Nevirapine (NVP) Resistance in Women with HIV-1 Subtype C, Compared with Subtypes A and D, after the Administration of Single-Dose NVP

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Objective. In the Human Immunodeficiency Virus (HIV) Network for Prevention Trials (HIVNET) 012 trial in Uganda, 6–8 weeks after single-dose nevirapine (SD-NVP), NVP resistance mutations were detected at a higher rate in women with HIV-1 subtype D than in women with subtype A. Here, we evaluate the rate of NVP resistance mutations in women with subtype C.

Methods. NVP resistance mutations were detected using the ViroSeq HIV-1 Genotyping System.

Results. The portion of women with any NVP resistance mutation was higher in those with subtype C (45/65 [69.2%] in the NVP and zidovudine trial, Malawi) than in those in the HIVNET 012 trial with either subtype A (28/144 [19.4%; P < .0001]) or subtype D (35/97 [36.1%; P < .0001]). In a multivariate model, subtype (C vs. A: odds ratio [OR], 8.73 [95% confidence interval [CI], 4.29–17.76]; C vs. D: OR, 3.38 [95% CI, 1.65–6.93]) and viral load at delivery (OR, 2.35 [95% CI, 1.62–3.40]) independently predicted NVP resistance mutations, but maternal age, parity, and time between SD-NVP and the 6–8-week visit did not.

Conclusions. The rate of NVP resistance mutations after SD-NVP was significantly higher in women with HIV-1 subtype C than in women with subtype A or D. Studies are needed to assess the clinical significance of this finding.

The HIV Network for Prevention Trials (HIVNET) 012 trial and subsequent studies have demonstrated that a single-dose nevirapine (SD-NVP) regimen is safe and effective for the prevention of mother-to-child transmission (pMTCT) of HIV-1 [1–5]. However, NVP resistance emerges in some women and infants after the administration of SD-NVP [6–12]. The emergence of NVP resistance after the administration of SD-NVP is promoted by the low genetic threshold for NVP resistance and the long half-life of NVP [13–15]. Factors associated with NVP resistance in women after the administration of SD-NVP include higher viral load before exposure to NVP, lower CD4+ cell count before exposure to NVP, increased pharmacokinetic exposure to NVP (e.g., longer half-life and decreased oral clearance of NVP), and the timing of the sample collection [6, 7, 10, 11, 16]. Results of a recent study suggest that previous exposure to SD-NVP may lower women’s virologic response to an NVP-containing treatment regimen [17]. Further studies are needed to assess whether...
previous exposure to SD-NVP compromises the efficacy of future antiretroviral treatment or the efficacy of SD-NVP in subsequent pregnancies.

In the HIVNET 012 trial, the probability of having NVP resistance was significantly higher in women with HIV-1 subtype D than in women with subtype A (36% vs. 19%; P = .0035) [6, 18]. This association was independent of baseline CD4+ cell count or baseline viral load. It is important to evaluate NVP resistance after the administration of SD-NVP in women with subtype C, because subtype C is the most common subtype found in women living in many resource-poor countries where SD-NVP and other short NVP-containing regimens for pMTCT are likely to be implemented. In the present study, we determine the rate of NVP resistance in Malawian women with HIV-1 subtype C who were administered SD-NVP in the NV and zidovudine (NVAZ) trial [19]. These data are compared with data from women with HIV-1 subtype A or D who were administered SD-NVP in the HIVNET 012 trial.

SUBJECTS, MATERIALS, AND METHODS

Study subjects. Women enrolled in the HIVNET 012 and NVAZ trials were antiretroviral drug naive before the administration of SD-NVP (supplied by Boehringer Ingelheim). The women received no other antiretroviral drugs during the months after delivery, which is consistent with the standard of care in Uganda and Malawi at the time the trials were performed. In the NVAZ trial, 889 women were administered SD-NVP when they presented for delivery, and NVP dosing was observed. Blood samples for resistance testing were collected 6–8 weeks after the administration of SD-NVP from 68 women consecutively enrolled at the beginning of the trial (in total, 889 women were enrolled in the NVAZ trial). Genotyping results were obtained for 67 of 68 women, all of whom had HIV-1 subtype C. Two women were excluded from the analysis, because the time that SD-NVP was administered was not documented. Four (6.2%) of the remaining 65 women were administered a second dose of NVP (e.g., because of premature labor or vomiting). Plasma samples obtained before the administration of SD-NVP from 40 women enrolled in the NVAZ trial were also genotyped in the present study.

In the HIVNET 012 trial, pregnant women were enrolled and were provided with a single dose of NVP to self-administer at the onset of labor. Adherence was assessed by interview and by counting remaining doses of the drug. Only women who reported the self-administration of SD-NVP or had SD-NVP administered by study personnel were included in the analysis. Genotyping results—documented in a study published elsewhere [6]—of plasma samples collected 6–8 weeks after the administration of SD-NVP were obtained for 279 of 306 women who were administered SD-NVP (all available samples): 147 women with subtype A, 98 women with subtype D, 6 women with subtype C, and 28 women with intersubtype recombinant HIV-1. The present study included the subset of 245 women who had either subtype A or subtype D. Four women were excluded from the analysis, because their visits scheduled for 6–8 weeks after delivery actually occurred >100 days after delivery. Five (3.5%) of the remaining 144 women with subtype A and 4 (4.1%) of the remaining 97 women with subtype D were administered a second dose of NVP. Plasma samples obtained before the administration of SD-NVP from 203 of 241 women in the HIVNET 012 trial who were included in the present study (all available samples)—117 with subtype A and 86 with subtype D—were also genotyped in the present study.

Viral load testing. Viral loads were determined using the AMPLICOR Monitor Test (version 1.5; Roche Diagnostics). The viral load at the time of delivery was available for all women in the NVAZ trial and for 228 (94.6%) of 241 women in the HIVNET 012 trial who were included in the present study.

The viral load at the time of delivery was available for all women in the NVAZ trial and for 228 (94.6%) of 241 women in the HIVNET 012 trial who were included in the present study. Two women in the NVAZ trial and 11 women in the HIVNET 012 trial had viral loads <400 RNA copies/mL (the lower detection limit of the assay) and were assigned viral loads of 200 RNA copies/mL, splitting the difference between 0 and 400 RNA copies/mL.

Table 1. Comparison of women in the nevirapine and zidovudine (NVAZ) trial with HIV-1 subtype C with women in the HIV Network for Prevention Trials (HIVNET) 012 trial with HIV-1 subtype A or D, by age, parity, viral load at delivery, and time between the administration of single-dose nevirapine (SD-NVP) and the 6–8-week visit.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Women in NVAZ with subtype C (n = 65)</th>
<th>Women in HIVNET 012 with subtype A (n = 144)</th>
<th>Women in HIVNET 012 with subtype D (n = 97)</th>
<th>Intersubtype comparison of NVP resistance, P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>25.0 (4.53)</td>
<td>24.9 (4.39)</td>
<td>24.2 (4.11)</td>
<td>.09</td>
</tr>
<tr>
<td>Parity, mean (SD)</td>
<td>3.11 (1.82)</td>
<td>3.38 (1.63)</td>
<td>3.03 (1.51)</td>
<td>.77</td>
</tr>
<tr>
<td>RNA copies/mL at delivery, median, log10</td>
<td>4.78</td>
<td>4.34</td>
<td>4.50</td>
<td>.63</td>
</tr>
</tbody>
</table>

| Time between administration of SD-NVP and 6–8-week visit, mean (SD), days | 43.3 (5.41) | 45.9 (8.89) | 45.1 (8.08) | .51 |

*P values from t tests were used to compare the difference between means. P values from the Wilcoxon rank sum test were used to compare the difference between medians.

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copies/mL. Assigning values of either 400 or 100 RNA copies/mL to these women did not change the findings.

**HIV-1 genotyping.** NVP resistance mutations were identified using the ViroSeq HIV-1 Genotyping System (Celera Diagnostics), as described elsewhere [6]. Phylogenetic analysis by use of MegAlign in the Lasergene suite of software (version 5.07/5.52; DNASTAR) and PHYLIP (version 3.572; available at: http://evolution.genetics.washington.edu/phylip.html) was performed to rule out mislabeling or cross-contamination of samples. Mutations were considered to be present when they were detected alone or in combination with wild-type (wt) HIV-1 sequences (mixtures).

**HIV-1 subtyping.** Subtyping was performed using nucleotide sequences corresponding to HIV-1 protease aa 1–99 and reverse transcriptase aa 1–324. Sequences were examined for intersubtype recombination using the Recombinant Identification Program [20]. Sequences without evidence of recombination were subtyped using PHYLIP, as described elsewhere [6].

**Statistical methods.** The probabilities of having NVP resistance and of having individual NVP resistance mutations were compared between subtypes by use of Fisher’s exact test. Subtype and other potential risk factors for NVP resistance were analyzed with univariate and multivariate logistic regression. Statistical analysis was performed using SAS (version 8.2; SAS Institute).

**GenBank accession numbers.** The GenBank accession numbers for the HIV-1 (subtype C) sequences from the NVAZ cohort are AY756823–AY56830, AY56832–AY756835, and AY56837–AY756889. The GenBank accession numbers for the HIV-1 (subtypes A and D) sequences from the HIVNET 012 cohort are available elsewhere [6].

**Study approval.** The human experimentation guidelines of the US Department of Health and Human Services and those of the authors’ institutions were followed in the conduct of this research. Informed consent was obtained from all women before they were enrolled in the HIVNET 012 and NVAZ trials.

**RESULTS**

We analyzed NVP resistance in 65 Malawian women with HIV-1 subtype C who were administered SD-NVP in the NVAZ trial (table 1). The HIV-1 genotypes in samples obtained before the administration of SD-NVP in 40 women in the NVAZ trial were all wt (no NVP resistance mutations were detected). In contrast, at least 1 NVP resistance mutation was detected in samples from 45 (69.2%) of 65 women 6–8 weeks after the administration of SD-NVP, and ≥2 NVP resistance mutations were detected in samples from 28 (43.1%) of these 65 women (table 2).

We compared the probability of having NVP resistance in the NVAZ cohort with the probability of having NVP resistance previously observed in 144 Ugandan women with subtype A and 97 Ugandan women with subtype D who were administered SD-NVP in the HIVNET 012 trial [6]. The HIV-1 genotypes detected in blood samples obtained before the administration of SD-NVP from 203 of those women were wt (no NVP re-
Table 3. Risk factors associated with detection of nevirapine (NVP) resistance mutations 6–8 weeks after the administration of single-dose NVP (SD-NVP).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>≥1 NVP resistance mutation</th>
<th>≥2 NVP resistance mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate model, crude OR (95% CI)</td>
<td>Multivariate model, adjusted OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>1.02 (0.96–1.07)</td>
<td>1.03 (0.96–1.10)</td>
</tr>
<tr>
<td>Age</td>
<td>0.93 (0.80–1.08)</td>
<td>0.97 (0.92–1.01)</td>
</tr>
<tr>
<td>Days between administration of SD-NVP and 6–8-week visit</td>
<td>0.95 (0.91–0.99)</td>
<td>0.97 (0.92–1.01)</td>
</tr>
<tr>
<td>Log_{10} viral load at delivery</td>
<td>2.47 (1.73–3.52)</td>
<td>2.35 (1.62–3.40)</td>
</tr>
<tr>
<td>HIV-1 subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C vs. A</td>
<td>9.32 (4.77–18.20)</td>
<td>8.73 (4.29–17.76)</td>
</tr>
<tr>
<td>C vs. D</td>
<td>3.99 (2.04–7.79)</td>
<td>3.38 (1.65–6.93)</td>
</tr>
<tr>
<td>D vs. A</td>
<td>2.34 (1.30–4.20)</td>
<td>2.58 (1.37–4.87)</td>
</tr>
</tbody>
</table>

* Univariate models test the association between NVP resistance and a single variable (age, parity, days between the administration of SD-NVP and resistance testing, viral load, or HIV-1 subtype). Multivariate models include all variables (covariates) that were associated with NVP resistance (P<.10) in the corresponding univariate model. CI, confidence interval; OR, odds ratio.
sistance mutations were detected). There were no significant differences in the mean maternal age or parity in women with subtypes A, C, or D (table 1). However, the median log-transformed viral load was significantly higher in women with subtype C, compared with that in women with subtype A or D, and the time between the administration of SD-NVP and the 6–8-week visit was significantly shorter in women with subtype C, compared with that in women with subtype A. Similar numbers of women with each subtype were administered 2 doses of NVP (5/144 [3.5%] women with subtype A, 4/97 [4.1%] women with subtype D, and 4/65 [6.2%] women with subtype C; P = .63).

At 6–8 weeks after the administration of SD-NVP, the portion of women with subtype C with at least 1 NVP resistance mutation (45/65 [69.2%]) was significantly higher than that of women with subtype A (28/144 [19.4%]; P < .0001) or D (35/97 [36.1%]; P < .0001) (table 2). The portion of women with ≥2 NVP resistance mutations was also higher in women with subtype C (28/65 [43.1%]) than in women with subtype A (12/144 [8.3%]; P < .0001) or D (16/97 [16.5%]; P < .0001). The most common NVP resistance mutations detected in all 3 subtypes were K103N and Y181C. The rates of detection of both mutations, as well as for Y188C, were significantly higher in women with subtype C than in women with subtypes A or D (in all cases, P < .03; in many cases, P < .0001). Other NVP resistance mutations (e.g., K101E, V106A/M, V108A/I, and G190A) were detected in some women. However, the numbers of women with those mutations were too small for meaningful statistical analysis.

Univariate and multivariate regression models tested the associations between detection of NVP resistance mutations 6–8 weeks after the administration of SD-NVP and HIV-1 subtype and other factors. Other factors examined included maternal age, parity, viral load at the time of delivery, and days between the administration of NVP and the 6–8-week visit (table 3). Multivariate analysis revealed that subtype C vs. A: OR, 8.73 [95% CI, 4.29–17.76]; C vs. D: OR, 3.38 [95% CI, 1.65–6.93]) and log_{10} viral load at delivery (OR, 2.35 log_{10} RNA copies/mL [95% CI, 1.62–3.40 log_{10} RNA copies/mL]) independently predicted having NVP resistance (detection of any NVP resistance mutation), but age, parity, and the time between the administration of SD-NVP and the 6–8-week visit did not. Similar associations were observed between those factors and detection of ≥2 NVP resistance mutations (table 3).

**DISCUSSION**

The present study provides a comparison of NVP resistance rates in women with HIV-1 subtype C versus subtypes A and D. Women with each subtype had similar demographic characteristics, such as age and parity, and a similar proportion of women in each group were administered >1 dose of NVP. We also controlled for viral load—which was significantly higher in women with subtype C, compared with that in women with subtypes A or D—and for the timing of sample collection. Testing was performed in a single laboratory, and identical methods of genotyping and sequence editing were used.

We found a significantly higher probability of having NVP resistance 6–8 weeks after the administration of SD-NVP in women with subtype C, compared with women with subtypes A or D. In multivariate models, women with subtype C had a dramatically higher (OR >3) risk of having single and multiple NVP resistance mutations, even after adjustments were made for age, parity, viral load at delivery, and the time between the administration of SD-NVP and the 6–8–week visit. The high probability of having NVP resistance observed in women with subtype C is of concern, because subtype C is the most common subtype found in women living in many countries likely to implement the HIVNET 012 regimen or other regimens for pMTCT that combine SD-NVP with other antiretroviral drugs. Some caution is needed, however, when data from different clinical trials are used to compare the rates of NVP resistance after the administration of SD-NVP. Other factors that were not examined in this study (e.g., factors that influence the rate of NVP metabolism or clearance, weight, and HIV-1 disease stage) may also have contributed to the higher rate of NVP resistance observed in the NVAZ cohort. Also, because we analyzed only a subset of women enrolled in the NVAZ trial, the frequency of NVP resistance we observed may not be representative of that in the entire NVAZ cohort.

Recent studies have demonstrated that the HIV-1 subtype can influence the type of mutations that emerge under drug pressure [21–24]. For example, the V106M mutation (rather than V106A) is selected by nonnucleoside reverse-transcriptase inhibitors in subtype C. Because novel mutations (not yet identified) in some subtypes may contribute to NVP resistance, genotyping studies such as the present one may tend to underestimate the true rate of NVP resistance in cohorts with non–subtype B infection.

The HIV-1 subtype has also been shown to influence the persistence of NVP-resistant strains after the administration of SD-NVP [25]. In a South African cohort, 55 (35%) of 155 women with subtype C who had NVP resistance mutations detected 7 weeks after the administration of SD-NVP still had the K103N mutation in their plasma 6 months after exposure to NVP, when a population-based sequencing assay was used [26]. This suggests that some NVP resistance mutations in subtype C viruses may have little or no detrimental effect on viral fitness. Recent studies have used more sensitive resistance assays to detect specific NVP resistance mutations in women after the administration of SD-NVP. Preliminary reports on the use of those assays show that NVP resistance mutations can be detected in many women who did not have NVP resistance mutations when population-based sequencing assays were used [27–30]. Because population-based
sequencing assays are relatively insensitive for the detection of NVP-resistant variants present at low levels, the higher rate of NVP resistance observed in women with subtype C versus those with subtypes A or D in the present study may reflect the selection of NVP resistance in a greater number of women with subtype C, selection of NVP-resistant variants at higher levels in the viral populations of those women, faster fading of NVP-resistant variants in women with subtype A or D, or a combination of these factors. Further studies, using both routine and more sensitive resistance assays, that include long-term follow-up are needed to compare the emergence and persistence of NVP resistance after the administration of SD-NVP in women with different HIV-1 subtypes.

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References

