Persistence of Nevirapine in Breast Milk after Discontinuation of Treatment

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The objective of this study was to serially quantitate the concentration of nevirapine in breast milk after discontinuation of treatment. Samples were collected from both breasts of a human immunodeficiency virus–infected patient for 3 weeks. Nevirapine was quantifiable for up to 17 days after discontinuation of therapy; total nevirapine concentrations remained above the 90% inhibitory concentration for 6 days, and no differences were observed between breasts.

In resource-poor regions, transmission of HIV infection through breast milk accounts for almost one-half of the cases of pediatric HIV infection. In these regions, alternatives to breast milk are, for the most part, unavailable, unaffordable, and unsafe. Antiretroviral treatment during lactation is currently being studied as a means to prevent transmission of HIV infection both by reducing virus in breast milk and by providing the infant with “pre-exposure” prophylaxis. However, there are few data on the pharmacokinetics of antiretrovirals in breast milk and the relative drug exposure to the suckling infant [1]. Moreover, for many pregnant women, antiretroviral therapy may be discontinued postpartum, when access to drugs is limited, or because it is not essential for their own health. High antiretroviral concentrations in the breast milk may lead to neonatal toxicity, whereas antiretroviral treatment that inadequately suppresses viral replication may result in rapid emergence of drug resistance mutations that can be transmitted to the infant.

Nevirapine (NVP) is a nonnucleoside reverse-transcriptase inhibitor (NNRTI) that is commonly administered alone or in combination regimens for the treatment of maternal HIV infection; it has been shown to be effective when used to help prevent mother-to-child transmission of HIV infection [2]. Studies of NVP concentrations in breast milk have revealed that NVP readily enters breast milk with a ratio of 60%–70% of that measured in maternal serum samples [3–5]. However, no studies have examined the long-term or steady-state pharmacokinetics of NVP in breast milk or whether concentrations vary between breasts. The objective of this study was to examine NVP concentrations and pharmacokinetics in breast milk for a prolonged period after discontinuation of treatment to ascertain penetration, protein binding, and persistence in this biological compartment.

Methods. Breast milk samples from both breasts were obtained daily from a single HIV-infected woman participating in an institutional review board–approved protocol studying the immunology of breast milk. She was being treated continuously, starting in the second trimester, with an antiretroviral regimen containing zidovudine, lamivudine, and NVP to prevent mother-to-child transmission of HIV infection. Therapy was discontinued 8-weeks postpartum, because the patient’s CD4 cell count was >500 cells/mm3 and antiretroviral therapy was not essential for her own health. Levels of total and free NVP were measured in expressed breast milk samples for 22 days after the patient’s last NVP dose (200 mg) using a simple extraction procedure and a high-performance liquid chromatographic method that was developed and validated in our laboratory specifically for breast milk, as described below. NVP concentrations and pharmacokinetic parameters were determined to ascertain protein binding, drug penetration, and persistence in this biological compartment.

Free NVP was obtained by initial ultrafiltration of whole breast milk samples using the Amicon Centrifree YM-30 filter system (Millipore). The resulting filtrate, which contained only unbound drug, was removed and subjected to a liquid-liquid extraction procedure, which was also applied to whole breast milk samples. NVP and internal standard (BIRH-414) were extracted using sodium hydroxide–buffered ethyl ether. After the addition of extraction solvent, samples were vortex-mixed and subsequently centrifuged. The aqueous layer was then frozen in a dry ice–isopropanol bath. The organic layer was removed and allowed to evaporate to dryness, and the resulting dried specimen residues were prepared for injection onto the high-performance liquid chromatographic system for analysis.

High-performance liquid chromatographic analysis was per-
formed using a chromatography system (Waters Alliance), including a 2695 Separations Module coupled to a 2487 Dual Wavelength UV Detector set to monitor 284 nm. Separation was achieved by reverse-phase liquid chromatography on a Microsorb MV C8 analytical column (Varian; diameter and length, 4.6 × 250 mm; particle size, 5 μm). The mobile phase consisted of 70% 50 mmol/L potassium phosphate buffer (pH, 3.0) and 30% acetonitrile, run at a flow rate of 1.0 mL/min. This assay is linear over the range of 10–5000 ng/mL. The intraday and interday precision (coefficient of variance) ranged from 1.1% to 6.1% and 3.5% to 4.7%, respectively, and the intraday and interday accuracy (percent of deviation) ranged from −8.2% to 1.2% and −5.7% to −1.8%, respectively.

All chromatographic integrations and calculations were performed using the Waters Millennium software (Waters). Calibration curves were obtained by weighted (1/concentration) linear regression analysis. For assay validation and data comparisons, statistical calculations were performed using Microsoft Excel (Microsoft) and S-PLUS 4.0 (Insightful). Standard noncompartmental pharmacokinetic analysis was performed using WinNonlin 4.01 (Pharsight).

**Results.** NVP is a highly lipophilic drug, remains essentially nonionized at physiological pH, and readily passes into the breast milk at concentrations slightly lower than those measured in plasma [3–5]. NVP was quantifiable in whole breast milk samples for up to 17 days after the final administration of the drug at concentrations of 17.8 ng/mL and 13.2 ng/mL in the right and left breast, respectively. Free NVP was quantifiable for up to 13 days after the final administration of the drug at concentrations of 17.8 ng/mL and 13.2 ng/mL in the right and left breast, respectively. The total NVP maximal concentration, half-life, and area under the curve (AUC) for the right and left breast were 2742 ng/mL and 2475 ng/mL, 69.2 h and 72.0 h, and 152.7 mg × L/h and 140.2 mg × L/h, respectively. The free NVP maximal concentration, half-life, and AUC for the right and left breast were 1378 ng/mL and 1660 ng/mL, 65.2 h and 50.8 h, and 85.6 mg × L/h and 94.5 mg × L/h, respectively. Total NVP clearance was also similar for both breasts, at 1.21 L/h for the right breast and 1.31 L/h for the left breast. In comparison, the median steady-state plasma maximal concentration, half-life, AUC, and 12-h concentration after administration of NVP (200 mg twice daily) were 5580 ng/mL, 16.5 h, 55.47 mg × L/h, and 3720 ng/mL [6], respectively.

The mean total and free NVP concentration data (by time) for breast milk from the right and left breasts are presented in figure 1. Total NVP concentrations remained above the estimated IC50 of 195 ng/mL (N. Parkin, K. Limoli, and L. Trinh, personal communication) for 6 days, whereas free NVP concentrations remained above the estimated IC50 of 161 ng/mL for 5 days and the estimated IC50 of 24 ng/mL [7] for 11 days. A recent study reported that, after a final administration of 200 mg of NVP to HIV-infected patients, the mean concentration in plasma samples reached 4200 ng/mL with a mean elimination half-life of 24.3 h [8]. NVP concentrations above the IC50 of wild-type HIV-1 were shown to persist in plasma specimens for a mean period of 7 days (range, 108–264 h) after discontinuation of treatment, which is comparable to our observations in breast milk. The duration of NVP persistence in biological fluids is important, because NNRTI class resistance emerges rapidly when NVP is administered as monotherapy [9–11]. Recently, a simple strategy has emerged to prevent NNRTI resistance by administering supplemental antiretroviral therapy to suppress viral replication until NVP concentrations are no longer sufficient to select for resistant HIV infection [12].

This is, to our knowledge, the first evaluation of NVP pharmacokinetics in breast milk after discontinuation of treatment. The persistence of NVP in human plasma has, however, been investigated after intrapartum single-dose NVP treatment was administered to Thai women [13]. Cressey et al. [13] found that NVP was detectable in human plasma samples for up to 21 days postpartum and had a mean elimination half-life of 72.5 h. The half-life of NVP in plasma samples described in that single-dose study is much longer than the half-life of 24.3 h after treatment discontinuation reported by Mackie et al. [8] but is similar to the results we obtained for NVP in breast milk (mean half-life of NVP in breast milk from both breasts, 70.6 h). It is known that continuous NVP therapy results in more rapid drug elimination through autoinduction, indicating a shorter interval in which NVP concentrations remain above the IC50. Because of the long elimination half-life we observed for NVP in breast milk (up to 72 h), the optimal duration of supplemental antiretroviral therapy for women who are breastfeeding may need to be determined to prevent the development...
of resistance mutations and transmission of resistant virus to the suckling infant. A recent study by Lee et al. [14] highlights the issue of NNRTI-resistant virus in breast milk. They reported that, after exposure to single-dose NVP, 65% of breast milk samples showed at least 1 mutation conferring resistance to NNRTIs. Additionally, in paired right and left breast milk samples, 36% had different patterns of NNRTI resistance mutations. NNRTI resistance was not observed in plasma samples before NVP exposure in the study; therefore, the selection of resistant virus in plasma and breast milk samples was likely to be the result of differences in viral replication and selective pressure in plasma and breast milk, in which decreasing NVP levels are maintained for many weeks.

This is also, to our knowledge, the first study to date that measures both free and total NVP concentrations in human breast milk. Our results reveal that NVP was 39% protein bound in breast milk, which is less than the 60%–70% binding observed in plasma [15, 16]. NVP is bound primarily to albumin in human plasma [17], as well as in breast milk; however, the albumin content in breast milk is much lower [18], which could account for the difference in protein binding seen in our study.

Ultimately, antiretroviral drugs present in breast milk may have beneficial as well as adverse effects for the infant. A recent study of 20 mother and infant pairs found median serum concentrations 40 times the IC50 in the suckling infants [4]. The NVP concentrations in breast milk reported in that study tend to be high, compared with our observations; however, the results we obtained were within the range of values reported for the 20 breast-feeding mothers (2000–10,000 ng/mL). NVP concentrations in breast milk, such as we observed, were higher than those generally believed to be necessary for prophylaxis of HIV infection and, therefore, may provide protection against transmission of HIV infection to uninfected infants through breast milk. These concentrations, however, may also be high enough to lead to drug-related toxicities in the suckling infant, especially early in life, when clearance mechanisms are not fully developed.

Conclusions. We present herein the first evaluation of the pharmacokinetics of NVP in breast milk that examined the persistence of total and free NVP concentrations in both breasts after discontinuation of treatment. Persistence of NVP in breast milk may have important clinical implications for the suckling infant. A simple, effective regimen administered over the duration of breast-feeding may be important in maintaining the decreased risk of mother-to-child transmission of HIV infection that has been seen in association with single-dose regimens. Our understanding of the persistence of NVP in breast milk may also be crucial when attempting to prevent the development of viral resistance in women and infants when NVP therapy is discontinued during breast-feeding.

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References

