Association of cord blood nevirapine concentration with reported timing of dose and HIV-1 transmission

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Background: To correlate nevirapine presence and concentration in cord bloods of infants born to HIV-1 infected women with report of timing of dose and HIV-1 transmission at 6 weeks of age.

Methods: All available cord blood samples from the infants of mothers enrolled in the HIVNET 012 trial who were randomly assigned to receive either nevirapine or zidovudine at the onset of labor were tested for a nevirapine concentration.

Results: Nevirapine was detected in the cord blood of 244 of 259 (94%) infants whose mothers reported they took nevirapine in labor more than 1 h before delivery and in 12 of 13 (92%) infants whose mothers reported they took nevirapine less than 1 h before delivery. The median nevirapine cord blood concentration was 1238 ng/ml [interquartile range (IQR), 905–1474 ng/ml] and 122 ng/ml (IQR, 64–321 ng/ml) for women who reported taking nevirapine more or less than 1 h before delivery, respectively ($P < 0.001$). The median nevirapine cord blood concentration of infants who were HIV-1 negative at birth, but positive at 6–8 weeks of age ($n = 11$), was 916 ng/ml (IQR, 737–1245 ng/ml) compared with 1192 ng/ml (IQR, 875–1471 ng/ml) for uninfected infants ($n = 236$).

Conclusions: Cord blood nevirapine concentration correlated well with report of nevirapine administration and timing of dose before delivery. The nevirapine cord blood concentration was modestly lower in infected infants, although the number of infants infected between birth and 6–8 weeks of age was small ($n = 11$). The high adherence rate in the HIVNET 012 study supports the efficacy, simplicity and deliverability of this regimen.

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Introduction

The HIVNET 012 trial in Uganda demonstrated that a single 200 mg tablet of nevirapine taken by an HIV-1-infected mother at the onset of labor and a 2 mg/kg dose of nevirapine oral suspension given to the infant within 72 h of birth could reduce the HIV transmission rate by 42% at 6 weeks of life compared with a very short course of zidovudine given during labor and the first week of life [1,2]. The efficacy of this regimen was sustained in this breastfeeding population up to 18 months of age [2]. A phase I/II study has shown that a single 200 mg oral dose of nevirapine has a median half-life of 61 h in pregnant women and can cross the placenta and be detected in cord blood within 1 h of ingestion of nevirapine [3]. This dose has been sufficient to maintain a plasma concentration of nevirapine above 100 ng/ml [approximately ten times the 50% inhibitory concentration (IC_{50}) needed to inhibit HIV-1 replication] in the infant 7 days after dosing [3,4]. Stringer *et al*. reported that HIV-1 perinatal transmission rates were higher in a study of HIV-1-infected women in Zambia if the period of time between ingestion of the nevirapine dose by the mother and delivery was less than 1 h [5]. Similarly, in the South African Intrapartum Nevirapine Trial (SAINT) study, the rate of intrapartum transmission was three times higher when the mother had received nevirapine less than 2 h before delivery [6]. In order to correlate the presence and concentration of nevirapine in the cord blood of infants born to HIV-1 infected women with self-reported timing of the maternal dose and HIV-1 transmission at 6 weeks of age, we measured the concentration of nevirapine in the cord blood samples from infants in the HIVNET 012 study. Approval for this study was obtained from both the institutional review boards at Johns Hopkins University and in Uganda.

Methods

Population

All cord blood samples available from the firstborn infants of mothers enrolled in the HIVNET 012 trial were assayed for nevirapine concentration. Firstborn is defined as the firstborn infant of singletons, twins or triplets which represented 97.7% of the infants born in the HIVNET 012 study. These samples were from 278 of 308 infants (90%) whose mothers were randomized to receive zidovudine, 275 of 311 infants (88%) whose mothers were randomized to receive nevirapine, and 16 of 18 (89%) infants whose mothers were randomized to placebo (Table 1). Of those infants whose mothers were randomized to receive nevirapine and were reported dosed, cord blood samples were available from all 11 infected infants who were HIV-1 polymerase chain reaction (PCR) negative at birth, but positive at 6 weeks of age and from 236 of 263 infants (90%) who were HIV-1 RNA PCR negative at birth and 6 weeks of age. Cord blood samples were also tested from 24 of 25 (96%) infants who were HIV-1 RNA or HIV-1 culture positive at birth. The HIV status of three infants was unknown.

Table 1. Detection of nevirapine (NVP) in cord bloods of firstborn HIVNET 012 infants.

	Zidovudine arm	Nevirapine arm	Placebo arm
Cord bloods tested	278/308 (90%)	275/311 (88%)	16/18 (89%)
NVP detected in cord blood	1/278 (< 1%)	256/275 (93%)	0/16 (0%)
Mother NVP dosed		256/272 (94%)	
Mother not NVP dosed		0/3	

Timing of dose

In the HIVNET 012 study, mothers were instructed to swallow a 200 mg tablet of nevirapine, which they had been given to take home with them, at the onset of labor and to come to the hospital for delivery. Date and time of taking the tablet and delivery was recorded by a nurse midwife based on self-report if not observed, or based on actual times if observed. The treatment groups did not differ in the proportion of mothers who reportedly received study drugs [302 of 308 (98.1%) of the zidovudine group and 306 of 311 (98.4%) of the nevirapine group] [2]. Infants received a 2 mg/kg oral dose of nevirapine suspension within 72 h of birth or at time of discharge whichever occurred earlier. Prior to admission to the hospital 185 (60.2%) of 307 mothers on the nevirapine arm had self-dosed (data missing on four mothers) compared with 187 (62.3%) of 300 mothers on the zidovudine arm (data missing on eight mothers). Of the 185 women who self-dosed nevirapine prior to admission to the hospital, cord bloods were available for 162 (88%) firstborn infants compared with 109 (92%) of 119 firstborn infants whose mothers took nevirapine after admission. The infants who were born within 1 h of the mother's ingestion of nevirapine were dosed with nevirapine as soon as possible after birth.

Nevirapine measurement

Cord blood samples were collected in vacutainer tubes with ethylenediamine tetra-acetic acid anticoagulant at the time of delivery. Nevirapine drug concentration was measured in 50 μl of cord blood plasma using a validated high performance liquid chromatography (HPLC) assay. The assay was a modification of an assay described by Pav *et al*. [7]. The changes involved the extraction method and the addition of a step gradient wash to the chromatography method. Plasma samples were applied to Waters HLB reverse-phase cartridges (Waters Inc., Milford, Massachusetts, USA), washed with an acid/base series, eluted, dried, and dissolved in a mobile phase. Calibration standards ranged from 25 to 10 000 ng/ml for nevirapine.

Quality control samples of nevirapine were interspersed between unknown samples. The mean correlation coefficient for the calibration curve for plasma nevirapine was 0.998 ± 0.001. The precision and accuracy for nevirapine measurement were high, with a coefficient of variation of $< 13\%$ within a run (intra-day) and $< 8\%$ between runs (inter-day). In order to assure specificity for values near the level of detection, any samples with an initial positive value < 200 ng/ml were repeated. At least two of two or two of three values must have been $>$ 25 ng/ml to be considered positive. The technologist performing the testing was blinded as to the study arm of the infant.

Statistical analysis

Comparisons of the percentage of infants who had detectable cord blood nevirapine levels were performed using an exact binomial test. The distribution of cord blood nevirapine concentrations was compared using the Wilcoxon rank sum test for unpaired analysis. Similar comparisons were also performed in those infants whose mothers reported receiving nevirapine less than 1 or 2 h before delivery versus those who received nevirapine more than 1 or 2 h before delivery. Transmission rates were computed using Kaplan–Meier estimates, and tests for differences in transmission up to 56 days via the log-rank test.

Results

The relationship between nevirapine cord blood concentration and the interval between maternal dosing and birth is presented for all patients in Fig. 1 and characterized by transmission status in Fig. 2.

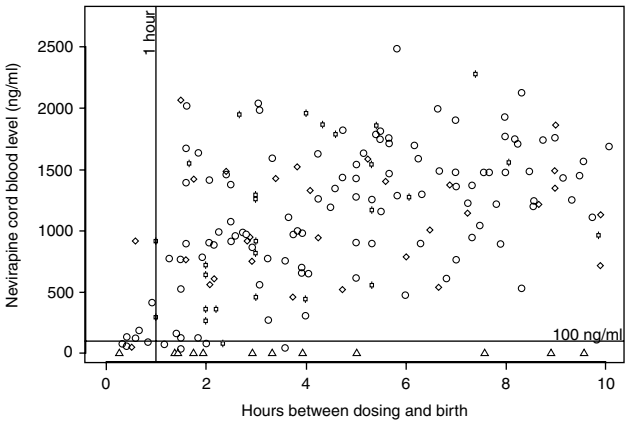

Fig. 1. Cord blood nevirapine concentration plotted against the time interval between maternal dosing and delivery. Concentrations below the assay limit of quantitation of 25 ng/ml are presented as open triangles. 100 ng/ml is 10 times the IC_{50} of nevirapine. Times longer than 10 h are not shown.

Nevirapine was detected in the cord blood of 256 of 272 (94%) of infants whose mothers reported they took a 200 mg tablet of nevirapine in labor (Table 1). Nevirapine was not detected in the cord blood of the three infants whose mothers were randomized to receive nevirapine, but reported not taking the tablet, for an overall validation dosing rate of 94% (259 of 275). In the 16 women whose cord blood nevirapine levels were undetectable, despite report of dosing, the median time between dosing and delivery was 4 h 27 min (range 15 min to 17 h 5 min). Nevirapine was detected in the cord blood of 150 of 162 (93%) infants whose mothers self-dosed prior to admission to the hospital compared with 105 of 109 (96%) infants whose mothers took nevirapine after admission. Nevirapine was not detected in the cord blood of 277 of 278 and 16 of 16 infants whose mothers were randomized to receive zidovudine or placebo, respectively.

Table 2 shows that nevirapine was detected in the cord blood of 244 of 259 (94%) infants whose mothers reported they took a 200 mg tablet of nevirapine in labor more than 1 h before delivery and in 12 of 13 (92%) infants whose mothers reported they took nevirapine less than 1 h before delivery. The median cord blood concentration of nevirapine was 1238 ng/ml [interquartile range (IQR), 905–1474 ng/ml] for women who reported taking nevirapine more than 1 h prior to delivery and was 122 ng/ml (IQR, 64–321 ng/ml) for women who reported taking nevirapine less than 1 h prior to delivery ($P < 0.001$).

The percentage of HIV-1-infected infants at 6–8 weeks of age (but HIV-1 RNA negative at birth) in the nevirapine arm with detectable cord blood nevirapine was 91% (10 of 11) compared with 94% (223 of 236) of infants who were uninfected at birth and at 6–8 weeks of age. Ten (91%) of 11 mothers of infants infected between birth and 6–8 weeks of age reported taking nevirapine more than 1 h before delivery (median, 4 h 32 min; range, 1 h 35 min–28 h 20 min) compared with 249 (95%) of 263 mothers of uninfected infants (median, 7 h 20 min; range, 1 h 10 min–41 h 10 min). The one mother of the infected infant who took nevirapine less than 1 h before delivery had a detectable cord blood level.

The median cord blood concentration of nevirapine of infants HIV infected at birth was similar to the concentration of those uninfected at 6–8 weeks of age (1313 versus 1192 ng/ml, respectively). This result is not surprising since those infants infected at birth probably became infected during pregnancy prior to receipt of nevirapine. The median cord blood concentration of nevirapine of infants who were HIV-1 negative at birth, but HIV-1 positive at 6-8 weeks of age was 916 ng/ml (IQR, 737–1245 ng/ml), which was modestly lower than the concentration of 1192 ng/ml (IQR, 875–1471 ng/ml) for uninfected infants at 6–8 weeks of age.

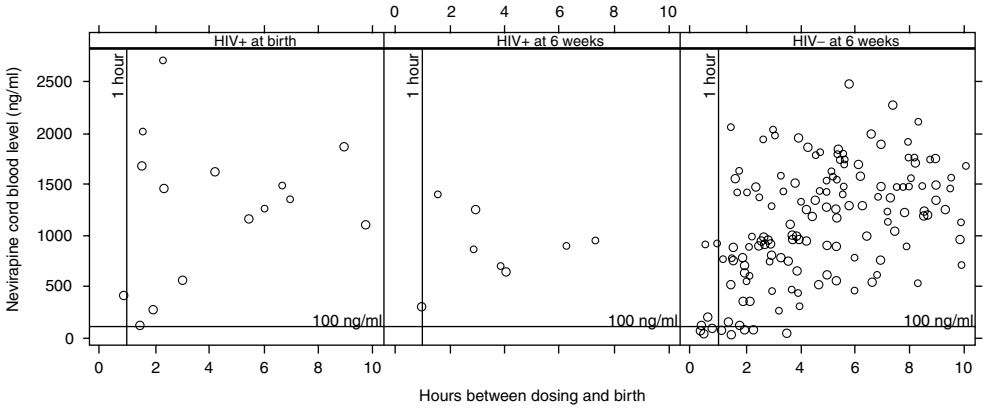

Fig. 2. Nevirapine concentrations and timing of mother's dose displayed by HIV transmission status at birth and 6 weeks. Concentrations below the assay limit of quantitation of 25 ng/ml are presented as open triangles. 100 ng/ml is 10 times the IC_{50} of nevirapine. Times longer than 10 h are not shown.

Table 2. Detection of nevirapine (NVP) in cord bloods of firstborn infants whose mothers were randomized to receive nevirapine in the HIVNET 012 study.

	HIV infected at birth	HIV infected at 6–8 weeks	HIV uninfected at 6–8 weeks	Dosed within 1 h of birth	Dosed at least 1 h before birth	Total
Mothers dosed[a]	25/25 (100%)	11/11 (100%)	263/272 (97%)	n/a	n/a	301/311 (97%)
Cord bloods tested	24/25 (96%)	11/11 (100%)	236/263 (90%)	13/16 (81%)	259/284 (91%)	272/301 (90%)
Cord bloods with detectable NVP	22/24 (92%)	10/11 (91%)	223/236 (94%)	12/13 (92%)	244/259 (94%)	256/272 (94%)
NVP level (ng/ml) median, (25%, 75%)	1313 (1118, 1658)	916 (737, 1245)	1192 (875, 1471)	122 (64, 321)	1238 (905, 1474)	1208 (877, 1472)

[a]HIV status not available for three infants.

In the 15 firstborn infants uninfected at birth in the nevirapine arm whose mothers received nevirapine less than 1 h before delivery, one infant became HIV infected between birth and 6–8 weeks despite a cord blood concentration of 292 ng/ml and receipt of nevirapine 20 min after birth, giving a post-delivery HIV transmission rate at 6–8 weeks of 6.7%, compared with 4.0% for those where nevirapine was taken more than 1 h before birth. In the 40 firstborn infants uninfected at birth where maternal dosing was less than 2 h before delivery, two infants became newly infected between birth and 6–8 weeks, giving a post-delivery transmission rate of 5.4%, compared with 3.9% where dosing was more than 2 h).

In 17 firstborn infants in the nevirapine arm who had no detectable virus at birth and had no detectable nevirapine in their cord blood, 16 of 17 received post-birth dosing. One of these 17 infants had become HIV-infected by 6–8 weeks of age (despite receiving nevirapine 9 h after birth) for a transmission rate of 5.9% [95% confidence interval (CI), 0, 16.4%] versus 10 of 232 (4.4%) [95% CI, 0.17, 7.1%], for those with detectable nevirapine in cord blood.

Discussion

In this study we confirm that the correlation between the level of nevirapine in the cord blood of infants born to HIV-1 infected women with the interval between maternal dosing and delivery is quite high, with the median cord blood concentration of nevirapine being approximately ten times higher for women who reported taking nevirapine more than 1 h prior to delivery [8]. Nevirapine was detected in the available cord blood of 94% of infants whose mothers were assigned to receive nevirapine in the study, and in 94 and 92% of infants whose mothers reported they actually took the nevirapine more or less than 1 h before delivery, respectively. These data indicate that nevirapine crosses the placenta very quickly after ingestion and that the mother's self-report of taking nevirapine was very reliable in the HIVNET 012 study. Likewise, the fact that nevirapine was not detected in the cord blood of 277 of 278 cord bloods of women assigned to receive zidovudine nor in 16 of 16 cord bloods of women assigned to receive placebo, indicates that there was minimal crossover of nevirapine between the arms of the study. Nevirapine (777 ng/ml) was detected in the cord blood of one of the 278 infants whose mothers reportedly received zidovudine. Specimen mix-up was unlikely to explain the discrepancy because nevirapine was also detected in the peripheral blood of both the mother and baby at delivery and birth. In addition, identity typing using microsatellite polymorphism testing (AmpFlSTR Profiler kit; Applied Biosystems, Foster City, California,

USA) of cord blood and peripheral blood specimens at birth, 7 days, and 6 weeks indicated they were from the same mother–infant pair. This mother's and infant's records clearly indicated they had received multiple doses of zidovudine which was also detected in the cord blood and baby's peripheral blood sample at birth using a qualitative HPLC assay. These findings suggest that the mother may have been inadvertently given nevirapine in addition to zidovudine during labor on admission to the hospital or accessed nevirapine herself from other sources.

The median cord blood concentration of nevirapine of 10 infants who were HIV-1 negative at birth, but positive at 6–8 weeks of age was 916 ng/ml. This value was moderately lower than the median concentration of 1192 ng/ml for 223 uninfected infants. Likewise, the time interval between ingestion of nevirapine and delivery tended to be longer for uninfected infants compared with infants infected between birth and 6–8 weeks of age. The SAINT study [6] reported higher transmission rates if women ingested nevirapine less than 2 h before delivery. We also observed a pattern of increased transmission where dosing occurred within 2 h before delivery, although it was not statistically significant. However, the number of mothers who ingested nevirapine less than 1 h before delivery was small (16) as was the number of infected infants so that our study is underpowered to address this issue definitively or to assess what minimum cord blood nevirapine concentration is needed to prevent transmission. Our ability to answer this latter assessment is also limited by the fact that transmission in this small number of infected infants diagnosed at 6–8 weeks of age may have occurred through breastfeeding several weeks after nevirapine was ingested and would no longer be detected. In addition, infants whose mothers ingested nevirapine less than 1 h before delivery received nevirapine immediately after birth which further confounds the analysis.

Thus, cord blood nevirapine concentration represents only one point in time prior to this infant dose and does not reflect the total area under the nevirapine time–dose curve in the infant, which would indicate the level of pre-exposure prophylaxis from the maternal dose (as shown by the cord blood concentration) and the level of post-exposure prophylaxis from the subsequent infant dose. It is likely that this measure might be more indicative of protection of the infant from infection than cord blood levels alone.

In any case, our data provide the distribution of nevirapine cord blood concentrations in a population of infants in which the efficacy of a single dose of nevirapine given to the mother and to the infant in the prevention of perinatal transmission was demonstrated. More importantly, our data support the feasibility of allowing women who receive antenatal care to self-administer the nevirapine tablet thereby assuring access to nevirapine before delivery and increasing the duration of nevirapine exposure to the infant in labor. The high adherence rate in the HIVNET 012 study supports the simplicity and deliverability of this regimen which allows HIV-infected pregnant women in resource-limited settings to self-administer the nevirapine tablet at labor onset. The lower efficacy rates of the single dose nevirapine regimen reported in some field programs may reflect lower adherence than that seen in the HIVNET 012 study [9].

It should be noted that the benefits of the HIVNET 012 nevirapine regimen should be weighed against the potential risk of nevirapine resistance which has been documented in a number of studies [10–13], although differences in clinical outcomes between women exposed or not exposed to single-dose nevirapine subsequently taking a nevirapine-containing treatment regimen have not been demonstrated and studies are underway that will address this issue. Thus, although there is concern about resistance for all regimens using nevirapine either alone or in combination for prevention of mother-to-child transmission, the efficacy and feasibility of the HIVNET 012 nevirapine regimen makes this a very attractive prevention strategy that, for a large number of HIV-1-infected pregnant women, is often the only option to prevent HIV transmission to their baby.

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