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Concentrations of tenofovir, lamivudine and efavirenz in mothers and children enrolled under the Option B-Plus approach in Malawi

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Objectives: To evaluate antiretroviral drug concentrations in mothers and infants enrolled under the Option B-Plus approach for the prevention of HIV mother-to-child transmission in Malawi and to assess the maternal virological response after 1 year of treatment.

Patients and methods: Forty-seven women and 25 children were studied. Mothers were administered during pregnancy a combination of tenofovir, lamivudine and efavirenz and continued it during breastfeeding (up to 2 years) and thereafter. Drug concentrations were evaluated in mothers (plasma and breast milk) at 1 and 12 months post-partum and in infants (plasma) at 6 and 12 months of age. Drug concentrations were determined using an LC-MS/MS validated methodology.

Results: In breast milk, tenofovir concentrations were very low (breast milk/maternal plasma ratio=0.08), while lamivudine was concentrated (breast milk/plasma ratio=3) and efavirenz levels were 80% of those found in plasma. In infants, median levels at 6 months were 24 ng/mL tenofovir, 2.5 ng/mL lamivudine and 86.4 ng/mL efavirenz. At month 12, median levels were below the limit of quantification for the three drugs. No correlation was found between drug concentrations and laboratory parameters or indices of growth. HIV-RNA >1000 copies/mL was seen at month 1 in 15% of the women and at month 12 in 8.5%. Resistance was found in half of the women with detectable viral load.

Conclusions: Breastfeeding infants under Option B-Plus are exposed to low concentrations of antiretroviral drugs. With this strategy, mothers had a good virological response 1 year after delivery.

Introduction

Besides the well-recognized benefits of the Option B-Plus approach, the life-long ART administration to all HIV-infected women irrespective of CD4+ count for the prevention of HIV mother-to-child transmission,[1,2] concerns remain on the possible exposure to antiretroviral drugs in the breastfeeding infants and on the long-term virological response of the women enrolled under this approach. The recommended drug combination for this strategy in Malawi is composed of tenofovir, lamivudine and efavirenz. The present study had the following objectives: (i) to determine tenofovir, lamivudine and efavirenz concentrations in mothers (plasma and breast milk) and infants enrolled under the Option B-Plus approach; (ii) to evaluate possible correlations between drug concentrations and laboratory parameters and infant growth; and (iii) to assess at two timepoints maternal virological response and emergence of resistance.

Patients and methods

The study was approved by the National Health Sciences Research Committee of Malawi (approval number NHSRC 905). Written informed consent was signed by all participants. In the study, conducted within the structures of the DREAM (Drug Resource Enhancement against AIDS and Malnutrition) Program of the S. Egidio Community, an Italian faith-based non-governmental organization, women received, according to the national policy, a regimen consisting of tenofovir, lamivudine and efavirenz starting as soon as possible after presentation to the antenatal clinic and continuing it indefinitely. Women were allowed to breastfeed for up to 2 years with a recommendation of maintaining exclusive breastfeeding for the first 6 months. The present analyses are based on study samples available from mothers (plasma and breast milk, site of Blantyre, 1 and 12 months after delivery) and infants (plasma, site of Balaka, at 6 and 12 months of age). Blood samples were collected in the morning while drugs were generally taken in the evening (generally between 6 and 8 pm).

Drug concentrations

Drug concentrations in maternal and infant plasma and whole breast milk were determined by LC-MS/MS systems using an Alliance HPLC system (Waters, Etten-Leur, The Netherlands). Specimens (100 μL) with added 10 μL of internal standard (1 μg/mL emtricitabine) were protein precipitated, filtered, evaporated to dryness and reconstituted in 0.05% formic acid in water/methanol (90:10, v/v); 20 μL was injected into the LC-MS/MS instrument. The method was validated as described elsewhere[3] and applied with limits of quantification (LOQ) at 5 ng/mL lamivudine, 10 ng/mL tenofovir (5 ng/mL in breast milk) and 50 ng/mL efavirenz; limits of detection (LOD) were 1.5 ng/mL lamivudine, 3 ng/mL tenofovir and 15 ng/mL efavirenz. Linearity ranged from LOQ to 2000 ng/mL for tenofovir and lamivudine and from LOQ to 5000 ng/mL for efavirenz in all samples. Imprecision was <10% and analytical recovery ranged between 73.1% and 85.3%. None of the analytes under investigation showed significant ion suppression/enhancement (<15% analytical signal suppression due to matrix effect).

Virological analyses

HIV-RNA was quantified in plasma and breast milk using the Versant kPCR 1.0 assay (Siemens Diagnostics, Deerfield, IL, USA). The presence of resistance mutations was assessed in samples with HIV-RNA >37 copies/mL (LOQ of the Versant assay) using the TruGene assay (Siemens Diagnostics). Mutations were classified according to the 2013 IAS-USA classification.

Data analysis

Results are presented as medians with IQRs and proportions. Pearson's coefficient was used to determine possible correlations between drug concentrations and laboratory or growth parameters.

For drug concentrations below the LOQ but above the LOD, a value of half of the LOQ was used in summary calculations.

Statistical analysis was performed using SPSS, version 22.0 (IBM, Somers, NY, USA), with the significance threshold set at 0.05.

Results

Study population

The study population included 47 mothers and 25 infants. Mothers' and infants' characteristics are reported in Table 1. Twenty-four mothers (51%) had previously received ART (5 were on chronic treatment at the beginning of the present pregnancy and 19 had received ART prophylaxis in previous pregnancies). Median duration of ART during pregnancy considering all women was 15.2 weeks (107 days). No child was HIV positive.

Drug concentrations

Maternal samples available for quantification of drug concentrations in plasma and breast milk included 33 samples at month 1 after delivery and 47 samples (all women) at month 12. Median levels are reported in Table 2. One patient had no detectable drug levels at month 1.

Considering drug concentrations at both timepoints, there was a significant direct correlation between tenofovir plasma concentrations and creatinine levels ($P=0.048$). All women had normal creatinine levels. No correlation was found between duration of ART and creatinine levels. No other correlation with laboratory parameters or indices of infant growth was found.

Median concentrations found in infants at the two timepoints are also reported in Table 2. Median levels at month 12 were below the LOQ for all drugs. No correlation was found between drug concentrations and laboratory parameters or indices of growth (both weight and height) at both timepoints.

Virological response and emergence of resistance

At month 1, 9 out of 33 (27%) women tested had detectable HIV-RNA in plasma (with a median level of 1308 copies/mL) and

Table 1. Patient characteristics

No. of women	47	No. of infants	25
Age (years), median (IQR)	26 (23–30)	No. of females (%)	13 (52%)
Patients with no previous exposure to ART, *n* (%)	23 (48.9)	Birth weight (kg), median (IQR)	3.28 (3.02–3.85)
Baseline CD4+ cell count (cells/mm^3), median (IQR)	469 (299–559)	Birth length (cm), median (IQR)	50 (48–53.5)
Baseline haemoglobin (g/dL), median (IQR)	10.9 (10.2–12.1)		
Baseline AST (U/L), median (IQR)	21 (15–27)	Month 6 weight (kg), median (IQR)	7.3 (6.4–7.9)
Baseline ALT (U/L), median (IQR)	16 (10–22)	Month 6 height (cm), median (IQR)	64 (62.5–66.8)
Baseline creatinine (mg/dL), median (IQR)	0.6 (0.5–0.7)	Month 6 haemoglobin (g/dL), median (IQR)	9.9 (9.3–10.7)
Pre-partum ART duration (days), median (IQR)	107 (68–143)	Month 6 ALT (U/L), median (IQR)	18 (12–23)
Month 1 weight (kg), median (IQR)	54.1 (49.6–62.0)	Month 6 AST (U/L), median (IQR)	27 (25–36)
Month 1 haemoglobin (g/dL), median (IQR)	11.4 (10.7–12.5)	Month 6 creatinine (mg/dL), median (IQR)	0.19 (0.16–0.28)
Month 1 AST (U/L), median (IQR)	23 (12–32)	Month 12 weight (kg), median (IQR)	8.8 (7.9–9.3)
Month 1 ALT (U/L), median (IQR)	25 (18–33)	Month 12 height (cm), median (IQR)	72 (70.5–74)
Month 1 creatinine (mg/dL), median (IQR)	0.4 (0.3–0.6)	Month 12 haemoglobin (g/dL), median (IQR)	9.6 (9.5–10.2)
Month 12 haemoglobin (mg/dL), median (IQR)	12.3 (11.4–13.1)	Month 12 ALT (U/L), median (IQR)	18 (13–22)
Month 12 AST (U/L), median (IQR)	26 (19–39)	Month 12 AST (U/L), median (IQR)	32 (28–35)
Month 12 ALT (U/L), median (IQR)	36 (27–52)	Month 12 creatinine (mg/dL), median (IQR)	0.19 (0.13–0.21)
Month 12 creatinine (mg/dL) median (IQR)	0.5 (0.4–0.6)		
Month 12 CD4+ cell count (cells/mm^3), median (IQR)	610 (510–815)		
Month 12 weight (kg), median (IQR)	52.0 (47.2–58.5)		

Table 2. Drug concentrations in mothers (plasma and breast milk) and infants (plasma) at the different timepoints

Antiretroviral, timepoint	Maternal plasma drug concentration (ng/mL), median (IQR)	Breast milk drug concentration (ng/mL), median (IQR)	Breast milk/maternal plasma ratio, median (IQR)[a,b]	Antiretroviral, timepoint	Infant plasma drug concentration (ng/mL), median (IQR)
Tenofovir				Tenofovir	
month 1 (*n*=33)	86.7 (73.7–102.6)	5.0 (0–6.1)	0.07 (0.06–0.08)	month 6 (*n*=25)	24 (0–51.6)
month 12 (*n*=47)	86.1 (60.1–115.3)	2.5 (0–5.5)	0.08 (0.05–0.11)	month 12 (*n*=25)	0 (0–29.9)
Lamivudine				Lamivudine	
month 1 (*n*=33)	159.3 (91.6–335.5)	537.1 (369.2–768.2)	3.29 (1.77–4.61)	month 6 (*n*=25)	2.5 (2.5–7.6)
month 12 (*n*=47)	155.1 (94.1–317.5)	429.6 (266.4–530.6)	2.35 (0.90–3.56)	month 12 (*n*=25)	0 (0–2.5)
Efavirenz				Efavirenz	
month 1 (*n*=33)	2109.3 (1624.1–3169.9)	1556.3 (855.5–2266.4)	0.76 (0.50–1.10)	month 6 (*n*=25)	86.4 (0–329.1)
month 12 (*n*=47)	2254.2 (1689.2–4798.6)	1824.2 (1050.1–4296.6)	0.86 (0.54–1.13)	month 12 (*n*=25)	0 (0–268.7)

[a]Median of individual ratios.
[b]Ratios were determined only when both concentrations were above the LOD.

among them 5 (15.2%) had values >1000 copies/mL. At month 12, 10 out of 47 (21.3%) women tested had detectable plasma HIV-RNA (with a median level of 439 copies/mL); of these, 4 (8.5%) had values >1000 copies/mL. There was no significant difference between drug concentrations in women with or without detectable HIV-RNA. HIV-RNA in breast milk was always under the LOD with the exception of two samples with 53 copies/mL (corresponding plasma value 940 copies/mL) and 55 copies/mL (corresponding plasma value of <37 copies/mL), respectively.

Drug resistance was present in 4 out of 9 patients with detectable viral load at month 1 and in 4 out of 10 patients (but in 2 cases the sequence was not obtained) with detectable viral load at month 12. Mutations were mainly NNRTI-associated mutations (K103N in four patients, G190A in two patients and M230L, Y188L, K101E, V108I and E138A in one patient each); the M184V mutation was also present in four patients. No K65R mutation was detected. There was no difference in drug concentrations between women with or without drug resistance. Having previously received ART and duration of ART were not associated with the emergence of resistance.

Discussion

Previous studies have assessed antiretroviral drug concentrations in breast milk and breastfeeding infants. For tenofovir, two studies[4,5] have shown very low concentrations in breast milk ranging from 17.6 ng/mL to undetectable levels in women studied 1 week after delivery. Our results, showing negligible passage of tenofovir into breast milk, are in accordance with these previous studies and add new information regarding long-term therapy, since in our study the assessments were done at 1 and 12 months. Reporting for the first time information about tenofovir plasma concentrations in breastfeeding infants, we found that both at 6 and 12 months the plasma drug concentrations were low. However, the median level of 24 ng/mL at 6 months suggests that a low clearance of the drug may occur in breastfeeding infants, since, given the very low concentrations seen in breast milk, we would expect even lower concentrations. However, no correlation between these drug concentrations and the

laboratory parameters or infant growth indices was seen. For lamivudine and efavirenz, our findings are in agreement with previous studies[6–13] showing significant concentrations of these drugs in breast milk and low levels in breastfeeding infants, especially after 6 months of age, reflecting maturation of the infant's metabolic clearance system.

The only significant association found between drug concentrations and laboratory parameters was between plasma maternal tenofovir concentrations and creatinine levels. Although all the mothers had values within the normal range, this finding merits attention and should be further explored in larger cohorts.

Although we found that a significant proportion of women had detectable viral load at the two timepoints, only a limited proportion had values >1000 copies/mL, indicating good virological response in the majority of women. Resistance mutations were present in about half of the women. No K65R mutations were found. In previous reports in patients failing tenofovir-containing regimens, the K65R mutation was present in significant proportions (between 23% and 65%) in subtype C patients.[14–17] Besides the low number of women that we studied, we can also hypothesize that a relatively short exposure to the drug may have played a role in our findings.

Our study has some limitations, including small sample size, the fact that maternal and infant samples were not collected from mother/child pairs and the fact that analyses of drug concentrations and virological response were performed at only two timepoints.

In conclusion, in our study we showed that during chronic therapy under the Option B-Plus approach, infants are exposed to low concentrations of antiretroviral drugs (well below the therapeutic concentrations of each of the drugs).[18–20] Mothers had a good virological response during chronic treatment under this approach, although further studies are needed to assess the response after the first year.

References

1 WHO. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach.* 2013. http://www.who.int/hiv/pub/guidelines/arv2013/download/en/index.html.

2 CDC. Impact of an innovative approach to prevent mother-to-child transmission of HIV—Malawi, July 2011–September 2012. *MMWR Morb Mortal Wkly Rep* 2013; **62**: 148–51.

3 Marchei E, Escuder D, Pallas CR *et al.* Simultaneous analysis of frequently used licit and illicit psychoactive drugs in breast milk by liquid chromatography tandem mass spectrometry. *J Pharm Biomed Anal* 2011; **55**: 309–16.

4 Benaboud S, Pruvost A, Coffie PA *et al.* Concentrations of tenofovir and emtricitabine in breast milk of HIV-infected women in Abidjan, Côte d'Ivoire, in the ANRS 12109 TEmAA Study, Step 2. *Antimicrob Agents Chemother* 2011; **55**: 1315–7.

5 Mirochnick M, Taha T, Kreitchmann R *et al.* Pharmacokinetics and safety of tenofovir in HIV-infected women during labor and their infants during the first week of life. *J Acquir Immune Defic Syndr* 2014; **65**: 33–41.

6 Shapiro RL, Holland DT, Capparelli E *et al.* Antiretroviral concentrations in breastfeeding infants of women in Botswana receiving antiretroviral treatment. *J Infect Dis* 2005; **192**: 720–7.

7 Corbett AH, Kayira D, White NR *et al.* Antiretroviral pharmacokinetics in mothers and breastfeeding infants from 6 to 24 weeks post-partum: results of the BAN study. *Antivir Ther* 2014; **19**: 587–95.

8 Mirochnick M, Thomas T, Capparelli E *et al.* Antiretroviral concentrations in breastfeeding infants of mothers receiving highly active antiretroviral therapy. *Antimicrob Agents Chemother* 2009; **53**: 1170–6.

9 Palombi L, Pirillo MF, Andreotti M *et al.* Antiretroviral prophylaxis for breastfeeding transmission in Malawi: drug concentrations, virological efficacy and safety. *Antivir Ther* 2012; **17**: 1511–9.

10 Schneider S, Peltier A, Gras A *et al.* Efavirenz in human breast milk, mothers', and newborns' plasma. *J Acquir Immune Defic Syndr* 2008; **48**: 450–4.

11 Gandhi M, Mwesigwa J, Aweeka F *et al.* Hair and plasma data show that lopinavir, ritonavir and efavirenz all transfer from mother to infant in utero, but only efavirenz transfers via breastfeeding. *J Acquir Immune Defic Syndr* 2013; **63**: 578–84.

12 Olagunju A, Bolaji OO, Amara A *et al.* Development, validation and clinical application of a novel method for the quantification of efavirenz in dried breast milk spots using LC-MS/MS. *J Antimicrob Chemother* 2015; **70**: 555–61.

13 Olagunju A, Bolaji O, Amara A *et al.* Breast milk pharmacokinetics of efavirenz and breastfed infants' exposure in genetically defined subgroups of mother-infant pairs: an observational study. *Clin Infect Dis* 2015; **61**: 453–63.

14 Skhosana L, Steegen K, Bronze M *et al.* High prevalence of the K65R mutations in HIV-1 subtype C infected patients failing tenofovir-based first-line regimens in South Africa. *PLoS One* 2015; **19**: e0118145.

15 Sunpath H, Wu B, Gordon M *et al.* High rate of K65R for antiretroviral therapy-naïve patients with subtype C HIV infections failing a tenofovir-containing first-line regimen. *AIDS* 2012; **26**: 1679–84.

16 Van Zyl GU, Liu TF, Claassen M *et al.* Trend in genotypic HIV-1 antiretroviral resistance between 2006 and 2012 in South African patients receiving first and second-line antiretroviral treatment regimens. *PLoS One* 2013; **8**: e67188.

17 Hoffmann CJ, Ledwaba J, Li JF *et al.* Resistance to tenofovir-based regimens during treatment failure of subtype C HIV-1 in South Africa. *Antivir Ther* 2013; **18**: 915–20.

18 Aurpibul L, Puthanakit T. Review of tenofovir use in HIV-infected children. *Pediatr Infect Dis J* 2015; **34**: 383–91.

19 Tremoulet AH, Capparelli EV, Patel P *et al.* Population pharmacokinetics of lamivudine in human immunodeficiency virus-exposed and -infected infants. *Antimicrob Agents Chemother* 2007; **51**: 4297–302.

20 Viljoen M, Gous H, Kruger HS *et al.* Efavirenz plasma concentrations at 1, 3 and 6 months post-antiretroviral therapy initiation in HIV type 1-infected South African children. *AIDS Res Hum Retrovir* 2010; **26**: 613–9.