Published in final edited form as:

Clin Pharmacol Ther. 2017 September ; 102(3): 520–528. doi:10.1002/cpt.664.

Antiretroviral therapy with efavirenz accentuates pregnancy-associated reduction of dihydroartemisinin-piperaquine exposure during malaria chemoprevention

Richard Kajubi^{1,2,†}, Liusheng Huang^{3,†}, Prasanna Jagannathan⁴, Nona Chamankhah³, Moses Were¹, Theodore. Ruel⁵, Catherine A. Koss⁴, Abel Kakuru¹, Norah Mwebaza^{1,2}, Moses Kanya¹, Diane Havlir⁴, Grant Dorsey⁴, Philip J. Rosenthal⁴, and Francesca T. Aweeka^{3,*}

¹Infectious Disease Research Collaboration, Makerere University College of Health Sciences- all in Kampala, Uganda ²Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences- all in Kampala, Uganda ³Department of Clinical Pharmacy, University of California, San Francisco, San Francisco General Hospital, San Francisco, CA, USA ⁴Department of Medicine, University of California, San Francisco, San Francisco General Hospital, San Francisco, CA, USA ⁵Department of Pediatrics, University of California, San Francisco, San Francisco General Hospital, San Francisco, CA, USA

Abstract

Dihydroartemisinin (DHA)-piperaquine is promising for malaria chemoprevention in pregnancy. We assessed impacts of pregnancy and efavirenz-based antiretroviral therapy on exposure to DHA and piperaquine in pregnant Ugandan women. Intensive sampling was performed at 28 weeks gestation in 31 HIV-uninfected pregnant women, in 27 HIV-infected pregnant women receiving efavirenz, and in 30 HIV-uninfected non-pregnant women. DHA peak concentration and area under the concentration time curve (AUC_{0–8hr}) were 50% and 47% lower, respectively, and piperaquine AUC_{0–21d} was 40% lower in pregnant women compared to non-pregnant women. DHA AUC_{0–8hr} and piperaquine AUC_{0–21d} were 27% and 38% lower, respectively in pregnant women receiving efavirenz compared to HIV-uninfected pregnant women. Exposure to DHA and piperaquine were lower among pregnant women and particularly in women on efavirenz, suggesting a need for dose modifications. The study of modified dosing strategies for these populations is urgently needed.

*Corresponding author: Francesca Aweeka, Pharm.D. Professor of Pharmacy, University of California, San Francisco, 1001 Potrero Avenue, San Francisco General Hospital, Building 100, room 157, San Francisco, CA 94110; phone: 415 476-0339; fax: 415 476-0307; fran.aweka@ucsf.edu.

†contributed equally to this work

CONFLICT OF INTEREST

The authors have no conflicts to disclose

AUTHOR CONTRIBUTIONS

L.H., P.J., G.D., P.J.R., and F.A. wrote the manuscript; N.W., M.K., D.H., G.D., P.J.R., and F.A. designed the research; R.K., L.H., M.W., T. R., C. K., A.K., and N.C. performed the research; L.H., P.J., N.C., and F.A. analyzed the data.

Keywords

malaria; HIV; antimalarial; antiretroviral; pharmacokinetics; DHA-piperaquine; artemisinin combination therapy; drug-drug interaction; IPTp; chemoprevention

BACKGROUND

The burdens of malaria and HIV infection are large and overlapping in sub-Saharan Africa. The World Health Organization (WHO) estimated 214 million cases of malaria in 2015 worldwide, resulting in 438,000 deaths, with 90% in sub-Saharan Africa(1). Sub-Saharan Africa is also home to 25 million people living with HIV(2).

Pregnant women represent one of the most vulnerable populations for malaria, with up to 41% reported to have evidence of placental malaria in regions of sub-Saharan Africa,(3) an estimate consistent with recent findings from our group in Uganda(4). Malaria during pregnancy is estimated to cause low birth weight in up to 20% of deliveries and more than 100,000 infant deaths annually.(5, 6) To reduce the burden of malaria, intermittent preventive therapy during pregnancy (IPTp), in which standard treatment doses of antimalarials are given intermittently, is endorsed for malaria-endemic regions of Africa by the WHO(7). Sulfadoxine-pyrimethamine (SP) is the standard of care for IPTp, but its efficacy is limited across much of Africa due to drug resistance(8), leading to study of artemisinin-based combination therapies (ACTs), which are first line malaria treatments, as alternative IPTp regimens. The ACTs combine a short-acting artemisinin that rapidly reduces parasite load with a long-acting partner drug that eradicates parasites and prevents new infection. The ACT favored for IPTp is dihydroartemisinin (DHA)-piperaquine, as this regimen benefits from the long (~3 We recently found that the burden of malaria in pregnancy was significantly lower among women who received IPTp with DHA-piperaquine compared to those who received SP(4, 10). To date, no pharmacokinetic data have been available for DHA-piperaquine when used as IPTp during pregnancy.

It is estimated that ~10% of pregnant African women are infected with HIV(11), and therefore require management with antiretroviral therapy (ART). Efavirenz (EFV)-based ART is recommended as first line ART by the WHO, including during pregnancy (12, 13). However, no information is available regarding impacts of ART on DHA-piperaquine exposure when used as chemoprevention during pregnancy.

DHA is metabolized by UDP-glucuronosyltransferase (UGT)(14). Piperaquine is metabolized by cytochrome p450 (CYP) isoenzymes, including CYP3A4/2C8.(15) Both pregnancy and EFV-based ART can cause induction of CYP isoenzymes and potentially UGT(16, 17). Although the pharmacokinetics of DHA-piperaquine as treatment for malaria has been evaluated in pregnancy, reports are conflicting(18–21) and pharmacokinetics may differ when antimalarial drugs are used to prevent malaria rather than to treat symptomatic disease(22). No studies are published on DHA-piperaquine-ART drug interactions during pregnancy, although clinically relevant changes are anticipated, given our prior research showing highly significant alterations in artemether, DHA, and lumefantrine exposure when artemether-lumefantrine, another ACT, is co-administered with ART in children (23, 24). An

appreciation of impacts of pregnancy and co-administered drugs on the exposure of DHA and piperaquine is needed in pregnant women to best design appropriately dosed IPTp interventions. We therefore performed an intensive pharmacokinetic study, in the context of two IPTp clinical trials in Uganda, that was aimed at delineating the impact of pregnancy and EFV-based ART on DHA-piperaquine pharmacokinetics in pregnant women.

RESULTS

Study profile

Screening and enrollment of study subjects from the parent trials is summarized in Figure 1 and baseline characteristics of those included in PK analyses are in Table 1. For pregnant women weights were comparable, but HIV-infected women were older ($p=0.001$). Non-pregnant women had lower weights ($p<0.01$) and higher hemoglobin levels ($p<0.001$) compared to pregnant women (Table 1). Non-pregnant women underwent intensive PK assessments at a median time of 39 weeks post-partum.

Capillary and venous concentration correlation

For pregnant women ($n=57$), the capillary plasma piperaquine median concentration (range) at 24hr post dose was 80.7 (16.7, 196) ng/mL, while the corresponding venous concentration was 72.2 (9.67, 200) ng/mL. For non-pregnant women ($n=30$), the capillary versus venous plasma piperaquine median concentration (range) at 24hr post dose was 115 (26.4, 292) ng/mL versus 104 (24.8, 251) ng/mL. The correlation equation determined from simultaneous venous and capillary plasma piperaquine measurements 24 hours post-dose for pregnant women was $\ln C_{\text{cap}} = 0.673 * \ln C_{\text{venous}} + 1.574$ ($n=57$), $r^2=0.705$, and for non-pregnant women was $\ln C_{\text{cap}} = 0.975 * \ln C_{\text{venous}} + 0.3201$ ($n=30$), $r^2=0.877$. Conversions were used for all capillary values to estimate the piperaquine AUC_{0-21d} .

Pharmacokinetics of DHA

Impact of pregnancy—To assess the impact of pregnancy on the pharmacokinetics of DHA, drug exposure in 31 HIV-uninfected pregnant women was compared to that in 29 HIV-uninfected non-pregnant women (Table 2; Figure 2a). DHA exposure was lower during pregnancy, with the C_{max} and AUC_{0-8hr} 50% and 47% lower, respectively ($p<0.0001$ for both). Likewise, the half-life of DHA was 17% shorter ($p=0.001$) and the C_{8hr} terminal concentration 55% lower ($p<0.0001$) in pregnant women. By paired analysis (comparing the same women during and after pregnancy ($n=27$)), the impacts of pregnancy on each PK parameter was nearly identical to that estimated by unpaired analysis (Table 2).

Impact of EFV-based ART during pregnancy—To assess the impact of EFV-based ART on the pharmacokinetics of DHA, drug exposure in 27 HIV-infected pregnant women receiving EFV-based ART was compared to that in 31 HIV-uninfected pregnant women. DHA exposure was lower in women receiving EFV-based ART, with the C_{max} and AUC_{0-8hr} 34% ($p=0.004$) and 27% ($p=0.009$) lower, respectively, than in pregnant women not on ART. In contrast, DHA half-life and C_{8hr} concentration were not significantly different between pregnant women receiving and not receiving ART.

Impact of pregnancy and EFV-based ART—We then estimated what the combined impact of pregnancy and EFV-based ART would be on DHA exposure, as we expected the impact of HIV disease itself to have minimal impact on DHA pharmacokinetics(25). Comparing HIV-infected pregnant women receiving EFV-based ART to HIV-uninfected non-pregnant women, all DHA parameters were lower in pregnant women, with the DHA C_{max} and AUC_{0-8hr} 67% ($p<0.0001$) and 61% ($p<0.0001$) lower, respectively, and the half-life and C_{8hr} concentration 21% ($p=0.0002$) and 60% ($p<0.0001$) lower, respectively. In addition, the time to reach C_{max} (T_{max}) was twice as long in HIV-infected pregnant women receiving EFV-based ART compared to HIV-uninfected non-pregnant women (2.0 vs. 1.0 hrs, $p=0.03$).

Pharmacokinetics of piperazine

Impact of pregnancy—To assess the impact of pregnancy on the pharmacokinetics of piperazine, drug exposure in 30 HIV-uninfected pregnant women was compared to that in 30 HIV-uninfected non-pregnant women (Table 3 and Figure 2B). Piperazine exposure was lower during pregnancy, with the AUC_{0-21d} reduced 40% ($p<0.0001$), a trend toward a lower C_{max} ($p=0.08$), and the half-life 23% shorter ($p=0.003$). By paired analysis (comparing the same women during and after pregnancy ($=27$)), the impacts of pregnancy on each PK parameter were again nearly identical to that estimated by unpaired analysis. In addition, terminal concentrations of piperazine on days 7, 14 and 21 were 17% to 34% lower during pregnancy using either unpaired or paired comparisons (Table 3).

Impact of EFV-based ART during pregnancy—To assess the impact of EFV-based ART on the pharmacokinetics of piperazine, exposure in 26 HIV-infected pregnant women receiving EFV-based ART was compared with that in 30 HIV-uninfected pregnant women not receiving ART. Piperazine exposure was lower in women receiving EFV-based ART, with the AUC_{0-21d} 38% lower ($p=0.0001$); however, there was no significant difference in piperazine C_{max} . Terminal concentrations of piperazine on days 7, 14 and 21 were 50% to 68% lower ($p<0.0001$ for all) and the piperazine half-life was 23% shorter ($p=0.01$) in those receiving EFV-based ART.

Impact of pregnancy and EFV-based ART—We then estimated what the combined impact of both pregnancy and EFV-based ART would be on piperazine exposure, again assuming minimal impact of HIV infection(25). Comparing HIV-infected pregnant women receiving EFV-based ART to HIV-uninfected non-pregnant women, all piperazine parameters (except T_{max}) were lower in HIV-infected pregnant women receiving EFV, with the C_{max} and AUC_{0-21d} 31% ($p=0.001$) and 62% ($p<0.0001$) lower, respectively, and the half-life 40% shorter ($p<0.0001$). Notably, terminal concentrations of piperazine on Days 7, 14 and 21 were 61%, 70% and 74% lower, respectively ($p<0.0001$ for all comparisons).

Correlation of piperazine terminal concentrations and AUC

Measurements of piperazine at 7, 14 or 21 days post-treatment may be used to monitor piperazine concentrations during large field studies, since evaluating the AUC is not practical. Therefore, we sought to determine if terminal concentrations were predictive of overall piperazine exposure by investigating associations between piperazine AUC_{0-21d}

and terminal concentrations. Piperazine day 7, 14, and 21 concentrations were highly correlated with AUC_{0-21d} (Pearson $r = 0.84, 0.81, 0.68$, respectively, all p values <0.0001).

Electrocardiographic findings

No clinical adverse events consistent with cardiotoxicity occurred during the course of the study. All pre-treatment and post-treatment QTc intervals were 450 msec by Fridericia's method. By Bazett's method, all pretreatment and 91% of post-treatment QTc intervals were 450 msec, with 7/80 post-treatment QTcB intervals 460 – 480 msec. Overall, there was a mean 17 msec increase in the QTc interval between baseline and 3–4 hours following the third dose using both correction methods ($p < 0.001$ when comparing pre- and post-treatment QTc intervals for both correction methods). There was no significant relationship between the change in QTcF interval and total number of prior DHA-piperazine doses, or between pregnant HIV-infected, uninfected, or non-pregnant women (Supplementary Table). Correlation between piperazine exposure and QTcF prolongation was not significant (Figure 3).

DISCUSSION

DHA-piperazine is a promising regimen for IPTp. We performed an intensive analysis of exposure to both components of the regimen when used as IPTp, considering impacts of pregnancy and concomitant treatment with EFV-based ART. We demonstrated significantly lower DHA and piperazine exposure in HIV-uninfected women during pregnancy compared to non-pregnant women, and in HIV-infected pregnant women receiving concomitant EFV-based ART compared to HIV uninfected pregnant women. These results suggest that pregnant women, and HIV-infected pregnant women on EFV-based ART, may experience inadequate protection from IPTp with DHA-piperazine unless dosing regimens are modified to compensate for the observed PK changes. Correlations between drug exposure and clinical outcomes are currently under investigation.

The impact of pregnancy on exposure to many drugs has been widely documented, with both increases and reductions in exposure reported (16, 26–28). We found that pregnancy was associated with ~50% decreases in both the peak concentration and AUC of DHA, suggesting reduced absorption, increased distribution or increases in total body clearance of the drug. DHA is primarily metabolized by UGT(14) and pregnancy is associated with reduced exposure of other UGT substrates(16). We found that pregnancy reduced the AUC_{0-21d} of piperazine by ~40%, with no change in peak concentration but a decrease in half-life of ~20%. As piperazine is metabolized by CYP3A4/2C8(15), these changes are likely due to the induction of CYP3A4 during pregnancy, as reported for other drugs(26, 27).

Our results differed from prior studies evaluating the impact of pregnancy on DHA-piperazine exposure. To our knowledge prior studies were carried out in the context of treatment for acute malaria, which can result in changes in PK(22), rather than in generally healthy individuals receiving IPTp, as in our study. For DHA, two reports in Thai pregnant women conflicted(20, 21) with one reporting reduced absorption, as we report here(21), and a second study reporting no change in peak or overall exposure(20). For piperazine, studies in Sudanese and Thai pregnant women reported little change in total exposure or the C_{max}

during pregnancy, but they did identify a more rapid decline in concentration during pregnancy, as we also observed.(18–21)

Co-administration of drugs with EFV-based ART results in many clinically relevant interactions due to induction of CYP3A4(32). We recently reported a 3.4 fold reduction in exposure to DHA (the active metabolite of artemether), and a 2.1 fold reduction in exposure to lumefantrine in HIV-infected children receiving EFV-based ART compared to HIV-uninfected children not receiving ART after they were treated for malaria with artemether-lumefantrine. These changes impacted on the risk of recurrent malaria(23). To our knowledge, this is the first study to report on potential interactions between EFV-based ART and DHA-piperaquine in pregnancy. By comparing HIV-infected pregnant women receiving EFV-based ART to HIV-uninfected pregnant women, we controlled for the effects of pregnancy, and we observed significant reductions in exposure to DHA and piperaquine among women receiving EFV-based ART. These reductions are expected to reduce the protective efficacy of IPTp with DHA-piperaquine in EFV treated women.

Comparing our results for both groups of pregnant women to the only other PK study of DHA-piperaquine as IPT, involving Thai non-pregnant adults, piperaquine terminal concentrations on days 14 and 21 were consistently lower in Ugandan pregnant women. Specifically, compared to a median trough value of ~25 ng/mL (representing a capillary concentration corrected from a venous value of 18.8 ng/mL), estimated in Thai adults at the end of a monthly or every two month dosing interval(33), mean concentrations on days 14 and 21 ranged from 3.8 to 15 ng/mL in Ugandan pregnant women. These results provide further evidence that pregnancy and EFV-based ART reduce exposure to piperaquine.

Of interest, assuming minimal impact of HIV disease itself(25), the impact of pregnancy and EFV-based ART on DHA and piperaquine exposure was estimated to be greater than either effect alone, suggesting the UGT and CYP induction effects of pregnancy and EFV are additive. Differentiating the impact of pregnancy and EFV helps inform specific recommendations for both HIV-uninfected and EFV-treated pregnant women and indicates that each factor must be considered separately for other treatment guidelines, for example for tuberculosis(32),(34). Metabolic induction of CYP and potentially UGT can be impacted by other intrinsic mechanisms, such as induction of drug transporters including p-glycoprotein(35, 36). An interplay of multiple factors may have resulted in the distinct effects of pregnancy and EFV on exposure to DHA and piperaquine.

Target concentrations for DHA or piperaquine when used for IPTp are not well defined. Recent trials in Uganda(4) and Kenya(10) showed excellent preventive efficacy of monthly IPTp with DHA-piperaquine, and monthly dosing was superior to every two month dosing(4), indicating that higher and consistent PK exposure is advantageous. However, monthly dosing with DHA-piperaquine did not eliminate malaria risks, and outcomes may be improved if dosing is adjusted to compensate for impacts of pregnancy and concomitant EFV. In non-pregnant Thai adults receiving DHA-piperaquine as IPT, piperaquine levels >31 ng/mL at the end of a dosing interval were deemed necessary to confer protection (corresponding to ~40 ng/mL for capillary values as we report here)(33). For our HIV-

uninfected and HIV-infected pregnant women, mean day 21 levels were only 11.8 and 3.8 ng/mL, suggesting inadequate exposure to afford reliable protection against malaria.

Regarding DHA-piperaquine toxicity, the main concern is a dose-dependent QTc prolongation associated with piperaquine. In a randomized study of a compressed, two-day regimen of DHA-piperaquine for malaria prevention in Cambodian male adults, 4 of 47 individuals receiving the regimen developed QTcF prolongation of >500 msec, leading to premature study termination(37). In comparison to individuals receiving placebo, individuals receiving the compressed regimen had a mean increase in QTcF of 46 msec post-treatment; a change significantly correlated with piperaquine peak concentrations(37). In our study of pregnant and non-pregnant women, standard dosing of DHA-piperaquine was associated with a modest QTcF prolongation of 17 msec, and this prolongation was not associated with pregnancy status, use of EFV-based ART, or the number of previous courses taken. Although we did not observe a significant correlation between piperaquine exposure and QT prolongation, the lack of correlation may be due to the lower peak levels observed within this population in comparison to those seen in male Cambodian adults. Importantly, no QTcF prolongations >450 msec and/or clinically significant arrhythmias were observed.

This study had some limitations. HIV-uninfected pregnant women who received DHA-piperaquine either monthly or every other month were compared to HIV-infected pregnant women who received the drug monthly. This design was considered acceptable since the majority of piperaquine is eliminated within 30 days. Indeed, if we limited the comparison to women receiving monthly dosing only, results were nearly identical, with all changes remaining significant (data not shown). Another limitation was that our control group was restricted to post-partum women, who may differ from other non-pregnant adults. However, women were at least 34 weeks post-partum so the physiological changes of pregnancy should have fully subsided. Also, venous and capillary concentration measurements were combined for estimates using simultaneous measurements made at 24 hours post-dosing, and it is possible that correlation estimates differed at other time points during the dosing interval. Lastly, this substudy had inadequate power to address pharmacodynamic outcomes. Although women with placental malaria had lower piperaquine Cmax and AUC than women without placental malaria, these differences were not statistically significant in this small group of women (Data not shown). The relationship between drug exposure and clinical outcomes in pregnancy is currently under additional study.

In summary, both pregnancy and EFV-based ART led to significant decreases in exposure to both DHA and piperaquine in pregnant women receiving DHA-piperaquine as IPTp. The study of modified dosing strategies, such as more frequent dosing for DHA-piperaquine when given as IPTp for pregnant women, including those receiving EFV, is urgently needed.

METHODS

Study Area and Patients

This study was carried out between December, 2014 and March, 2016 in Tororo, Uganda, a region with historically high malaria transmission intensity(38). Eligible participants included a) *HIV-uninfected pregnant women* enrolled in a randomized controlled trial that

compared SP given every 8 weeks, DHA-piperaquine given every 8 weeks, and monthly DHA-piperaquine as IPTp for malaria during pregnancy; b) *HIV-infected pregnant women* on EFV-based ART enrolled in a similar randomized controlled trial that compared daily TS to daily TS plus monthly DHA-piperaquine, and c) *HIV-uninfected non-pregnant women*, studied when at least 12 weeks post-partum, who were preferentially enrolled if previously enrolled in group (a). Pregnant women were enrolled in the parent trials between 12 and 28 weeks gestation as confirmed by ultrasonography, with co-enrollment into the intensive pharmacokinetics (PK) study prior to 28 weeks gestation. Protocol details and results for the parent trial for HIV-uninfected pregnant women were reported previously(4).

The trial was funded by the National Institutes of Health (4P01HD059454-09 and R01AI117001; ClinicalTrials.gov number, NCT02163447). Procedures were 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.

Study Design

Consenting pregnant women from both longitudinal trials were enrolled into the intensive PK study prior to their 28 week study visit. All women underwent intensive PK procedures around their 28 week visit. HIV-uninfected women were approached again for reenrollment post-partum, to provide a control group of non-pregnant adults. Although unblinding for treatment group did not occur prior to enrollment into the PK study, only those randomized to a DHA-piperaquine regimen had results evaluated for the intensive PK study.

For pregnant women randomized to either every 8 week or monthly DHA-piperaquine, a standard dose (3 tablets [40 mg DHA and 320 mg piperaquine, 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. HIV-uninfected non-pregnant women were administered a single standard 3-dose DHA-piperaquine regimen at least 12 weeks post-partum. HIV-infected pregnant women enrolled in the intensive PK study were required to be receiving EFV-based ART, which consisted of standard single-tablet regimen of EFV (600 mg), tenofovir disoproxil fumarate (300 mg) and lamivudine (300 mg) once daily, with ART dosing in the morning.

For all study subjects, sampling for intensive PK occurred before and after the 3rd daily dose of DHA-piperaquine (considered day 2 of dosing). Venous samples were collected pre-dose (0hr), and 0.5, 1, 2, 3, 4, 6, 8, and 24 hours post-dose. Capillary samples were collected at 24 hours and 4, 7, 14, and 21 days post-dose for piperaquine measurements. Venous and capillary samples collected simultaneously 24 hours post-dose were used to establish correlations between capillary and venous piperaquine concentration results. Concentrations of DHA and piperaquine were determined using high performance liquid chromatography tandem mass spectrometry, as previously described(39, 40). For DHA, the calibration range was 0.5–200 ng/ml, the lower limit of quantification (LLOQ) was 0.5 ng/ml, and the coefficient of variation (CV%) was <10% for quality control (QC) concentrations. For piperaquine, the original method was modified to lower the calibration range to 0.5–50 ng/ml and a new method with a calibration range of 10–1000 ng/mL was developed; the

LLOQ was 0.5 ng/mL and the CV was <10% for QC concentrations. The primary outcome was plasma PK parameters for DHA and piperazine, which included the area-under-the-plasma concentration versus time curve to 8 hours for DHA ($AUC_{0-8\text{ hr}}$) and to 21 days for piperazine ($AUC_{0-21\text{d}}$), maximal concentration (C_{max}), time to C_{max} (T_{max}), and elimination half-life ($t_{1/2}$). Additionally terminal concentrations were determined, which included the concentration of DHA at 8 hours ($C_{8\text{hr}}$) and the concentrations of piperazine at days 7, 14 and 21 ($C_{7\text{d}}$, $C_{14\text{d}}$, $C_{21\text{d}}$). Non-compartmental analysis was carried out using WinNonlin® 6.4 (Certara L.P., Princeton, NJ, USA) via the linear up-log down trapezoidal rule. Results below the LLOQ were treated as missing data except for the pre-dose drug concentration, which was set at 0 if below LLOQ.

The correlation between capillary and venous plasma concentration results was evaluated by linear regression after natural log transformation of the data using STATA SE 12.1 (StataCorp, College Station, TX, USA). Capillary concentration results for piperazine were converted to predicted venous values using the resulting correlation equation for estimation of $AUC_{0-21\text{d}}$. $C_{7\text{d}}$, $C_{14\text{d}}$, $C_{21\text{d}}$ piperazine concentrations were reported as non-adjusted capillary concentrations.

Electrocardiogram monitoring

12 lead electrocardiograms (ECGs) were carried out prior to the first dose and 3 – 4 hours following the 3rd dose in all subjects for safety assessments. QT and RR intervals were measured manually using calipers and the corrected QT interval (QTc) calculated using

Fridericia's formula ($QTcF, \frac{QT}{\sqrt[3]{RR}}$) and Bazett's formula ($QTcB, \frac{QT}{\sqrt{RR}}$).

Statistical Analysis

STATA® version SE12.1 (StataCorp, College Station, TX, USA) was used for analyses. Using measures of observed mean AUC and standard deviations from our own studies, at least 24 subjects on active DHA-piperazine for each study group were required to detect a difference in mean AUC between groups of 29.5% for piperazine and 31% for DHA with 80% power and a significance level (alpha) of 0.05 using a two-sided two-sample t-test (coefficients of variation [CV] for AUC for piperazine=35% and DHA=38%). For PK parameters, Wilcoxon signed-rank test for paired analysis or rank sum test were used. Data were presented as geometric means (GM) or median as appropriate. For ECG analyses, pre-treatment and post-treatment comparisons of QTc intervals were performed using paired t-tests, and comparisons between groups were performed using unpaired t-tests. Correlations between changes in the QTcF interval and piperazine exposure were assessed using Spearman's correlation (R_s). Statistical significance was considered a two-sided adjusted p value <0.017, in respect to comparison between 3 groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported primarily by 4P01HD059454-09 and R01AI117001 funded by the National Institutes of Health (NIH). Additional support was provided by the UCSF Center for AIDS Research P30 AI022763. The investigators would like to thank staff of the Infectious Disease Research Collaboration (IDRC, based in Kampala, Uganda) including Catherine Tugaineyo and Bridget Nzarubara, as well as Tamara Clark and Melissa Conrad at UCSF. In addition, we would like to thank the research staff of Dr. Aweeka's laboratory at UCSF including Ms. Florence Marzan, Ms. Camilla Forsman, Mr. David Gingrich and Mr. Vincent Cheah for their excellent analytical work for all PK results. 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.

References

1. World Health Organization. World Malaria Report 2015. 2015. (<http://www.who.int/malaria/publications/world-malaria-report-2015/wmr2015-without-profiles.pdf?ua=1>, 2015)
2. World Health Organization. [Accessed August 16 2016] Number of people (all ages) living with HIV estimates by WHO region. 2015. <<http://apps.who.int/gho/data/view.main.22100WHO?>>
3. Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC. Estimated risk of placental infection and low birthweight attributable to *Plasmodium falciparum* malaria in Africa in 2010: a modelling study. *Lancet Glob Health*. 2014; 2:e460–7. [PubMed: 25103519]
4. Kakuru A, et al. Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. *N Engl J Med*. 2016; 374:928–39. [PubMed: 26962728]
5. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg*. 2001; 64:28–35.
6. Guyatt HL, Snow RW. The epidemiology and burden of *Plasmodium falciparum*-related anemia among pregnant women in sub-Saharan Africa. *Am J Trop Med Hyg*. 2001; 64:36–44. [PubMed: 11425176]
7. World Health Organization. updated WHO policy recommendations: intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP). 2012.
8. Naidoo I, Roper C. Drug resistance maps to guide intermittent preventive treatment of malaria in African infants. *Parasitology*. 2011; 138:1469–79. [PubMed: 21835078]
9. Tarning J, et al. Population pharmacokinetics of piperaquine after two different treatment regimens with dihydroartemisinin-piperaquine in patients with *Plasmodium falciparum* malaria in Thailand. *Antimicrob Agents Chemother*. 2008; 52:1052–61. [PubMed: 18180343]
10. Desai M, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. *Lancet*. 2015; 386:2507–19. [PubMed: 26429700]
11. Chaponda EB, Chandramohan D, Michelo C, Mharakurwa S, Chipeta J, Chico RM. High burden of malaria infection in pregnant women in a rural district of Zambia: a cross-sectional study. *Malar J*. 2015; 14:380. [PubMed: 26423819]
12. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. Geneva, Switzerland: 2013.
13. Tsague L, Abrams EJ. Commentary: Antiretroviral treatment for pregnant and breastfeeding women--the shifting paradigm. *AIDS (London, England)*. 2014; 28(Suppl 2):S119–21.
14. Ilett KF, et al. Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. *Drug Metab Dispos*. 2002; 30:1005–12. [PubMed: 12167566]
15. Lee TM, et al. In vitro metabolism of piperaquine is primarily mediated by CYP3A4. *Xenobiotica; the fate of foreign compounds in biological systems*. 2012; 42:1088–95. [PubMed: 22671777]
16. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy: how does drug disposition change during pregnancy and what are the mechanisms that cause such changes? *Drug Metab Dispos*. 2013; 41:256–62. [PubMed: 23328895]

17. Michaud V, et al. Induction of CYP2C19 and CYP3A activity following repeated administration of efavirenz in healthy volunteers. *Clinical pharmacology and therapeutics*. 2012; 91:475–82. [PubMed: 22318618]
18. Adam I, Tarning J, Lindegardh N, Mahgoub H, McGready R, Nosten F. Pharmacokinetics of piperazine in pregnant women in Sudan with uncomplicated Plasmodium falciparum malaria. *Am J Trop Med Hyg*. 2012; 87:35–40. [PubMed: 22764289]
19. Hoglund RM, et al. A population pharmacokinetic model of piperazine in pregnant and non-pregnant women with uncomplicated Plasmodium falciparum malaria in Sudan. *Malar J*. 2012; 11:398. [PubMed: 23190801]
20. Rijken MJ, et al. Pharmacokinetics of dihydroartemisinin and piperazine in pregnant and nonpregnant women with uncomplicated falciparum malaria. *Antimicrob Agents Chemother*. 2011; 55:5500–6. [PubMed: 21947392]
21. Tarning J, et al. Population pharmacokinetics of dihydroartemisinin and piperazine in pregnant and nonpregnant women with uncomplicated malaria. *Antimicrob Agents Chemother*. 2012; 56:1997–2007. [PubMed: 22252822]
22. Pukrittayakamee S, et al. A study of the factors affecting the metabolic clearance of quinine in malaria. *Eur J Clin Pharmacol*. 1997; 52:487–93. [PubMed: 9342585]
23. Parikh S, et al. Antiretroviral Choice for HIV Impacts Antimalarial Exposure and Treatment Outcomes in Ugandan Children. *Clin Infect Dis*. 2016; 63:414–22. [PubMed: 27143666]
24. Achan J, et al. Antiretroviral agents and prevention of malaria in HIV-infected Ugandan children. *N Engl J Med*. 2012; 367:2110–8. [PubMed: 23190222]
25. van Oosterhout JJ, et al. Pharmacokinetics of Antituberculosis Drugs in HIV-Positive and HIV-Negative Adults in Malawi. *Antimicrob Agents Chemother*. 2015; 59:6175–80. [PubMed: 26248378]
26. Dawes M, Chowienczyk PJ. Drugs in pregnancy. Pharmacokinetics in pregnancy. *Best Pract Res Clin Obstet Gynaecol*. 2001; 15:819–26. [PubMed: 11800526]
27. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. *Front Pharmacol*. 2014; 5:65. [PubMed: 24772083]
28. Anderson GD. Using pharmacokinetics to predict the effects of pregnancy and maternal-infant transfer of drugs during lactation. *Expert Opin Drug Metab Toxicol*. 2006; 2:947–60. [PubMed: 17125410]
29. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J*. 2003; 3:136–58. [PubMed: 12815363]
30. Stek AM, et al. Reduced lopinavir exposure during pregnancy. *AIDS (London, England)*. 2006; 20:1931–9.
31. HHS Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States. 2016
32. Dooley KE, Flexner C, Andrade AS. Drug interactions involving combination antiretroviral therapy and other anti-infective agents: repercussions for resource-limited countries. *J Infect Dis*. 2008; 198:948–61. [PubMed: 18713054]
33. Lwin KM, et al. Randomized, double-blind, placebo-controlled trial of monthly versus bimonthly dihydroartemisinin-piperazine chemoprevention in adults at high risk of malaria. *Antimicrob Agents Chemother*. 2012; 56:1571–7. [PubMed: 22252804]
34. Regazzi M, Carvalho AC, Villani P, Matteelli A. Treatment optimization in patients co-infected with HIV and Mycobacterium tuberculosis infections: focus on drug-drug interactions with rifamycins. *Clin Pharmacokinet*. 2014; 53:489–507. [PubMed: 24777631]
35. Sahasrabudhe V, Zhu T, Vaz A, Tse S. Drug metabolism and drug interactions: potential application to antituberculosis drugs. *J Infect Dis*. 2015; 211(Suppl 3):S107–14. [PubMed: 26009614]
36. Srinivas NR. Pharmacokinetic Interaction of Rifampicin with Oral Versus Intravenous Anticancer Drugs: Challenges, Dilemmas and Paradoxical Effects Due to Multiple Mechanisms. *Drugs in R&D*. 2016; 16:141–8. [PubMed: 27098526]

37. Manning J, et al. Randomized, double-blind, placebo-controlled clinical trial of a two-day regimen of dihydroartemisinin-piperaquine for malaria prevention halted for concern over prolonged corrected QT interval. *Antimicrob Agents Chemother*. 2014; 58:6056–67. [PubMed: 25092702]
38. Kanya MR, et al. Malaria transmission, infection, and disease at three sites with varied transmission intensity in Uganda: implications for malaria control. *Am J Trop Med Hyg*. 2015; 92:903–12. [PubMed: 25778501]
39. 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. [PubMed: 23795928]
40. Kjellin LL, Dorsey G, Rosenthal PJ, Aweeka F, Huang L. Determination of the antimalarial drug piperaquine in small volume pediatric plasma samples by LC-MS/MS. *Bioanalysis*. 2014; 6:3081–9. [PubMed: 25529877]

STUDY HIGHLIGHTS

What is the current knowledge on the topic?

DHA-piperaquine is a promising regimen for malaria chemoprevention for vulnerable populations, but the impact of pregnancy, and of EFV-based ART, on DHA-piperaquine exposure is not known.

What question did this study address?

This study addressed the impact of pregnancy and EFV-based ART on DHA-piperaquine exposure when used as intermittent preventative therapy (IPTp) during pregnancy.

What this study adds to our knowledge?

By comparing DHA and piperaquine exposure between HIV-uninfected non-pregnant, HIV-uninfected pregnant, and HIV-infected pregnant women on EFV-based ART, this study found that pregnancy and EFV-based ART both significantly reduced the exposure of DHA and piperaquine when used as IPTp.

How this might change clinical pharmacology or translational science

Our results suggest that DHA-piperaquine may be underdosed in target populations, and that the study of modified dosing strategies, including more frequent dosing for DHA-piperaquine when given as IPTp for pregnant women, and especially those receiving EFV, is urgently needed.

Figure 1.

Enrollment and completion of intensive PK studies from trials evaluating DHA-piperaquine as IPTp for malaria; DP denotes DHA-piperaquine, SP sulfadoxine-pyrimethamine, EFV-ART, efavirenz- based antiretroviral therapy, PK pharmacokinetics, DHA, dihydroartemisinin, PQ piperaquine

Figure 2.

Plasma concentration-time profile of DHA (DHA) (A) and piperazine (B) in HIV-uninfected pregnant women (black line) and HIV-infected pregnant women stabilized on EFV-based ART (red line) and HIV-uninfected postpartum women (green line). Data is represented as median (IQR).

Figure 3.
Correlation of changes in the QTcF interval and pharmacokinetic exposure of piperaquine.
AUC denotes area under the concentration versus time curve to 21 days.

Table 1

Baseline characteristics of study participants at time of PK study enrollment. Data represent median (range).

	At 28 weeks gestational age during pregnancy			Non-pregnant
	HIV-uninfected (no ART), n=31	HIV-infected (EFV), n=27	HIV-uninfected, n=30	
Age (yr)	23 (18, 31)	30 (18, 43) *	24 (19, 32)	
Weight (kg)	57.5 (45.2, 83.2)	57.6 (43.7, 72.8)	52.9 (38.5, 72.9) *	
Height (cm)	163 (150, 179)	163 (147, 176)	162 (148, 174)	
Hemoglobin (g/dL)	12.0 (10.3, 16.8)	11.6 (8.1, 19.2)	13.9 (11.8, 16.3) *	
Weeks post-partum			38.5 (34, 54)	

* There were significant differences in demographic parameters when compared to other two groups ($p < 0.01$, using Wilcoxon rank sum test). ART, antiretroviral therapy; EFV, efavirenz-based ART

Table 2

Impact of pregnancy and EFV-based ART on the pharmacokinetics of DHA

	During pregnancy		Non-pregnant		pregnant/non-pregnant (HIV-uninfected, no ART)	Ratio EFV/no ART (all pregnant)	EFV and pregnant/non-pregnant
	HIV-uninfected (no ART)	HIV-infected (EFV)	HIV-uninfected (no ART)	HIV-uninfected (no ART)			
	n=31	n=27	n=29***		Paired (n=27)	All subjects	
C _{max} (ng/mL)	181 (145, 226)	120 (99.8, 145)	363 (304, 432)		0.52 (0.0001)	0.50 (<0.0001)	0.33 (<0.0001)
T _{max} (hr)	1.07 (1.00, 2.00)	2.00 (1.02, 3.00)	1.03 (1.00, 1.98)		1.02 (0.47)	1.04 (0.33)	1.94 (0.03)
t _{1/2} , hr	1.24 (1.15, 1.33)	1.17 (1.08, 1.27)	1.49 (1.37, 1.62)*		0.84 (0.004)**	0.83 (0.001)	0.79 (0.0002)
AUC _{0-8hr} , hr-ng/mL	401 (327, 493)	292 (243, 352)	754 (662, 860)		0.54 (<0.0001)	0.53 (<0.0001)	0.39 (<0.0001)
C _{8hr} , ng/mL	3.37 (2.61, 4.35)	2.94 (2.22, 3.90)	7.45 (5.96, 9.31)		0.45 (0.0002)	0.45 (<0.0001)	0.40 (<0.0001)

Note: ART, antiretroviral therapy; EFV, efavirenz-based ART; C_{max}, maximal concentration, T_{max}, time to reach maximal concentration, t_{1/2}, drug elimination half life, AUC, area under concentration-time curve; C_{8hr} DHA concentration at 8 hours post 3rd dose. Data are presented as geometric mean (95% confidence interval) except for T_{max}, which is reported as median (interquartile range). P-value is calculated with Stata@ 12.1 using Wilcoxon rank sum test (or signed rank test for paired analysis) Significance level: α=0.017(0.05/3);

* n= 28.

**

n= 25. Paired represents same HIV-uninfected women enrolled during pregnancy and after pregnancy.

One subject excluded due to all samples <LLOQ.

Table 3
Impact of pregnancy and EFV-based ART on the pharmacokinetics of piperazine

	During pregnancy			Non-pregnant		Ratio	
	HIV-uninfected (no ART)	HIV-infected (EFV)		HIV-uninfected	pregnant/non-pregnant (HIV-uninfected, no ART)	EFV/no ART (all pregnant)	EFV and pregnant/non-pregnant
	n=30***	n=26‡	n=30		Paired (n=27)	All subjects	
C _{max} , ng/mL	391 (323, 474)	342 (285, 411)	499 (393, 633)		0.82 (0.12)	0.78 (0.08)	0.69 (0.001)
T _{max} , hr	3.11 (3.00, 4.03)	3.99 (2.03, 5.98)	3.06 (2.07, 4.03)		1.01 (0.91)	1.02 (0.62)	1.30 (0.64)
t _{1/2} , hr	161 (143, 183)	124 (104, 149)	208 (187, 232)*		0.79 (0.012)**	0.77 (0.003)	0.60 (<0.0001)
AUC ₀₋₂₄ , hr·µg/mL	10.6 (8.84, 12.7)	6.60 (5.57, 7.83)	17.6 (15.1, 20.7)		0.61 (0.0001)	0.60 (<0.0001)	0.38 (<0.0001)
C _{7d} , ng/mL	30.5 (25.9, 36.0)	15.1 (13.0, 17.6)	39.0 (32.3, 47.2)		0.79 (0.03)	0.78 (0.07)	0.39 (<0.0001)
C _{14d} , ng/mL	15.0 (12.4, 18.1)	6.67(5.44, 8.19)	22.6 (18.7, 27.3)		0.68 (0.004)	0.66 (0.007)	0.30 (<0.0001)
C _{21d} , ng/mL	11.8 (10.2, 13.6)	3.75 (2.77, 5.08)	14.5 (12.2, 17.1)		0.83 (0.05)	0.81 (0.016)	0.26 (<0.0001)

Note: ART, antiretroviral therapy; EFV, efavirenz-based ART; C_{max}, maximal concentration, T_{max}, time to reach maximal concentration, t_{1/2}, drug elimination half life, AUC, area under concentration-time curve, AUC was calculated using piperazine concentrations from venous plasma with conversion of capillary to venous plasma concentrations when necessary; C_{7d}, C_{14d}, and C_{21d} are actual capillary plasma concentrations at day 7, 14, and 21 post the 3rd dose. Data are presented as geometric mean (95% confidence interval) except for T_{max}, which is reported as median (interquartile range). P-value is calculated with Stata® 12.1 using Wilcoxon rank sum test (or signed rank test for paired analysis) Significance level: alpha=0.017(0.05/3);

* n=29.

** n=26. Paired represents same HIV-uninfected women enrolled antepartum and postpartum.

*** one subject excluded since missing day 21 PK sample.

‡ one subject excluded since missing day 14 and 21 samples