

Emergence and persistence of nevirapine (NVP) resistance in breast milk after single-dose NVP administration

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INTRODUCTION

HIV-infected women often have detectable nevirapine (NVP) resistance after receiving single-dose NVP (sdNVP) for prevention of mother-to-infant HIV transmission (MTCT). Emergence of NVP resistance in maternal plasma after sdNVP is associated with high maternal pre-NVP plasma viral load, low maternal pre-NVP CD4 cell count, HIV subtype (C>D>A), and pharmacokinetic factors associated with NVP exposure. NVP is transferred to breast milk (BM) after sdNVP exposure, and NVP-resistant HIV can be transmitted to infants by breastfeeding. We analyzed NVP resistance in BM from Ugandan women following sdNVP administration.

METHODS

Source of samples

Samples were obtained from 51 women enrolled in an observational study in Kampala, Uganda (BF Study, 2003-2004). Women received sdNVP; they did not receive any other antiretroviral drugs. We tested samples from 17 women whose infants were HIV-infected by 6 weeks of age and 34 women whose infants were HIV-uninfected at 6 weeks of age.

Laboratory methods performed in the BF study

BM supernatants were centrifuged to remove lipids and were stored at -70°C. Plasma HIV viral loads were measured using the Roche AMPLICOR Monitor test kit v1.5. BM viral loads were measured using the Roche AMPLICOR Ultrasensitive Monitor test kit after extracting HIV RNA using the Boom method.

Laboratory methods performed in the resistance sub-study

HIV genotyping was performed using the ViroSeq™ HIV-1 Genotyping System v2.7; a nested PCR procedure was used to amplify BM samples with <500 copies/ml HIV RNA. HIV subtypes were determined by phylogenetic analysis of *pol* region sequences. NVP concentrations were determined using a liquid chromatography tandem mass spectroscopy assay (lower limit of quantification: 10 ng/ml).

Statistical methods

Statistical significance for comparison of proportions and medians was tested using Fisher's Exact test and the Wilcoxon test, respectively. Statistical tests were done using the R Software, against a two-sided 0.05 alpha significance level. For analysis, plasma viral loads <400 copies/ml were assigned a value of 200 copies/ml, plasma viral loads >750,000 copies/ml were assigned a value of 750,000 copies/ml, and BM viral loads <50 copies/ml were assigned a value of 25 copies/ml.

RESULTS

Genotyping results were obtained for 30 (58.8%) of 51 BM samples and 30 paired plasma samples (15A, 1C, 12D, 2 intersubtype recombinant). 12/30 (40%) BM samples had at least one NVP resistance mutation; most had K103N and/or Y181C (Table 1). NVP resistance was detected in BM from 4/11 (36.4%) transmitters vs. 8/19 (42.1%) non-transmitters (p=1.0). Five women had NVP resistance in plasma only, 4 had NVP resistance in BM only, 8 had NVP resistance in both samples, and 13 did not have NVP resistance detected in either sample. 5/8 women with NVP resistance in both samples had different mutations in the two samples (Table 1). NVP resistance was still detected in BM from 4/10 evaluable women by 10 weeks post-partum.

We analyzed the association of BM resistance at 4 weeks with clinical and laboratory factors (Table 2). There was a non-significant association of NVP resistance in plasma and BM (p=0.06). We did not find an association of BM resistance with median maternal pre-NVP viral load, white blood cell count in BM, or CD4 cell count, median BM viral load at 4 weeks, HIV subtype, or BM sodium >10 mmol/L at 1 or 4 weeks; none of the women had clinical mastitis.

At 1 week post-partum, NVP was detected (>10 ng/ml) in all 30 plasma samples and in 28/29 (96.6%) BM samples. At 2 weeks post-partum, NVP was detected in 21/29 (72.4%) plasma samples and 16/23 (69.6%) BM samples, and detection of NVP in BM was associated with detection of NVP in plasma (p<0.001). Median NVP concentrations in plasma BM were similar at 1 and 2 weeks. NVP was not detected in plasma and BM by 4 weeks post-partum.

ID	Plasma 4 weeks	Breast milk 4 weeks	Breast milk 10 weeks
64	K103N	WT	
117	V106A	WT	
52	G190A	WT	
5	K103N+G190A	WT	
79	Y181C+Y188C	WT	
42	WT	G190A	WT
17	WT	Y188C	WT
8	WT	Y181C	Failed
69	WT	K103N	WT
39	Y181C	Y181C	WT
3	Y181C+Y188C	Y188C	K103N
23	K103N+Y181C	Y181C	WT
77	K103N+Y181C	K103N	WT
30	K103N+Y181C+Y188C	K103N+Y181C+Y188C	K103N+G190A
13	K103N+V106A+Y188C	K103N	No Sample
24	K103N+Y181C+Y188C+G190A	K103N+Y181C+Y188C+G190A	K103N
22	K103N+V106A+Y181C+G190A	K103N+V106A+G190A	K103N

Table 1. NVP resistance mutations detected in plasma and BM samples

Thirteen women had no NVP resistance mutations detected in plasma or breast milk. Results are shown for the 17 women who had at least one NVP resistance mutation detected in either sample. Wild type (WT) indicates no resistance mutations detected.

Factor	N	BM Resistance	No BM Resistance	P value
Number of women	30	12	18	
Plasma NVP resistance at 4 weeks	30	8/12 (66.7%)	5/18 (27.8%)	0.06 ^c
Median pre-NVP viral load (plasma)	30	1002	1035	0.33 ^b
Median pre-NVP CD4 cell count	30	0.371	0.341	0.75 ^b
Median pre-NVP BM WBC	30	6.90	5.85	0.18 ^c
Median BM viral load at 4 weeks	30	352	200	0.37 ^b
BM sodium ≥ 10mmol/L at 1 week	22	7/11 (64%)	3/11 (27%)	0.20 ^c
BM sodium ≥ 10mmol/L at 4 weeks	19	5/10 (50%)	2/9 (22%)	0.35 ^c
HIV Subtype^a	30			0.10 ^c
A		4/12 (33.3%)	11/18 (61.1%)	
C		1/12 (8.3%)	0/18 (0%)	
D		5/12 (41.7%)	7/18 (38.9%)	
Intersubtype recombinant		2/12 (16.7%)	0/18 (0%)	
Non-A		8/12 (66.7%)	7/18 (38.9%)	0.26 ^c
Non-D		7/12 (58.3%)	11/18 (61.1%)	1.00 ^c
Pharmacokinetic analysis^d				
Detection of NVP in plasma at 1 week	30	12/12 (100%)	18/18 (100%)	1.00 ^c
Detection of NVP in plasma at 2 weeks	29	8/11 (72.7%)	13/18 (72.2%)	1.00 ^c
Median [NVP] in plasma at 1 week (range)	30	133 (16-379)	168 (20-744)	0.37 ^b
Median [NVP] in plasma at 2 weeks (range)	29	16 (<10-46)	16 (<10-83)	0.98 ^b
Detection of NVP in BM at 1 week	29	11/12 (91.7%)	17/17 (100%)	0.41 ^c
Detection of NVP in BM at 2 weeks	23	8/10 (80%)	8/13 (61.5%)	0.40 ^c
Median [NVP] in BM at 1 week (range)	29	82 (<10-226)	132 (31-576)	0.14 ^b
Median [NVP] in BM at 2 weeks (range)	23	15 (<10-33)	14 (<10-64)	0.70 ^b

Table 2. Factors associated with NVP resistance in BM 4 weeks after sdNVP

^a P value for all subtypes; ^b Wilcoxon test; ^c Fisher's exact test; ^d [NVP]=NVP level, NVP levels below 10 ng/ml were below the limit of quantification of the assay.

CONCLUSIONS

We detected NVP resistance in 40% of BM samples collected 4 weeks after sdNVP. We did not identify any clinical or laboratory factors associated with detection of NVP resistance in BM, possibly due to small sample size. Maternal sdNVP administration (alone or with other antiretroviral drugs) is often used for prevention of MTCT in resource-limited settings. Recent studies show that use of extended NVP infant prophylaxis reduces post-natal HIV transmission. Further studies are needed to evaluate whether emergence of NVP-resistant HIV in BM increases the risk of post-natal transmission in infants receiving NVP-based regimens for prevention of MTCT, or is associated with transmission of NVP-resistant strains to infants during breastfeeding.