Suboptimal Nevirapine Steady-State Pharmacokinetics During Intrapartum Compared With Postpartum in HIV-1–Seropositive Ugandan Women

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Background: Conflicting data exist regarding the effect of pregnancy on steady-state nevirapine pharmacokinetics (PK), although steady-state nevirapine concentrations during pregnancy have never been characterized in sub-Saharan Africa.

Methods: This was a longitudinal intensive PK study in Ugandan pregnant women receiving nevirapine-based antiretroviral therapy. Participants underwent intensive 12-hour PK sampling during the second trimester (T2; n = 4), third trimester (T3; n = 15) and 6 weeks postpartum (PP; n = 15). HIV-1 RNA was performed within 2 weeks of each visit. Nevirapine C12 above 3000 ng/mL was classified as optimal based on the suggested value for therapeutic drug monitoring.

Results: The pharmacokinetics of nevirapine were influenced by pregnancy, demonstrated by a 20% reduction in the maximum concentration, minimum concentration (C12), and area under the curve between T3 and PP visits ($P = 0.001$, $P = 0.011$ and $P = 0.005$, respectively). Ten subjects (66.7%) had C12 values <3000 ng/mL during T3. Of these participants, 7 partcipant's C12 concentrations increased to >3000 ng/mL during the PP visit. HIV-1 RNA were <1000 copies per milliliter at T3 and <400 copies per milliliter at PP in all patients.

Conclusions: Nevirapine exposure was reduced in Ugandan women during their third trimester compared with the same women PP, however, HIV RNA remained <1000 copies per milliliter. The long-term impact of intermittent suboptimal nevirapine concentrations during pregnancy is unknown.

Key Words: antiretroviral agents, HIV, pregnancy, pharmacokinetics, Sub-Saharan Africa

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BACKGROUND

Vertical transmission of HIV is a significant public health challenge in sub-Saharan Africa. In 2007, it was estimated that 1.8 million African children were infected with HIV, mostly acquired from their mothers during pregnancy, childbirth, and breastfeeding.[1] In an effort to prevent new infections, many African countries have adopted prevention strategies, including routine screening for HIV during pregnancy and provision of antiretroviral drugs to HIV-infected pregnant women.[2] One of the most widely used antiretroviral drugs in these countries is nevirapine (NVP), a nonnucleoside reverse transcriptase inhibitor used in prevention of mother to child transmission (PMTCT) programs because of its low cost and high potency against the HIV-1 virus.[3,4]

In developing countries, recommended options for PMTCT in women who do not qualify for combination antiretroviral therapy (ART) for their own disease (typically those with CD4 >250 cells/mm^3) have included the use of nucleoside reverse transcriptase inhibitors (NRTIs) during and after pregnancy, along with a single dose of NVP administered during labour.[5] However, due to concerns about the emergence of NVP-associated resistance in mothers and infected newborns and better potency of ART compared with NRTIs plus single-dose NVP, regimens containing single-dose NVP are gradually being replaced by NVP-based ART.[6–8]

After oral administration, NVP undergoes rapid absorption, wide distribution, and extensive metabolism by hepatic microsomal oxidases (predominantly by 3A4 and 2B6 isoforms of the cytochrome P450 enzyme system, CYP450).[9] Furthermore, NVP is an inducer of CYP450, and therefore, it enhances its own metabolism over time (autoinduction). Consequently,

NVP pharmacokinetics during chronic dosing differs from the pharmacokinetics after a single dose, demonstrated by a shorter elimination half-life.[10] In pregnancy, drug pharmacokinetics may be modified by changes in the rate and extent of absorption; alterations in plasma protein concentrations; increased volume of distribution; changes in elimination related to stimulation or inhibition of hepatic metabolism secondary to physiologic hormonal changes; and compartmentalization, and biotransformation and elimination of drugs by the fetus or placenta.[11–14]

During pregnancy, the steady-state pharmacokinetics of some antiretroviral drugs metabolized by CYP450 have been shown to be be altered, and dosage adjustments are required for some drugs, most notably the protease inhibitor class of drugs.[15–18] Understanding the impact of pregnancy on the pharmacokinetics of NVP is important to ensure adequate drug levels to suppress HIV replication and prevent transmission to the fetus, particularly in resource-limited settings where NVP is a cornerstone of PMTCT therapy. This study compares the steady-state pharmacokinetics of NVP during the second trimester and third trimesters of pregnancy to postpartum (PP) concentrations in HIV-1–seropositive Ugandan women on NVP-based ART regimens.

METHODS

This was an intensive 3-phase pharmacokinetic study using patients as their own controls. Ethical approval was granted by the National HIV/AIDS Research Committee (Approval number, ARC 060). The trial was registered with the Uganda National Council for Science and Technology and on www.clinicaltrials.gov (protocol ID: NCT00616252).

Study Subjects

HIV-1 antibody–seropositive pregnant women receiving ART for at least 1 month were recruited from 2 clinics within Mulago hospital complex (Adult Infectious Diseases Clinic and Mulago PMTCT Clinic). All participants provided written informed consent before screening. The consent was administered in English and one local language (Luganda). Pregnant women were suitable for enrollment if they were between 18 and 39 years of age and receiving NVP-based ART for a minimum of 1 month. Subjects were excluded if they were anemic (haemoglobin <9.0 g/dL), had significant impairment of renal function (serum creatinine >2.5 times the upper limit of normal) or hepatic function (serum transaminases >5 times the upper limits of normal). Subjects who used herbal medicines or any medication known to be inducers or inhibitors of CYP450 metabolism or who had significant intercurrent illness were also excluded.

Pharmacokinetic Sampling

Eligible study subjects were scheduled for pharmacokinetic evaluations at the private ward of Mulago hospital for a second trimester visit between weeks 20–24 of gestation (T2), third trimester visit between weeks 32–36 (T3), and after 6 weeks from their delivery date for a PP visit. Patients enrolled in their late second trimester or during the third trimester had only T3 and PP sampling. All patients received NVP 200 mg twice daily for a minimum of 2 weeks before their first pharmacokinetic sampling visit. Patients who did not complete the PP visit were excluded from pharmacokinetic analysis. A standardized breakfast was provided on the morning of each pharmacokinetic evaluation. Antiretroviral adherence assessments were conducted using structured questionnaires in English and Luganda, as appropriate. Dosing times in the 3 days before the pharmacokinetic sampling visit were recorded. NVP 200 mg, lamivudine 150 mg plus zidovudine 300 mg, or stavudine 30 mg were administered under direct observation on the sampling visit. Twelve hour pharmacokinetic sampling was conducted at the following time points: predose (0 hour), 1, 2, 3, 4, 6, 8, 10, and 12 hours postdose. Venous whole blood (4 mL) was collected into lithium heparin vacutainers and transported within 1 hour of collection to the Makerere University–Johns Hopkins University Core Research Laboratory, Kampala. Plasma samples were obtained by centrifugation at 5000 rpm for 10 minutes and stored in 2 mL cryovials at $-70°C$.

Safety Assessments

A complete blood count, serum electrolytes, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum bilirubin, and albumin were performed at screening and at each pharmacokinetic sampling visit. CD4 count and HIV-1 RNA test results were obtained within 2 weeks of the T2, T3, and PP pharmacokinetic sampling visits. Safety and tolerability of NVP-based ART was evaluated based on both physical examinations and laboratory test results and characterized using the US Division of AIDS table for grading the severity of adult and pediatric adverse events.[19]

Analytical and Pharmacokinetic Methods

NVP assays were performed at the Department of Pharmacology, University of Liverpool, using reversed-phase high-performance liquid chromatography with ultraviolet detection. The lower limit of quantification for NVP was 450 ng/mL.

Demographic data were entered into Epidata Entry version 2.0 (The Epidata Association, 34 DK 5230, Odense M, Denmark) and exported to STATA version 9.2 (Statacorp, 2006; College Station, TX) for analysis by noncompartmental methods. NVP plasma pharmacokinetic parameters [maximum plasma concentration, (C_{max}); time to C_{max}, T_{max}; and 12 hour concentration (C_{12})] were derived. A threshold of 3000 ng/mL was used to classify NVP C_{12} as optimal or suboptimal. This is the threshold proposed for therapeutic drug monitoring of NVP, and patients with concentrations below this value may be at increased risk for virologic failure.[20,21] Area under the plasma concentration–time curve from 0 to 12 hours ($AUC_{0–12}$) and elimination half-life ($t_{1/2}$) were calculated using STATA.

Intra-individual differences in NVP pharmacokinetic parameters (T2 or T3 pharmacokinetics versus PP pharmacokinetics) were evaluated by calculating geometric mean ratios (GMRs) and 90% confidence intervals (CIs). The CIs were determined using logarithms of the individual geometric mean values; the calculated values were then expressed as linear values. If both upper and lower 90% CI of the GMRs for NVP pharmacokinetic parameters were between 0.80 and 1.25, no difference was assumed.[22] The Wilcoxon signed-rank

test was performed to compare T2 or T3 pharmacokinetic parameters with corresponding PP values. A P value ≤ 0.05 was considered statistically significant. Interindividual and intra-individual variability of NVP concentrations in plasma was assessed by calculating the coefficient of variation (CV) using the formula CV = SD/arithmetic mean × 100.

RESULTS

Patients

Sixteen participants were enrolled, however, 1 did not attend her PP visit and was excluded from the pharmacokinetic analysis. Participants were of median age (interquartile range) 29 (27–32) years. Other participant characteristics are described in Table 1. Thirteen subjects were receiving zidovudine, lamivudine plus NVP, whereas 2 participants received stavudine, lamivudine plus NVP. One subject interrupted ART on her own initiative for 3 weeks after delivery but before her PP visit date, however, PP sampling was conducted 4 weeks after she restarted ART. No other participant reported nonadherence within 3 days of the pharmacokinetic sampling visits.

Thirteen participants were on cotrimoxazole prophylaxis, and 2 were on dapsone. Other concomitant medications used by study participants at baseline include folic acid,[4] supplemental iron,[4] paracetamol,[1] magnesium sulphate,[1] and multivitamin preparations.[1] Six patients were on ART for their own disease before pregnancy. Four of these patients were eligible for T2 pharmacokinetic sampling based on gestational age. Nine patients were commenced on NVP-based ART for PMTCT during the index pregnancy at a median (range) gestational age of 28 (17–35) weeks. For these patients, median (range) duration on ART before T3 sampling was 6 (4–16) weeks.

NVP Pharmacokinetics

NVP plasma concentration time curves during T2, T3, and PP are shown in Figure 1. A comparison of NVP steady-state pharmacokinetic parameters intrapartum versus PP is shown in Table 2. The T3 C_{12}, C_{max}, and AUC were lower than the corresponding parameters for PP [GMR and 90% CI: 0.79 (0.70 to 0.90), 0.80 (0.73 to 0.88), and 0.79 (0.72 to 0.88), respectively]. Three (75%) subjects had C_{12} <3000 ng/mL in T2. All 3 subjects had C_{12} hour concentrations that increased to >3000 ng/mL at the PP visit. Ten subjects (66.7%) had concentrations <3000 ng/mL in T3. Of these subjects, 7 had C_{12} hour concentrations that increased to >3000 ng/mL during the PP visit. Four subjects (26.7%) had C_{12} hour concentrations <3000 ng/mL in PP. The NVP C_{12} concentrations for individual patients during the 3 sampling occasions are illustrated in Figure 2.

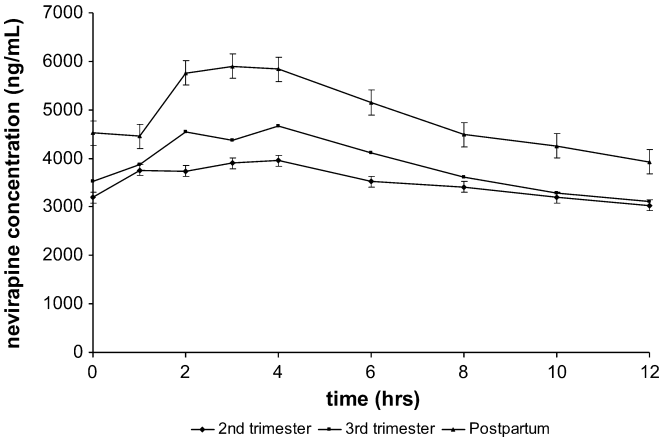

FIGURE 1. Nevirapine plasma concentration–time curve during pregnancy and postpartum.

Across the study participants, the mean CV for the C_{12} and AUC of NVP was 21%, 50%, and 39%; and 10%, 40%, and 30% in T2, T3, and PP, respectively. Intra-individual CV ranged from 2 to 48% for C_{12} and 1 to 42% for AUC.

Virologic and Safety Assessments

All the 16 enrolled participants were included in the safety analysis. All 4 participants who underwent T2 sampling and 2 who were enrolled during T3 had commenced NVP-based ART before the index pregnancy and had undetectable HIV-1 RNA measurements (<400 copies/mL). Of the remaining 10 participants who initiated ART during T3, HIV-1 RNA measured >400 copies per milliliter in 4 participants (range 417–533 copies/mL) during the T3 visit. Median (range) duration on NVP-based ART at the time of HIV RNA measurement was 4 (2–8) weeks for these participants. NVP C_{12} measured <3000 ng/mL in 2 of the 4 participants with detectable HIV RNA. All subjects' had HIV RNA <400 copies per milliliter during their PP visit.

There were no grade 2 or higher alanine aminotransferase or aspartate aminotransferase elevations during the study. Grade 3 or higher adverse events included 1 patient with transient neutropenia (grade 3), 1 intrauterine fetal death, and 1 congenital anomaly (pre-axial polydactyly). These events were considered unlikely to be related to the study medications.

TABLE 1. Characteristics of Subjects at Screening, in the Second and Third Trimesters, and PP

Variable	Second Trimester, n = 4	Third Trimester, n = 15	PP, n = 15
Weight (kg)	59 ± 7	61 ± 6	57 ± 6
Body mass index (kg/m^2)	23 ± 2	24 ± 3	23 ± 3
Hemoglobin (g/dL)	12 ± 1.2	12 ± 1.9	13 ± 0.9
CD4 count (cells/mm^3)	191 ± 43	297 ± 122	365 ± 142
HIV-1 RNA < 400	4/4 (100%)	11/15 (73%)	15/15 (100%)
ALT IU/L	12 ± 4	12 ± 5	22 ± 8
AST IU/L	21 ± 7	20 ± 5	26 ± 8

Age, 30 ± 4 years; ethnicity, black African (all participants).
Data presented as means ± SD and proportions (percentages).
ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

DISCUSSION

NVP pharmacokinetics were lower in Ugandan women during pregnancy compared with PP. Statistically significant reductions for C_{12}, C_{max}, and AUC were observed in late

TABLE 2. NVP Steady-State Pharmacokinetic Parameters

NVP PK Parameter (90% CI)	Second Trimester, GM (90% CI), (n = 4)	Third Trimester, GM (90% CI) (n = 15)	PP, GM (90% CI) (n = 15)	Second Trimester Versus PP, GMR (90% CI)	*P*	Third Trimester Versus PP, GMR (90% CI)	*P*
C_{12} (ng/mL)	3029 (2798 to 3350)	3117 (2679 to 4139)	3930 (3477 to 4861)	0.87 (0.74 to 1.02)	0.273	0.79* (0.70 to 0.90)	0.011
C_{max} (ng/mL)	4780 (4494 to 5165)	5251 (4661 to 6287)	6567 (5929 to 7688)	0.77 (0.67 to 0.88)	0.144	0.80* (0.73 to 0.88)	0.001
T_{max} (hr)	4.23 (3.52 to 6.47)	2.61 (2.37 to 4.43)	2.83 (2.59 to 3.27)	1.50 (0.98 to 2.27)	0.454	0.92 (0.67 to 1.27)	0.708
AUC_{0-12} ($ng \cdot hr^{-1} \cdot mL^{-1}$)	42,988 (41,267 to 45,052)	47,329 (41,574 to 58,891)	59,404 (53,597 to 69,582)	0.76 (0.70 to 0.83)	0.068	0.80* (0.72 to 0.88)	0.005

*Statistically significant (Wilcoxon signed-rank test).
C_{12}, NVP concentration 12 hours postdosing, GM, geometric mean, GMR, geometric mean ratio, PK, pharmacokinetics, T_{max}, time to C_{max}.

pregnancy. The 90% CIs of the GMRs for C_{max} and AUC did not fall within the 0.8–1.25 interval indicating a difference in NVP exposure between the pregnant and nonpregnant state. Our findings contrast with results of an analysis of pharmacokinetic data from 26 women who were enrolled in 2 separate studies in the United States.[23] In that analysis, NVP exposure and minimum concentrations were found to be similar intrapartum and PP, and no significant differences for AUC were observed. Unlike the present study, abbreviated pharmacokinetic sampling strategies were used with sampling performed over either 6 or 8 hours, and samples for C_{12} were not obtained.

Two cross-sectional studies, conducted on therapeutic drug monitoring samples of patients using NVP during pregnancy, have also reported significant differences in NVP concentrations between pregnant women and other nonpregnant patients.[24,25] Absolute NVP concentrations in our study were similar to those found by von Hentig et al[24] but seem lower than those reported by Nellen et al.[25] Nellen et al[25] concluded that patients of African origin were likely to have higher NVP concentrations and therefore maintain therapeutic concentrations despite pregnancy. This conclusion is not supported by our data and point out the importance of recognizing the pharmacogenomic differences seen between patients in different regions of Sub-Saharan Africa. In our study, most participants with subtherapeutic concentrations in late pregnancy subsequently attained therapeutic levels after delivery. Based on this observation, it is plausible that lower NVP concentrations during pregnancy reflect a physiologic effect of pregnancy on NVP pharmacokinetics. One possible explanation is increased activity of CYP450 during pregnancy resulting in enhanced NVP elimination.[11] However, other pregnancy-related factors may also contribute.

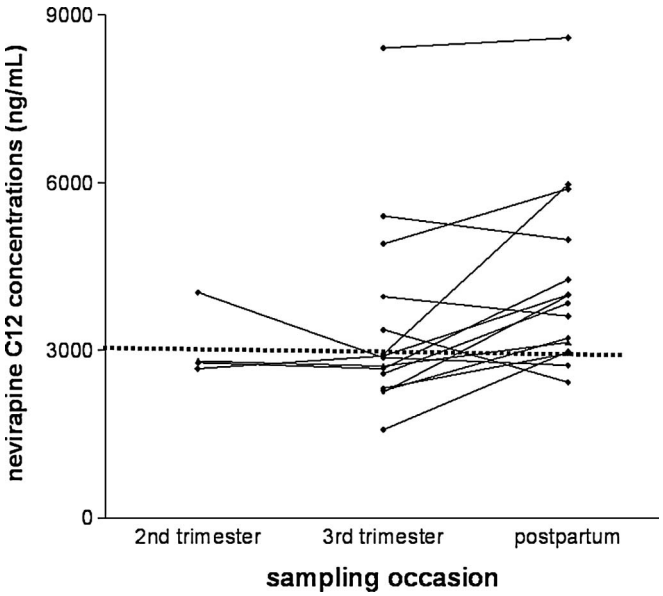

FIGURE 2. Nevirapine C12 at second trimester, third trimester, and postpartum sampling visits.

We used a minimum threshold of 3000 ng/mL to define NVP concentrations as achieving optimal or suboptimal levels. For patients infected with wild type virus, concentrations below this value are likely to remain effective since the IC_{50} of NVP is about 100 times below this threshold.[10] Also, the NRTI components of the NVP-based ART regimen may prevent development of resistance by contributing to antiretroviral efficacy. Although the current dose of NVP may be sufficient for Ugandan women infected with wild-type virus, dosing recommendations can only be made after validating the minimum effective concentration of NVP in the Ugandan context.

We found wide inter-individual variability in NVP C_{12} across the participants in both intrapartum and PP (CV = 21%–50%). NVP metabolism is mediated by CYP450 isoenzymes, which are characterized by marked variability in expression (CYP3A4, CYP2B6, and CYP3A5). Interindividual variability of NVP pharmacokinetics may be explained, in part, by single nucleotide polymorphisms resulting in altered activity of these enzymes.[26–28] Some of these polymorphisms have been demonstrated in Ugandan patients.[29] Unfortunately, pharmacogenetic testing is expensive and it is unlikely to be relevant to clinical practice in Africa in the near future. This emphasizes the importance of carrying out pharmacokinetic research in different patient populations to characterize the pharmacokinetics and optimal drug dosing in diverse groups.

For pregnant women, attaining and maintaining virologic suppression is crucial for PMTCT. In our study, adequate virologic suppression was achieved in the 9 participants who were initiated on NVP-based ART for PMTCT. By the third trimester sampling visit, all participants had HIV RNA <1000 copies per milliliter, a threshold associated with HIV transmission as low as 1%.[30] Moreover, all women had HIV RNA <400 copies per milliliter PP suggesting maintained virologic suppression, despite low

intrapartum NVP concentrations in most subjects. The encouraging short-term virologic data in this study should be interpreted with caution. It is not known if reductions in NVP concentrations intrapartum can alter long-term efficacy of NVP-based ART. Furthermore, this study observed virologic responses during a single pregnancy, but women in sub-Saharan Africa may have repeated pregnancies after the commencement of NVP-based ART and, therefore, be exposed to repeated periods of suboptimal NVP exposure.

A further consideration is the safety of continuous NVP therapy during pregnancy. In our participants, NVP-based ART was well tolerated, with no evidence of hepatotoxicity during or after pregnancy. However, our study design and sample size were inadequate to assess safety, particularly at initiation of therapy. On average, our participants had received NVP-based ART for about 1 month at enrollment. By that time, it is possible that other patients at the recruitment sites could have experienced early toxicity to NVP or even discontinued NVP-based ART. Nonetheless, our data is consistent with those from larger African studies, which also support the use of NVP-based ART in appropriately selected pregnant women.[31,32]

In summary, NVP concentrations were lower and subtherapeutic concentrations were common during pregnancy. Adequate virologic suppression was achieved during and after pregnancy. Based on these findings, NVP-based ART is recommended for HIV-1–infected women during pregnancy. However, the effect of suboptimal NVP concentrations during pregnancy on the durability of NVP-based ART needs to be elucidated. To date, few pharmacokinetic studies have been conducted in Africa. This study demonstrates that intensive pharmacokinetic studies are feasible in special populations living in resource-limited settings. Given resource constraints in many academic units in Africa, academic and research partnerships with a Global Health focus provide an important channel for this to be achieved.

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