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Stavudine (d4T) concentrations in women receiving post-partum antiretroviral treatment and their breastfeeding infants

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Abstract

First-line antiretroviral treatment regimens in resource-limited settings used in breastfeeding mothers often include stavudine (d4T). Limited data describing d4T concentrations in breast milk

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Conflicts of Interest

None of the authors has a conflict of interest.

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CONTRIBUTION OF AUTHORS

All authors contributed to preparation of the manuscript. In addition, individual authors had the following contributions to the study:

J Fogel Designed the study, prepared samples for ARV testing, performed resistance testing, analyzed data, wrote the manuscript.

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are available. We analyzed d4T concentrations in 52 mother-infant pairs using ultra-performance liquid chromatography-tandem mass spectrometry (lower limit of quantification: 5 ng/ml in plasma, 20 ng/ml in breast milk). Median (interquartile range) d4T concentrations were 86 (36–191) ng/ml in maternal plasma, 151 (48–259) ng/ml in whole milk, 190 (58–296) ng/ml in skim milk, and <5 (<5–<5) ng/ml in infant plasma. While d4T is concentrated in breast milk relative to maternal plasma, the infant d4T dose received from breast milk is very small and not clinically significant.

Keywords

stavudine concentrations; breast milk; mother-to-child transmission; HIV

INTRODUCTION

Access to antiretroviral (ARV) drugs is increasing in many resource-limited settings. Breastfeeding HIV-infected mothers may receive ARV regimens for HIV treatment or for prevention of mother-to-child HIV transmission. Nursing infants whose mothers take ARV drugs may receive subtherapeutic doses of maternally-administered ARV drugs via breast milk [1–4]. This may lead to development of ARV drug resistance in infants who are HIV infected [5–7]. Data describing breast milk transfer exist for some ARV drugs, including nevirapine (NVP), lamivudine (3TC), zidovudine (ZDV), efavirenz, nelfinavir, and indinavir, but limited data are available for stavudine (d4T) [1–4, 8]. While the World Health Organization (WHO) recently recommended against including d4T in ARV treatment regimens because of side-effects, such as lactic acidosis and mitochondrial toxicity, d4T-based treatment regimens are still widely used in many resource-limited settings [9]. In Malawi, d4T-based ARV regimens are recommended for both infants and adults, including lactating women [10].

In the PEPI-Malawi trial, HIV-infected women with CD4 cell counts <250 cells/μl who were eligible for ARV therapy (ART) according to WHO guidelines at the time initiated therapy post-partum while nursing [11, 12]. Most of those women received NVP, 3TC, and d4T, which is the first-line ART regimen in Malawi. We analyzed d4T concentrations in maternal plasma and breast milk from PEPI-Malawi trial participants who initiated ART by 6 months post-partum, and in plasma from their breastfeeding infants.

METHODS

Samples used for analysis

ARV drug concentrations were measured in maternal plasma, breast milk, and infant plasma collected in the PEPI-Malawi trial. The main objective of the PEPI-Malawi trial (2004–2009) was to compare three ARV regimens to prevent postnatal HIV transmission: (1) single dose nevirapine (sdNVP) plus one week of daily zidovudine (ZDV, control), (2) control plus daily NVP from day 8 to 14 weeks of age (extended NVP), and (3) control plus daily NVP and ZDV from day 8 to 14 weeks of age (extended NVP/ZDV) [11]. Women whose infants were HIV-uninfected were counseled to exclusively breastfeed for 6 months, and women whose infants became HIV-infected were counseled to breastfeed as long as possible, based on WHO recommendations at the time. Study visits took place at birth, 3, 6, 9, and 14 weeks and then every three months up to 18–24 months post-partum to monitor safety and to test infants for HIV infection. Women who met WHO criteria for ART initiation during the post-partum period received NVP (200 mg), 3TC (150 mg), and d4T (30 mg if <60kg, 40 mg if >60kg) twice daily. This treatment was administered outside of the PEPI-Malawi trial. Maternal ARV drug use was recorded on a structured questionnaire. However, the exact date

of ART initiation was not consistently recorded. Post-partum visits usually occurred in the morning; the exact time of maternal dosing and sample collection were not recorded. In this report, HIV-infected infants who also received ART were excluded from the analysis after ART initiation.

Laboratory methods

Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to measure ARV concentrations and was performed at the Clinical Pharmacology Analytical Laboratory at Johns Hopkins University School of Medicine. The instrumentations used were AB-Sciex API4000 triple quadrupole mass spectrometer (Foster City, CA) interfaced with a Waters Acquity UPLC (Milford, MA). Maternal plasma samples (50 μ l) were assayed for d4T and NVP concentrations and infant plasma samples (50 μ l) were assayed for d4T concentrations. Breast milk (both whole milk and breast milk supernatant/skim milk) were also assayed for d4T. Skim milk was prepared by centrifuging whole milk at 3000 rpm for 15 minutes at room temperature; the upper lipid layer was discarded and the supernatant was transferred to a clean tube. The process was repeated 2–4 times to ensure complete removal of lipids. The lower limit of detection (below quantification limit [BQL]) was 5 ng/ml in plasma and 20 ng/ml in breast milk. Samples with NVP concentration below the limit of assay quantification are referred to as undetectable. Precision within the assay for plasma was <7% and accuracy was <7%. Precision within the assay for whole and skim breast milk was <11% and accuracy was <6%. Deuterated d4T was added to all samples as an internal standard followed by protein precipitation with acetonitrile. Samples were vortexed and centrifuged at 3000xg for 15 minutes. The organic layer was dried and reconstituted with mobile phase prior to injection. All drugs and internal standards were separated on an Acquity UPLC HSS C18 column (2.1 $\times$ 50mm, 1.8 μ m) using methanol and water containing 0.1% acetic acid as the mobile phases with a gradient elution. Detection was through electrospray ionization in the positive multiple reaction monitoring mode.

HIV drug resistance was assessed by analyzing infant plasma (100 μ l) using the ViroSeq HIV Genotyping System (ViroSeq, Celera, Alameda, CA) and was performed at the HIV Genotyping Laboratory at Johns Hopkins University School of Medicine.

Statistical Analysis

Detection of NVP in maternal plasma was used as a surrogate measure of maternal drug adherence because of the long half-life of NVP in plasma which, if not detected, provides evidence that NVP doses had not been taken within 2 weeks. Women with no NVP detected in plasma were presumed to be poorly adherent, and they and their infants were excluded from the analysis. Concentrations of d4T that were below the quantification limit (BQL) were assigned values of 2.5 ng/ml for plasma and 10 ng/ml for breast milk. Fisher's exact test compared proportions and Wilcoxon rank sum test compared medians between two variables. Data were analyzed using SAS 9.3 (SAS Institute, Inc, Cary, NC).

Ethical considerations

Written informed consent was obtained from all women for participation in the PEPI-Malawi trial. The trial was approved by Institutional Review Boards in Malawi and the U.S., including the U.S. Centers for Disease Control and Prevention.

GenBank Accession Numbers

Sequences analyzed in this report have the following GenBank Accession numbers: HM635507, HM635530, HM635536, HM635636, HM635500, HM635630, HM635521, HM635599, HM635489, HM635481, HM635559, and HM635558.

RESULTS

Study cohort

Overall, 246 women who were enrolled in the PEPI-Malawi trial initiated ART by 6 months post-partum; 221 (89.8%) were nursing at the time of ART initiation. Eighty-three mother-infant pairs had samples available from all four sample types (maternal plasma, whole milk, skim milk, and infant plasma) from the same visit. Thirty-one women were excluded from analysis; 10 women did not have sufficient plasma volumes available for testing and 21 women had undetectable NVP-concentrations in their plasma. Fifty-two mother-infant pairs had d4T results obtained from all four sample types. We compared clinical and laboratory characteristics of the 52 mother/infant pairs with d4T results with the 169 nursing mother/infant pairs not included in the study (Table). There was no significant difference between the groups in terms of maternal viral load or CD4 cell count at enrollment, maternal sdNVP administration, maternal report of ART use by 3 months post-partum, infant HIV-infection prior to maternal ART initiation, or infant study regimen (Table).

Detection and quantification of d4T in study samples

Concentrations of d4T in maternal plasma, whole milk, skim milk, and infant plasma samples were measured using the first available set of samples collected after the mother reported that she had started ART (median: 1.5 months after the mother reported ART initiation, range: 0–7.5 months). For the 52 mother-infant pairs with results from all four sample types, d4T was detected in 46 (88.5%) of the maternal plasma samples, 44 (84.6%) of the whole milk samples, and 45 (86.5%) of the skim milk samples. In contrast, d4T was only detected in seven (13.5%) of the infant plasma samples. The median concentrations of d4T were 86 ng/ml in maternal plasma (interquartile range [IQR]: 36–191 ng/ml), 151 ng/ml in whole milk (IQR: 48–259 ng/ml), 190 ng/ml in skim milk (range: 58–296 ng/ml), and BQL (<5 ng/ml) in infant plasma (IQR: BQL-BQL ng/ml, Figure). The median ratio of d4T concentration in skim milk and whole milk was 1.12 (range 0.90–2.41). The median ratio of d4T concentration in whole milk and maternal plasma was 1.45 (range 0.17–5.44). The median ratio of maternal plasma and infant plasma of the seven infants with detectable d4T concentrations was 0.05 (0.01–0.15). The estimated median daily infant d4T dose from breast milk (whole milk) was 22.7 µg/kg, assuming a daily breast milk intake of 150 ml/kg/day.

ARV drug resistance

Fifteen (28.8%) of the 52 infants analyzed were HIV-infected; 12 (80.0%) of those infants were HIV-infected before the mother reported ART initiation. HIV genotyping results were obtained from 12 (80.0%) of 15 infants. HIV from all 12 infants had mutations associated with resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs). In addition, HIV from five (41.7%) of the 12 infants had mutations associated with resistance to nucleoside reverse transcriptase inhibitors (NRTIs, one infant had M184V, three infants had K65R, and one infant had T69N and L74V). No d4T-specific thymidine analog mutations were detected.

DISCUSSION

This is the largest study to date reporting concentrations of d4T in plasma and breast milk from lactating women and in plasma from their breastfeeding infants. The majority of maternal plasma and breast milk samples had detectable concentrations of d4T (only 11–15% of plasma and breast milk samples had d4T concentrations below the limit of quantification [<5 ng/ml]). D4T is concentrated in breast milk, with whole milk d4T concentrations on average 45% greater than in plasma. The estimated infant median daily exposure to d4T from breast milk was 22.7 $\mu\text{g/kg}$, which corresponds to approximately 1% of the recommended pediatric treatment dose of 2 mg/kg/day [13]. Only seven (13.5%) of 52 infants studied had detectable d4T concentrations; all seven of those infants had d4T concentrations of <10 ng/ml, which falls in the lower limit of the range of the 50% inhibitory concentration (IC₅₀) for d4T for wild-type HIV (2–897 ng/ml) [14]. The low concentrations of d4T detected in the infants reflects the small dose of d4T from breast milk and the short plasma d4T half-life, which averages 2.2 hours in 1-week-old infants and 1.5 hours in 6-week-old infants [15]. One limitation of the study is that data on the time of dosing were not available, which precluded more detailed analysis of the kinetics of d4T transfer from plasma to breast milk and the resulting infant d4T exposure. We are only aware of one prior study that described d4T concentrations in maternal and infant plasma [16]. In that study, d4T concentrations were undetectable (<5 ng/ml) in eight serum samples collected from four infants who were exposed to maternally-administered d4T [16]. In another study, d4T was detected in breast milk from 2 women [17].

All HIV-infected infants in this study were analyzed for drug resistance mutations. All infants had NNRTI resistance mutations detected. The infants had several sources of NNRTI exposure, including maternal and infant sdNVP, infant NVP prophylaxis regimen (up to 14 weeks), and maternal NVP-containing ART. Any of these exposures could have contributed to the acquisition of NNRTI resistance mutations. In contrast, none of the HIV-infected infants had d4T-specific thymidine analog mutations. Other NRTI mutations were observed, such as M184V and K65R. M184V increases susceptibility to d4T and is often selected after 3TC exposure. The K65R mutation is selected by several NRTIs; however, in this study it was likely selected by 3TC in the maternal ART regimens. Selection of K65R by d4T was less likely because of the low concentrations of d4T detected in infant plasma. In a previous study of ARV drug resistance in HIV-infected infants from the PEPI-Malawi trial who were exposed to maternal ART, we detected only one of 37 infants with a mutation that may have been selected by exposure to maternally-administered d4T (D67N); however, that mutation is not sufficient to cause resistance to d4T [5]. The absence of d4T mutations in infants is supported by the limited transfer of d4T from breast milk and the little antiviral effect it has on infants as the majority of infants have d4T concentrations below the in vitro IC₅₀ of d4T.

In conclusion, our data demonstrate that while breast milk d4T concentrations generally exceed those in maternal plasma, the d4T dose provided via breast milk is very small and does not result in clinically significant d4T exposure in breastfeeding infants whose mothers are receiving d4T.

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References

1. Mirochnick M, Thomas T, Capparelli E, et al. Antiretroviral concentrations in breast-feeding infants of mothers receiving highly active antiretroviral therapy. *Antimicrob Agents Chemother.* 2009; 53:1170–1176. [PubMed: 19114673]
2. Schneider S, Peltier A, Gras A, et al. Efavirenz in human breast milk, mothers', and newborns' plasma. *J Acquir Immune Defic Syndr.* 2008; 48:450–454. [PubMed: 18614925]
3. Shapiro RL, Holland DT, Capparelli E, et al. Antiretroviral concentrations in breast-feeding infants of women in Botswana receiving antiretroviral treatment. *J Infect Dis.* 2005; 192:720–727. [PubMed: 16088821]
4. Weidle PJ, Zeh C, Martin A, et al. Nelfinavir and its active metabolite, hydroxy-t-butylamidenelfinavir (M8), are transferred in small quantities to breast milk and do not reach biologically significant concentrations in breast-feeding infants whose mothers are taking nelfinavir. *Antimicrob Agents Chemother.* 55:5168–5171. [PubMed: 21876052]
5. Fogel J, Li Q, Taha TE, et al. Initiation of antiretroviral treatment in women after delivery can induce multiclass drug resistance in breastfeeding HIV-infected infants. *Clin Infect Dis.* 2011; 52:1069–1076. [PubMed: 21460326]
6. Zeh C, Weidle PJ, Nafisa L, et al. HIV-1 drug resistance emergence among breastfeeding infants born to HIV-infected mothers during a single-arm trial of triple-antiretroviral prophylaxis for prevention of mother-to-child transmission: a secondary analysis. *PLoS Med.* 2011; 8:e1000430. [PubMed: 21468304]
7. Lidstrom, J.; Guay, LA.; Musoke, P., et al. Multi-class Drug Resistance Arises Frequently in HIV-infected Breastfeeding Infants Whose Mothers Initiate Highly Active Antiretroviral Therapy (HAART) Post-partum [Abstract T-124]. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. 2010.
8. Colebunders R, Hodossy B, Burger D, et al. The effect of highly active antiretroviral treatment on viral load and antiretroviral drug levels in breast milk. *AIDS.* 2005; 19:1912–1915. [PubMed: 16227801]
9. World Health Organization (WHO). [Accessed: March 12, 2012] Antiretroviral therapy for HIV infection in adults and adolescents. 2010. Available at: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf.
10. Ministry of Health M. [Accessed: March 12, 2012] Clinical Management of HIV in Children and Adults. 2011. Available at: http://www.who.int/hiv/pub/guidelines/malawi_art.pdf
11. Kumwenda NI, Hoover DR, Mofenson LM, et al. Extended antiretroviral prophylaxis to reduce breast-milk HIV-1 transmission. *N Engl J Med.* 2008; 359:119–129. [PubMed: 18525035]
12. Taha TE, Kumwenda J, Cole SR, et al. Postnatal HIV-1 transmission after cessation of infant extended antiretroviral prophylaxis and effect of maternal highly active antiretroviral therapy. *J Infect Dis.* 2009; 200:1490–1497. [PubMed: 19832114]
13. Kaul S, Kline MW, Church JA, et al. Determination of dosing guidelines for stavudine (2',3'-didehydro-3'-deoxythymidine) in children with human immunodeficiency virus infection. *Antimicrob Agents Chemother.* 2001; 45:758–763. [PubMed: 11181356]
14. Riddler SA, Anderson RE, Mellors JW. Antiretroviral activity of stavudine (2',3'-didehydro-3'-deoxythymidine, D4T). *Antiviral Res.* 1995; 27:189–203. [PubMed: 8540743]
15. Wade NA, Unadkat JD, Huang S, et al. Pharmacokinetics and safety of stavudine in HIV-infected pregnant women and their infants: Pediatric AIDS Clinical Trials Group protocol 332. *J Infect Dis.* 2004; 190:2167–2174. [PubMed: 15551216]
16. Liotta, GPM.; Andreotti, M.; Sagno, J-B.; Doro Altan, A.; Marchei, E.; Marazzi, MC.; Vella, S.; Palombi, L.; Giuliano, M. Drug concentrations in breastfeeding infants of women receiving ARV for the prevention of postnatal transmission in Malawi. 18th Conference on Retroviruses and Opportunistic Infections; Boston, MA. 2011.

17. Rezk NL, White N, Bridges AS, et al. Studies on antiretroviral drug concentrations in breast milk: validation of a liquid chromatography-tandem mass spectrometric method for the determination of 7 anti-human immunodeficiency virus medications. *Ther Drug Monit.* 2008; 30:611–619. [PubMed: 18758393]

Figure. Stavudine (d4T) concentrations in maternal plasma, breast milk, and infant plasma (PEPI-Malawi trial, 2004–2009)

The figure shows stavudine concentrations in maternal plasma, whole milk, skim milk and infant plasma from the PEPI-Malawi trial. Results are shown for 52 mother-infant pairs. For each sample type, the median (closed square), interquartile range (IQR, box), lower inner fence (first quartile [Q1] – $1.5 \times \text{IQR}$) and upper inner fence (third quartile [Q3] + $1.5 \times \text{IQR}$), whiskers), outliers (greater than [Q3] + $1.5 \times \text{IQR}$, open circle) and extremes (greater than [Q3] + $3 \times \text{IQR}$, asterisk) are shown. Abbreviations: d4T: stavudine; N: number; PL: plasma; % BQL: percentage of samples that had d4T concentrations below the quantification limit of the assay (<5 ng/ml for plasma and <20 ng/ml for breast milk). Concentrations of d4T that were below the quantification limit (BQL) were assigned values of 2.5 ng/ml for plasma and 10 ng/ml for breast milk.

Table

Clinical and laboratory characteristics of nursing mothers who initiated antiretroviral treatment by 6 months post-partum (PEPI-Malawi trial, 2004–2009) ^a.

Variable ^a	Mothers included in the analysis (N=52)	Mothers not included in the analysis (N=169 ^b)	P value ^c
Maternal CD4 cell count at delivery	159.0 (100, 196)	151.0 (96, 193)	0.45 ^d
Maternal log ₁₀ HIV viral load at delivery	4.3 (3.6, 4.9)	4.6 (4.0, 4.9)	0.15 ^d
Maternal sdNVP exposure	35 (67.3%)	98 (44.6%)	0.26
Infant HIV-infected prior to maternal ART initiation	5 (9.6%)	5 (2.9%)	0.06
Mother reported ART use by 3 months	35 (67.3%)	110 (65.1%)	0.87
Infant regimen	19 (36.5%)	44 (25.6%)	0.21
Control	19 (36.5%)	62 (36.1%)	
Extended NVP	14 (26.9%)	66 (38.4%)	
Extended NVP+ZDV			

Footnotes for Table.

^a Abbreviations: sdNVP: single dose nevirapine; ART: antiretroviral therapy; NVP: nevirapine; ZDV: ziduvudine.

^a Maternal CD4 cell count and log₁₀ HIV viral load data are shown as median (interquartile range). Other data are shown as the number and percentage of participants in each group.

^b A total of 169 women and 172 infants were not included in the analysis.

^c P-values are based on Fisher's exact tests, unless otherwise indicated.

^d Wilcoxon rank sum tests.