

Concentrations of Tenofovir and Emtricitabine in Breast Milk of HIV-1-Infected Women in Abidjan, Côte d'Ivoire, in the ANRS 12109 TEmAA Study, Step 2[∇]

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The aim was to evaluate emtricitabine (FTC) and tenofovir (TFV) neonatal ingestion through breast milk. Median TFV and FTC breast milk doses represented 0.03% and 2%, respectively, of the proposed oral infant doses. Neonatal simulated plasma concentrations were extremely low for TFV but between the half-maximal inhibitory concentration and the adult minimal expected concentration for FTC. The rare children who will acquire HIV despite TDF-FTC therapy will need to be monitored for viral resistance acquisition.

For prevention of mother-to-child transmission (PMTCT) of HIV in resource-limited settings, single-dose nevirapine (sd-NVP) has been commonly administered at the start of labor up to now according to previous World Health Organization (WHO) guidelines. However, the use of sdNVP results in resistance mutations in 15 to 70% of women, at 4 to 6 weeks postpartum, compromising the success of subsequent treatments with NVP in mothers and children (1, 7). Adding a single dose of tenofovir disoproxil fumarate (TDF)-emtricitabine (FTC) reduced these resistance mutations by half (2, 10). TDF and FTC placental transfer has already been studied (4, 5), but no data were reported so far on the transfer of these drugs after birth through breast-feeding. Breast-feeding cessation or replacement feeding introduced under routine circumstances is no longer recommended by WHO, as these interventions are often associated with increased mortality and morbidity (13). Thus, it is important to assess to what extent maternal TDF and FTC are transmitted through breast milk to infants. Furthermore, TDF-FTC-based fully suppressive antiretroviral regimens represent now a recommended approach for PMTCT in pregnant, delivering, and breast-feeding women (14).

Five Ivorian mothers included in the ANRS 12109 TEmAA (Tenofovir/Emtricitabine in Africa and Asia) study and who chose to exclusively breast-feed their infant were administered one tablet of NVP (200 mg) plus two tablets of TDF (300 mg)-FTC (200 mg) at the start of labor and one TDF-FTC daily tablet for 7 days postpartum. The protocol was approved by the Ethics Committee of the country (Cote d'Ivoire). The women and the children's fathers were asked to sign an in-

formed consent form. Maternal blood and milk samples were collected concomitantly after drug administration on days 1, 2, 3, and 7 after delivery. A liquid-liquid extraction procedure was performed using 300 μ l of methanol-dichloromethane (10/16, vol/vol) containing 0.1% hydrochloric acid added to 100 μ l of milk samples. After vortexing and centrifugation at 20,000 \times g at +4°C for 20 min, the upper phase was withdrawn and evaporated under nitrogen to dryness. The sample was then reconstituted with 100 μ l of mobile phase, and 40 μ l of the extract was injected in the analytical system. TFV and FTC concentrations were measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method previously described (9) and adapted for FTC. Limits of quantitation were 4.9 and 9.5 ng/ml for TFV and FTC, respectively. Maximal and minimal milk concentrations were observed 4 to 5 h and more than 19 h after drug administration, respectively. Using the median of the maximal and minimal milk concentrations and assuming that the mean volume of milk ingested by a neonate is around 500 ml per day, minimal and maximal doses ingested via breast-feeding were estimated and compared to the proposed neonatal oral dose. Finally, TFV-FTC neonatal concentrations resulting from the estimated minimal and maximal milk doses were simulated using a previously published population pharmacokinetics (PK) model (4, 5) and compared to the half-maximal inhibitory concentration (IC₅₀) and to the minimal expected trough concentrations in adults (C_{min}) (4, 5, 12).

Sixteen breast milk TFV and FTC samples were collected from 10 min to 21 h after maternal administration, at the same time \pm 30 min as the maternal blood samples. FTC maximal and minimal concentrations in breast milk (medians; interquartile ranges [IQRs] shown in parentheses) were 679 (658 to 743) ng/ml and 177 (105 to 253.5) ng/ml, respectively. TFV maximal and minimal concentrations in breast milk were 14.1 (11.60 to 16.25) ng/ml and 6.8 (5.83 to 8.75) ng/ml, respectively. Median (minimal and maximal) doses ingested via breast-feed-

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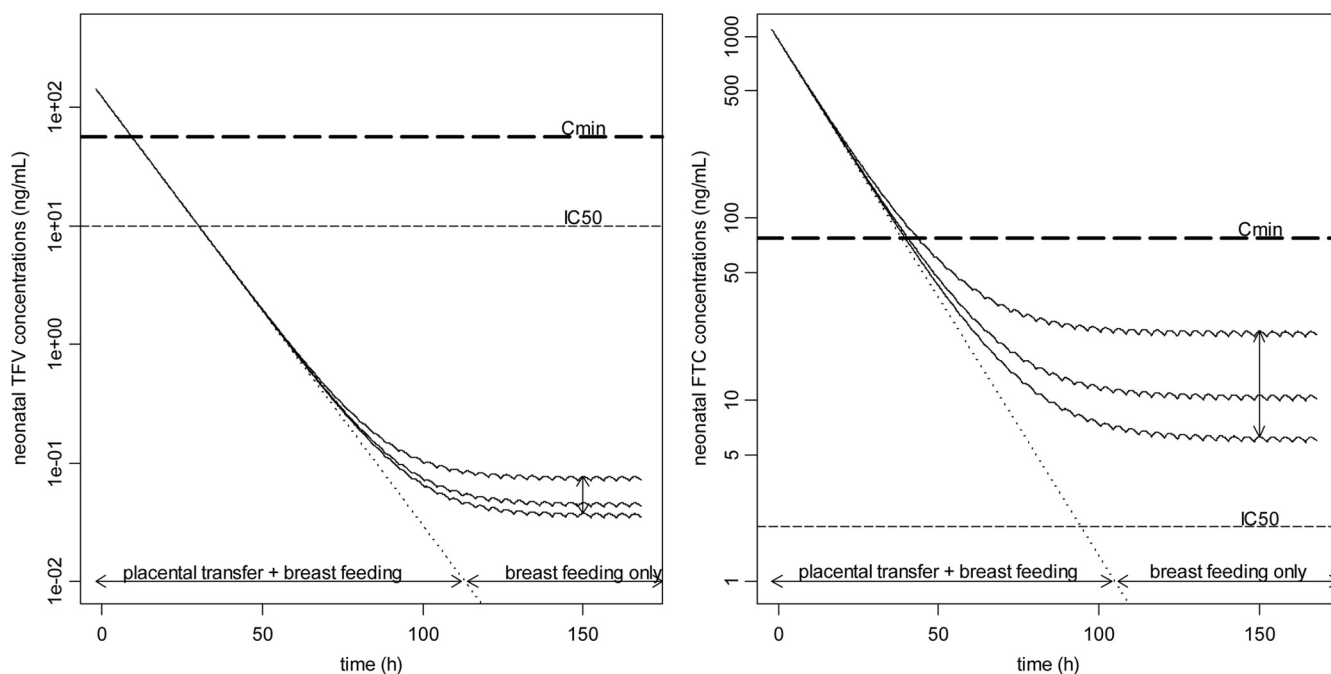


FIG. 1. Neonatal tenofovir (TFV) (left panel) and emtricitabine (FTC) (right panel) concentrations during the first week of life, from placental transfer only (dotted curve) and from both placental transfer and breast-feeding (solid curves). Data are from the ANRS 12109 TEmAA study (Step 2), Abidjan, Côte d'Ivoire, 2009. Note that, up to approximately 110 h, concentrations resulted from placental transfer and breast-feeding; thereafter, neonatal concentrations resulted only from breast-feeding (every 4 h). The arrow links the concentrations with the median, minimal, and maximal breast-feeding doses. The lower horizontal dashed line corresponds to the IC_{50} (0.01 mg/liter for TFV and 0.002 mg/liter for FTC), and the upper horizontal dashed line corresponds to the minimal expected trough concentrations in adults (0.056 mg/liter for TFV and 0.077 mg/liter for FTC).

ing by a neonate weighing 3 kg would thus be 126 (89 and 340) μ g/day for FTC and 4.2 (3.4 and 7.1) μ g/day for TFV. These amounts represent 2% (1 and 6%) and 0.03% (0.02 and 0.05%) of the oral FTC and TFV proposed doses for neonates, respectively (4, 5).

Finally, neonatal concentrations resulting from these calculated doses were simulated from delivery up to 7 days (Fig. 1). After 110 h, the simulated concentrations corresponded only to the drug ingested via breast-feeding. For TFV, concentration profiles were much lower than the lowest IC_{50} found (128 to 266 times) (3, 4). For FTC, they were 3.2 to 12 times higher than the IC_{50} and 3.2 to 12 times lower than the C_{min} (5, 12).

This is to our knowledge the first report on FTC and TFV transfer in human breast milk. Data were already available for lamivudine, nevirapine, and zidovudine: infant median daily doses received from breast milk corresponded to 2%, 4%, and 0.1%, respectively, of the recommended infant daily doses (8).

These results suggest that variability in breast milk concentrations during the dosing interval was relatively small and essentially due to the difference in time intervals between drug administration and sampling. Indeed the maximal doses ingested via breast milk for TFV and FTC were two and four times higher, respectively, than the minimal ones, a finding which is consistent with other antiretroviral studies in breast milk. In lactating rhesus macaques, TFV maximal concentration was three to four times higher than the trough concentration (11). In human, lamivudine and nevirapine milk concentrations were also relatively flat during a dosing interval (8).

For the simulation, the drugs were assumed to be bioavail-

able from breast milk to neonate. While this assumption is reasonable for FTC, it is less likely for TFV, which needs the disoproxil esters to cross membranes easily. Thus, simulated neonate TFV concentrations were probably overestimated, and the neonate exposure reaches, at the most, the simulated concentrations. The very low neonatal TFV simulated concentrations were unlikely to produce toxicities or select for resistant viruses, whereas the FTC dose ingested via breast milk produced significant concentrations, since the simulated neonatal concentrations were between the IC_{50} and the C_{min} . Thus, if FTC is administered only to the mother, the infant could be exposed to subtherapeutic concentrations and thus be at risk for the emergence of resistance, compromising his future therapeutic options.

In conclusion, this study is the first report on human TFV-FTC breast milk transfer. Median TFV and FTC breast milk doses represented 0.03% and 2%, respectively, of the proposed oral infant dose. Neonatal simulated plasma concentrations were extremely low for TFV but between the IC_{50} and the adult C_{min} for FTC. While TDF-FTC-based antiretroviral therapy is being rolled out for adult and PMTCT indications (14), the rare infected children who will acquire HIV despite such fully suppressive drug regimens will need to be monitored for the acquisition of viral resistance, as already shown for lamivudine in the NVP (SWEN)-Uganda study (6).

REFERENCES

- Arrive, E., et al. 2007. Ghent Group on HIV in Women and Children. Prevalence of resistance to nevirapine in mothers and children after single-

- dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. *Int. J. Epidemiol.* **36**:1009–1021.
2. **Chi, B. H., et al.** 2007. Single-dose tenofovir and emtricitabine for reduction of viral resistance to non-nucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial. *Lancet* **370**:1698–1705.
 3. **Gilead Sciences, Inc.** 2003. Viread (tenofovir disoproxil fumarate) tablet product labeling. Gilead Sciences, Inc., Foster City, CA.
 4. **Hirt, D., et al.** 2009. ANRS 12109. Population pharmacokinetics of tenofovir in HIV-1-infected pregnant women and their neonates (ANRS 12109). *Clin. Pharmacol. Ther.* **85**:182–189.
 5. **Hirt, D., et al.** 2009. Population pharmacokinetics of emtricitabine in human immunodeficiency virus type 1-infected pregnant women and their neonates. *Antimicrob. Agents Chemother.* **53**:1067–1073.
 6. **Lidstrom, J., et al.** 2010. Multi-class drug resistance arises frequently in HIV-infected breastfeeding infants whose mothers initiate HAART postpartum, poster 920. Program Abstr. 17th Conf. Retrovir. Opportunistic Infect. San Francisco, CA, 16 to 19 February 2010.
 7. **Lockman, S., et al.** 2007. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. *N. Engl. J. Med.* **356**:135–147.
 8. **Mirochnick, M., et al.** 2009. Antiretroviral concentrations in breast-feeding infants of mothers receiving highly active antiretroviral therapy. *Antimicrob. Agents Chemother.* **53**:1170–1176.
 9. **Pruvost, A., et al.** 2009. Pilot pharmacokinetic study of human immunodeficiency virus-infected patients receiving tenofovir disoproxil fumarate (TDF): investigation of systemic and intracellular interactions between TDF and abacavir, lamivudine, or lopinavir-ritonavir. *Antimicrob. Agents Chemother.* **53**:1937–1943.
 10. **Stringer, J. S., et al.** 2010. Effectiveness of non-nucleoside reverse-transcriptase inhibitor-based antiretroviral therapy in women previously exposed to a single intrapartum dose of nevirapine: a multi-country, prospective cohort study. *PLoS Med.* **7**:e1000233.
 11. **Van Rompay, K. K., M. Hamilton, B. Kearney, and N. Bischofberger.** 2005. Pharmacokinetics of tenofovir in breast milk of lactating rhesus macaques. *Antimicrob. Agents Chemother.* **49**:2093–2094.
 12. **Wang, L. H., J. Begley, J. Y. Feng, J. Quinn, and F. Rousseau.** 2002. Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once daily dosing, poster 4546. XIV Int. AIDS Conf. Barcelona, Spain, July 2002.
 13. **World Health Organization.** 2009. Rapid advice: revised WHO principles and recommendations on infant feeding in the context of HIV—November 2009. World Health Organization, Geneva, Switzerland. http://www.who.int/child_adolescent_health/documents/9789241598873/en/.
 14. **World Health Organization.** 2010. Rapid advice: use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. World Health Organization, Geneva, Switzerland. <http://www.who.int/hiv/pub/mtct/advice/en/index.html>.