 9	n = 8	n = 10
Age (median, years; range)	3.5 (1.1–7.9)	4.9 (2.3–6.7)	5.3 (3.3–6.3)	4.0 (2.3–5.8)	5.0 (2.4–6.7)
Weight[b] (median, kg; range)	13.7 (10.0–27.0)	16.5 (10.7–20.5)	17.5 (12.3–18.0)	12.6 (10.9–20.5)	17.0 (10.7–19.4)
Parasite density at diagnosis (geometric mean μL^{-1}; 95% CI)	14 387 (8893–23 273)	18 373 (7857–42 965)	22 628 (5483–93 386)	50 147 (19 791–127 062)*	6822 (987–47 176)
Gametocytes present at diagnosis	10.8%	18.5%	11.1%	37.5%	10.0%
Hemoglobin at diagnosis (median, g/dL; IQR)	10.9 (9.9–11.6)	10.7 (9.9–11.7)	10.4 (10.2–10.9)	10.0 (9.4–11.3)	11.5 (10.6–12)*
Total artemether dose (median, mg/kg; range)	11.4 (8.0–16.0)	12.7 (8.1–16.0)	13.3 (8.1–16.0)	11.0 (8.6–13.8)	13.0 (8.5–14.6)
Parasite clearance slope half-life (median, hours; IQR)[c]	2.80 (2.38–3.36)	3.51 (2.98–4.03)	3.44 (3.04–4.01)[d]	3.59 (2.46–6.17)[d]	3.48 (2.40–4.03)
Intensive Pharmacokinetics Cohort (n = 142 Children)[e]					
		HIV-Infected			
Variable	HIV-Uninfected	HIV-Infected	Efavirenz-Based ART	Nevirapine-Based ART	Lopinavir/Ritonavir-Based ART
	n = 51	n = 91	n = 31	n = 30	n = 30
Age (median, years; range)	3.8 (1.4–7.7)	5.0 (1.4–8.6)	6.0 (3.1–8.6)	5.0 (1.4–8.0)	4.5 (1.6–7.8)
Weight (median, kg; range)	14.6 (9.8–26)	16.4 (7.7–30)	18.0 (11.4–25.1)	16.4 (8.5–30.0)	15.8 (7.7–23.4)
Parasite density at diagnosis (geometric mean μL^{-1}; 95% CI)	11 956 (6708–21 309)	7742 (4777–12 549)	10 993 (4922–24 554)	7368 (2911–18 646)	5665 (2398–13 382)
Hemoglobin at diagnosis (median, g/dL; IQR)	11.6 (9.6–14.0)	10.4 (9.7–10.9)	12.4 (10.6–14.3)	12.2 (9.8–14.1)	13.1 (9.5–14.6)
Total artemether dose (median, mg/kg; range)	11.6 (8.1–16.0)	12.4 (8.2–16.0)	12.4 (8.4–16.0)	12.2 (8.3–15.4)	13.0 (8.2–15.9)

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range; PK, pharmacokinetics.

NOTE: Children could only participate in each cohort for a single episode of malaria, and no episodes contributed data to both cohorts.

*Signifies that *P* value <.05 for comparison of parameter of interest between parasite clearance and intensive PK cohorts.

[a]96.6% of HIV-infected children were on daily trimethoprim-sulfamethoxazole prophylaxis.

[b]Note that children must have been ≥10 kg to be eligible for parasite clearance study enrollment, but could be ≥6 kg for intensive cohort enrollment.

[c]Comparison parasite clearance slope half-lives of HIV-infected (n = 22) and HIV-uninfected children (n = 77), $P = .003$.

[d]n = 7 for parameter estimate.

[e]Data from the intensive cohort has been previously published and is provided here to enable comparison between the demographics of the parasite clearance cohort.

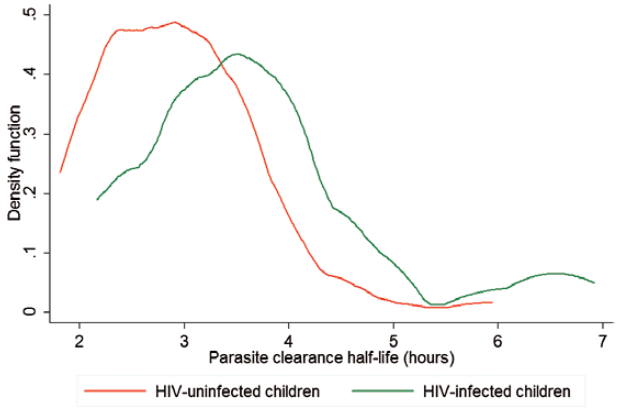

Figure 1. Kernal density distribution of parasite clearance slope-half life by human immunodeficiency virus (HIV) status. Median parasite clearance half-life was 3.51 vs 2.80 hours in HIV-infected and HIV-uninfected children, respectively ($P = .003$).

significantly correlated (Pearson correlation r = 0.12; $P = .22$), and controlling for baseline parasite density, parasite clearance remained significantly different between HIV-infected and HIV-uninfected children ($P = .0004$). Other clearance parameters (PC50, PC90, PC95, PC99) were also significantly different based on HIV status (Supplemental Table 1). Sample sizes were too small to assess differences in parasite clearance parameters among HIV-infected children on the 3 different ARTs.

Pharmacokinetics of Artemether and Dihydroartemisinin in Human Immunodeficiency Virus-Uninfected Children

Of the n = 110 participants in the parasite clearance cohort, n = 107 children had sufficient data to estimate 1 or more post-1st dose PK parameters for artemether and DHA. Pharmacokinetic parameters for the post-1st dose parasite clearance cohort and post-6th dose intensive PK cohort are in Table 2. Comparing post-6th dose to post-1st dose PK parameters of artemether in HIV-uninfected children, it is revealed that the C_{max} decreased by approximately 4-fold and the $AUC_{0\text{-}8hr}$ by over 3-fold ($P < .0001$ for both) (Figure 2). In contrast, DHA exposure increased over the course of the dosing regimen, with a 1.2-fold increase in C_{max} ($P = .001$) and 1.8-fold increase in $AUC_{0\text{-}8hr}$ ($P = .0003$). Overall, the geometric mean-combined artemisinin AUC decreased by 39% from post-1st dose to post-6th dose (558 versus 340 hr•ng/mL, respectively; $P = .0001$; Supplemental Table 2).

Pharmacokinetics of Artemether and Dihydroartemisinin in Human Immunodeficiency Virus-Infected Children on Antiretroviral Therapy

In HIV-infected children, exposure to artemisinins after the 1st dose of AL was different depending on the concomitant antiretroviral regimen (Table 2; Figure 2). For children on EFV- and NVP-based regimens, the post-1st dose $AUC_{0\text{-}8hr}$ for artemether is >6-fold and 3-fold lower than the post-1st dose AUC in HIV-uninfected children ($P < .0001$ and $P < .001$, respectively). The C_{max} was also significantly lower for those on EFV- and NVP-based regimens after the 1st dose compared with HIV-uninfected children.

Dihydroartemisinin exposure after the 1st dose of AL was ~2-fold lower for those children on EFV-based regimens compared with HIV-uninfected children ($P = .03$). For those on LPV/r-based regimens, no difference in exposure to artemether or DHA after the 1st dose of AL compared with HIV-uninfected children was seen ($P = .97$ and $P = .61$, respectively). None of the antiretroviral regimens significantly impacted C_{max} values compared with HIV-uninfected children.

We also assessed whether the use of concomitant antiretrovirals affected the changes in artemisinin exposure over the treatment interval. For artemether, children on LPV/r- and NVP-based regimens both exhibited a 4-fold reduction in $AUC_{0\text{-}8hr}$ over the course of the treatment interval ($P = .0002$ and $P = .002$, respectively), as well as significant decreases in C_{max}. However, for DHA, there were no significant changes in exposure (AUC or C_{max}) over the treatment interval in children receiving antiretrovirals.

The combined artemisinin AUC was 46% lower in those on NVP, and 51% lower in those on LPV/r after the last dose, compared with after the 1st dose (Supplemental Table 2). Furthermore, although the combined AUC remained stable over the treatment interval for children on EFV, overall exposure to artemether plus DHA after the 1st dose of AL was significantly lower in children on EFV than exposure seen in HIV-uninfected children ($P < .0001$), children on LPV/r ($P = .0096$), but not children on NPV ($P = .11$) (Supplemental Table 2).

Determinants of Parasite Clearance

In simple regression analysis, a greater post-1st dose artemether AUC ($P = .0498$), but not DHA AUC ($P = .80$) or combined AUC ($P = .08$), was associated with faster parasite clearance (Table 3 and Supplemental Table 3). Because parasite clearance half-life was significantly longer in HIV-infected children, and post-1st dose exposure to artemether and DHA was significantly lower in HIV-infected children on ART compared with HIV-uninfected children ($P < .001$), we further assessed whether the longer parasite clearance half-life in HIV-infected children was mediated by lower artemether AUC or DHA AUC. The inclusion of artemether or DHA AUC in the regression model led to only a 1.6% and 4% change in the regression coefficient of HIV status, respectively, and the effect of artemether or DHA was insignificant in this model, suggesting that the effect of HIV status on parasite clearance was independent of post-1st dose artemether and DHA exposure. Furthermore, we assessed whether the association between artemisinin exposure and parasite clearance was modified by HIV status by examining the interaction effect of HIV and artemisinin AUC in multiple regression. In this analysis, a significant interaction was noted between HIV status and each PK exposure variable (artemether,

Table 2. Artemether and DHA Exposure After the First and Last AL Dose in HIV-Uninfected and HIV-Infected Children on Antiretroviral Therapy

	Artemether							
	$AUC_{0\ to\ 8\ hours}$ (hr•ng/mL)		Ratio[a]		C_{max} (ng/mL)		Ratio[a]	
Regimen	After 1st Dose	After 6th Dose	6th Dose/1st Dose	1st Dose ART/ 1st Dose No ART	After 1st Dose	After 6th Dose	6th Dose/1st Dose	1st Dose ART/1st Dose No ART
No ART (HIV-uninfected)	386 (320, 465) n = 77	120 (96, 149) n = 51	0.31 ($P < .0001$)	N/A	133 (110, 162) n = 80	35 (27, 46) n = 51	0.26 ($P < .0001$)	N/A
Efavirenz	63 (19, 214) n = 9	48 (36, 64) n = 31	0.76 ($P = .47$)	0.16 ($P < .0001$)	25.0 (8.1, 77.6) n = 9	15.3 (11.2, 21.0) n = 31	0.61 ($P = .21$)	0.19 ($P < .0001$)
Lopinavir/ ritonavir	382 (221, 660) n = 9	91 (63, 131) n = 30	0.24 ($P = .0002$)	0.99 ($P = .97$)	116.2 (63.5, 212.9) n = 10	26.4 (17.0, 40.8) n = 30	0.23 ($P = .0007$)	0.87 ($P = .64$)
Nevirapine	137 (65, 289) N = 8	36 (25, 53) N = 30	0.26 ($P = .002$)	0.35 ($P = .001$)	42.3 (18.1, 98.7) n = 8	10.2 (6.9, 15.2) n = 30	0.24 ($P = .0017$)	0.32 ($P = .0008$)
	Dihydroartemisinin							
No ART (HIV-uninfected)	121 (99, 149) N = 77	212 (172, 261) N = 51	1.75 ($P = .0003$)	N/A	39.4 (32.4, 48.0) n = 79	66.9 (52.1, 859) n = 51	1.69 ($P = .001$)	N/A
Efavirenz	54 (18, 161) N = 9	63 (45, 88) N = 31	1.17 ($P = .69$)	0.45 ($P = .028$)	22.7 (8.5, 60.8) n = 9	24.1 (16.6, 35.1) n = 31	1.06 ($P = .88$)	0.58 ($P = .09$)
Lopinavir/ ritonavir	143 (70, 291) N = 9	170 (126, 231) N = 30	1.19 ($P = .58$)	1.21 ($P = .61$)	42.7 (20.6, 88.5) n = 10	55.5 (38.0, 81.0) n = 30	1.30 ($P = .65$)	1.08 ($P = .79$)
Nevirapine	176 (93, 334) N = 8	137 (100, 186) N = 30	0.78 ($P = .44$)	1.45 ($P = .26$)	54.3 (23.4, 125.8) n = 8	45.2 (32.0, 64.8) n = 30	0.83 ($P = .48$)	1.38 ($P = .34$)

Abbreviations: AUC, area under the curve; AL, artemether-lumefantrine; ART, antiretroviral therapy; DHA, dihydroartemisinin; HIV, human immunodeficiency virus; N/A, not applicable; PK, pharmacokinetics.

[a]All PK parameters expressed as geometric means with 95% confidence intervals. Statistical comparisons performed using *t* test on log-transformed AUCs and C_{max} values.

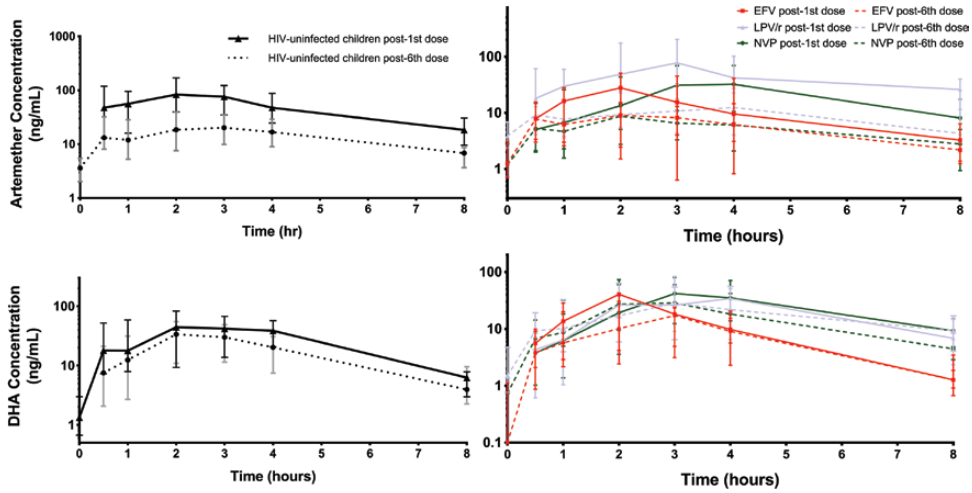

Figure 2. Artemether and dihydroartemisinin exposure after the 1st and last dose of artemether-lumefantrine in human immunodeficiency virus (HIV)-uninfected children and HIV-infected children on 3 different antiretroviral regimens. Solid lines represent post-1st dose concentration-time curves, and dotted lines represent post-6th dose concentration-time curves. Colors represent HIV-uninfected children (black), and HIV-infected children on efavirenz (EFV; red), nevirapine (NVP; green), and lopinavir/ritonavir (LPV/r)-based regimens (light blue).

DHA, and the combined post-1st dose AUC; $P = .039$, $P = .038$, $P = .016$ for respective interaction terms), indicating that the relationship between drug exposure and parasite clearance was impacted by HIV status (Table 3, Supplemental Figure 1, and Supplemental Table 3). In HIV-uninfected children, a trend towards a decrease in parasite clearance half-life with increasing artemether AUC was seen ($P = .09$), compared with an upward trend in HIV-infected children ($P = .19$), although neither was statistically significant. Additional covariates, such as age, parasite density, and hemoglobin at diagnosis, were not significantly associated with parasite clearance half-life. Sample sizes were too small to assess the interaction of HIV and PK in individual antiretroviral groups on parasite clearance.

DISCUSSION

Our results support previous reports of rapid rates of parasite clearance in Africa after AL, indicating it is unlikely that artemisinin resistance had emerged at our site. However, our findings suggest other areas of concern: (1) HIV-infected children demonstrate a slight, albeit significantly slower rate of early parasite clearance than HIV-uninfected children, independent of baseline parasite density; (2) combined artemether and DHA exposure after the last AL dose is dramatically lower than after the first dose; and(3) artemether and DHA exposure are exceedingly low throughout the dosing regimen in children on EFV-based ART (compared with children not on ART or on NVP and LPV/r). Specifically, for HIV-infected children on EFV, this slower rate of parasite clearance, coupled with low artemisinin exposure throughout the dosing interval, places these children at risk of treatment failure. In addition, for almost all children, the dramatic declines in total artemisinin exposure over the treatment interval may put them at higher risk of failure. As noted by others, we further suggest that such low drug exposure, particularly coupled with alterations in immunity (those with HIV), present conditions conducive to the de novo selection and transmission of resistance [1, 21, 25].

In a recent meta-analysis of early parasitological response in African patients, independent predictors of day 3 smear positivity included higher baseline parasitemia, fever, severe anemia,

Table 3. Linear Regression Analysis on Log-Transformed Parasite Clearance Slope Half-Life

Covariate		Unadjusted Coefficient ± SE	*P* Value	Adjusted Coefficient ± SE	*P* Value
HIV Infection					
	Infected	0.211 ± 0.063	.001	0.038 ± 0.106	.72
	Uninfected	Ref		Ref	
Artemether AUC (unit = 100 hr•ng/mL)		−0.019 ± 0.01	.0498	0.039 ± 0.023	.09
HIV* artemether AUC interaction				0.053 ± 0.025	.037
Age (years)		0.013 ± 0.019	.49	0.010 ± 0.020	.603
Hemoglobin (g/dL)[a]		−0.020 ± 0.021	.35	−0.033 ± 0.022	.127
Parasite density (per 10^4 parasites/μL)[a]		−0.004 ± 0.004	.22	−0.005 ± 0.003	.155

Abbreviations: AUC, area under the curve; HIV, human immunodeficiency virus; SE, standard error; Ref, reference.

*Adjusted linear regression analysis included the covariates age, hemoglobin at baseline, and log-transformed parasite density.

use of certain ACTs, and living in low/moderate transmission settings [13]. Several of these factors, as well as studies of age and parasite clearance, suggest that antimalarial immunity plays an important role in early parasitological responses [10, 11]. Our findings further support this notion, because HIV-infected children displayed longer parasite clearance half-lives, even when controlling for baseline parasite density [22]. Two other studies lend support to our findings. A study in Eastern Uganda using once-daily smears found that HIV-infected children (n = 38) had a higher risk of a positive smear on day 2, but not on day 1, compared with HIV-uninfected children [26]. A more recent study in Tanzania, also using daily smears, similarly found that a higher proportion of HIV-infected children had positive smears on days 2 and 3, compared with HIV-uninfected children [27]. Mechanistic insight may be gleaned from a study suggesting that parasite destruction by splenic macrophages is impaired in HIV-malaria coinfected patients [28]. A related, but distinct explanation may be the delayed removal of dead parasites in those with HIV, a possibility that we are unable to answer with our study.

In a univariate analysis, greater artemether exposure ($P = .05$), but not DHA exposure, was associated with a shorter parasite clearance half-life. The relationships among HIV status, artemether/DHA exposure, and parasite clearance half-life was further assessed by both mediation and moderation analyses, results that supported a primary role of HIV status in modifying the delay in clearance, rather than variability in artemether/DHA exposure. Other studies have assessed the potential direct impact of artemisinin exposure on parasite clearance, although they have largely focused on artesunate [3, 12, 29–33]. Although these studies have largely found no clear associations of parasitologic responses to PK parameters, an early AL dose-finding study found that higher artemether and DHA AUCs were associated with a decrease in parasite clearance time. Other studies have found either that lower doses of artesunate or the use of ACTs with lower relative doses of artemisinin (for example, AL versus DHA-piperaquine) negatively impacted parasite clearance times [11–13].

Combined exposure to artemether and DHA, both of which are biologically active, decreased by 39% to 51% between the 1st and 6th dose in all groups of children, aside from those on EFV. We also noted increases in DHA exposure in HIV-uninfected children after the 6th dose compared with the 1st dose. One explanation for this decrease in artemether exposure and increase in DHA is autoinduction; an increase in metabolism of artemether through its own induction of CYP enzymes, as postulated by others [34–36] The use of CYP inducers and inhibitors in our study afforded an opportunity to further interrogate the potential role of CYP modulation in explaining the decrease in artemether exposure. Our results do not demonstrate a significant impact of either concomitant CYP3A4 inhibitors (LPV/r) or inducers (EFV or NVP) on the multidose reductions in exposure to artemether, perhaps suggesting a relatively minor role of CYP3A4 autoinduction in explaining this phenomenon. However, we are unable to comment on the involvement of other CYP enzymes (ie, CYP2B6), less impacted by the studied antiretrovirals, in explaining these changes [36]. An alternative hypothesis is the notion of a "disease effect," whereby exposure is increased in the setting of malaria compared with the healthy/recovered state. Antimalarial exposure has been found to be altered in the setting of malaria infection, possibly due to reduced first-pass effects or clearance in acute disease [20, 37, 38]. Although it is difficult to find support for or against this notion in our study, notable previous arguments against a disease effect are findings that exposure to artemisinins decreased with repeated dosing even in healthy volunteers (ie, in the absence of disease) [34, 39, 40].

Regardless of the mechanism, the major concern is the impact of this reduction in artemisinin exposure on AL efficacy. Furthermore, a theoretical concern is that such low exposure, particularly in those individuals who are hyperparasitemic or initially dosed too low (for example, young children and pregnant women [25]), may produce high-risk scenarios for the emergence or selection of resistance [1]. In our study, these theoretical concerns are particularly notable in HIV-infected children on antiretrovirals, a group that demonstrates significantly impaired antmalarial immunity. A case-in-point: children on NVP-based antiretrovirals start out with 3-fold lower exposure to artemether compared with HIV-uninfected children, and exposure further declines by the 6th dose ($AUC_{0\text{-}8hr}$ 36 vs 120 hr•ng/mL, respectively). However, perhaps of greatest concern is the situation of children on EFV-based regimens, a group for which we and others have recommended close monitoring for treatment failure after AL use [2, 21]. In these individuals, combined exposure to artemether and DHA was 4.0-fold and 3.0-fold lower after the 1st and 6th dose, respectively, compared with HIV-uninfected children [21]. Together with the 4-fold lower post-6th dose exposure to the long-acting partner drug lumefantrine, lower levels of antimalarial immunity, and higher risk of recurrent malaria after treatment with AL in those with EFV [21], these children appear to be among the highest risk groups for increased morbidity and a theoretical increased risk of de novo selection of drug resistance.

Although our study raises several concerns, there are several notable limitations. For the assessment of parasite clearance, we used the validated PCE tool [22]. However, our smear frequency was insufficient to adequately describe the lag phase in many individuals, and in those individuals, slope half-life may be overestimated [22]. In addition, although we demonstrated a delay in parasite clearance in HIV-infected children, the overall range in parasite clearance half-lives was narrow (interquartile range, 2.4–3.6 hours) and limited our ability to assess for associations between PK exposure and parasite clearance. With respect to our assessment and comparison of PK

exposure, due to logistical concerns and adherence to limits of blood volume collections in pediatric patients, we used 2 concurrently enrolled groups of participants for obtaining post-1st and post-6th dose artemether/DHA-intensive PK parameters. Importantly, however, our 2 cohorts were comparable (Table 1), and any differences were unlikely to have significantly impacted our results. Lastly, while we did not genotype for Kelch 13 mutations, studies by other groups suggests that mutations linked to delayed parasite clearance in Asia were absent in sub-Saharan Africa during the time of our study [6].

CONCLUSIONS

Although the delay in parasite clearance in our Ugandan children is well within the 5-hour half-life-threshold that currently defines delayed parasite clearance in Southeast Asia (only 3 of 99 children had >5-hour half-life), the delay in clearance half-life in the setting of HIV infection, the decrease combined artemisinin exposure with repeated dosing, and the low exposure in HIV-infected children on ART raise several areas for concern regarding the potential for reduced efficacy, as well as theoretical situations conducive to the emergence and/or spread of drug resistance. This is of primary concern for those individuals on EFV, and it further supports the urgent need to find alternative dosing regimens in EFV-treated children to improve exposure to both artemether and lumefantrine, or the consideration of LPV/r-based regimens as first-line in HIV-infected children.

Supplementary Data

Supplementary material is available at *Open Forum Infectious Diseases* online.

Acknowledgments

We thank Dr. Kasia Stepniewska from the Worldwide Antimalarial Resistance Network for assistance in generating the parasite clearance parameters using the Parasite Clearance Estimator Tool. We also thank Dr. Moses Kamya for his leadership in the Infectious Disease Research Collaboration ([IDRC] based in Kampala, Uganda) and expert consultation. Moreover, we thank the staff at the IDRC including Abel Kakura, Francis Orukan, Catherine Tugaineyo, and Bridget Nzarbara. Most importantly, we are grateful to the families in Tororo who participated in all study procedures, without whose support this study would not have been possible.

Disclaimer. The contents of the manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health (NIH).

Financial support. This work was supported by grant R01 HD068174 funded by the NIH, National Institute of Child Health and Human Development.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. White NJ, Pongtavornpinyo W, Maude RJ, et al. Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. Malar J **2009**; 8:253.
2. World Health Organization. *Guidelines for the Treatment of Malaria. Third edition*. Geneva: World Health Organization; **2015**.
3. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med **2009**; 361:455–67.
4. Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med **2014**; 371:411–23.
5. Takala-Harrison S, Jacob CG, Arze C, et al. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis **2015**; 211:670–9.
6. Taylor SM, Parobek CM, DeConti DK et al. Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in sub-Saharan Africa: a molecular epidemiologic study. J Infect Dis **2015**; 211:680–8.
7. World Health Organization. Status report on artemisinin and ACT resistance. Geneva: World Health Organization; **2015**.
8. Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature **2014**; 505:50–5.
9. Amaratunga C, Sreng S, Suon S, et al. Artemisinin-resistant *Plasmodium falciparum* in Pursat province, western Cambodia: a parasite clearance rate study. Lancet Infect Dis **2012**; 12:851–8.
10. Ndour PA, Lopera-Mesa TM, Diakité SA, et al. *Plasmodium falciparum* clearance is rapid and pitting independent in immune Malian children treated with artesunate for malaria. J Infect Dis **2015**; 211:290–7.
11. Das D, Price RN, Bethell D, et al. Early parasitological response following artemisinin-containing regimens: a critical review of the literature. Malar J. **2013**; 12:125.
12. WWARN Parasite Clearance Study Group; Abdulla S, Ashley EA, et al. Baseline data of parasite clearance in patients with falciparum malaria treated with an artemisinin derivative: an individual patient data meta-analysis. Malar J **2015**; 14:359.
13. Dahal P, d'Alessandro U, Dorsey G et al. Clinical determinants of early parasitological response to ACTs in African patients with uncomplicated falciparum malaria: a literature review and meta-analysis of individual patient data. BMC Med **2015**; 13:212.
14. Hastings IM, Kay K, Hodel EM. How robust are malaria parasite clearance rates as indicators of drug effectiveness and resistance? Antimicrob Agents Chemother **2015**; 59:6428–36.
15. Svensson US, Ashton M. Identification of the human cytochrome P450 enzymes involved in the in vitro metabolism of artemisinin. Br J Clin Pharmacol **1999**; 48:528–35.
16. Li XQ, Björkman A, Andersson TB, et al. Identification of human cytochrome P(450)s that metabolise anti-parasitic drugs and predictions of in vivo drug hepatic clearance from in vitro data. Eur J Clin Pharmacol **2003**; 59:429–42.
17. van Agtmael MA, Gupta V, van der Wösten TH, et al. Grapefruit juice increases the bioavailability of artemether. Eur J Clin Pharmacol **1999**; 55:405–10.
18. Hassan Alin M, Ashton M, Kihamia CM, et al. Multiple dose pharmacokinetics of oral artemisinin and comparison of its efficacy with that of oral artesunate in falciparum malaria patients. Trans R Soc Trop Med Hyg **1996**; 90:61–5.
19. Ezzet F, Mull R, Karbwang J. Population pharmacokinetics and therapeutic response of CGP 56697 (artemether + benflumetol) in malaria patients. Br J Clin Pharmacol **1998**; 46:553–61.
20. Newton P, Suputtamongkol Y, Teja-Isavadharm P, et al. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. Antimicrob Agents Chemother **2000**; 44:972–7.
21. Parikh S, Kajubi R, Huang L, et al. Antiretroviral choice for HIV impacts antimalarial exposure and treatment outcomes in Ugandan children. Clin Infect Dis **2016**; 63:414–22.
22. Worldwide Antimalarial Resistance Network (WWARN). Parasite Clearance Estimator. **2012**; Available at: http://www.wwarn.org/tools-resources/toolkit/analyse/parasite-clearance-estimator-pce. Accessed 11 April 2016.
23. Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. Malar J **2011**; 10:339.
24. Huang L, Olson A, Gingrich D, Aweeka FT. Determination of artemether and dihydroartemisinin in human plasma with a new hydrogen peroxide stabilization method. Bioanalysis **2013**; 5:1501–6.
25. Barnes KI, Watkins WM, White NJ. Antimalarial dosing regimens and drug resistance. Trends Parasitol **2008**; 24:127–34.
26. Muhindo MK, Kakuru A, Jagannathan P, et al. Early parasite clearance following artemisinin-based combination therapy among Ugandan children with uncomplicated *Plasmodium falciparum* malaria. Malar J **2014**; 13:32.
27. Smart LR, Orgenes N, Mazigo HD, et al. Malaria and HIV among pediatric inpatients in two Tanzanian referral hospitals: a prospective study. Acta Trop **2016**; 159:36–43.
28. Joice R, Frantzreb C, Pradham A, et al. Evidence for spleen dysfunction in malaria-HIV co-infection in a subset of pediatric patients. Mod Pathol **2016**; 29:381–90.
29. Kyaw MP, Nyunt MH, Chit K, et al. Reduced susceptibility of *Plasmodium falciparum* to artesunate in southern Myanmar. PLoS One **2013**; 8:e57689.
30. Simpson JA, Agbenyega T, Barnes KI, et al. Population pharmacokinetics of artesunate and dihydroartemisinin following intra-rectal dosing of artesunate in malaria patients. PLoS Med **2006**; 3:e444.
31. Das D, Tripura R, Phyo AP, et al. Effect of high-dose or split-dose artesunate on parasite clearance in artemisinin-resistant falciparum malaria. Clin Infect Dis **2013**; 56:e48–58.

32. Djimdé AA, Tekete M, Abdulla S, et al. Pharmacokinetic and pharmacodynamic characteristics of a new pediatric formulation of artemether-lumefantrine in African children with uncomplicated *Plasmodium falciparum* malaria. Antimicrob Agents Chemother **2011**; 55:3994–9.
33. Zaloumis SG, Tarning J, Krishna S, et al. Population pharmacokinetics of intravenous artesunate: a pooled analysis of individual data from patients with severe malaria. CPT Pharmacometrics Syst Pharmacol **2014**; 3:e145.
34. Ashton M, Hai TN, Sy ND, et al. Artemisinin pharmacokinetics is time-dependent during repeated oral administration in healthy male adults. Drug Metab Dispos **1998**; 26:25–7.
35. Salman S, Bendel D, Lee TC, et al. Pharmacokinetics of a novel sublingual spray formulation of the antimalarial drug artemether in African children with malaria. Antimicrob Agents Chemother **2015**; 59:3208–15.
36. Simonsson US, Jansson B, Hai TN, et al. Artemisinin autoinduction is caused by involvement of cytochrome P450 2B6 but not 2C9. Clin Pharmacol Ther **2003**; 74:32–43.
37. Hien TT, Davis TM, Chuong LV, et al. Comparative pharmacokinetics of intramuscular artesunate and artemether in patients with severe falciparum malaria. Antimicrob Agents Chemother **2004**; 48:4234–9.
38. McGready R, Phyo AP, Rijken MJ, et al. Artesunate/dihydroartemisinin pharmacokinetics in acute falciparum malaria in pregnancy: absorption, bioavailability, disposition and disease effects. Br J Clin Pharmacol **2012**; 73:467–77.
39. Lefèvre G, Bindschedler M, Ezzet F, et al. Pharmacokinetic interaction trial between co-artemether and mefloquine. Eur J Pharm Sci **2000**; 10:141–51.
40. van Agtmael MA, Gupta V, van der Graaf CA, van Boxtel CJ. The effect of grapefruit juice on the time-dependent decline of artemether plasma levels in healthy subjects. Clin Pharmacol Ther **1999**; 66:408–14.