Antiretroviral Concentrations in Breast-Feeding Infants of Women in Botswana Receiving Antiretroviral Treatment

Roger L. Shapiro,1,4 Diane T. Holland,3 Edmund Capparelli,5 Shahin Lockman,1,3 Ibou Thior,1 Carolyn Wester,1 Lisa Stevens,1 Trevor Peter,1 Max Essex,1 James D. Connor,5 and Mark Mirochnick4

1Department of Immunology and Infectious Diseases, Harvard School of Public Health, 2Division of Infectious Diseases, Beth Israel Deaconess Medical Center, 3Infectious Disease Unit, Brigham and Women’s Hospital, and 4Department of Pediatrics, Boston University School of Medicine, Boston, Massachusetts; 5Division of Clinical Pharmacology and Developmental Therapeutics, University of California, San Diego, Department of Pediatrics, San Diego

Background. The magnitude of infant antiretroviral (ARV) exposure from breast milk is unknown.

Methods. We measured concentrations of nevirapine, lamivudine, and zidovudine in serum and whole breast milk from human immunodeficiency virus type 1 (HIV-1)–infected women in Botswana receiving ARV treatment and serum from their uninfected, breast-feeding infants.

Results. Twenty mother-infant pairs were enrolled. Maternal serum concentrations of nevirapine were high (median, 9534 ng/mL at a median of 4 h after nevirapine ingestion). Median breast-milk concentrations of nevirapine, lamivudine, and zidovudine were 0.67, 3.34, and 3.21 times, respectively, those in maternal serum. The median infant serum concentration of nevirapine was 971 ng/mL, at least 40 times the 50% inhibitory concentration and similar to peak concentrations after a single 2-mg/kg dose of nevirapine. The median infant serum concentration of lamivudine was 28 ng/mL, and the median infant serum concentration of zidovudine was 123 ng/mL, but infants were also receiving zidovudine prophylaxis.

Conclusions. HIV-1 inhibitory concentrations of nevirapine are achieved in breast-feeding infants of mothers receiving these ARVs, exposing infants to the potential for beneficial and adverse effects of nevirapine ingestion. Further study is needed to understand the impact of maternal ARV treatment on breast-feeding HIV-1 transmission, infant toxicity, and HIV-1 resistance mutations among infected infants.

As access to antiretrovirals (ARVs) improves throughout the developing world, optimal infant feeding strategies for HIV-infected women receiving these medications will require reevaluation. In the absence of maternal or infant ARVs, mother-to-child transmission (MTCT) of HIV from breast-feeding occurs in ~9%–16% of breast-fed infants [1], which represents >40% of all transmission among these infants [1, 2]. However, in many areas of the developing world, formula feeding is impractical and may be associated with increased morbidity and mortality [3]. Alternate strategies—such as the administration of prophylactic ARVs to infants [4] or of highly active antiretroviral therapy (HAART) to mothers [5]—are being considered to prevent MTCT from breast-feeding.

The presence of ARVs in breast milk may reduce MTCT either through the direct inhibition of local HIV replication or by providing infant prophylaxis. In small studies that did not report the time between drug administration and breast-milk sample collection, nevirapine, lamivudine, and zidovudine were detected in breast milk.
milk from nursing mothers at levels near or above plasma concentrations [6]. No previous studies have directly measured concentrations of ARVs in breast-feeding infants of women being treated with these drugs, and it is unknown whether infant plasma concentrations of nevirapine, lamivudine, and zidovudine ingested through breast milk may exceed the IC₅₀ thought to be required for infant MTCT prophylaxis. The present study was designed to determine serum and breast-milk concentrations of nevirapine, lamivudine, and zidovudine among 20 breast-feeding women, and in serum from their infants, at a known time after maternal drug ingestion.

SUBJECTS AND METHODS

Study Population
We studied a subset of 20 women and their infants enrolled in an ongoing randomized, clinical trial for the prevention of MTCT at 4 sites in Botswana. Known as the Mashi Study (meaning “milk” in Setswana), the study began enrolling subjects in March 2001 and completed the enrollment of 1200 HIV-infected women in October 2003. The Mashi Study made use of a factorial design to determine whether (1) a single dose of nevirapine given to mothers and infants provides additional MTCT prevention in the setting of maternal and infant zidovudine therapy and (2) extended prophylactic zidovudine given to breast-feeding infants prevents MTCT, compared with formula feeding. Breast milk was collected at delivery and 2 weeks, 2 months, and 5 months after delivery from breast-feeding women. In August 2002, the first part of the study was modified to provide all infants with both zidovudine and single-dose nevirapine. Beginning in October 2002, HAART (initiated with nevirapine, zidovudine, and lamivudine) became available through the Botswana Government Antiretroviral Treatment Program and was offered to women with CD₄ cell counts <200 cells/mm³ or with AIDS-defining illnesses and to all infected infants.

The first 20 women and their infants who were eligible to participate in the substudy of concentrations of ARVs were enrolled if they were enrolled in the Mashi Study and had been randomized to the arm of breast-feeding plus infant zidovudine prophylaxis, had been receiving HAART continuously for at least 6 weeks, and had agreed to provide samples of breast milk and serum and of infant serum at the 2- or 5-month postpartum visit. All women were still breast-feeding and able to express milk at the time of sample collection, and no infants were receiving ARVs other than prophylactic zidovudine. All women were receiving lamivudine (150 mg twice daily) and nevirapine (200 mg twice daily) at the time of sample collection. Eighteen of 20 women were receiving zidovudine (300 mg twice daily), and 2 women were receiving stavudine (30 mg twice daily). Sufficient maternal serum was available to determine concentrations of nevirapine, lamivudine, and zidovudine in all 20 women. Sufficient breast milk was available to determine concentrations of nevirapine and zidovudine in all 20 women and concentrations of lamivudine in 18 women.

All infants had received continuous zidovudine prophylaxis since the time of birth; at the time of sample collection, they were receiving either 4 mg/kg 3 times daily (if collection occurred at 2 months) or 6 mg/kg 3 times daily (if collection occurred at 5 months). All infants had previously received a single dose of 6 mg of nevirapine at birth. Sufficient infant serum was available to determine concentrations of nevirapine, lamivudine, and zidovudine in all 20 infants.

Study Procedure
The study was reviewed and approved by an institutional review board (Harvard School of Public Health) and by the Health Research Unit (Botswana). A separate informed consent was signed by all participants. At either the 2- or the 5-month postpartum visit, mothers completed a questionnaire to record their ARV dosing history (date and time of the last 3 doses), the time of the last infant feeding, and the time of the last infant dose of prophylactic zidovudine. Twenty milliliters of breast milk and 1 mL of infant blood were drawn as part of routine Mashi Study follow-up or for the purposes of the present study. An additional 3 mL of maternal blood was drawn for the purposes of the study. The time of collection was documented for all samples. Maternal demographic data were collected as part of the Mashi Study.

All samples were transported to the Botswana-Harvard HIV Reference Laboratory on the day of collection. At the laboratory, serum and breast-milk samples were frozen at −70°C before shipment to the Pediatric Pharmacology Research Laboratory at the University of California, San Diego (UCSD) for pharmacokinetic analysis.

Drug Assays from Blood and Breast Milk

**Serum.** Infant and maternal serum concentrations of zidovudine were measured by use of a validated EIA. The calibration range for the assay was 5–1250 ng/mL. Assay sensitivity was 10 ng/mL. Intra- and interday precisions were 7.4%–13.6% coefficients of variation (CVs), and accuracy ranged from −8.6% to 9.9%. Infant serum concentrations of lamivudine and nevirapine were determined by use of a validated liquid chromatography–mass spectrometry assay, because of small volumes. Assay sensitivity was 7 ng/mL for lamivudine and 16 ng/mL for nevirapine. Maternal serum concentrations of lamivudine and nevirapine were measured by validated reversed-phase high-pressure liquid chromatography (RP-HPLC) assays with UV detection. Assay sensitivity was 32 ng/mL for lamivudine and 43 ng/mL for nevirapine.

**Breast milk.** The percentage of fat in each breast-milk sam-
Table 1. Concentrations of antiretrovirals (ARVs) in maternal serum and breast milk and in serum from breast-feeding infants.

<table>
<thead>
<tr>
<th>ARV</th>
<th>Maternal serum concentration, median, ng/mL</th>
<th>Breast-milk concentration, median, ng/mL</th>
<th>Breast milk:serum ratio, median (no.)</th>
<th>Infant serum concentration, median, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVP</td>
<td>9534</td>
<td>6795</td>
<td>0.67 (19)</td>
<td>971</td>
</tr>
<tr>
<td>3TC</td>
<td>678</td>
<td>1828</td>
<td>3.34 (18)</td>
<td>28</td>
</tr>
<tr>
<td>ZDV</td>
<td>58</td>
<td>207</td>
<td>3.21 (17)</td>
<td>123</td>
</tr>
</tbody>
</table>

a IC$_{50}$ for nevirapine (NVP), 24 ng/mL; IC$_{50}$ for lamivudine (3TC), 550 ng/mL; and IC$_{50}$ for zidovudine (ZDV), 5 ng/mL [7].

b Median of individual breast milk:serum ratios for each participant.
c Results exclude 2 women who were not receiving ZDV.
d All infants also received prophylactic ZDV.

The median length of time that women had received continuous HAART was 108 days (range, 68–222 days). The median age of women participating in the study was 30 years (range, 25–41 years), their median weight was 59 kg (range, 40–75 kg), and their median pretreatment CD4+ cell count was 167 cells/mm$^3$ (range, 65–191 cells/mm$^3$). All infants had received continuous zidovudine prophylaxis since birth and a single dose of nevirapine after birth.

The median time between ingestion of the last dose of ARVs and collection of maternal blood samples and breast milk was 4.0 h (range, 1.0–8.5 h). All participants reported adherence with their 3 most recent ARV doses and had ingested all 3 drugs at the scheduled 12-h interval. All infants were breast-feeding, and the median time between their most recent feeding and blood sampling was 40 min (range, 0 min–3.1 h). The median time between the infants’ receipt of their last dose of zidovudine prophylaxis and blood sampling was 5.4 h (range, 30 min–9.5 h).
Concentrations of ARVs in each sample were measured in both skim and whole milk. The average ratio (± SD) of the concentration of each ARV in skim milk to that of whole milk was 77% (± 14%) for nevirapine, 94% (± 13%) for lamivudine, and 116% (± 24%) for zidovudine. We therefore used whole-milk concentrations for all analyses. Where values for whole-milk concentrations were unavailable (3 for lamivudine and 1 for nevirapine), the skim-milk concentration was converted to approximate the value for whole milk by use of the median whole milk:skim milk ratio for each drug.

The median maternal serum and breast-milk concentrations of nevirapine, lamivudine, and zidovudine and the median breast milk:serum ratio for each are shown in table 1. Breast-milk concentrations of nevirapine, lamivudine, and zidovudine were a median of 0.67 (range, 0.24–1.01 ng/mL), 3.34 (range, 0.87–12.61 ng/mL), and 3.21 (range, 0.58–9.52 ng/mL) times those in serum, respectively.

Figure 1 shows the drug concentrations in serum and breast milk for each participant, by time between drug ingestion and sample collection. Drug concentration was not significantly associated with maternal weight for any of the ARVs. Figure 2 shows the breast milk:serum ratio, by time between drug ingestion and sample collection, for all women with detectable drug concentrations in both breast milk and serum. The lamivudine breast milk:serum ratio varied widely and increased significantly as the interval between drug ingestion and sample collection increased (P < .05). There was less variation in the nevirapine breast milk:serum ratios, but there was a trend toward a decrease as the time since the last dose increased (P = .09). The 2 women who were receiving stavudine rather than...
Figure 2. Breast milk:serum drug-concentration ratios for nevirapine (NVP), lamivudine (3TC), and zidovudine (ZDV), by hours between dosing and sample collection.

zidovudine at the time of sampling are included in figure 1 but are not included in figure 2. Each had detectable serum concentrations of stavudine and undetectable serum concentrations of zidovudine (represented in figure 1 by serum zidovudine values below the limit of detection). However, despite having stopped zidovudine 10 to 15 weeks previously, each had a detectable concentration of zidovudine in her breast milk (151 and 52 ng/mL, respectively). A third woman who reported adherence with her prescribed ARVs had undetectable levels of nevirapine and zidovudine and low levels of lamivudine in her serum (86 ng/mL), with undetectable nevirapine and moderate concentrations of lamivudine and zidovudine in her breast milk (420 and 165 ng/mL, respectively). The median serum concentrations of nevirapine, lamivudine, and zidovudine in breast-feeding infants are also shown in table 1. The median concentration of nevirapine in breast-feeding infants was 971 ng/mL (range, 16–2191 ng/mL), which is 40 times the IC_{50} (24 ng/mL) [7]. The median infant serum concentration of nevirapine was 10% of maternal values (range, 0%–20%). The median infant concentration of lamivudine was 28 ng/mL (range, 14–53 ng/mL), which is 5% of the IC_{50} (550 ng/mL) [7]. The median infant concentration of lamivudine was 123 ng/mL (range, 14–3302 ng/mL), which is 25 times the IC_{50} (5 ng/mL) [7]. Infant serum concentrations of zidovudine were a median of 2.5 times high-
er than the maternal concentrations of zidovudine for each mother-infant pair. Figure 3 shows the concentrations of nevirapine and lamivudine in serum for each infant by the time between last feeding and sample collection. For zidovudine, prophylactic dosing complicated the relationship between serum concentration and the time between last feeding and sample collection.

In these 20 infants, all of whom also received nevirapine at birth and oral zidovudine throughout breast-feeding, 3 serious or life-threatening episodes of neutropenia and 1 episode of serious anemia were reported during the breast-feeding period. No serious or life-threatening chemical abnormalities were reported. An additional infant had a severe skin rash 3 days after birth.

**DISCUSSION**

To our knowledge, this is the first study to evaluate concentrations of ARVs among breast-feeding infants of women receiving HAART and is the largest study to date of concentrations of ARVs in breast milk. Our findings demonstrate serum concentrations of nevirapine in these breast-feeding women in Botswana that are \( \sim 60\% \) higher than those expected; concentrations of nevirapine, lamivudine, and zidovudine in breast milk that are similar to or higher than those in serum; and HIV-1 inhibitory concentrations of nevirapine in serum from breast-feeding infants. Concentrations of nevirapine in infant serum were comparable to peak levels achieved after the administration of a single dose of 2 mg/kg of nevirapine at birth [8] and were well above the levels thought to be necessary to provide prophylaxis against HIV infection. Concentrations of lamivudine in infant serum were only 5% of the reported IC\(_{50}\) [7]. However, the IC\(_{50}\) reported by the manufacturer gives a broader range of values [9], and we cannot rule out the possibility that lamivudine has some inhibitory effect at the concentrations detected. Infants in the study were all receiving prophylactic zidovudine in addition to breast-feeding, and our results demonstrate serum concentrations of zidovudine that were above the target prophylactic value. The accepted target level for prophylaxis is \( >10\times \) the IC\(_{50}\) based on the successful HIVNET 012 study [10], in which prophylactic concentrations of nevirapine were maintained above this level. The actual lower limit of the serum (and intracellular) concentration needed for MTCT prophylaxis is not known.

In addition to providing infant prophylaxis, maternal HAART may reduce MTCT via breast-feeding by directly inhibiting viral replication in breast milk. The breast-milk concentrations of nevirapine, lamivudine, and zidovudine in our study were at or above serum concentrations, which provides support for the direct inhibition of virus in this compartment. The breast milk:maternal serum drug-concentration ratios were higher than those previously reported for lamivudine and zidovudine and were similar to previous reports for nevirapine. Nevirapine has a long serum half-life, and the trend toward a small decrease in the breast milk:serum drug-concentration ratio over time implies that its half-life in breast milk is similar to or slightly less than that in serum. However, the breast milk:serum drug-concentration ratios seen for lamivudine and possibly for zidovudine suggest decreased clearance from breast milk, compared with serum (figure 2), which has also been demonstrated with zidovudine in mice [11].

Although it is the largest study to date, our study included only 20 breast-feeding women and their infants and included only 1 sampling point per woman and infant. This limited our ability to fully explore the relationships between dosing interval and concentration and between feeding interval and concentration. However, because breast-feeding occurs frequently during the day and because of the high concentrations of all drugs found in breast milk, it is likely that infants in our study were receiving a fairly constant dose of ARVs with each feeding. Larger studies with more mothers and infants, sampled at more time points, would help clarify these relationships.

Our results raise the possibility that infant exposure to nevirapine and, possibly, to lamivudine and zidovudine from breast milk is sufficient for both the beneficial and adverse effects of these drugs. The concentrations of nevirapine that we observed were higher than those generally believed to be necessary for
prophylaxis of HIV infection, and this may provide protection against HIV infection via breast milk in uninfected infants. These concentrations may also expose infants to the risk of drug-related toxicities. One infant had a rash 3 days after birth (and had also received oral nevirapine at birth), and 4 of the infants had either severe or life-threatening neutropenia or anemia at some point during breast-feeding. However, all infants were also receiving oral zidovudine, and the overall rate of hematologic toxicities may not have differed from that in infants exposed to oral zidovudine alone. A full assessment of hematologic toxicities, mitochondrial toxicities, hypersensitivity reactions, and other adverse events among infants with exposure to ARVs via breast milk requires a much larger sample size, and monitoring for such events is indicated, at least until more data are available.

The concentrations of ARVs that we detected in infant serum are also of concern for causing drug resistance among HIV-infected infants. The levels are lower than those achieved in a fully suppressive treatment regimen but are likely to be high enough to select for resistant viruses. Thus, a breast-feeding HIV-infected infant whose mother is receiving nevirapine and lamivudine (and, possibly, zidovudine, although the contribution from breast-feeding could not be directly measured in our study) may be at high risk for developing resistance mutations to these drugs. This risk must be weighed against the health benefits to the mother from maternal HAART during breast-feeding and the potential for maternal HAART to reduce MTCT via breast-feeding in most infants.

We cannot explain why serum concentrations of nevirapine were high in the mothers in our study, although we believe that these high maternal concentrations were at least partly responsible for the high levels in infant serum. The median maternal serum concentration of nevirapine of 9534 ng/mL in our study is 60% higher than the expected maternal concentration of nevirapine 4 h after dosing (the median in our study) to be high enough to select for resistant viruses. Therefore, breast-feeding HIV-infected infants whose mothers are receiving nevirapine and lamivudine (and, possibly, zidovudine, although the contribution from breast-feeding could not be directly measured in our study) may be at high risk for developing resistance mutations to these drugs. This risk must be weighed against the health benefits to the mother from maternal HAART during breast-feeding and the potential for maternal HAART to reduce MTCT via breast-feeding in most infants.

In conclusion, we have demonstrated high concentrations of nevirapine in maternal serum; breast-milk concentrations of nevirapine, lamivudine, and zidovudine similar to or higher than those in serum; and HIV-1 inhibitory concentrations of nevirapine among breast-feeding infants. Infants receiving zidovudine through direct prophylaxis as well as breast-feeding had serum concentrations above the target value for prophylaxis against HIV infection. These data suggest that breast-feeding infants of women receiving nevirapine, lamivudine, and zidovudine may be exposed to both the beneficial and adverse effects of these drugs. When the mother is receiving HAART with these agents, ARV administration to the infant may not be required for the prevention of MTCT via breast-feeding. Further study is needed to better understand the pharmacokinetics of these drugs in both the serum and breast milk of women in the developing world, the impact of maternal HAART on MTCT via breast-feeding, potential toxicities in infants exposed to ARVs from breast milk, and the effect of exposure to ARVs from breast milk on the occurrence of HIV resistance mutations among infected infants.

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References