Persistence of nevirapine in breast milk and plasma of mothers and their children after single-dose administration

Andrea Kunz1*, Monika Frank2, Kizito Mugenyi3, Rose Kabasinguzi3, Astrid Weidenhammer1, Michael Kurowski4, Charlotte Kloft2 and Gundel Harms1

1Institute of Tropical Medicine and International Health, Charité—University Medicine Berlin, Berlin, Germany; 2Institute of Pharmacy, Martin-Luther-Universitaet Halle-Wittenberg, Halle, Germany; 3MoH/GTZ PMTCT Project western Uganda, Fort Portal, Uganda; 4Therapia HIV-Lab, Auguste-Viktoria Hospital, Berlin, Germany

Received 12 June 2008; returned 30 July 2008; revised 9 September 2008; accepted 26 September 2008

Objectives: Nevirapine is widely used in the developing world for the prevention of mother-to-child transmission (PMTCT) of HIV. A single mutation in the HIV genome is sufficient to lead to significant nevirapine resistance. Persistence of low-level drug concentrations in body compartments can foster resistance formation. In this study, concentration–time courses of nevirapine after single-dose administration were analysed over an extended post-partum period.

Patients and methods: Breast milk and plasma samples of 62 HIV-positive Ugandan mother–child pairs who had received single-dose nevirapine were collected at delivery and 1, 2 and 6 weeks post-partum. Nevirapine concentrations were quantified by LC/tandem-mass-spectrometry using a quantification limit of 15 ng/mL, and a population pharmacokinetic (PK) analysis was performed.

Results: Concentration–time profiles in breast milk, maternal plasma and child plasma showed similar shapes. At week 1, median nevirapine concentrations were 164 ng/mL in maternal plasma, 114 ng/mL in breast milk and 183 ng/mL in child plasma. The population PK model predicted nevirapine concentrations >10 ng/mL (IC50 for nevirapine) for 13 days in breast milk, 14 days in maternal plasma and 18 days in child plasma in 80% of the samples.

Conclusions: Nevirapine concentrations were present for 2–3 weeks in the three compartments. The concentrations are probably sufficiently high to protect most breastfed children from HIV transmission during the first 2 weeks. The long presence of slowly decreasing levels of nevirapine is likely to induce resistance formation. Post-natal addition of antiretrovirals for 1 week only, as recommended in the current PMTCT guidelines, will not suffice to avoid nevirapine resistance formation.

Keywords: HIV, breastfeeding, population pharmacokinetics, Uganda

Introduction

Vertical transmission accounts for almost 20% of all new HIV infections in sub-Saharan Africa.1 The antiretroviral drug nevirapine is widely used in resource-limited areas to reduce the associated risk of intra-partum transmission.2,3 Since breastfeeding is the norm in sub-Saharan Africa, there is an additional risk of 10% to 20% for post-natal transmission.4 The duration and levels of nevirapine in breast milk and plasma of mothers and children after nevirapine administration may have implications for both post-natal transmission of HIV and the emergence of resistant virus. Nevirapine, like other non-nucleoside reverse transcriptase inhibitors (NNRTIs), displays a long half-life, and one single mutation is sufficient to generate a high degree of resistance.5–16 Nevirapine-associated resistance mutations were shown to result in a reduced efficacy of a subsequent NNRTI-containing antiretroviral long-term treatment if the interval between nevirapine prophylaxis and start of treatment was <6 months.17–19

So far, nevirapine concentrations in breast milk after a single dose for periods longer than 1 week have not been analysed. For plasma, such data are available using assays that detect nevirapine levels >50 ng/mL only.5,6

In this study in rural western Uganda, we analysed nevirapine concentrations in three compartments, breast milk, plasma of

*Corresponding author. Tel: +49-30-30116-700; Fax: +49-30-30116-710; E-mail: andrea-ursula.kunz@charite.de

© The Author 2008. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
mothers and plasma of children, over a period of 6 weeks after single-dose administration. We used a sensitive HPLC/tandem mass spectrometry assay able to detect nevirapine concentrations as low as 15 ng/mL. Since in a peripheral, rural setting in Eastern Africa, as in this study, extensive sampling is often impossible and some samples were not available, a population pharmacokinetic (PK) analysis approach was chosen to exemplify the maintenance period of nevirapine concentrations.

Patients and methods

HIV-positive pregnant women participating in an intervention for the prevention of mother-to-child transmission (PMTCT) of HIV in western Uganda were consecutively included in the study together with their children if they had given informed consent and delivered at Fort Portal District Hospital (Kabarole District, Uganda). The study was approved by the National Council of Science and Technology of Uganda. All participating women took a 200 mg nevirapine tablet (Boehringer Ingelheim, Ingelheim, Germany) within 48 h before delivery, and all newborns received 2 mg/kg nevirapine syrup under observation within 72 h after birth. Women who took antiretroviral treatment instead of nevirapine prophylaxis were not eligible for the study. Enrolment took place between September 2003 and September 2004. Breast milk samples of the left and right breast and plasma samples of mothers and children were taken at delivery and 1, 2 and 6 weeks after maternal nevirapine intake. Whole breast milk and plasma specimens were frozen at −80°C.

Concentrations of total nevirapine in plasma and breast milk were determined by a validated LC/tandem-mass-spectrometry method according to the criteria set by the FDA.20,21 For breast milk, a full validation was performed using replacement milk. All HIV-1-negative control samples were processed (frozen–thawed) in the same way as the patients’ samples. The recovery of nevirapine from replacement milk when compared with standard solutions (water/acetonitrile, 50:50) exceeded 99%. Nevirapine samples retained over 94% of their concentration at room temperature within 24 h. Accuracy and precision of the analytical procedure were evaluated using three nevirapine concentrations of quality control samples assayed in eight replicates. The concentrations of the quality control samples were back-calculated from daily calibration curves. Intra-day accuracy for nevirapine concentrations was 99% (2500 ng/mL), 101% (500 ng/mL) and 110% (100 ng/mL), respectively. The coefficients of variation (CVs) for nevirapine in breast milk were 7% (2500 ng/mL), 8% (500 ng/mL) and 11% (100 ng/mL), respectively. The relative SD criteria for repeatability (within-day precision) were fulfilled. With each run, a duplicate 9-point standard calibration curve was analysed containing nevirapine at concentrations ranging from 20 to 5000 ng/mL. The lower quantification limit for nevirapine was 15 ng/mL. Correlations were determined using Spearman’s rank correlation coefficient.

Concentration–time profiles were created in WinNonlinTM, version 5.2 (Pharsight, Mountain View, CA, USA). The geometric mean was applied. The population PK analyses were performed using the non-linear mixed-effects modelling approach implemented in the software NONMEMTM (version V, level 1.1, Globomax LLC, San Francisco, CA, USA). All samples with quantifiable nevirapine concentrations were included. An integrated maternal PK model was developed including maternal plasma and breast milk data considering the drug transfer from plasma to breast milk (Figure 1a). The PK model for nevirapine plasma concentrations of the newborns considered all possibilities for the children to encounter nevirapine: maternal nevirapine reaching the fetal organism through the blood/placenta barrier, newborns’ own oral intake of nevirapine at delivery and the nevirapine dose children received via breast milk (Figure 1b). The model-building process was guided by the

![Figure 1. Schematic structural pharmacokinetic model of nevirapine for data of mother (a) (K12, gastrointestinal absorption rate constant for drug administration to mother; V2, volume of central compartment for maternal plasma; V3, volume of peripheral compartment for breast milk; CL, clearance; and Q, inter-compartmental clearance from blood to breast milk) and of child (b) (K12, gastrointestinal absorption rate constant for drug administration to child; K32, plasma/placenta–plasma/breast milk transfer rate constant; F, ‘bioavailability’ resulting from the mother’s dose via placenta and breast milk; V2, volume of central compartment for child plasma; and CL, clearance).](image-url)
it was thereby significantly improved (decrease in OFV gated on all PK parameters and was added to the final PK model if it was thereby significantly improved (decrease in OFV >3.84)).

The long-term exposure of nevirapine concentrations for differently behaving individuals in the three compartments was simulated in NONMEM™ using the final population PK models with typical administrations: mothers, 200 mg nevirapine tablet; children, 6.2 mg nevirapine syrup 8.5 h after maternal nevirapine intake. Based on these simulations, the percentage of individuals with nevirapine concentrations >10 ng/mL being the 50% inhibitory concentration (IC50)13 of HIV wild-type rate constant (K12) was set to the previously reported value of 1.66 h−1.25 As the bioavailability of the oral dose was unknown,

### Results

Sixty-two women fulfilled the inclusion criteria. The median age and weight of the women were 26 years (IQR 23–30 years) and 56 kg (IQR 52–62 kg), respectively. At delivery, the median CD4 count was 516 cells/mm³ (IQR 361–684 cells/mm³) and the median HIV-1 viral load was 7433 copies/mL (IQR 2553–41 872 copies/mL). The median interval between maternal nevirapine intake and delivery was 5.2 h (IQR 3.0–9.9 h), while the median interval between maternal and child nevirapine intake was 8.5 h (IQR 4.2–17.3 h). Nevirapine concentrations between left and right breasts highly correlated at all time points (r = 0.87–0.96; P < 0.0001); therefore the mean nevirapine concentration was used.

At delivery, the median nevirapine concentration was 1755 ng/mL (IQR 1338–2043 ng/mL) in maternal plasma, 1012 ng/mL (IQR 657–1364 ng/mL) in breast milk and 1300 ng/mL (IQR 942–1690 ng/mL) in plasma of newborns. The nevirapine concentration in plasma of newborns correlated with the elapsed time between maternal nevirapine intake and delivery (r = 0.37; P = 0.006).

At week 1, the median interval between sample collection and maternal nevirapine intake was 7.7 days (IQR 7.0–8.1 days). All nevirapine concentrations were >IC50 (maternal plasma: median 164 ng/mL, IQR 75–378 ng/mL; breast milk: median 114 ng/mL, IQR 75–213 ng/mL; and child plasma: median 183 ng/mL, IQR 120–257 ng/mL).

At week 2, the median interval between sample collection and maternal nevirapine intake was 14.8 days (IQR 14.1–15.7 days). Considering only those samples taken 13–16 days after maternal nevirapine intake, 51.4% of the maternal plasma (median 15 ng/mL; IQR <15–50 ng/mL), 56.2% of the breast milk (median 17 ng/mL, IQR <15–35 ng/mL) and 61.3% of the children plasma samples (median 22 ng/mL; IQR <15–45 ng/mL) contained detectable nevirapine concentrations. At week 6, nevirapine was not measurable in any specimen.

Concentration–time profiles of nevirapine in the three compartments showed similar shapes with slightly lower mean nevirapine concentrations in breast milk than in maternal plasma. (Figure 2). The semi-logarithmic plots indicated one slope only, suggesting a monophasic elimination of nevirapine.

### Concentrations of nevirapine in breast milk and plasma of mothers and children

At delivery, the median interval between maternal nevirapine intake and maternal sample collection was 5.8 h (IQR 3.5–12.4 h) for maternal plasma and 28.0 h (IQR 20.1–40.9 h) for breast milk. Blood samples from newborns were collected after maternal nevirapine intake but before the newborn received nevirapine, and in all but one case within 1 h after birth (median 5 min, IQR 0–10 min). The median interval between maternal nevirapine intake and delivery was 5.2 h (IQR 3.0–9.9 h), while the median interval between maternal and child nevirapine intake was 8.5 h (IQR 4.2–17.3 h). Nevirapine concentrations between left and right breasts highly correlated at all time points (r = 0.87–0.96; P < 0.0001); therefore the mean nevirapine concentration was used.

At delivery, the median nevirapine concentration was 1755 ng/mL (IQR 1338–2043 ng/mL) in maternal plasma, 1012 ng/mL (IQR 657–1364 ng/mL) in breast milk and 1300 ng/mL (IQR 942–1690 ng/mL) in plasma of newborns. The nevirapine concentration in plasma of newborns correlated with the elapsed time between maternal nevirapine intake and delivery (r = 0.37; P = 0.006).

At week 1, the median interval between sample collection and maternal nevirapine intake was 7.7 days (IQR 7.0–8.1 days). All nevirapine concentrations were >IC50 (maternal plasma: median 164 ng/mL, IQR 75–378 ng/mL; breast milk: median 114 ng/mL, IQR 75–213 ng/mL; and child plasma: median 183 ng/mL, IQR 120–257 ng/mL).

At week 2, the median interval between sample collection and maternal nevirapine intake was 14.8 days (IQR 14.1–15.7 days). Considering only those samples taken 13–16 days after maternal nevirapine intake, 51.4% of the maternal plasma (median 15 ng/mL; IQR <15–50 ng/mL), 56.2% of the breast milk (median 17 ng/mL, IQR <15–35 ng/mL) and 61.3% of the children plasma samples (median 22 ng/mL; IQR <15–45 ng/mL) contained detectable nevirapine concentrations. At week 6, nevirapine was not measurable in any specimen.

Concentration–time profiles of nevirapine in the three compartments showed similar shapes with slightly lower mean nevirapine concentrations in breast milk than in maternal plasma. (Figure 2). The semi-logarithmic plots indicated one slope only, suggesting a monophasic elimination of nevirapine.

### Results

Sixty-two women fulfilled the inclusion criteria. The median age and weight of the women were 26 years (IQR 23–30 years) and 56 kg (IQR 52–62 kg), respectively. At delivery, the median CD4 count was 516 cells/mm³ (IQR 361–684 cells/mm³) and the median HIV-1 viral load was 7433 copies/mL (IQR 2553–41 872 copies/mL). The median interval between maternal nevirapine intake and delivery was 5.2 h (IQR 3.0–9.9 h), while the median interval between maternal and child nevirapine intake was 8.5 h (IQR 4.2–17.3 h). Nevirapine concentrations between left and right breasts highly correlated at all time points (r = 0.87–0.96; P < 0.0001); therefore the mean nevirapine concentration was used.

At delivery, the median nevirapine concentration was 1755 ng/mL (IQR 1338–2043 ng/mL) in maternal plasma, 1012 ng/mL (IQR 657–1364 ng/mL) in breast milk and 1300 ng/mL (IQR 942–1690 ng/mL) in plasma of newborns. The nevirapine concentration in plasma of newborns correlated with the elapsed time between maternal nevirapine intake and delivery (r = 0.37; P = 0.006).

At week 1, the median interval between sample collection and maternal nevirapine intake was 7.7 days (IQR 7.0–8.1 days). All nevirapine concentrations were >IC50 (maternal plasma: median 164 ng/mL, IQR 75–378 ng/mL; breast milk: median 114 ng/mL, IQR 75–213 ng/mL; and child plasma: median 183 ng/mL, IQR 120–257 ng/mL).

At week 2, the median interval between sample collection and maternal nevirapine intake was 14.8 days (IQR 14.1–15.7 days). Considering only those samples taken 13–16 days after maternal nevirapine intake, 51.4% of the maternal plasma (median 15 ng/mL; IQR <15–50 ng/mL), 56.2% of the breast milk (median 17 ng/mL, IQR <15–35 ng/mL) and 61.3% of the children plasma samples (median 22 ng/mL; IQR <15–45 ng/mL) contained detectable nevirapine concentrations. At week 6, nevirapine was not measurable in any specimen.

Concentration–time profiles of nevirapine in the three compartments showed similar shapes with slightly lower mean nevirapine concentrations in breast milk than in maternal plasma. (Figure 2). The semi-logarithmic plots indicated one slope only, suggesting a monophasic elimination of nevirapine.
clearance and volumes of distribution have to be reported as relative parameters. The relative total distribution volume (plasma + breast milk) was estimated to be 104.3 L, suggesting a large distribution throughout the body. The relative clearance of 1.45 L/h indicated a low elimination capacity for nevirapine resulting in a long half-life of 50.3 h. Variability between the mothers in relative clearance was moderate with a CV of 29%. The small relative standard errors of the fixed- and random-effect parameters ranging between 5% and 27% suggest a high precision of all parameters. The appropriateness of the final model was also demonstrated by a GOF plot.

Population PK analysis of child data

For the 113 child plasma samples, the two routes of obtaining nevirapine via mothers (blood/placenta and breast milk) were combined. The ‘bioavailability’ for the maternal dose via placenta and breast milk was estimated in the population PK analysis as being 14% (Table 1). The plasma/placenta–plasma/breast milk transfer rate constant (K32) was 4.5 h\(^{-1}\). The relative clearance of 0.27 L/h and volume of distribution of the plasma compartment of 22.7 L resulted in a half-life for nevirapine in plasma of children of 59.4 h. The GOF plot indicates that the model was acceptable for the description of the data.

Simulations

Maximum concentrations of nevirapine for differently eliminating individuals were 2449–2472 ng/mL in maternal plasma (Figure 3a) and 1540–1593 ng/mL in breast milk (Figure 3b), and were reached at ~0.3 and 3 h in plasma and breast milk, respectively. In newborns, the peak concentrations of nevirapine in plasma were reached ~4 h after maternal nevirapine intake (Figure 3c). The newborns’ own nevirapine dosing at 8.5 h after maternal intake caused a moderate but sudden increase in nevirapine concentration. While the impact of the inter-individual variability in elimination capacity was rather small in the onset phase (Figure 3a–c), it increased in the terminal phase (Figure 3d–f). In the three compartments, time periods until nevirapine concentrations declined to <10 ng/mL ranged between 270 and 620 h (median 17 days) for maternal plasma, 262 and 595 h (median 16 days) for breast milk and 384 and 512 h (median 19 days) for child plasma.

In Figure 4, the sigmoidal curves revealed a similar pattern for breast milk and maternal plasma with 80% of the mothers having nevirapine concentrations of <10 ng/mL for 2–3 weeks. Eighty percent of the children remained at nevirapine concentrations <10 ng/mL for 18 days.

Discussion

Nevirapine prophylaxis is the most common practice to prevent intra-partum transmission of HIV in resource-limited settings. In this study, we aimed to analyse the duration of nevirapine in breast milk, as well as in plasma of mothers and children who received a single dose for PMTCT of HIV. Samples were collected from pregnant women/mothers and children participating in a PMTCT intervention in a peripheral East African setting. Since in such a setting extensive sampling was not possible, the population analysis approach for nevirapine concentrations was highly attractive.

The simulations predicted total nevirapine concentrations >10 ng/mL in 80% of the mothers and children for 2–3 weeks.

Table 1. Population pharmacokinetic estimates of nevirapine obtained from the final models (maternal plasma and breast milk, and child plasma)

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Units</th>
<th>Population estimate, mother</th>
<th>RSE (%)</th>
<th>Population estimate, child</th>
<th>RSE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K12 h(^{-1})</td>
<td>1.66 fixed</td>
<td>—</td>
<td>1.66 fixed</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>F(^0) %</td>
<td>—</td>
<td>14.2</td>
<td>36.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V2/F L</td>
<td>9.0</td>
<td>26.7</td>
<td>22.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3/F L</td>
<td>95.3</td>
<td>5.1</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q/F L/h</td>
<td>122</td>
<td>18.9</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K32 h(^{-1})</td>
<td>—</td>
<td>4.5</td>
<td>60.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F L/h</td>
<td>1.45</td>
<td>5.1</td>
<td>0.27</td>
<td>41.9</td>
<td></td>
</tr>
</tbody>
</table>

| **Random effects** |
| CL/F % CV | 29.0 | 19.2 | 16.6 | 53.1 |
| V2/F % CV | — | — | 23.2 | 35.9 |
| F\(^0\) % CV | — | — | 19.8 | 28.7 |

| **Residual variability, coefficient of variation or standard deviation for** |
| proportional part % CV | 37.8 | 6.0 | 43.8 | 20.5 |
| additive part ng/mL | 0.01 fixed | — | 2 fixed | — |

CV, coefficient of variation; CL, clearance; F, bioavailability; F\(^0\), ‘bioavailability’ resulting from the mother’s dose via placenta and breast milk; K12, gastrointestinal absorption rate constant; K32, plasma/placenta–plasma/breast milk transfer rate constant; Q, inter-compartmental clearance from blood to breast milk; RSE, relative standard error (standard error divided by population estimate × 100; for the random effects parameters, RSE is related to the corresponding variance scale); V2, volume of distribution (plasma); and V3, volume of distribution (breast milk).
Figure 3. Simulated concentration–time courses for maternal plasma (a and d), breast milk (b and e) and child plasma (c and f). Linear scaled axis illustrates the different nevirapine concentration pattern in the ‘onset’ phase at delivery (a–c) and the semi-logarithmic scaled plots show the different patterns in the terminal phase including a vertical line at the IC$_{50}$ value (d–f).
Nevirapine in breast milk and plasma

Figure 4. Percentage of individuals with nevirapine (NVP) concentration above the IC50 over time.

Thus, nevirapine concentrations were detectable for a long time, even when considering that 60% of nevirapine in plasma and 40% of nevirapine in breast milk are bound by protein26,27 and only the unbound part is active.

The chosen PK models were appropriate and the typical maternal PK data such as clearance of 1.45 L/h were consistent with a prior population analysis performed for maternal plasma in Thai women (clearance = 1.40 L/h) and other studies analysing nevirapine single-dose PKs.5,7,8 In comparison with the elimination of nevirapine after long-term treatment,28,29 the elimination of nevirapine after single-dose intake was slower. We attribute this finding to the described mechanism of autoinduction of hepatic cytochrome P450, which leads to an accelerated metabolism of nevirapine over time.25

The expression of cytochrome CYP3A4, one of the main nevirapine-metabolizing enzymes, is very low during fetal development and only increases after birth.30,31 This might explain why the elimination capacity of nevirapine in newborns was lower when compared with their mothers (Table 1).

To prevent intra-partum transmission of HIV, it is desirable that newborns have the highest nevirapine levels at birth. We found that the nevirapine concentration in plasma of newborns at delivery correlated with the elapsed time between maternal nevirapine intake and delivery (r = 0.37; P = 0.006). The plasma/placenta–plasma/breast milk transfer rate constant was high at 4.5 h⁻¹, suggesting a high transport via these barriers. Accordingly, the maximum concentration of nevirapine in plasma of children was achieved ~4 h after maternal nevirapine intake. As shown in Figure 3(c), the newborns’ own nevirapine dosing caused only a moderate increase in nevirapine concentration. Obviously, the concentration in the newborns’ plasma predominately resulted from the mother’s dose, i.e. from placenta and breast milk transfer. This observation supports the rationale for the recommended timing of maternal administration of nevirapine at the onset of labour, hence, clearly before approaching the maternity unit for delivery and before labour has progressed to an advanced stage.

An important aspect is the possible impact of long-term nevirapine concentrations in breast milk on post-natal HIV infection. The nevirapine transfer from maternal plasma to breast milk was rapid and occurred to a large extent as can be concluded from the large values of the two drug transfer rate constants from plasma to milk compartment and vice versa (13.57 and 1.28 h⁻¹) and the high ratio of 10.6. Consequently, HIV is exposed to the influence of nevirapine in the breast milk compartment before newborns start to ingest breast milk. This is noteworthy because breastfed children ingest up to 10⁶ cell-free HIV particles and ~25 000 HIV-infected cells daily, and high levels of HIV in breast milk were shown to be significantly associated with transmission of HIV via breastfeeding.32–38 Even higher HIV levels were found in colostrum and early milk (0–10 days) compared with mature breast milk.35,36 Accordingly, the risk of post-natal HIV transmission seems to be especially high in the early period of breastfeeding.39–41 Therefore, the long persistence of nevirapine concentrations in the breast milk compartment during the early, risky period of breastfeeding may be beneficial. In therapeutic regimens, nevirapine concentrations >3000 ng/mL are postulated to achieve durable viral suppression without selection of nevirapine resistance mutations.32,44 In vitro, concentrations as low as 10 ng/mL have been shown to effectively reduce the replication of the HIV wild-type.24 Accordingly, HIV-RNA was shown to be suppressed in breast milk over 3 weeks after single-dose nevirapine.44 Furthermore, nevirapine is not only effective in inhibiting viral replication in cells, but also directly impedes the reverse transcriptase in cell-free virions.45 This mode of action may additionally contribute to a reduction of infectivity of breast milk, even if HIV virions are still present in this compartment. We conclude that single-dose nevirapine reduces the risk of post-natal transmission of HIV caused by breastfeeding.

However, the long duration of slowly decreasing levels of nevirapine predisposes for resistance mutations. As recently shown, 65% of the 20 sequenceable breast milk samples of 32 women exhibited resistance mutations 8 weeks after nevirapine single-dose intake.46 Hence, a child infected post-natally after nevirapine prophylaxis is at risk of acquiring a resistant virus. This resistant virus could fuel the cellular reservoir and persist for years.47 Since nevirapine-associated resistance mutations fade over time,1,14 the risk of acquiring a resistant virus through breast milk is probably highest during the early post-natal phase. In contrast, a child infected in utero or intra-partum acquires wild-type virus initially and is at risk of developing resistant virus as a result of the persisting nevirapine concentrations. Resistant virus, irrespective of the mechanism by which it evolved, will negatively affect the future treatment options of those children. The initial data on treatment responses of infants to NNRTI-containing highly active antiretroviral treatment after nevirapine single-dose prophylaxis point to a higher rate of virological failure.70

Post-natal transmission of HIV still poses an unsolved problem. In settings where infants are breastfed, post-natal antiretroviral prophylaxis with either extended nevirapine or a combination of antiretroviral drugs is considered. Extended nevirapine administration to children for 6 weeks after birth led to lower transmission risk compared with single-dose nevirapine (6.9% versus 9%) at month 6, a difference that is not statistically significant.48 Similarly, nevirapine resistance formation was observed to be higher in children who received an extended nevirapine regimen when compared with those who received single-dose nevirapine.49 The rationale for extended nevirapine regimens beyond the single dose, therefore, has to be carefully balanced. Combinations of antiretrovirals, which represent the
focus of several ongoing trials, may be more promising to prevent post-natal transmission while at the same time reducing resistance formation.\textsuperscript{50,51}

In this study, nevirapine was detectable in breast milk and plasma of mothers and children for a considerable period, which will almost inevitably have an effect on the quantity and quality of HIV: the known protective intra-partum effect and the potentially beneficial effect on post-natal transmission of nevirapine are hampered by the likely emergence of resistant virus.

To prevent resistance formation, the latest PMTCT guidelines recommend the additional post-natal administration of zidovudine/lamivudine for 1 week.\textsuperscript{52} Although resistance formation was shown to be reduced using this regimen, nevirapine mutations were still detectable in 6 of 68 (9%) mothers by population-based sequencing, and this percentage was even higher using sensitive allele-specific PCR.\textsuperscript{53–55} According to the long presence of nevirapine concentrations shown in this study, the duration of zidovudine/lamivudine would have to be extended to a considerably longer period than 1 week in order to avoid nevirapine resistance formation. Extension of zidovudine/lamivudine administration does, however, bear the risk of NRTI-resistant virus selection.

Acknowledgements

We are indebted to the women who participated in this study with their children. We wish to thank the counsellors, nurses, midwives and other staff for their participation in sample collection and Silver Mashate for laboratory technical support.

Funding

The study was supported by the German Ministry for Economic Cooperation and Development through the project PN 01.2029.5 (Prevention of Mother-to-Child Transmission of HIV) and by a grant of the H.W. & J. Hector Stiftung, Germany.

Transparency declarations

M. K. has served as a consultant to, has been a speaker for and received research grants from the manufacturers of antiretroviral drugs (Boehringer Ingelheim, Gilead, BMS, Roche, MSD, Abbott and GSK). C. K. received research grants from Boehringer Ingelheim. All other authors declare no conflict of interest.

References

Nevirapine in breast milk and plasma


47. Ghosn J, Pellegrin I, Goujard C et al. HIV-1 resistant strains acquired at the time of primary infection massively fuel the cellular reservoir and persist for lengthy periods of time. AIDS 2006; 20: 159–70.


