Effects of Short-Course Zidovudine on the Selection of Nevirapine-Resistant HIV-1 in Women Taking Single-Dose Nevirapine

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Single-dose nevirapine (sdNVP) has been widely used in resource-limited settings to reduce mother-to-child-transmission (MTCT) of HIV-1 because of its simplicity and low cost [1]. However, sdNVP frequently selects HIV-1–resistant mutants in mothers [2], which may diminish the efficacy of subsequent NVP-containing antiretroviral treatment (ART) [3], and for this reason, alternative regimens for the prevention of MTCT (pMTCT) are currently preferred [4].

One pMTCT regimen endorsed by the World Health Organization (WHO) is sdNVP combined with zidovudine (ZDV) from ≥14 weeks gestation and ZDV and lamivudine (3TC) for a week postpartum [4]. This regimen is effective for preventing MTCT [5] and reducing postpartum NVP resistance [6]. However, it is not clear whether short-course ZDV alone, without 3TC, could similarly reduce NVP resistance, either by increasing the genetic barrier to resistance or reducing peripartum viral load when NVP selective pressure is highest. In studies reporting NVP resistance among women taking sdNVP and short-course antepartum ZDV only, NVP-resistance rates ranged from 17% to 75% [3, 7–9]. However, none of these studies included a comparison group of women who did not take ZDV.

We compared the selection of NVP-resistant HIV-1 in women receiving sdNVP with or without short-course ZDV in Beira, Mozambique. Since 2001, Mozambican guidelines endorsed sdNVP to prevent MTCT. In 2006, during enrollment of pregnant women into our observational study of HIV-1 drug resistance, short-course ZDV (initiated ≥32 weeks gestation) and continuing for 1 week postpartum was recommended (postpartum ZDV/3TC was implemented in early 2008). As clinics began offering ZDV, 2 cohorts of women, those who did and did not take ZDV, were created. We hypothesized that women who received ZDV in addition to sdNVP would have lower rates of selecting NVP-resistant HIV-1 mutants relative to those who received sdNVP alone.

METHODS

Study Design
An observational prospective cohort study of pregnant women in pMTCT programs in Beira, Mozambique, enrolled HIV-1–infected women who did not qualify for lifelong ART (criteria for lifelong ART = CD4 lymphocyte count <350 cells/μL or WHO clinical stage 4). One of 2 pMTCT regimens were prescribed by medical providers based on the national guidelines on the date women presented for prenatal care: sdNVP (200 mg) during labor, or sdNVP during labor with ZDV (300 mg orally 2 times a day) starting ≥32 weeks gestation and continuing for 1 week postpartum. Participants were excluded from this analysis if they took >1 dose of NVP, took any antiretroviral (ARV) medication other than sdNVP with or without short-course ZDV, or had NVP resistance prior to...
delivered. Blood was collected from women prior to delivery, and at 2, 4, 6, and 8 weeks, and 3, 4, 5, 6, 8, 10, 12, 18, and 24 months postpartum.

The study was approved by the Institutional Review Board of Seattle Children’s Hospital (Seattle, WA) and the Mozambique Ministry of Health (Maputo, Mozambique). Informed consent was obtained from all mothers prior to enrollment.

Measures
NVP-resistant HIV-1 mutants were evaluated in peripheral blood mononuclear cells with an oligonucleotide ligation assay (OLA) adapted to detect 4 common mutations in HIV-1 subtype C pol (K103N, V106M, Y181C, and G190A) [10]. HIV-1 DNA concentration was determined by amplification of gag. Predelivery samples, and 2 specimens from 2–8 weeks postdelivery (4- and 6-week specimens favored), were tested for NVP resistance. If NVP resistance was detected, subsequent samples were used to evaluate mutant decay.

Plasma HIV-1 RNA concentrations were measured at the first postpartum visit scheduled at 2 weeks after delivery (UltraSensitive AMPLICOR, Roche; or an in-house assay [11]). CD4 lymphocyte counts were abstracted from clinic records. Ingestion of sdNVP and use of other ARV medication were determined by participants’ self-report and confirmed with clinical records.

Statistical Analyses
The proportion with NVP-resistant HIV-1 between 2–8 weeks postpartum was determined, and 95% confidence intervals (CIs) estimated using the exact binomial method. NVP resistance was compared between those who did and did not take ZDV using χ²-square (or Fisher exact) tests. Among those with resistance, peak mutant concentrations were compared in women who did and did not take ZDV using the Wilcoxon rank-sum test; and in women taking no ZDV, ZDV antepartum-only, and ZDV both ante- and postpartum, using the Kruskal-Wallis test. Associations between NVP resistance and ZDV exposure were evaluated with logistic regression, with adjustment for first postpartum viral load and last antepartum CD4 lymphocyte count. Among women with NVP-resistance mutations who took ZDV, the association between ZDV duration and peak mutant proportion was evaluated using Spearman rank correlation coefficient. Finally, among those with NVP resistance, the time to <2% (undetectable) mutant status was measured, and compared among those with and without ZDV exposure using the logrank test. A sensitivity analysis evaluated whether missed study visits affected this interval.

RESULTS
Of 201 pregnant women enrolled between June 2005 and May 2008, 64 participants remained in the analyses; most exclusions were due to loss to follow-up before (n = 74) or after (n = 21) delivery, or no sdNPV (n = 20). Thirty-one (48.4%) participants also took short-course ZDV for a median of 43 days (range 12, 86); all of these took ZDV antepartum (median 37 days; range 6, 78), and 21 also took ZDV postpartum (median 7 days; range 2, 28). While ZDV for pMTCT was introduced in 2006, 42.6% enrolled after 2006 did not take ZDV, most likely because HIV and prenatal care were suboptimally integrated. No differences in sociodemographic and clinical characteristics were detected between participants that did and did not take short-course ZDV (Table 1).

NVP Resistance and ZDV Exposure
Thirty-five (54.7%) of the 64 participants had NVP-resistance detected 2–8 weeks postpartum (data available on request). NVP-resistance mutations were fewer among those taking ZDV (n = 11/31, 35.5%; 95% CI 19.2, 54.6) compared to sdNVP only (n = 24/33, 72.7%; 95% CI 54.5, 86.7; χ² P = .003). Resistance at each codon was more common in the sdNVP-only group, although only statistically significant (P < .05) for the K103N and Y181C mutations (Figure 1). Among those with NVP-resistance mutations ≤8 weeks postpartum, the median peak mutant concentration was 16.2% (n = 35; range 2.0, 91.2), with codon K103N having the highest median peak (n = 29, median 15.4%; range 2.0, 91.2) followed by G190A (n = 15, median 11.2%; range 2.9, 83.8), V106M (n = 8, median 5.1%; range 2.5, 16.2), and Y181C (n = 13, median 3.9%; range 2.0, 18.0). Among those with NVP resistance, median peak mutant concentrations tended to be lower among those taking ZDV, although this was only significant for the G190A codon (median 4.1% vs 13.5%, P = .05), likely due to low numbers of participants.

In multivariate analyses adjusted for the first postpartum plasma HIV-1 RNA load and CD4 lymphocyte count prior to delivery, short-course ZDV remained associated with fewer NVP-resistance mutations compared with sdNVP only (odds ratio [OR] 0.2, 95% CI .06, .7). However, the multivariate model revealed a significant (P < .05) interaction between ZDV and the first postpartum viral load; viral load was only associated with NVP resistance among those who did not take ZDV (Supplementary Figure 1).

NVP Resistance and ZDV Timing/Duration
The proportion of participants with NVP resistance was similar among women taking ZDV both ante- and postpartum (n = 21, 33.3%; 95% CI 11.3, 55.3) and antepartum only (n = 10, 40.0%; 95% CI 3.1, 76.9; Fisher exact, P = 1.0). Both groups had less NVP resistance compared to participants without ZDV, although this was significant only for those taking ZDV both ante- and postpartum (OR 0.2 for ZDV ante- and postpartum vs no ZDV; 95% CI .05, .7; P = .005; OR 0.3 for ZDV antepartum-only vs no ZDV; 95% CI .05, 1.2;
P = .06). Among those with NVP-resistance, peak concentration of mutants were similar in all groups (P > .10, Kruskal-Wallis), although comparisons were limited by low numbers of participants.

Among women taking ZDV, NVP-resistance was not associated with the total, antepartum, or postpartum duration of ZDV exposure (Wilcoxon rank-sum P = .26, .26, and .93, respectively). In the women with NVP resistance, the peak concentration of mutant virus was also not associated with ZDV duration overall (n = 11, Spearman ρ = 0.33; P = .32), antepartum (n = 11, Spearman ρ = 0.53; P = .09), or postpartum (n = 7, Spearman ρ = 0.54; P = .22).

**NVP Resistance Decay and ZDV Exposure**

Follow-up specimens from the 35 women with NVP resistance ≤8 weeks postpartum were available through a median of 12 months postpartum (range 1–24). In most of these women (n = 24/35, 68.6%; 95% CI 50.7, 83.1), mutant viruses decayed below the limit of detection (<2%) at a median of 6 months postpartum (range 2–24). The time to first undetectable specimen did not differ by ZDV status (logrank P = .99).

Sensitivity analyses assessing the impact of missing observations showed similar results. Notably, after NVP-resistance mutations decayed to <2%, mutations were detected at low concentrations in subsequent specimens from 2 women without documentation of additional NVP ingestion, at 18 and 24 months postpartum.

A reverse Kaplan-Meier curve examining NVP resistance over time since sdNVP for all (n = 64) subjects suggests that 96.8% had <2% mutant by 12 months postpartum (Supplementary Figure 2). Two women did not achieve mutant-free status by 12 months postpartum: 1 (ZDV group) had no specimens >12 months (lost to follow-up), and the other (no ZDV group) had a mutant-free specimen at 24 months.

**DISCUSSION**

In this observational study, the addition of short-course ZDV for a median of 6 weeks duration to sdNVP was associated with a substantial reduction in postpartum selection of NVP-resistant HIV-1. While not randomized, women’s baseline characteristics were similar regardless of ZDV use, and ZDV
use was most influenced by the prevailing guidelines during their pregnancy.

Postpartum plasma viral load was associated with the selection of resistant viruses, but only among subjects who did not take ZDV. The lesser rate of NVP resistance across all plasma viral loads in women taking ZDV illustrates the greater genetic barrier to selecting resistance posed by combining ZDV with sdNVP. ZDV resistance requires multiple mutations and transpires over weeks to months [12], making it unlikely that an individual would harbor HIV-1 variants with mutations conferring both ZDV and NVP resistance either spontaneously or as a result of short-term ZDV exposure, as in this study.

Our study may not have observed the maximal effect of ZDV, as a third of the women did not continue ZDV postpartum as recommended by national guidelines. After delivery, as intracellular ZDV concentrations wane, selective pressure of NVP could select mutant viruses, diminishing the effect of ZDV. While continuing ZDV postpartum should theoretically be superior at preventing NVP resistance compared with antepartum ZDV only, our comparisons were limited by the low number of subjects.

In the majority of women with NVP resistance, mutant viruses decayed below detection by 12 months postpartum, similar to prior studies [2, 13]. While the rate of decay did not vary by ZDV exposure, the ZDV group had a greater proportion of mutant-free women across all time points. Of note, a few women had very low concentrations of NVP-resistant mutants detected after becoming undetectable on at least one earlier time point. We did not evaluate whether these mutants were replication competent, although our study of Thai women suggests that the clinically significant threshold concentration of NVP mutants detected by OLA is >5% [14].

Our study has several limitations. First, exclusions and variable follow-up limited the power of our comparative analyses. Second, the short duration of ZDV use limited our examination of whether longer antepartum ZDV durations select ZDV-resistant viruses and reduce its barrier to NVP resistance. Third, a single viral load approximately 2 weeks postpartum may be an imperfect surrogate for viral replication between 0–3 weeks postpartum during NVP selective pressure. Fourth, self-report of ARV medications other than sdNVP is subject to recall bias. Finally, predelivery specimens were missing from 12 participants: 8 had no postpartum NVP resistance and were likely wild-type prepartum, and 4 (3 sdNVP only, 1 ZDV+sdNVP) had NVP resistance postpartum that decayed briskly to undetectable levels, making antepartum NVP resistance unlikely. Repeating analyses excluding these 4 participants did not substantially change our findings.

In conclusion, short-course (median 6 weeks) ZDV reduced selection of NVP resistance in women taking sdNVP for pMTCT. To our knowledge, no study has directly compared rates of NVP resistance between short-course ZDV and the current WHO-recommended postpartum combination of ZDV and 3TC. While 3TC has relatively few toxicities and is

![Image of Figure 1](image-url)

**Figure 1.** The proportion of mothers with detectable NVP resistance mutations 2–8 weeks postpartum, by exposure to ZDV. *P value calculated by \( \chi^2 \) test of independence. **P value calculated by Fisher exact test.
inexpensive, dispensing the regimen postpartum is impractical when women deliver at home, and selection of 3TC resistance with this combination [6, 15] could threaten the efficacy of later ART. These issues provide a rationale for trials to evaluate short-course ZDV alone compared to postpartum ZDV and 3TC following sdNVP.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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