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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[1], **Michelle S. McConnell**[2], **Flavia Matovu**[3], **Jessica D. Church**[1], **Danstan Bagenda**[3,4], **Leila Khaki**[1], **Paul Bakaki**[3,5], **Michael C. Thigpen**[2], **Chineta Eure**[2], **Mary Glenn Fowler**[1,2], and **Susan H. Eshleman**[1]

[1]*Dept. of Pathology, Johns Hopkins Univ. School of Medicine, Baltimore, MD, USA* [2]*Epidemiology Branch, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA* [3]*Makerere Univ. - Johns Hopkins Univ. Research Collaboration, Kampala, Uganda* [4]*Makerere Univ, School of Public Health, Uganda* [5]*Dept. of Epidemiology and Biostatistics, Case Western Reserve Univ., Cleveland, OH, USA*

Abstract

Single dose (SD) nevirapine (NVP) significantly reduces HIV mother-to-child transmission. We analyzed NVP resistance after SD NVP in 57 previously SD NVP-naïve women, 34 SD NVP-experienced women, and 17 HIV-infected infants. The proportion of women with resistance, 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-naïve versus SD NVP-experienced women. Repeated use of SD NVP to prevent HIV transmission does not appear to influence NVP resistance.

INTRODUCTION

Nevirapine (NVP)-resistant HIV variants can emerge in HIV-infected women and infants who receive single dose (SD) NVP for 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]. Emergence and persistence of NVP resistance in women after first and second use of SD NVP has been associated with high baseline (pre-NVP) viral load and HIV-1 subtype (D>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 suggest that it may compromise future antiretroviral therapy of women and HIV-infected children with a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen if therapy is started within 6–12 months of SD NVP administration [12,13].

Note:: Dr. Mary Glenn Fowler performed this work while employed at Centers for Disease Control and Prevention. Her current affiliation is Johns Hopkins Univ. School of Medicine. Dr. Paul Bakaki worked on this study while employed at the Makerere Univ.-Johns Hopkins Research Collaboration. His current affiliation is Case Western Reserve Univ.

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Conflict of Interest Statement

None of the authors has a commercial or other association that might pose a conflict of interest with the following exception: Dr. Susan Eshleman is a co-inventor of the LigAmp assay and Johns Hopkins University has filed a patent application with the US-Patent and Trademark Office. The inventors may receive royalty payments if the patent is awarded and licensed.

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It is not known whether repeated use of SD NVP increases 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 in one or more pregnancies during 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 emergence and persistence of NVP-resistant strains in SD NVP-naïve 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 two groups, and from their infants, at fixed times after SD NVP administration.

METHODS

Study Cohort

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 in women who had received SD NVP in a prior pregnancy, or were SD NVP-naïve, and to evaluate NVP resistance in these two 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-naïve women and SD NVP-experienced women. Women received SD NVP in labor, and infants received SD NVP within 72 hours of birth. HIV viral 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.

HIV genotyping and subtyping

Methods for HIV genotyping using the ViroSeq HIV Genotyping System (version 2.6, Alameda, CA) and HIV subtyping are described in previous reports [1,7]. Sequencing was performed using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Analysis of NVP-resistance mutations using the LigAmp assay

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 1.0% for Y181C.

Statistical methods

Statistical analyses included the computation of point estimates and their respective measures of variation, and the between group comparison of proportions, means and medians based on 2-sided statistical tests. Fishers Exact test was used for comparison of proportions. P-values associated with comparisons of medians were based on two-sample Wilcoxon Rank Sum test. Normal approximations for P-values were computed, unless data allowed for exact calculation of P-values (i.e. less than 50 discrete finite values). P-values for comparisons of means were based on the 2-sample Welch Students t-test. Analyses were done using the R Statistical package (R Development Core Team, Vienna, Austria) [14].

Ethical considerations

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 U.S. Centers for Disease Control and Prevention in Atlanta, GA.

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RESULTS

Characteristics of women in the sub-study

The prospective part of the RP study enrolled 105 HIV-infected pregnant women, 65 who did not receive SD NVP in a prior pregnancy (SD NVP-naïve) and 40 who received SD NVP or pMTCT in one or more previous pregnancies (SD NVP-experienced). Plasma samples collected 6 weeks post-partum were available from 102 (97.1%) of the 105 women, and HIV genotyping was successful for 91 (89.2%) of the 102 samples (57 SD NVP-naïve and 34 SD NVP-experienced women). Therefore, 91 women were included in the resistance sub-study described in this report. The 34 NVP-experienced women had one (N=30), two (N=3), or three (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 to 74.8 months IQR = 16.8–42.3). There were no significant differences in baseline viral load or baseline CD4 cell count in the SD NVP-naïve versus SD NVP-experienced women in this sub-study. 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 in the NVP-experienced group (Table 1).

NVP resistance in women

We first analyzed samples collected prior to SD NVP administration (at 28–36 weeks gestation). None of the 91 samples had NVP-resistance mutations detected with the ViroSeq system. We also used the LigAmp assay for analysis of K103N in these samples. LigAmp results were obtained for 89 women. One woman with subtype G HIV was not tested, and one baseline sample failed to amplify. K103N was detected in only 3 (3.4%) of 89 women, one SD NVP-experienced woman and two SD NVP-naïve women. The NVP-experienced woman received two doses of NVP in a prior pregnancy, one dose two weeks before delivery due to false labor, and one 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-naïve woman had 1.6% K103N prior to delivery in the RP study; that woman had received an extra dose of NVP 12 days prior to delivery due to premature labor. The other SD NVP-naïve woman, who had no prior history of SD NVP exposure, had 0.7% K103N.

We next analyzed plasma samples collected 6 weeks after SD NVP in the RP study. Using the ViroSeq system, we detected NVP resistance mutations in a similar proportion of SD NVP-naïve versus SD NVP-experienced women (Table 2). The types of mutations detected in these two groups were also similar (Table 2). Interestingly, none of the women with two or three prior exposures to SD NVP had resistance detected. Samples from 90 women were analyzed using the LigAmp assay (excluding the woman with subtype G). Using this assay, K103N was detected in 26 (45.6%) of 57 SD NVP-naïve women and 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 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 in the proportion of SD NVP-naïve women versus 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.

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NVP resistance in infants

Samples from 6 weeks of age were available for 17 of 20 HIV-infected infants in the RP study. A similar proportion of infants born to SD NVP-naïve 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, footnote). Among the seven infants who had NVP resistance mutations detected at 6 weeks, five had a 6-month sample available for testing, and three of those infants had a NVP resistance mutation detected by ViroSeq at 6 months (two born to SD-NVP experienced women, one born to a SD NVP-naïve woman). One infant, who had K103N+Y181C at 6 weeks, had Y181C detected at 6 months. In two infants, the same mutation was detected at 6 weeks and 6 months (one with V106M and one with Y181C). Infant samples from 6 weeks of age were also analyzed using the LigAmp assay to detect and quantify K103N, Y181C, and G190A. LigAmp results were consistent with results obtained with the ViroSeq system (above). The mutations detected by LigAmp were present at levels less than 10% with one exception: G190A was detected at 60% in one infant. LigAmp did not detect any NVP-resistance mutations that were not detected by ViroSeq. In two samples, mutations detected with ViroSeq were not detected with LigAmp (one sample had nucleotide polymorphisms at the LigAmp oligonucleotide binding sites; one sample used an alternative codon for Y181C).

DISCUSSION

We found no significant difference in the proportion of women with NVP resistance or the pattern of resistance mutations in SD NVP-naïve versus SD NVP-experienced women following SD NVP administration in the RP study. At 6 weeks, 23.5% of SD NVP-experienced women had one or more NVP resistance mutation detected by ViroSeq. This was nearly identical to the proportion of SD NVP-naïve 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-naïve 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 four women in the RP study who had multiple prior exposures to SD NVP had NVP resistance mutations detected at 6 weeks with either ViroSeq or LigAmp. At 6 months, the proportion of women with K103N detected by LigAmp was also similar in SD NVP-naïve versus SD NVP-experienced women. The median %K103N 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 NVP-experienced group. However, our retrospective study of the HIVNET 012 cohort found no difference in detection of K103N in women by 2 years after first versus subsequent SD NVP use [9].

This report provides the first data comparing NVP resistance in HIV-infected infants born to SD NVP-naïve vs. SD NVP-experienced women following SD NVP prophylaxis. In this study of 17 infants, we did not find a difference in NVP resistance between these two groups. The proportion of infants with NVP resistance at 6 weeks in this study (7/17=41%) was also similar to the proportion of infants with NVP exposure in the HIVNET 012 cohort (11/24=46%), where all women were SD NVP-naïve before receiving SD NVP prophylaxis [1]. One limitation of this 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-

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containing regimens [13]. Data in this 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, since SD NVP remains the only option for pMTCT in many resource-limited settings.

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REFERENCES

1. Eshleman SH, Mracna M, Guay LA, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS 2001;15:1951–1957. [PubMed: 11600822]
2. Johnson JA, Li JF, Morris L, et al. Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. J Infect Dis 2005;192:16–23. [PubMed: 15942889]
3. Flys T, Nissley DV, Claasen CW, et al. Sensitive drug resistance assays reveal long-term persistence of HIV-1 variants with the K103N nevirapine (NVP) resistance mutation in some women and infants after single dose NVP: HIVNET 012. J Infect Dis 2005;192:24–29. [PubMed: 15942890]
4. Martinson NA, Morris L, Gray G, et al. Selection and persistence of viral resistance in HIV-infected children after exposure to single-dose nevirapine. J Acquir Immune Defic Syndr 2007;44:148–153. [PubMed: 17117145]
5. Flys TS, Donnell D, Mwatha A, et al. Persistence of K103N-containing HIV-1 variants after single dose nevirapine for prevention of HIV-1 mother-to-child transmission. J Infect Dis 2007;195:11–15.
6. Palmer S, Boltz V, Martinson N, et al. Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. Proc Natl Acad Sci USA 2006;103:7094–7099. [PubMed: 16641095]
7. Eshleman SH, Guay LA, Mwatha A, et al. Characterization of nevirapine (NVP) resistance mutations in women with subtype A vs. D HIV-1 6–8 weeks after single dose NVP (HIVNET012). J Acquir Immune Defic Syndr 2004;35:126–130. [PubMed: 14722443]
8. Flys TS, Chen S, Jones DC, et al. Quantitative analysis of HIV-1 variants with the K103N resistance mutation after single dose nevirapine in women with HIV-1 subtypes A, C and D. J Acquir Immune Defic Syndr 2006;42:61–63. [PubMed: 16639341]
9. Flys T, Mwatha A, Guay LA, et al. Detection of K103N in Ugandan women after repeated exposure to single dose nevirapine. AIDS 2007;21:1–6. [PubMed: 17148962]
10. Martinson NA, Ekouevi DK, Dabis F, et al. Transmission rates in consecutive pregnancies exposed to single-dose nevirapine in Soweto, South Africa and Abidjan, Cote d'Ivoire. J Acquir Immune Defic Syndr 2007;45:206–209. [PubMed: 17438480]
11. McConnell MS, Bakaki P, Eure C, et al. Effectiveness of repeat single-dose nevirapine for prevention of mother-to-child transmission of HIV-1 in repeat pregnancies, Uganda. J Acquir Immune Defic Syndr. 2007In Press
12. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med 2004;351:229–240. [PubMed: 15247339]

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13. Lockman S, Shapiro RL, Smeaton LM, et al. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. N Engl J Med 2007;356:135–147. [PubMed: 17215531]

14. R Development Core Team. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2007. ISBN 3-900051-07-0 http://www.R-project.org.

15. Chi BH, Sinkala M, Stringer EM, et al. Early clinical and immune response to NNRTI-based antiretroviral therapy among women with prior exposure to single-dose nevirapine. AIDS 2007;21:957–964. [PubMed: 17457089]

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Table 1

Characteristics of SD NVP-naïve vs. SD NVP-experienced women in the Repeat Pregnancy (RP) Study, Uganda, 2004–2006.

	SD-NVP naïve	SD-NVP experienced	P value
Number of women	57	34	--
Mean age (SD [a])	27 (4.5)	27 (4.5)	0.91 [c]
Median parity (IQR) [b]	2 (1–3)	3 (2–4)	0.89 [d]
# (%) of women whose infants were diagnosed with HIV infection by 6 weeks of age	11 (19.2)	4 (11.8)	0.40 [e]
Mean baseline HIV viral load: $\log_{10}$ copies/ml (SD)	4.14 (0.82)	4.29 (0.85)	0.50 [c]
Median baseline CD4 cell count (cells per mm^3)	459 (253–619)	346 (159–632)	0.51 [d]
HIV-1 *pol* subtype			0.09 [f]
A	26 (45.6%)	23 (67.6%)	0.052 [g]
D	19 (33.3%)	8 (23.5%)	0.35 [g]
C	2 (3.5%)	--	0.53 [g]
G	--	1 (2.9%)	0.37 [g]
R (recombinant)	10 (17.5%)	2 (5.9%)	0.20 [g]

[a] SD: standard deviation.

[b] Parity includes pregnancies and still births; IQR: interquartile range.

[c] Welch Two sample t-test.

[d] Wilcoxon Rank Sum Test with continuity correction.

[e] P value is for association between groups (Fishers Exact Test).

[f] P value is for the overall association across all *pol* subtypes (Fishers Exact Test).

[g] P values are for each *pol* subtype (Fishers Exact Test).

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Table 2

Detection of NVP resistance mutations in SD NVP-naïve vs. SD NVP-experienced women and their infants in the Repeat Pregnancy (RP) Study, Uganda, 2004–2006.

Maternal SD NVP status prior to NVP dosing in the RP study	SD-NVP naïve	SD-NVP experienced	P value
Analysis of Maternal Samples			
VIROSEQ RESULTS (6 weeks)			
Detection of ≥ 1 NVP resistance mutation	13/57 (22.8%)	8/34 (23.5%)	1.00 [d]
Detection of ≥ 2 NVP resistance mutations [a]	8/57 (14.0%)	3/34 (8.8%)	0.59 [d]
# (%) women with:			
K101E	1 (1.8%)	2 (5.9%)	0.55 [d]
K103N	11 (19.3%)	5 (14.7%)	0.78 [d]
K103N+K103T	0	1 (2.9%)	0.37 [d]
V106A	1 (1.8%)	1 (2.9%)	1.00 [d]
Y181C	8 (14.0%)	3 (8.8%)	0.53 [d]
Y188C	4 (7.0%)	0	0.29 [d]
G190A	3 (5.3%)	1 (2.9%)	1.00 [d]
LIGAMP RESULTS			
6 weeks			
# (%) ≥ 0.5% K103N	26/57 (45.6%)	15/33 (45.5%)	1.00 [d]
Median % K103N [b]	3.2% K103N	8.3% K103N	0.27 [e]
6 months [c]			
# (%) ≥ 0.5% K103N	10/57 (17.5%)	7/32 (21.9%)	0.78 [d]
Median % K103N [b]	1.2% K103N	5.0% K103N	0.24 [e]
12 months [c]			
# (%) ≥ 0.5% K103N	2/56 (3.6%)	4/31 (12.9%)	0.18 [d]
Median % K103N [b]	9.2% K103N	11.9% K103N	1.00 [e]
Analysis of Infant Samples			
VIROSEQ RESULTS			
Detection of ≥ 1 NVP resistance mutation	4/11 (36.4%)	3/6 (50%)	0.64 [d]
Mutations detected [f]			
K103T	1		
V106M	1		
Y181C	2	1	
G190A		1	
K103N+Y181C		1	

[a] SD NVP-naïve: K103N+Y181C (n=3), K101E+K103N+Y181C+Y188C (n=1), K103N+Y181C+Y188C (n=1), K103N+G190A (n=1), K103N+V106A +Y181C+G190A (n=1), K103N+Y181C+Y188C+G190A (n=1). SD NVP-experienced: K103N/T+Y181C (n=1), K101E+K103N+Y181C (n=1), K101E +K103N+G190A (n=1).

[b] Among women with K103N detected.

[c] Women were only tested at 6 and 12 months if K103N was detected at the previous study visit. One SD NVP-experienced woman with K103N detected at 6 weeks did not have a 6- month sample available for testing. One SD NVP-naïve woman and one SD NVP-experienced woman with K103N detected at 6 months did not have a 12-month sample available for testing.

[d] Fishers Exact Test

[e] Wilcoxon Rank Sum test with continuity correction

[f] Seven infants had NVP resistance mutations at 6 weeks. HIV genotyping results were available for five of the corresponding mothers; three of those women had evidence of NVP resistance: (1) a SD NVP-naïve woman whose infant had Y181C had K101E+K103N+Y181C+Y188C, (2) a SD NVP-naïve woman whose infant had V106M had K103N+G190A, and (3) a SD NVP-experienced woman whose infant had G190A had K101E+K103N +G190A.

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