Nevirapine Resistance in Women and Infants after First versus Repeated Use of Single-Dose Nevirapine for Prevention of HIV-1 Vertical Transmission

Tamara S. Flys, Michelle S. McConnell, Flavia Matovu, Jessica D. Church, Danstan Bagenda, Leila Khaki, Paul Bakaki, Michael C. Thigpen, Chineta Eure, Mary Glenn Fowler, and Susan H. Eshleman

Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland; Epidemiology Branch, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia; Makerere University–Johns Hopkins University Research Collaboration and Makerere University School of Public Health, Kampala, Uganda

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Single-dose (SD) nevirapine (NVP) significantly reduces mother-to-child transmission of human immunodeficiency virus (HIV). We analyzed NVP resistance after receipt of SD NVP in 57 previously SD NVP–naive women, in 34 SD NVP–experienced women, and in 17 HIV-infected infants. The proportion of women infected with variants with resistance mutations, the types of mutations detected, and the frequency and level of K103N were similar in the two groups of women at 6 weeks and 6 months post partum. NVP resistance was detected in a similar proportion of infants born to SD NVP–naive versus SD NVP–experienced women. Repeated use of SD NVP to prevent HIV transmission does not appear to influence NVP resistance.

Nevirapine (NVP)–resistant HIV variants can emerge in HIV-infected women and infants who receive single-dose (SD) NVP for the prevention of HIV-1 mother-to-child transmission (pMTCT) [1, 2] and can persist in women and infants for a year or more after SD NVP exposure [3–6]. The emergence and persistence of NVP resistance in women after the first and second use of SD NVP has been associated with high baseline (pre-NVP) viral load and HIV-1 subtype (D more than A) [7–9]. Prior use of SD NVP for pMTCT does not appear to compromise the effectiveness of SD NVP in subsequent pregnancies [10, 11], but some studies have suggested that it may compromise future antiretroviral therapy in women and HIV-infected children receiving a nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based regimen if therapy is started within 6–12 months of SD NVP administration [12, 13].

It is not known whether repeated use of SD NVP increases the selection or persistence of NVP-resistant HIV. We recently analyzed NVP resistance in Ugandan women who first received SD NVP in the HIVNET 012 trial and then received SD NVP for pMTCT during 1 or more pregnancies over a 5-year follow-up period [9]. In that study, samples were collected at annual visits after the initial SD NVP exposure. In this report, we compared the emergence and persistence of NVP-resistant strains in SD NVP–naive versus SD NVP–experienced Ugandan women in the Repeat Pregnancy (RP) Study. This allowed us to examine NVP resistance in samples collected from the women in these 2 groups, as well as from their infants, at fixed times after SD NVP administration.

Methods. Women and infants were enrolled in an observational study, the RP Study, at the Mulago Hospital and the Makerere University–Johns Hopkins University Clinic in Kampala, Uganda [11]. The major aims of the RP Study were to compare transmission rates between women who had received SD NVP during a prior pregnancy and those who were SD NVP naïve and to evaluate NVP resistance in these 2 groups. Women were asked about prior SD NVP use at study enrollment, and prior SD NVP administration was verified by review of clinic and hospital records whenever possible. The prospective part of the RP Study enrolled age-matched SD NVP–naive women and SD NVP–experienced women. Women received SD NVP during labor, and infants received

* Present affiliations: Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland (M.G.F.); Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio (P.B.).

Reprints or correspondence: Dr. Susan Eshleman, Johns Hopkins Medical Institutions, Dept. of Pathology, Ross Building 646, 720 Rutland Ave., Baltimore, MD 21205 (eshleman@jhmi.edu).
SD NVP within 72 h of birth. HIV load and CD4 cell count were measured in the RP Study. We tested samples from women and HIV-infected infants enrolled in the prospective group of this study.

Methods for HIV genotyping using the ViroSeq HIV Genotyping System (version 2.6; Celera) and HIV subtyping have been described elsewhere [1, 7]. Sequencing was performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Specific NVP resistance mutations were detected and quantified using the LigAmp assay [5]. Different ligation oligonucleotides were used for subtypes A, C, and D. The assay cutoff for mutation detection was 0.5% for K103N and G190A and was 1.0% for Y181C.

Statistical analyses included the computation of point estimates and their respective measures of variation as well as the between-group comparison of proportions, means, and medians on the basis of 2-sided statistical tests. Fisher’s exact test was used for comparison of proportions. P values associated with comparisons of medians were based on the 2-sample Wilcoxon rank-sum test. Normal approximations for P values were computed, unless data allowed for exact calculation of P values (i.e., <50 discrete finite values). P values for comparisons of means were based on the Welch 2-sample t test. Analyses were done using the R statistical package (R Development Core Team) [14].

Written informed consent was obtained from all women for participation in the RP Study. The study was approved by institutional review boards at the Uganda Virus Research Institute in Uganda and the US Centers for Disease Control and Prevention in Atlanta, Georgia.

Results. The prospective part of the RP Study enrolled 105 HIV-infected pregnant women, 65 who did not receive SD NVP during a prior pregnancy (SD NVP naive) and 40 who received SD NVP for pMTCT during 1 or more previous pregnancies (SD NVP experienced). Plasma samples collected 6 weeks post partum were available for 102 (97.1%) of the 105 women, and HIV genotyping was successful for 91 (89.2%) of the 102 samples (57 SD NVP–naive and 34 SD NVP–experienced women). Therefore, 91 women were included in the resistance substudy described in this report. The 34 SD NVP–experienced women had 1 (n = 30), 2 (n = 3), or 3 (n = 1) prior SD NVP exposures. The median time between the most recent prior SD NVP exposure and receipt of SD NVP in these women was 31.2 months (range, 10.3–74.8 months; interquartile range, 16.8–42.3 months). There were no significant differences in baseline viral load or baseline CD4 cell count between the SD NVP–naive and SD NVP–experienced women in this substudy. These groups also were similar in terms of age, parity, and transmission status for the current pregnancy. There was a trend toward a higher portion of women with subtype A infection in the SD NVP–experienced group (table 1).

Table 1. Characteristics of single-dose (SD) nevirapine (NVP)–naive vs. SD NVP–experienced women in the Repeat-Pregnancy (RP) Study—Uganda, 2004–2006.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Naive (n = 57)</th>
<th>Experienced (n = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>27 (4.5)</td>
<td>27 (4.5)</td>
<td>.91a</td>
</tr>
<tr>
<td>Parity, median (IQR)b</td>
<td>2 (1–3)</td>
<td>3 (2–4)</td>
<td>.89</td>
</tr>
<tr>
<td>Women whose infants were diagnosed with HIV infection by 6 weeks of age, no. (%)</td>
<td>11 (19.2)</td>
<td>4 (11.8)</td>
<td>.40</td>
</tr>
<tr>
<td>Baseline viral load, log10 copies/mL</td>
<td>4.14 (0.82)</td>
<td>4.29 (0.85)</td>
<td>.50</td>
</tr>
<tr>
<td>Baseline CD4 cell count, median (IQR), cells/mm³</td>
<td>459 (253–619)</td>
<td>346 (159–632)</td>
<td>.51</td>
</tr>
<tr>
<td>HIV-1 pol subtype, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>26 (45.6)</td>
<td>23 (67.6)</td>
<td>.052</td>
</tr>
<tr>
<td>D</td>
<td>19 (33.3)</td>
<td>8 (23.5)</td>
<td>.35</td>
</tr>
<tr>
<td>C</td>
<td>2 (3.5)</td>
<td>. . . .</td>
<td>.53</td>
</tr>
<tr>
<td>G</td>
<td>. . . .</td>
<td>1 (2.9)</td>
<td>.37</td>
</tr>
<tr>
<td>R (recombinant)</td>
<td>10 (17.5)</td>
<td>2 (5.9)</td>
<td>.20</td>
</tr>
</tbody>
</table>

NOTE. Data are mean (standard deviation) values, unless otherwise specified. IQR, interquartile range.

a By the Welch 2-sample t test.
b Includes pregnancies and still births.
c By the Wilcoxon rank-sum test with continuity correction.
d For the association between groups (Fisher’s exact test).
e For the overall association across all pol subtypes (Fisher’s exact test).
f For each pol subtype (Fisher’s exact test).
NVP during a prior pregnancy, 1 dose 2 weeks before delivery due to false labor and 1 dose at delivery. Her baseline (pre-NVP) sample in the RP Study was collected 7–8 months later; that sample had 33.5% K103N. One SD NVP–naive woman had 1.6% K103N before delivery in the RP Study; that woman had received an extra dose of NVP 12 days before delivery because of premature labor. The other SD NVP–naive woman, who had no history of SD NVP exposure, had 0.7% K103N.

We next analyzed plasma samples collected 6 weeks after SD NVP administration in the RP Study. Using the ViroSeq system, we detected NVP resistance mutations in a similar proportion of SD NVP–naive and SD NVP–experienced women (table 2).
types of mutations detected in these 2 groups were also similar (table 2). Interestingly, none of the women with 2 or 3 prior exposures to SD NVP had resistance detected. Samples from 90 women were analyzed using the LigAmp assay (excluding the woman infected with subtype G). By this assay, K103N was detected in 26 (45.6%) of 57 SD NVP–naive women and in 15 (45.5%) of 33 SD NVP–experienced women (table 2). The median level of K103N detected was slightly higher in the SD NVP–experienced group; however, the difference was not statistically significant (table 2).

We examined the persistence of K103N among women who had K103N detected at 6 weeks (table 2). Analysis of samples collected at 6 and 12 months was limited to women who had detectable K103N at the prior study visit. Forty women who had K103N detected at 6 weeks had a 6-month sample available for analysis. There was no significant difference between the proportion of SD NVP–naive women and the proportion of SD NVP–experienced women with detectable K103N at 6 months. Fifteen women who had K103N detected at 6 months had a 12-month sample available for analysis. A greater proportion of SD NVP–experienced women had detectable K103N at 12 months. However, that difference was not statistically significant.

Samples from 6 weeks of age were available for 17 of 19 HIV-infected infants in the RP Study. A similar proportion of infants born to SD NVP–naive versus SD NVP–experienced women had a NVP resistance mutation detected (table 2). There was no apparent relationship between the mutations detected in these infants and the mutations detected in their mothers (table 2). Of the 7 infants who had NVP resistance mutations detected at 6 weeks, 5 had a 6-month sample available for testing, and 3 of those infants had a NVP resistance mutation detected by ViroSeq at 6 months (2 born to SD NVP–experienced women and 1 born to a SD NVP–naive woman). One infant, who had K103N + Y181C at 6 weeks, had Y181C detected at 6 months. In 2 infants, the same mutation was detected at 6 weeks and 6 months (1 with V106M and 1 with Y181C). Infant samples from 6 weeks of age were also analyzed by the LigAmp assay to detect and quantify K103N, Y181C, and G190A. LigAmp results were consistent with results obtained with the ViroSeq system described above. The mutations detected by LigAmp were present at levels <10%, with 1 exception: G190A was detected at 60% in 1 infant. LigAmp did not detect any NVP resistance mutations that were not detected by ViroSeq. In 2 samples, mutations detected by ViroSeq were not detected by LigAmp (1 sample had nucleotide polymorphisms at the LigAmp oligonucleotide binding sites, and 1 sample used an alternative codon for Y181C).

Discussion. We found no significant difference in the proportion of women in whom NVP resistance was detected or the pattern of resistance mutations in SD NVP–naive versus SD NVP–experienced women after SD NVP administration in the RP Study. At 6 weeks, 23.5% of SD NVP–experienced women had 1 or more NVP resistance mutation detected by ViroSeq. This was nearly identical to the proportion of SD NVP–naive women with NVP resistance detected by the same method in this study (22.8%) and in the HIVNET 012 trial (25% of 279 women) [7]. At 6 weeks, the proportion of SD NVP–experienced women with K103N detected by LigAmp (45.5%) was nearly identical to the proportion of SD NVP–naive women with K103N detected by the same method in this study (45.6%) and in the HIVNET 012 trial (47.1%) [8]. Notably, none of the 4 women in the RP Study who had multiple prior exposures to SD NVP had NVP resistance mutations detected at 6 weeks by either ViroSeq or LigAmp. At 6 months, the proportion of women with K103N detected by LigAmp was also similar in SD NVP–naive versus SD NVP–experienced women. The median K103N level was slightly higher at 6 weeks and 6 months in the SD NVP–experienced group, but these differences were not statistically significant. We did observe a trend toward increased detection of K103N at 12 months in the SD NVP–experienced group. However, our retrospective study of the HIVNET 012 cohort found no difference in K103N detection in women by 2 years after the first SD NVP use versus subsequent SD NVP use [9].

This report provides the first data comparing NVP resistance in HIV-infected infants born to SD NVP–naive versus SD NVP–experienced women after SD NVP prophylaxis. In this study of 17 infants, we did not find a difference in NVP resistance between these 2 groups. The proportion of infants with NVP resistance at 6 weeks in this study (7/17 [41.2%]) was also similar to the proportion of infants with NVP resistance in the HIVNET 012 cohort (11/24 [45.8%]), in which all women were SD NVP naive before receiving SD NVP prophylaxis [1]. One limitation of the present study was the small sample size, which limited the power to detect significant differences in resistance between these groups.

Recent studies suggest that treatment with an NNRTI-containing regimen may still be effective in SD NVP–exposed women [12, 13, 15], provided that treatment is not initiated too soon after SD NVP exposure [13]. However, in HIV-infected infants, who may require antiretroviral treatment at an early age, prior SD NVP exposure may compromise the efficacy of NNRTI-containing regimens [13]. Data in the present report suggest that repeated use of SD NVP for pMTCT will not further compromise the efficacy of antiretroviral treatment of women and HIV-infected infants. This is reassuring, given that SD NVP remains the only option for pMTCT in many resource-limited settings.

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