Title

Efavirenz-Based Antiretroviral Therapy but Not Pregnancy Increased Unbound Piperaquine Exposure in Women during Malaria Chemoprevention

Authors

Hong, Howard
Aslam-Mir, Usman
Kajubi, Richard
et al.

Publication Date

2023-03-14

DOI

10.1128/aac.01427-22

Data Availability

The data associated with this publication are in the supplemental files.

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

PHARMACOLOGY

Efavirenz-Based Antiretroviral Therapy but Not Pregnancy Increased Unbound Piperaquine Exposure in Women during Malaria Chemoprevention

Howard Hong,[a] Usman Aslam-Mir,[a] Richard Kajubi,[b] Erika Wallender,[a] Norah Mwebaza,[b] Grant Dorsey,[c] Philip J. Rosenthal,[c] Francesca T. Aweeka,[a] Liusheng Huang[a]

[a]Drug Research Unit, Department of Clinical Pharmacy, University of California, San Francisco, California, USA
[b]Infectious Disease Research Collaboration, Makerere University College of Health Sciences, Kampala, Uganda
[c]Department of Medicine, University of California, San Francisco, California, USA

ABSTRACT Dihydroartemisinin-piperaquine (DP) is highly effective for malaria chemoprevention during pregnancy, but the standard dosing of DP that is used for nonpregnant adults may not be optimal for pregnant women. We previously reported that the pharmacokinetic exposure of total piperaquine (PQ; both bound and unbound to plasma proteins) is reduced significantly in the context of pregnancy or efavirenz (EFV)-based antiretroviral therapy (ART). However, as PQ is >99% protein-bound, reduced protein binding during pregnancy may lead to an increase in the pharmacologically active unbound drug fraction (f_u), relative to the total PQ. We investigated the impact of pregnancy and EFV use on the f_u of PQ to inform the interpretation of pharmacokinetics. Plasma samples from 0 to 24 h after the third (final) DP dose were collected from pregnant women at 28 weeks gestation who were receiving or not receiving EFV-based ART as well as from women 34 to 54 weeks postpartum who were not receiving EFV-based ART, who served as controls. Unbound PQ was quantified via ultrafiltration and liquid chromatography-tandem mass spectrometry, with f_u being calculated as $PQ_{unbound}/PQ_{total}$. The geometric mean f_u did not differ between pregnant and postpartum women ($P = 0.66$), but it was 23% ($P < 0.01$) greater in pregnant women receiving EFV-based ART, compared to that in postpartum women who were not receiving EFV-based ART. The altered drug-protein binding, potentially due to the displacement of PQ from plasma proteins by EFV, resulted in only a 14% lower unbound PQ exposure ($P = 0.13$) in the presence of a 31% lower total PQ exposure ($P < 0.01$), as estimated by the area under the concentration time curve from 0 to 24 h post-last dose in pregnant women who were receiving EFV-based ART. The results suggest that the impact of pregnancy and EFV-based ART on the exposure and, in turn, the efficacy of PQ for malaria prevention may not be as significant as was suggested by the changes in the total PQ exposure. Further study during the terminal elimination phase (e.g., on day 28 post-dose) would help better characterize the unbound PQ exposure during the full dosing interval and, thus, the overall efficacy of PQ for malaria chemoprevention in this special population.

KEYWORDS unbound piperaquine, malaria, pregnancy, efavirenz, drug-drug interaction, ultrafiltration

Malaria remains one of the most challenging infectious diseases in the world. In 2020, the World Health Organization (WHO) estimated that over 95% of the 241 million global cases and 96% of the 627,000 malaria deaths were in Africa (1). In particular, pregnant women are among the most at-risk groups, with nearly 34% of pregnant women in Africa experiencing placental malaria, which has been linked to low birthweight and >100,000 infant deaths annually (2, 3). To decrease the burden of malaria during pregnancy, the WHO

Address correspondence to Liusheng Huang, liusheng.huang@ucsf.edu.

The authors declare no conflict of interest.

Received 21 October 2022
Returned for modification 12 December 2022
Accepted 13 February 2023

currently recommends intermittent preventive therapy during pregnancy (IPTp) (i.e., chemoprevention) with sulfadoxine-pyrimethamine (SP) in malaria regions of endemicity in Africa (4). However, the resistance of malaria parasites to this regimen has arisen, leading to the search for an effective alternative IPTp regimen (5).

Dihydroartemisinin-piperaquine (DP), which is an artemisinin-based combination therapy, had efficacy superior to that of SP in preventing malaria infections when provided as IPTp (6–8). Standard treatment doses of DP (120 mg dihydroartemisinin/960 mg piperaquine once daily for 3 days), which were developed for nonpregnant adults, have been tested on a monthly basis for IPTp in clinical trials. Although these studies found that monthly IPTp-DP was superior to monthly IPTp-SP for malaria prevention, breakthrough malaria parasitemia was occasionally detected. In addition, pregnancy decreases the pharmacokinetic (PK) exposure of PQ, which is the long-acting partner drug that is largely responsible for the long-term protective efficacy of DP (9). To fully prevent parasitemia in all pregnant women, PK/pharmacodynamic (PD) studies during pregnancy identified protective total PQ concentrations. Based on this, revised IPT-DP dosing regimens were proposed to maintain these protective PQ concentrations (8). Furthermore, for pregnant women with HIV who were receiving efavirenz (EFV)-based antiretroviral therapy (ART), our PK studies showed that EFV, which enhances drug metabolism, reduced the total PQ concentrations (9). As parasitemia outcomes during pregnancy are not available for pregnant women who require EFV, revised dosing regimens that are based on the total PQ concentration targets for pregnant women who are not receiving EFV have also been proposed for this population (10). However, if protein binding during pregnancy and/or with concomitant EFV differs from that which was observed in nonpregnant adults, this could impact the interpretation of the changes in the total drug concentrations and the dosing recommendations for these groups.

All previous PK/PD studies of PQ have used measurements of the total PQ (plasma protein bound PQ + unbound PQ) (8–12). However, it is the unbound drug that traverses biological membranes and exerts pharmacological effects against malaria parasites. During pregnancy, the fraction of the drug that is unbound (f_u) to plasma proteins may be greater than that in nonpregnant adults due to the increased body fluid and the potential displacement of drugs by steroid and/or placental hormones. This factor is especially relevant for highly protein-bound drugs (13), such as PQ (>99% bound) (14). If the PQ f_u increases during pregnancy, this may offset, at least in part, the decrease in the total PQ exposure during pregnancy that we previously reported, as lower total drug concentrations may be sufficient to maintain the same level of unbound PQ in pregnant women as in nonpregnant adults. Furthermore, concomitant EFV exposure may cause drug-drug interactions by competitively displacing PQ from plasma proteins, as EFV is also >99% protein-bound in plasma (15, 16). To further understand the optimal DP dose for IPTp, it is essential to assess the unbound PQ after DP dosing during pregnancy. Leveraging data and samples from our previous intensive PK study (ClinicalTrials registration number NCT02163447) (9), we studied whether pregnancy and EFV impact the unbound PQ exposure in pregnant women.

RESULTS

Profile of study participants. A total of 88 (31 HIV-negative pregnant women, 27 HIV-positive pregnant women, and 30 HIV-negative postpartum women) participants were included for this sample analysis (Fig. 1). The demographic characteristics of these participants were reported previously (9) and are summarized in Table 1. The HIV-positive pregnant women were older than were the HIV-negative pregnant women (median age of 30 years versus 23 years, $P < 0.01$), but their weights were similar. So, an impact of the age difference on the drug binding proteins is not expected. The HIV-negative postpartum women served as the nonpregnant control group and underwent a PK assessment at 34 to 54 weeks postpartum. Their median weight was 8% lower than those of the two pregnant groups ($P < 0.01$), and their hemoglobin level was 20% higher than that observed in

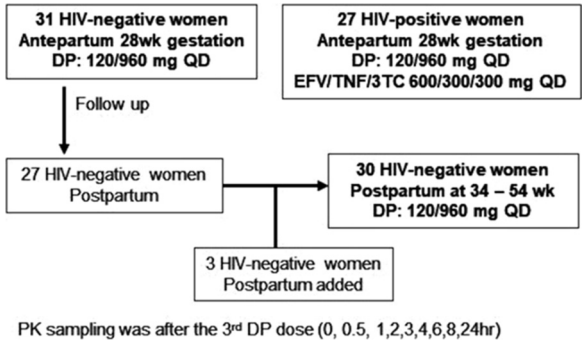

FIG 1 Schematic overview of the intensive PK study. DP, dihydroartemisinin-piperaquine; EFV, efavirenz; TNF, tenofovir; 3TC, lamivudine; QD, once daily; PK, pharmacokinetics.

the HIV-positive pregnant women who were receiving EFV-based ART and 16% higher than that observed in the HIV-negative pregnant women (Table 1).

Measurement of unbound PQ concentrations. A total of 275 plasma samples from 31 HIV-negative pregnant women, 237 plasma samples from 27 HIV-positive pregnant women receiving EFV-based ART, and 268 plasma samples from 30 postpartum women were analyzed (Table S1). The unbound PQ concentrations ranged from 0.040 to 11.1 ng/mL, with all sample results exceeding the lower limit of quantification (0.02 ng/mL).

Pharmacokinetics for unbound PQ. The calculated PK parameters for the unbound PQ are presented in Table 2. For comparison, the PK parameters for the total PQ are recalculated using the same plasma samples as were used for the unbound PQ, and these are included in Table 2. Of note, compared to our prior report of the total drug PK (9), two additional subjects were included (one HIV-negative pregnant woman and one HIV-positive pregnant woman who was receiving EFV-based ART), as these participants had samples available from 0 to 24 h but had insufficient samples to be a part of our prior study (9).

Impact of pregnancy. As we observed for the total PQ exposure, the unbound PQ exposure was also lower during pregnancy than in the postpartum women, and this was to a similar magnitude. Specifically, the unbound PQ peak concentration (Cmax) was 18.9% ($P = 0.20$) lower, and the area under the concentration-time curve from time zero to 24 h after the third dose ($AUC_{0-24\ h}$) was 16.3% ($P = 0.12$) lower in the pregnant women. None of these reductions reached statistical significance, as was true for the PK parameters for the total drug (Table 2). To further evaluate the unbound PQ exposure, we calculated the fraction of unbound PQ at each time point. The geometric mean f_u was 0.533% in the antepartum women and 0.520% in the postpartum women ($P = 0.66$).

Impact of EFV during pregnancy. To evaluate the impact of EFV on the PK exposure of unbound PQ, the exposure in the HIV-positive pregnant women who were concomitantly receiving EFV-based ART was compared with that in the HIV-negative pregnant women who were not receiving EFV-based ART (Table 2). The unbound PQ Cmax and $AUC_{0-24\ h}$ were similar in the two groups. The mean Cmax was 2.40 versus 2.41 ng/mL, and the mean $AUC_{0-24\ h}$ was 24.2 versus 23.6 h.ng/mL in the pregnant women with versus without EFV-based ART. However, considering the reduction of the total PQ Cmax (−18%) and $AUC_{0-24\ h}$ (−18%) values, f_u was 20% higher ($P < 0.01$), and the ratio of unbound

TABLE 1 Baseline characteristics of study participants[a]

Characteristic	Antepartum at 28 weeks gestation		Postpartum 34 to 54 weeks
	HIV-positive ($n = 27$)	HIV-negative ($n = 31$)	HIV-negative ($n = 30$)
Concomitant drugs	EFV-based ART	No ART	No ART (control)
Age (years)	30 (18, 43)[b]	23 (18, 31)	24 (19, 32)
wt (kg)	57.6 (43.7, 72.8)	57.5 (45.2, 83.2)	52.9 (38.5, 72.9)[b]
Hemoglobin levels (g/dL)	11.6 (8.1, 19.2)	12.0 (10.3, 16.8)	13.9 (11.8, 16.3)[b]

[a]The data represent the median (range). EFV, Efavirenz; ART, antiretroviral therapy.
[b]Denotes a statistically significant difference ($P < 0.017$, Wilcoxon rank-sum test), compared to the same parameter for the other two groups.

TABLE 2 Impact of pregnancy and EFV-based ART on the pharmacokinetics of piperaquine[a]

PK parameter	P alone GM; 95% CI (*n* = 31)	P+EFV GM; 95% CI (*n* = 27)	NP GM; 95% CI (*n* = 30)	P/NP GMR (*P* value)	P+EFV/P GMR (*P* value)	P+EFV/NP GMR (*P* value)
Total PQ						
Cmax, ng/mL	404 (332, 491)	333 (277, 400)	497 (392, 630)	0.813 (0.13)	0.824 (0.067)	0.670 (<0.01)
T_{max}, hr	3.08 (3.00, 4.03)	4.00 (2.03, 5.98)	3.06 (2.07, 4.03)	1.01 (0.71)	1.30 (0.78)	1.31 (0.65)
$AUC_{0-24\ h}$, hr·ng/mL	4,580 (3,850, 5,440)	3,770 (3,250, 4,380)	5,490 (4,420, 6,810)	0.834 (0.11)	0.823 (0.019)	0.687 (<0.01)
Free PQ						
Cmax, ng/mL	2.41 (1.83, 3.19)	2.40 (1.88, 3.07)	2.97 (2.29, 3.85)	0.811 (0.20)	1.00 (0.95)	0.808 (0.14)
T_{max}, hr	3.98 (3.02, 4.03)	4.00 (2.98, 4.07)	3.08 (3.00, 4.07)	1.29 (0.71)	1.01 (0.81)	1.30 (0.72)
$AUC_{0-24\ h}$, hr·ng/mL	23.6 (18.8, 29.5)	24.2 (19.1, 30.5)	28.2 (21.8, 36.5)	0.837 (0.12)	0.975 (0.87)	0.858 (0.13)
f_u (%)[b]	0.533 (0.510, 0.557)	0.641 (0.602, 0.682)	0.520 (0.501, 0.539)	1.03 (0.66)	1.20 (<0.01)	1.23 (<0.01)

[a]The data represent the geometric mean (GM) with a 95% confidence interval (CI), except for T_{max}, which is the median with the interquartile range (IQR). P, pregnancy; P+EFV, pregnancy plus efavirenz-based antiretroviral therapy; NP, nonpregnant postpartum control group.
[b]f_u was calculated as $C_{unbound}/C_{total}$ with *n* = 268 for the NP group, 275 for the P alone group, and 237 for the P+EFV group.

$AUC_{0-24\ h}$/total $AUC_{0-24\ h}$ was 24% higher ($P < 0.01$) in the pregnant women who were receiving EFV-based ART, compared to those who were not receiving EFV-based ART.

Impact of pregnancy and EFV. To examine the combined impact of pregnancy and EFV on the unbound PQ exposure, we compared the exposure in HIV-positive pregnant women who were receiving EFV-based ART with that in HIV-negative postpartum women. Although the total PQ Cmax was reduced by 33% ($P < 0.01$) and the $AUC_{0-24\ h}$ was reduced by 31% ($P < 0.01$), the reduction of unbound PQ exposure was less and did not reach statistical significance: the unbound PQ Cmax was 19% lower ($P = 0.14$), and the $AUC_{0-24\ h}$ was 14% lower ($P = 0.13$). Accordingly, the f_u increased by 23%, and the ratio of unbound PQ $AUC_{0-24\ h}$ to total PQ $AUC_{0-24\ h}$ increased by 25%, partially compensating for the reduction in the total PQ exposure in the HIV-positive pregnant women who were receiving EFV-based ART. The concentration-time profiles of the unbound PQ and the total PQ are presented in Fig. 2.

DISCUSSION

Our team previously reported that both pregnancy and EFV independently reduced PQ exposure (9). However, that study considered the total PQ exposure, and it is the free drug that exerts pharmacological effects. Here, we evaluated the PK exposure of free PQ in the same cohort that we studied previously for the total PQ (i.e., 58 pregnant women and 30 postpartum women who were receiving DP for malaria prevention). Surprisingly, in contrast to some other drugs, we did not detect a significant difference in PQ f_u between pregnant and postpartum women, as the total and unbound PQ exposures both decreased to a similar magnitude. Considering the impact of EFV, the total PQ exposure was lower, but the unbound PQ exposure did not change in pregnant women who were receiving EFV-based ART, compared to pregnant women who were not receiving EFV-based ART, and this resulted from a significant increase in f_u. In summary, EFV, but not pregnancy, increased the fraction of unbound PQ.

Pregnancy is expected to increase the fraction of the unbound drug due to the reduced drug-protein binding during pregnancy, especially during the third trimester. Although we did not find that pregnancy altered the fraction of unbound PQ, pregnancy increases the f_u of lopinavir (17–19), darunavir (20), and phenytoin (21). In the case of phenytoin, dose adjustments based on the decrease of the total phenytoin concentration caused toxicity (22).

A major finding in this study was the significantly greater fraction of unbound PQ in pregnant women who were receiving concomitant EFV-based ART, compared to those who were not receiving EFV-based ART. The f_u was 20% greater than that observed in the pregnant women who were not receiving EFV-based ART and 23% greater than that observed in the postpartum women. Because of the increased f_u, the unbound PQ $AUC_{0-24\ h}$ in the pregnant women who were receiving EFV-based ART was the same as that observed in the pregnant women without EFV-based ART, despite an 18% lower

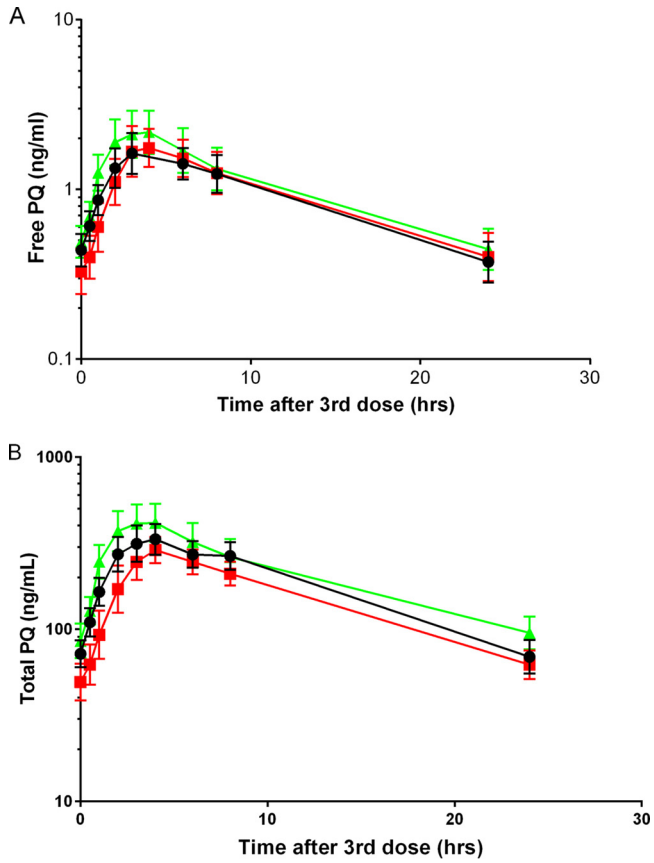

FIG 2 The concentration-time curve of unbound PQ (A) and total PQ (B) in HIV-positive pregnant women receiving concomitant EFV-based ART (red line), HIV-negative pregnant women (black line), and postpartum women (green line). The data represent the geometric mean (95% CI).

total PQ $AUC_{0-24\ h}$, and it decreased by only 14%, despite a 31% lower total PQ $AUC_{0-24\ h}$ compared to that observed in the postpartum women who were not receiving EFV-based ART. These results suggest the competitive displacement of PQ from plasma proteins by EFV. Like PQ, EFV is highly (approximately 99%) bound to plasma proteins (15, 16) so that competition for binding sites may release PQ into plasma, which thereby leads to an increase in PQ f_u. Displacement drug-drug interactions have been reported for other highly protein-binding drugs (e.g., a recent study reported that the f_u of dolutegravir in patients who were receiving concomitant valproic acid increased by 145% on day 14 after drug administration) (23).

Our study had two important limitations. First, the unbound PQ concentrations were only measured up to 24 h after the last dose of DP. Although the reduction of the total PQ exposure, as estimated via the $AUC_{0-21\ d}$ was 40% in pregnancy, 38% under EFV-based ART, and 62% in pregnant women under EFV-based ART (9), the total PQ exposure was reduced by a lower magnitude (<20%) for the first 24 h and did not reach statistical significance in the context of either pregnancy or EFV-based ART. Only the combined impact of pregnancy and EFV caused a significant reduction of the total PQ Cmax (−33%, $P < 0.01$) and $AUC_{0-24\ h}$ (−31%, $P < 0.01$) values. A future study will explore a new method by which to separate unbound PQ with only a 10 μL plasma sample volume, which will allow us to quantify the unbound PQ in capillary plasma samples, thereby enabling the study of unbound PQ across additional time points during the terminal phase of drug elimination, when a significant reduction of PQ concentrations is observed due to pregnancy and EFV, but the protective concentrations must be maintained. Another limitation was the larger variation of the unbound PQ measurement, compared to that of the total PQ measurement, which compromised statistical power in this study. Factors causing

variation in unbound drug measurements using ultrafiltration include nonspecific binding to filter devices, temperature, and pH (24). We used benzalkonium chloride (BAK)-treated filter devices to overcome nonspecific binding, and samples were incubated and centrifuged at 37°C to mimic physiological conditions, but we did not control the pH during the analysis, which might explain the larger variation that was observed in the spiked plasma analysis than that which was observed in the working solution analysis. Of note, the inter-individual coefficient of variation was 51 to 58% for the total PQ Cmax, 55 to 77% for the unbound PQ Cmax, and 28 to 44% for the f_u value (Tables S1–3).

In summary, this was the first study of unbound PQ PK exposure. The unbound PQ fraction remained largely the same in pregnant and postpartum women, but it was significantly greater in pregnant women who were receiving a concomitant administration of EFV-based ART. Since the chemopreventive efficacy of PQ relies on the maintenance of the minimum protective concentration, the clinical implications of the current findings remain to be determined. Further study of the unbound PQ at the terminal phase of PQ elimination is needed.

MATERIALS AND METHODS

Study population. This study was carried out between December of 2014 and March of 2016 in Tororo, Uganda. Eligible participants included (i) HIV-negative pregnant women (prior to 28 weeks gestation), (ii) HIV-negative postpartum women (at least 12 weeks postpartum), and (iii) HIV-positive pregnant women who were receiving EFV-based ART (prior to 28 weeks gestation) and were enrolled in clinical trials. The protocol details and results for the parent trials have been previously reported (9, 25, 26). The trials were conducted in accordance with the ethical standards of the responsible committee on human experimentation of Makerere University, the Uganda National Council of Science and Technology, and the University of California, San Francisco. The trials were registered at ClinicalTrials.gov (NCT02163447 and NCT02282293).

Study design. Consenting pregnant women underwent intensive PK procedures around their 28-week visit. A standard three-dose DP regimen (120 mg DHA and 960 mg PQ, Duo-Cotecxin, Holley-Cotec, once daily for 3 consecutive days with or without food) was administered in the clinic at the time of 28-week gestational visits for both groups of pregnant women. HIV-negative women were approached again for reenrollment, postpartum, and they were administered a single, standard three-dose DP regimen at least 12 weeks postpartum to provide nonpregnant control samples. The HIV-positive pregnant women who were enrolled in the intensive PK study were required to be receiving EFV-based ART that consisted of a standard single-tablet regimen of EFV (600 mg), tenofovir disoproxil fumarate (300 mg), and lamivudine (300 mg) once daily (Fig. 1).

Pharmacokinetic sample collection and analysis. Venous plasma samples were taken before (predose) and after the patients' last doses at 0.5, 1, 2, 3, 4, 6, 8, and 24 h. Finger stick capillary samples were collected at days 4, 7, 14, and 21 post-dose for total PQ measurement, but the unbound PQ concentrations were not determined for the capillary plasma samples due to their limited sample volumes.

Unbound PQ concentrations were determined using a previously reported method, with some modifications (27). We observed a decrease of concentration overnight if the PQ solutions were below 10 ng/mL. Therefore, the calibrators (0.02 to 5 ng/mL PQ) and the quality controls that were spiked in blank plasma filtrate, and the corresponding working solutions that were spiked in 10% acetonitrile 0.5% formic acid were all prepared freshly before the analysis of the samples. Samples above 5 ng/mL were repeated with the dilution of the sample filtrate by 4 to 10-fold. We found that the sample temperature and the delayed addition of samples in the BAK-treated filter devices caused variation in the PQ measurements. Therefore, plasma samples were added into the filter devices immediately after BAK-treatment, centrifuged at 37°C, and processed along with both spiked plasma and working PQ solutions as controls in batches of 12 to 24 devices. During the sample analysis, the precision (CV%) values of the quality controls that were spiked directly in blank plasma filtrate at 0.06, 0.6, and 4 ng/mL PQ were 13% ($n = 46$), 11% ($n = 44$), and 10% ($n = 42$), respectively. The precision values of the spiked solutions at 0.1 and 0.6 ng/mL PQ undergoing ultrafiltration were 12.8% ($n = 25$) and 10.7% ($n = 56$), respectively. The mean recovery values of the ultrafiltration of the spiked solutions at 0.1 and 0.6 ng/mL PQ were 94.5% ($n = 25$) and 89.5% ($n = 56$), respectively. These data demonstrated acceptable precision (<15%) and good recovery of ultrafiltration during the analysis of the samples. The CV% values from the spiked plasma samples at 80 and 800 ng/mL total PQ undergoing ultrafiltration were higher, being measured as 29.7% ($n = 48$) and 37.5% ($n = 48$), respectively.

Pharmacokinetic and statistical analyses. A noncompartmental analysis was carried out using Phoenix WinNonlin version 8.3.1 (Certara, Princeton, NJ, USA) via the linear up-log down trapezoidal rule. The PK parameters for the unbound PQ included the area-under-the-plasma concentration versus time curve to 24 h ($AUC_{0-24\ h}$), maximal concentration (Cmax), time to Cmax (T_{max}), and fractions of the unbound PQ concentrations (f_u). As the prior PK parameters for the total drug were based on the PQ concentrations from 0 to 21 days, a recalculation of the total drug exposure was made to allow for the comparison to the unbound PQ exposure, using the same PK time range (0 to 24 h after the third dose) (9). In addition, to pair samples fully with all free PQ sample measurements, the total PQ concentrations were treated as missing data if the unbound PQ concentrations were not available due to insufficient or missing samples. The f_u value was calculated using the unbound PQ concentration divided by the corresponding total PQ concentration at each time

point: $f_u = C_{unbound}/C_{total}$. The concentration-time curve was plotted using GraphPad Prism 6 (GraphPad software, San Diego, CA, USA).

Stata version SE14.1 was used for the statistical analyses. The sample size was determined in the total PQ PK study in the parent trials (9). At least 24 subjects on DP for each study group were required to detect a difference in the mean AUC between groups of 29.5% with 80% power and a significance level (α) of 0.05, using a two-sided, two-sample *t* test, based on an observed coefficient of variation for the total PQ AUC from our own studies (approximately 35%). For the PK parameters, the Wilcoxon rank-sum test (or signed-rank test for the paired analysis) was used. The data are presented as geometric means (GM) or medians, as appropriate.

Data availability. The data that were used in this study, including the individual concentrations and the PK parameters, are provided in the supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

We thank Florence Marzan and Lisa Wong at the Drug Research Unit for their administrative support. We also thank the staff of the Infectious Disease Research Collaboration and the women in Tororo who participated in all of the study procedures. This work was funded by the National Institutes of Health (grant numbers R21 AI153848 [to L.H.], R01 AI117001 [to P.J.R. and F.T.A.], and P01 HD059454 [to G.D.]). We thank Fei Jiang for the statistical consultant service, which was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI (grant number UL1 TR001872).

REFERENCES

1. WHO. 2021. World malaria report. WHO. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021.
2. Steketee RW, Nahlen BL, Parise ME, Menendez C. 2001. The burden of malaria in pregnancy in malaria-endemic areas. Am J Trop Med Hyg 64:28–35. https://doi.org/10.4269/ajtmh.2001.64.28.
3. Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC. 2014. Estimated risk of placental infection and low birthweight attributable to Plasmodium falciparum malaria in Africa in 2010: a modelling study. Lancet Glob Health 2:e460-7–e467. https://doi.org/10.1016/S2214-109X(14)70256-6.
4. WHO. 2014. WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP). https://www.who.int/publications/i/item/WHO-HTM-GMP-2014.4.
5. Walker PG, Floyd J, Ter Kuile F, Cairns M. 2017. Estimated impact on birth weight of scaling up intermittent preventive treatment of malaria in pregnancy given sulphadoxine-pyrimethamine resistance in Africa: a mathematical model. PLoS Med 14:e1002243. https://doi.org/10.1371/journal.pmed.1002243.
6. Kajubi R, Ochieng T, Kakuru A, Jagannathan P, Nakalembe M, Ruel T, Opira B, Ochokoru H, Ategeka J, Nayebare P, Clark TD, Havlir DV, Kamya MR, Dorsey G. 2019. Monthly sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in pregnancy: a double-blind, randomised, controlled, superiority trial. Lancet 393:1428–1439. https://doi.org/10.1016/S0140-6736(18)32224-4.
7. Briggs J, Ategeka J, Kajubi R, Ochieng T, Kakuru A, Ssemanda C, Wasswa R, Jagannathan P, Greenhouse B, Rodriguez-Barraquer I, Kamya M, Dorsey G. 2019. Impact of microscopic and submicroscopic parasitemia during pregnancy on placental malaria in a high-transmission setting in Uganda. J Infect Dis 220:457–466. https://doi.org/10.1093/infdis/jiz130.
8. Savic RM, Jagannathan P, Kajubi R, Huang L, Zhang N, Were M, Kakuru A, Muhindo MK, Mwebaza N, Wallender E, Clark TD, Opira B, Kamya M, Havlir DV, Rosenthal PJ, Dorsey G, Aweeka FT. 2018. Intermittent preventive treatment for malaria in pregnancy: optimization of target concentrations of dihydroartemisinin-piperaquine. Clin Infect Dis 67:1079–1088. https://doi.org/10.1093/cid/ciy218.
9. Kajubi R, Huang L, Jagannathan P, Chamankhah N, Were M, Ruel T, Koss CA, Kakuru A, Mwebaza N, Kamya M, Havlir D, Dorsey G, Rosenthal PJ, Aweeka FT. 2017. Antiretroviral therapy with efavirenz accentuates pregnancy-associated reduction of dihydroartemisinin-piperaquine exposure during malaria chemoprevention. Clin Pharmacol Ther 102:520–528. https://doi.org/10.1002/cpt.664.
10. Wallender E, Vucicevic K, Jagannathan P, Huang L, Natureeba P, Kakuru A, Muhindo M, Nakalembe M, Havlir D, Kamya M, Aweeka F, Dorsey G, Rosenthal PJ, Savic RM. 2018. Predicting optimal dihydroartemisinin-piperaquine regimens to prevent malaria during pregnancy for human immunodeficiency virus-infected women receiving efavirenz. J Infect Dis 217:964–972. https://doi.org/10.1093/infdis/jix660.
11. Benjamin JM, Moore BR, Salman S, Page-Sharp M, Tawat S, Yadi G, Lorry L, Siba PM, Batty KT, Robinson LJ, Mueller I, Davis TM. 2015. Population pharmacokinetics, tolerability, and safety of dihydroartemisinin-piperaquine and sulfadoxine-pyrimethamine-piperaquine in pregnant and nonpregnant Papua New Guinean women. Antimicrob Agents Chemother 59:4260–4271. https://doi.org/10.1128/AAC.00326-15.
12. Moore BR, Benjamin JM, Auyeung SO, Salman S, Yadi G, Griffin S, Page-Sharp M, Batty KT, Siba PM, Mueller I, Rogerson SJ, Davis TM. 2016. Safety, tolerability and pharmacokinetic properties of coadministered azithromycin and piperaquine in pregnant Papua New Guinean women. Br J Clin Pharmacol 82:199–212. https://doi.org/10.1111/bcp.12910.
13. Celestin MN, Musteata FM. 2021. Impact of changes in free concentrations and drug-protein binding on drug dosing regimens in special populations and disease states. J Pharm Sci 110:3331–3344. https://doi.org/10.1016/j.xphs.2021.05.018.
14. EMA. 2011. Eurartesim assessment report. https://www.ema.europa.eu/documents/assessment-report/eurartesim-epar-public-assessment-report_en.pdf. Accessed 6 February 2023.
15. Boffito M, Back DJ, Blaschke TF, Rowland M, Bertz RJ, Gerber JG, Miller V. 2003. Protein binding in antiretroviral therapies. AIDS Res Hum Retroviruses 19:825–835. https://doi.org/10.1089/088922203769232629.
16. Avery LB, Sacktor N, McArthur JC, Hendrix CW. 2013. Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent: applying the law of mass action to predict protein-free drug concentration. Antimicrob Agents Chemother 57:1409–1414. https://doi.org/10.1128/AAC.02329-12.
17. Aweeka FT, Stek A, Best BM, Hu C, Holland D, Hermes A, Burchett SK, Read J, Mirochnick M, Capparelli EV, International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) P1026s Protocol Team. 2010. Lopinavir protein binding in HIV-1-infected pregnant women. HIV Med 11:232–238. https://doi.org/10.1111/j.1468-1293.2009.00767.x.
18. Fayet-Mello A, Buclin T, Guignard N, Cruchon S, Cavassini M, Grawe C, Gremlich E, Popp KA, Schmid F, Eap CB, Telenti A, Biollaz J, Decosterd LA, Martinez de Tejada B, The Swiss HIV Cohort Study and The Mother & Child HIV Cohort Study. 2013. Free and total plasma levels of lopinavir during

pregnancy, at delivery and postpartum: implications for dosage adjustments in pregnant women. Antivir Ther 18:171–182. https://doi.org/10.3851/IMP2328.
19. Patterson KB, Dumond JB, Prince HA, Jenkins AJ, Scarsi KK, Wang R, Malone S, Hudgens MG, Kashuba AD. 2013. Protein binding of lopinavir and ritonavir during 4 phases of pregnancy: implications for treatment guidelines. J Acquir Immune Defic Syndr 63:51–58. https://doi.org/10.1097/QAI.0b013e31827fd47e.
20. Schalkwijk S, Ter Heine R, Colbers A, Capparelli E, Best BM, Cressey TR, Greupink R, Russel FGM, Molto J, Mirochnick M, Karlsson MO, Burger DM. 2019. Evaluating darunavir/ritonavir dosing regimens for HIV-positive pregnant women using semi-mechanistic pharmacokinetic modelling. J Antimicrob Chemother 74:1348–1356. https://doi.org/10.1093/jac/dky567.
21. Tomson T, Lindbom U, Ekqvist B, Sundqvist A. 1994. Epilepsy and pregnancy - a prospective-study of seizure control in relation to free and total plasma-concentrations of carbamazepine and phenytoin. Epilepsia 35:122–130. https://doi.org/10.1111/j.1528-1157.1994.tb02921.x.
22. Imam SH, Landry K, Kaul V, Gambhir H, John D, Kloss B. 2014. Free phenytoin toxicity. Am J Emerg Med 32:1301.e3–1301.e4. https://doi.org/10.1016/j.ajem.2014.03.036.
23. Bollen PDJ, Prins HAB, Colbers A, Velthoven-Graafland K, Rijnders BJA, de Vries-Sluijs T, van Nood E, Nouwen J, Bax H, de Mendonca Melo M, Verbon A, Burger DM, Rokx C. 2021. The dolutegravir/valproic acid drug-drug interaction is primarily based on protein displacement. J Antimicrob Chemother 76:1273–1276. https://doi.org/10.1093/jac/dkab021.
24. Toma CM, Imre S, Vari CE, Muntean DL, Tero-Vescan A. 2021. Ultrafiltration method for plasma protein binding studies and its limitations. Processes 9:382. https://doi.org/10.3390/pr9020382.
25. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B, Olwoch P, Ategeka J, Nayebare P, Clark TD, Feeney ME, Charlebois ED, Rizzuto G, Muehlenbachs A, Havlir DV, Kamya MR, Dorsey G. 2016. Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy. N Engl J Med 374:928–939. https://doi.org/10.1056/NEJMoa1509150.
26. Natureeba P, Kakuru A, Muhindo M, Ochieng T, Ategeka J, Koss CA, Plenty A, Charlebois ED, Clark TD, Nzarubara B, Nakalembe M, Cohan D, Rizzuto G, Muehlenbachs A, Ruel T, Jagannathan P, Havlir DV, Kamya MR, Dorsey G. 2017. Intermittent preventive treatment with dihydroartemisinin-piperaquine for the prevention of malaria among HIV-infected pregnant women. J Infectious Diseases 216:29–35. https://doi.org/10.1093/infdis/jix110.
27. Huang L, Sok V, Aslam-Mir U, Marzan F, Whalen M, Rosenthal PJ, Aweeka F. 2022. Determination of unbound piperaquine in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry. J Chromatogr Open 2:100042. https://doi.org/10.1016/j.jcoa.2022.100042.