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

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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.

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CONFLICT OF INTEREST

The authors have no conflicts to disclose

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 $\ln C_{cap}=0.673*\ln C_{venous}+1.574$ (n=57), $r^2=0.705$, and for non-pregnant women was $\ln C_{cap}=0.975*\ln C_{venous}+0.3201$ (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 pharamacokinetics(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 piperaquine

Impact of pregnancy—To assess the impact of pregnancy on the pharmacokinetics of piperaquine, drug exposure in 30 HIV-uninfected pregnant women was compared to that in 30 HIV-uninfected non-pregnant women (Table 3 and Figure 2B). Piperaquine 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 piperaquine 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 piperaquine, exposure in 26 HIV-infected pregnant women receiving EFV-based ART was compared with that in 30 HIV-uninfected pregnant women not receiving ART. Piperaquine 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 piperaquine C_{max}. Terminal concentrations of piperaquine on days 7, 14 and 21 were 50% to 68% lower (p<0.0001 for all) and the piperaquine 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 piperaquine 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 piperaquine 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 piperaquine on Days 7, 14 and 21 were 61%, 70% and 74% lower, respectively (p<0.0001 for all comparisons).

Correlation of piperaquine terminal concentrations and AUC

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

and terminal concentrations. Piperaquine day 7, 14, and 21 concentrations were highly correlated with $AUC_{0–21\ d}$ (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-piperaquine doses, or between pregnant HIV-infected, uninfected, or non-pregnant women (Supplementary Table). Correlation between piperaquine exposure and QTcF prolongation was not significant (Figure 3).

DISCUSSION

DHA-piperaquine 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 piperaquine 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-piperaquine 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 piperaquine by ~40%, with no change in peak concentration but a decrease in half-life of ~20%. As piperaquine 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-piperaquine 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 piperaquine, 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 piperaquine, which included the area-under-the-plasma concentration versus time curve to 8 hours for DHA (AUC $_{0-8\ hr}$) and to 21 days for piperaquine (AUC$_{0-21d}$), 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$_{8hr}$) and the concentrations of piperaquine at days 7, 14 and 21 (C$_{7d}$, C$_{14d}$, C$_{21d}$). 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 piperaquine were converted to predicted venous values using the resulting correlation equation for estimation of AUC$_{0-21d}$. C$_{7d}$, C$_{14d}$, C$_{21d}$ piperaquine 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, $\frac{QT}{\sqrt[2]{RR}}$).

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-piperaquine for each study group were required to detect a difference in mean AUC between groups of 29.5% for piperaquine 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 piperaquine=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 piperaquine 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

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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 EFZ-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.
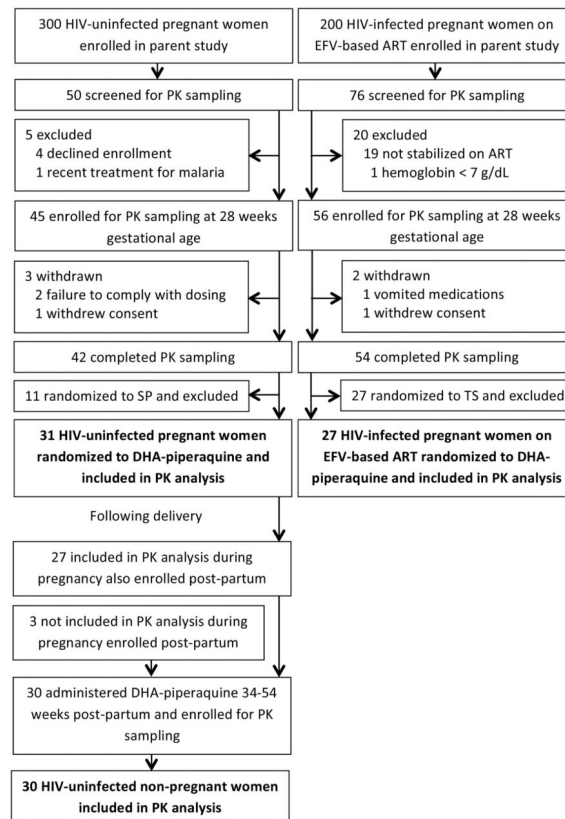
Figure 1.
Enrollment and completion of intensive PK studies from trials evaluating DHA-piperaquine as IPTp for malaria; DP denotes DHA-piperaquine, SP sulfadoxine-pyrimethamine, EFV-ART, efavirenz- based antiretroviral therapy, PK pharmacokinetics, DHA, dihydroartemisinin, PQ piperaquine
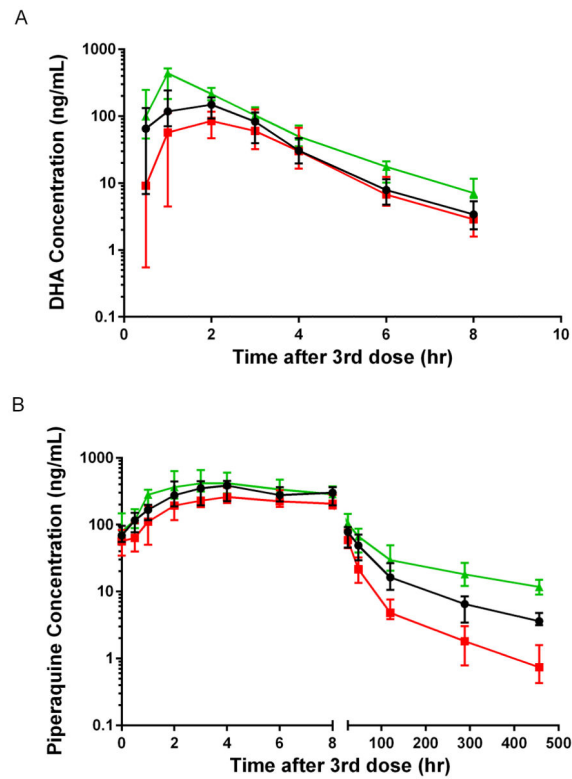
A

B

Figure 2.
Plasma concentration-time profile of DHA (DHA) (A) and piperaquine (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).
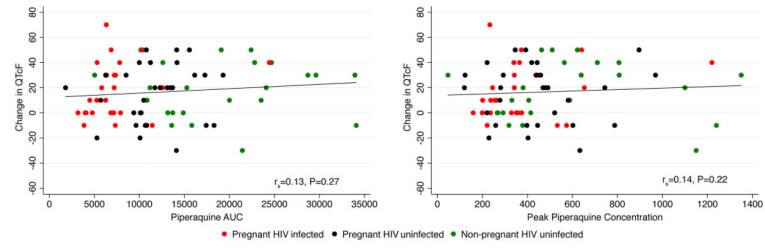
Figure 3.
Correlation of changes in the QTcF interval and pharmacokinetic exposure of piperaquine.
AUC denotes area under the concentration versus time curve to 21 days.

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	
	HIV-uninfected (no ART)	HIV-infected (EFV)	HIV-uninfected (no ART)			EFV/no ART (all pregnant)	EFV and pregnant/non-pregnant
				Paired (n=27)	All subjects		
	n=31	n=27	n=29***				
C_{max} (ng/mL)	181 (145, 226)	120 (99.8, 145)	363 (304, 432)	0.52 (0.0001)	0.50 (<0.0001)	0.66 (0.004)	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)	1.87 (0.09)	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)	0.94 (0.20)	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)	0.73 (0.009)	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)	0.87 (0.51)	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,; C8hr 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: $\alpha=0.017(0.05/3)$;

*
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 piperaquine

| | 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)	0.88 (0.13)	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)	1.28 (0.84)	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)	0.77 (0.01)	0.60 (<0.0001)
AUC_{0-d21}, 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)	0.62 (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)	0.50 (<0.0001)	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)	0.45 (<0.0001)	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)	0.32 (<0.0001)	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 piperaquine 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