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Timing of the maternal drug dose and risk of perinatal HIV transmission in the setting of intrapartum and neonatal single-dose nevirapine

Jeffrey S. A. Stringer[a,b,c], **Moses Sinkala**[a,c,d], **Victoria Chapman**[a], **Edward P. Acosta**[a], **Grace M. Aldrovandi**[a], **Victor Mudenda**[b,e], **Julia P. Stout**[a,c], **Robert L. Goldenberg**[a,c], **Rosemary Kumwenda**[f], and **Sten H. Vermund**[a,c]

[a]Schools of Medicine and Public Health, University of Alabama at Birmingham, Birmingham, Alabama, USA

[b]University of Zambia School of Medicine, Lusaka, Zambia.

[c]Centre for Infectious Disease Research in Zambia

[d]Lusaka Urban District Health Management Board, Zambian Ministry of Health, Lusaka, Zambia.

[e]University Teaching Hospital, Lusaka, Zambia.

[f]United Nations Development Program, Lusaka, Zambia.

Abstract

Context: Single-dose intrapartum and neonatal nevirapine (NVP) reduces perinatal HIV transmission and is in increasingly common use throughout the developing world.

Objective: We studied risk factors for perinatal transmission in the setting of NVP.

Design and setting: A prospective cohort study at two public obstetrical clinics in Lusaka, Zambia.

Patients and methods: In a volunteer sample of HIV-infected pregnant women and their newborns, the women received a 200 mg oral dose of NVP at the onset of labor; their infants received 2 mg/kg of NVP syrup within 24 h of birth. The main outcome measure was the infant HIV infection status at 6 weeks of life, determined by DNA polymerase chain reaction.

Results: Only 31 of 278 (11.2%) infants were infected at 6 weeks. In logistic regression, viral load exceeding the median [adjusted odds ratio (AOR), 3.1; 95% confidence interval (CI), 1.1–8.7] and 1 h or less elapsing between NVP ingestion and delivery (AOR, 5.0; 95% CI, 1.8–14) were associated with transmission. Women delivering within 1 h of NVP ingestion had a lower mean drug concentration (351 versus 942 ng/ml; $P < 0.001$) and were more likely to have a 'sub-therapeutic' NVP level of less than 100 ng/ml (56 versus 20%; $P < 0.001$) than those who delivered more than 1 h post-ingestion. However, concentrations < 100 ng/ml were not more likely to be associated with transmission than concentrations ≥ 100 ng/ml (12.9 versus 11.7%; $P = 0.8$). We did not identify a threshold concentration below which risk of transmission increased.

Conclusions: We confirmed low perinatal transmission rates with single-dose NVP. At least 1 h of pre-delivery NVP prophylaxis was a critical threshold for efficacy.

Correspondence to Jeff Stringer, 5977 Mwembeshi Road, PO Box 34681, Northmead, Lusaka, Zambia. Tel: 260 1 293 661; fax: 260 1 293 661 extension 148; e-mail: stringer@uab.edu.

Keywords

HIV; perinatal; nevirapine; timing; Zambia

Introduction

Each year, nearly 2.5 million children are born to HIV-infected mothers. In the absence of intervention, 35–40% of exposed infants will become infected [1,2]. Intrapartum and neonatal single-dose nevirapine (NVP) can reduce the risk of intrapartum transmission by about half, and this benefit persists throughout the breastfeeding period [3]. In the Ugandan HIVNET 012 trial that established the efficacy of NVP [4], a single 200 mg dose was self-administered by a pregnant mother upon labor onset and a second 2 mg/kg oral dose was given to the infant within 72 h of delivery. The choice of this specific dosing derived from prior pharmacokinetic studies and aimed to achieve infant plasma levels of 100 ng/ml, ten times the *in vitro* 50% inhibitory concentration (IC_{50}) of the drug [5,6]. The choice to have women self-administer the medication upon labor onset was intended both to ensure prophylactic drug levels earlier in labor and to avoid the possibility of missed doses among those women who might deliver at home or at a non-participating facility.

Some NVP implementation programs – such as an Elizabeth Glaser Pediatric AIDS Foundation-sponsored demonstration project in the Democratic Republic of Congo [7] and a program proposed nationally for Botswana [8] – have elected to administer NVP upon presentation to the labor unit, rather than issuing the tablet for self-administration at labor onset. While such an approach would probably result in a shorter average interval between maternal drug ingestion and delivery of the infant, the effect of this later drug administration on NVP efficacy is not known. In addition, the hypothetical ideal pre-infant dosing NVP concentration of 100 ng/ml continues to serve as a target in the planning of new investigations [9] but has not been validated *in vivo* [10].

We sought to confirm the protective efficacy of the HIVNET 012 NVP regimen in our setting and to understand risk factors for perinatal transmission in women receiving NVP. Because of their potential impact on strategies for program implementation, we were particularly interested in whether maternal dose timing and/or cord blood drug concentration would predict perinatal HIV transmission in a field setting.

Methods

We conducted a prospective clinical trial in Lusaka, Zambia to evaluate strategies for administering perinatal NVP. The primary aim of the trial (which has been reported elsewhere [11,12]) was to compare the use of NVP prophylaxis with and without HIV testing in an antenatal setting of very high HIV prevalence. Its primary outcomes were uptake (the proportion of women who accept the strategy when offered) and adherence (the proportion of women who actually ingest the NVP tablet at labor onset.) In an effort to maximize the study's generalizability, we used liberal inclusion and few exclusion criteria. Participating women were given a single 200 mg oral dose of NVP either at 36 weeks of gestation, with instructions to ingest it at the onset of labor, or upon presentation in established labor, if not previously enrolled in the antenatal clinic. Their infants received a 2 mg/kg dose of NVP syrup at discharge from the delivery facility, typically the following morning, but never more than 24 h later. The study protocol mandated immediate dosing of infants that were delivered within 2 h of maternal NVP ingestion, but data on compliance with this aspect of the protocol were not collected. Timing of maternal NVP ingestion was determined by self-report for those women enrolled prior to

presentation to the labor ward (n = 134) or by midwife observation for those women enrolled at the labor ward (n = 144).

We collected maternal serum in the third trimester, fetal cord blood and amniotic membranes at delivery, and infant blood spots at 1 day and 6 weeks of life. We assessed maternal HIV status with a dual rapid test algorithm that has been previously validated in our setting [13] (Determine HIV-1/2, Abbott Laboratories, Abbott Park, Illinois, USA; and Capillus HIV-1/HIV-2, Trinity Biotech, Wicklow Co, Ireland). We estimated the maternal CD4+ lymphocyte with an enzyme-linked immunoassay (TRAx CD4; Innogenetics, Atlanta, Georgia, USA) performed on lysed whole blood. This assay has excellent correlation with results from flow cytometry/hematology when the CD4+ lymphocyte count is $\geq 200 \times 10^3$ cells/l [14]. We screened for syphilis with a non-treponenal test (RPR Immunotrep; Omega Diagnostics, Alloa, Scotland) and treated all patients with positive results with intramuscular benzathine penicillin as per the protocol used in the Lusaka Urban District Clinics.

At delivery, fetal umbilical cord blood was collected in a heparinized vacuum vial. The specimen was immediately centrifuged and the plasma drawn off and frozen at −70°C. Fetal membranes were removed from their placental attachment, and a rectangular section that included the rupture site prepared. They were then rolled and placed in 10% neutral-buffered formalin. Once fixed, the membranes were embedded in paraffin, sectioned at 5 μm, and stained with standard Harris hematoxylin and eosin. Chorioamnionitis was diagnosed by examining at least 10 high-powered microscopy fields. Acute and chronic chorioamnionitis were quantified separately by recording the average number of polymorphonuclear leukocytes (acute) and monocytes (chronic) per high-powered field (hpf). We used the following classification to describe the degree of chorioamnionitis: 0–9 cells per hpf as none/minimal inflammation; 10–29 cells as intermediate inflammation; 30 or more cells as severe inflammation [15]. All slides were read by the same pathologist (V.M.) who was blinded to infant infection status.

We performed a quantitative assay for NVP using a method developed at the University of Alabama at Birmingham Clinical Pharmacology Infectious Diseases Laboratory. NVP was isolated from 0.1 ml of plasma by simple liquid extraction. NVP and plasma interferences in the extract were then separated by isocratic reversed phase high-performance liquid chromatography assay (Microsorb MV C8, 4.6 × 250 mm, 5 μm; Varian Inc., Palo Alto, California, USA) with UV detection at 284 nm. Detector responses of unknown patient samples were compared to known concentrations of an internal standard (5,11-dihydro-6H-11-ethyl-4-methyl-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-one, $C_{14}H_{14}N_4O$; supplied by Boehringer Ingelheim Pharmaceuticals, Inc. (Ingelheim, Germany) to provide a quantitative measure of NVP levels in the patient specimens. This assay is capable of measuring concentrations in the range of 25–10 000 ng NVP per ml plasma [16].

All virologic assays were performed at the University of Alabama at Birmingham in a laboratory certified by the virology quality assurance program of the AIDS Clinical Trials Group (ACTG; US National Institutes of Health.) Quantitative plasma HIV-1 RNA was assessed with the Roche Amplicor kit version 1.5 (Roche Diagnostics, Indianapolis, Indiana, USA.) Infant HIV infection was diagnosed by HIV DNA polymerase chain reaction (PCR) of peripheral blood mononuclear cells from whole blood collected on filter paper [17]. Each specimen was subjected to at least two independent amplifications. We did not confirm the diagnosis of infant HIV infection with a second specimen.

To assess the comparability of various demographic factors between transmitters and non-transmitters, continuous variables were log transformed when indicated and analyzed by either unpaired, two-tailed Student *t* tests or the Wilcoxon rank sum test (for non-parametric data.)

Dichotomous variables were analyzed by chi-square or Fisher's exact tests and Taylor series 95% confidence intervals were generated. Multivariable logistic regression analyses were performed with variables that were significant at the $P \leqslant 0.1$ level in univariate analysis using the LOGISTIC procedure, SAS System release 8.01 for Windows (SAS Institute, Cary, North Carolina, USA) Infants were determined to be HIV infected if the 6-week specimen was positive for HIV DNA. Timing of infection was categorized as *intrauterine* if both the birth and 6-week specimens were positive or *intrapartum/early postpartum* if the birth specimen was negative but the 6-week specimen was positive [18]. Some risk factors (e.g., viral load) would be expected to influence both intrauterine and intrapartum/early postpartum transmission. Others (e.g., timing of the maternal NVP dose) could only be expected to affect intrapartum/early postpartum transmission. Whereas our primary analyses were performed using the general outcome of infant infection (positive 6-week specimen), some sub-analyses were performed using intrapartum/early postpartum infection as the outcome (i.e., intrauterine infections were excluded.) In the case of twin gestations – which were few owing to our enrollment of women late in the third trimester after most multiple gestations had been detected and referred to the teaching hospital – the infection status of only the first born infant was considered. The study was approved by the University of Alabama at Birmingham Institutional Review Board and by the University of Zambia Research Ethics Committee; all participants provided informed consent.

Results

Between September 2000 and May 2001, we enrolled 430 HIV-infected women in the clinical trial, to whom 433 infants were live born. There were four sets of twins, six fresh stillbirths, and one macerated stillbirth. Six infants died between days 1 and 42 of life. Since women were not required to have their infant tested for HIV to participate in the study, perinatal transmission data are not available on all woman–infant pairs. The 278 pairs (65%) in whom transmission data are available did not differ from other 152 pairs (35%) with respect to any demographic or laboratory measure studied (Table 1).

Risk factors for perinatal transmission

Thirty-one (11.2%) singleton or first-born twin infants were infected at 6 weeks of life. We judged six infants (2.2%) to have been infected intrauterine and 25 infants (9.0%) infected intrapartum/early postpartum. Transmitting mothers did not differ from non-transmitting mothers with respect to age, body mass index, parity, history of stillbirth, marital status, educational status, or family income (Table 2). Factors associated in univariate analysis with perinatal transmission included: alcohol use in the index pregnancy [relative risk (RR), 2.2; 95% confidence interval (CI), 1.2–4.3], report of mixed rather than exclusive breastfeeding (RR, 5.1; 95% CI, 2.5–10.5), viral load greater than the median for the cohort (RR, 2.4; 95% CI, 1.03–5.5), clinical chorioamnionitis (RR, 4.7; 95% CI, 1.7–13), and less than 1 h elapsing between NVP ingestion and delivery (RR, 2.4; 95% CI, 1.2–4.8).

As the timing of the maternal NVP dose and breastfeeding practices would not be expected to affect intrauterine transmission, we repeated the above univariate analysis for these factors using the outcome of intrapartum/early postpartum transmission. Whereas less than 1 h elapsing between NVP ingestion and delivery remained significant in this analysis (RR, 3.3; 95% CI, 1.6–7.0), report of mixed rather than exclusive early postpartum breastfeeding did not (RR, 2.3; 95% CI, 0.63–8.5.)

In logistic regression analyses, we examined the contribution of those factors associated with transmission in univariate analysis (NVP dose timing, maternal viral load, exclusive breastfeeding, clinical chorioamnionitis, and alcohol use) as well as a factor shown in other settings to be predictive of transmission [4] (maternal CD4 cell count). Women who delivered

within 1 h of NVP ingestion, women who did not breastfeed exclusively, and women whose viral load was higher than the median were all more likely to have transmitted their infection by 6 weeks of infant life (Table 3). When the analysis was restricted to intrapartum/early postpartum transmissions, only the late NVP dosing variable remained significantly predictive. When exclusive breastfeeding was removed from the model altogether, late NVP dosing remained highly predictive of both overall transmission [adjusted odds ratio (AOR), 3.7; 95% CI, 1.4–10.1] and intrapartum/early postpartum transmission (AOR, 5.5; 95% CI, 1.9–16.)

Concentration of NVP in the cord blood

Women who delivered within 1 h of drug ingestion had a lower average cord blood NVP concentration than those in whom more than an hour elapsed (351 ± 805 versus 942 ± 797 ng/ml; $P < 0.001$). They were likewise more likely to have a 'sub-therapeutic' drug level (56 versus 20%; $P < 0.001$). However, cord blood NVP concentrations less than 100 ng/ml were not more likely to be associated with transmission than concentrations greater than 100 ng/ml (12.9 versus 11.7%; $P = 0.8$). When the data-set was limited to only those women in whom cord blood NVP concentration were in the 'sub-therapeutic' range of less than 100 ng/ml (n = 62), the mean drug concentration was not different between transmitters and non-transmitters (19.6 ± 27.2 versus did not 18.8 ± 24.2 ng/ml; $P = 0.9$). We did not identify a threshold concentration below which risk of transmission increased.

Exclusive breastfeeding

Only 10 women (3.6%) reported at the 6-week study visit having fed their infant something in addition to breast milk. Five of these 10 transmitted their infection: three intrauterine and two intrapartum/early postpartum (Table 4). Women who exclusively breastfed their infants did not differ from those who did not with respect to age, parity, family income, CD4+ lymphocyte estimate, viral load, or body mass index (data not shown). Exclusively breastfed infants weighed slightly less than non-exclusively breastfed infants (3057 ± 368 versus 3155 ± 630 g; $P = 0.4$). However exclusively breastfed infants were less likely to have been diagnosed with clinical chorioamnionitis at delivery (1.1 versus 10%; $P = 0.1384$), and to have had evidence of very severe acute chorioamnionitis on histological examination (defined as 200 or more acute inflammatory cells per high-powered microscopy field; 12.2 versus 37.5%; $P = 0.037$).

Discussion

In this first peer-reviewed report of the HIVNET 012 intrapartum and neonatal single-dose NVP regimen [4] since the original clinical trial, we describe an essentially identical rate of transmission to that observed in the Ugandan study. When compared with a contemporaneous population of Zambian infants who did not receive NVP prophylaxis [12], the efficacy estimate of the regimen appears substantial (11.1 versus 20.0%; 49% reduction; $P = 0.062$). In addition to confirming the conference reports that viral load represents an important risk factor for perinatal transmission in women and infants receiving single-dose NVP, [4,19] our data suggest that ingestion of NVP within 1 h of delivery also represents an important risk factor.

A limitation of our study design, and thus the interpretation of these data, is that we did not collect specific information on timing of the infant NVP dose. We therefore cannot confirm that study personnel actually complied with the protocol and immediately dosed all infants that were delivered within 2 h of maternal drug ingestion. In addition, since we did not confirm infant infection status with a second specimen, it is possible that we over-diagnosed a few infections. However, we expect that our use of multiple separate PCR amplifications and the documented very high specificity (> 98%) of the method used [17] would mitigate against false-positive results. A third limitation of the results we present here is the use of a

contemporaneous (but not randomized) control group to estimate the efficacy of the NVP regimen.

In order to insure that the highest proportion of HIV-infected women in the population were able to take NVP at labor onset, investigators from the original clinical trial issued the tablet in antenatal care for self-administration at labor onset. With such an approach, therapy is not directly observed, probably resulting in lower adherence to the single-dose intervention [11]. However, this strategy does allow drug access to those women who receive antenatal care, but do not deliver at a participating facility, and has been adopted by many implementation programs, including our own in Lusaka. An alternative approach, which has been adopted by various programs, including initiatives in the Democratic Republic of Congo and Botswana [7,8], would be to administer the drug upon presentation in labor. Since therapy is directly observed, adherence to the single dose intervention can be maximized. However, such an approach might result in a shorter average interval between drug ingestion and delivery, and by extension, a greater proportion of women who would not receive at least 1 h of pre-delivery NVP prophylaxis, a critical threshold for effectiveness in our study.

Although we did not identify a minimum cord blood NVP concentration below which the drug's prophylactic efficacy was lost, our study is underpowered to address this question definitively. However, possible explanations for this observation other than type II statistical error include: (1) plasma values may give incomplete or misleading information about drug concentrations in the compartments important for perinatal prophylaxis; (2) the hypothetical target concentration of 100 ng/ml may exceed that actually necessary to effect adequate prophylaxis; or (3) a single non-steadystate NVP cord blood concentration taken at delivery is an imprecise surrogate for what is thought to be most important, the area under the NVP dose–time curve. Although we did not undertake formal pharmaco-kinetic modeling for this analysis, it is interesting to note that cord NVP concentration continued to be non-predictive of transmission even when all factors pertinent to the area under the dose–time curve (maternal weight, infant birth weight, time of ingestion) were considered simultaneously in multivariable analysis (data not shown.)

The association between non-exclusive breastfeeding and overall perinatal transmission is puzzling. It would seem unlikely that exclusive breastfeeding would play such an important role in early postpartum transmission, and indeed impossible for it to affect viral transmission *in utero*. It is conceivable, as has been proposed by others [20], that this risk factor is being influenced by some other, as yet unrecognized factor(s). However, neither we nor the group that originally described the association [21] were able to identify any such confounders. Perhaps inability to exclusively breastfeed is a marker for maternal illness, infant failure-to-thrive, or a propensity to transmit that we have not measured or do not completely understand.

These data have policy implications for the planning of perinatal NVP implementation programs. We propose that counseling of women enrolled in NVP prophylaxis programs should emphasize recognition of the signs of labor onset, and focus on the importance of prompt drug self-administration once labor is suspected. This advice may be particularly important in multiparous women in whom delivery can be precipitous. Those programs that elect to administer the drug upon presentation to the delivery unit should encourage early presentation to the labor ward and make an effort to administer the drug as soon as possible after admission. A program that combines these two approaches; that is, seeks to provide NVP antepartum for onset-of-labor self-administration as well as providing labor room dosing for all women not receiving NVP earlier, is likely to be most effective.

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Table 1

Selected characteristics of mother–infant pairs included and not included in the analysis[a].

Characteristic	Mother–infant pairs included (n = 278)	Mother–infant pairs not included (n = 152)
Median age (range)	25 (17–43)	25 (18–40)
Median parity (range)	2 (0–7)	2 (0–7)
Used alcohol during pregnancy, %	30	32
Mean HIV knowledge score[b] (SD)	8.5 (1.2)	8.6 (1.1)
Mean body mass index, kg/m^2 (SD)	24.9 (3.4)	25.1 (3.2)
Mean CD4 cells × 10^3/l	372	376
Mean log viral load	4.3	4.2
Infant birthweight, g (SD)	3059 (380)	2963 (448)

[a] $P > 0.05$ for all comparisons.

[b] Sum of correct answers to 10 true/false questions about HIV and its transmission.

Table 2

Univariate predictors of infant infection at 6 weeks of age.

Characteristic	Transmitters (n = 31)	Non-transmitters (n = 247)	*P*
Maternal characteristics			
Mean age, years	25.0	25.0	0.87
Mean body mass index, kg/m^2 (SD)	24.1	25.0	0.16
Multiparous, %	74.2	81.0	0.37
Prior stillbirth, %	9.7	9.3	0.95
Did not breastfeed exclusively, %	16.1	2.0	0.0022
Married, %	90.3	93.5	0.45
Mean education, years	7.0	6.5	0.35
Mean family weekly income, US$	13.8	14.1	0.92
Used alcohol during pregnancy, %	48.4	27.3	0.016
Infant characteristics			
Birth weight, g (SD)	2987 (471)	3068 (367)	0.36
Birth weight < 25%ile, %	25.8	14.5	0.10
Clinical chorioamnionitis, %	6.5	1.0	0.06
Histologic severe chronic chorioamnionitis, %	32	20	0.18
Histologic severe acute chorioamnionitis, %	44	45	0.90
Maternal laboratory values			
CD4 cells × 10^3/l	320	379	0.14
Nevirapine concentration (ng/mL)	753	904	0.23
Nevirapine concentration < 100 ng/ml, %	26	24	0.80
Log viral load	4.6	4.2	0.075
Viral Load higher that the median, %	71	48	0.034
Positive RPR test, %	18	12	0.34
Maternal delivery time values			
Time from nevirapine dose to delivery, h (SD)	8.2 (10.3)	11.9 (15.8)	0.09
⩽ 1 h between nevirapine ingestion to delivery, %	30.0	13.5	0.018
Time from membrane rupture and delivery, h (SD)	1.5 (3.2)	2.2 (7.0)	0.33

Table 3

Results of two multivariable logistic regression analyses of factors associated with intrapartum/early postpartum transmission and cumulative perinatal transmission at 6 weeks of age.

Factor	Intrapartum/early postpartum transmission[a]	Cumulative transmission at 6 weeks of life
Did not breastfeed exclusively, %	2.5 (0.26–23.7)	13.5 (2.4–74)
Clinical chorioamnionitis	*b*	5.7 (0.085–386)
CD4 estimate above median	0.63 (0.22–1.8)	1.1 (0.42–2.8)
Viral load higher that the median, %	2.0 (0.68–5.6)	3.1 (1.1–8.7)
Used alcohol during pregnancy, %	2.1 (0.77–6.0)	2.2 (0.82–5.6)
⩽ 1 h between nevirapine ingestion and delivery, %	5.8 (2.0–17)	5.0 (1.8–14)

Values are adjusted odds ratio (95% confidence interval).

[a] Timing of infection was categorized as intrauterine if the birth specimen was positive for HIV DNA by polymerase chain reaction or intrapartum/early postpartum if the birth specimen was negative but the 6-week specimen was positive. The cumulative transmission rate describes all infants in whom the 6-week specimen was positive, irrespective of the status of the birth specimen.

[b] Insufficient sample size to include clinical chorioamnionitis in logistic model.

Table 4

Association of exclusive breastfeeding with intrauterine, intrapartum/early postpartum, and total transmission.

	Intrauterine transmission[a]	Intrapartum/early postpartum transmission	Cumulative transmission at 6 weeks of life
Exclusive breastfeeders (n = 266)[b]	3 of 266 (1.1%)	23 of 266 (8.7%)	26 of 266 (9.8%)
Non-exclusive breastfeeders (n = 10)	3 of 10 (30%)	2 of 10 (20%)	5 of 10 (50%)
	$P \leqslant 0.0001$	$P = 0.2263$	$P = 0.0022$

[a] See caption of Table 3 for explanation of transmission timing categories.

[b] Breast feeding practice data were not obtained from two women, neither of whom transmitted their infection.