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ARTICLE

Effect of 7 Days of Phenytoin on the Pharmacokinetics of and the Development of Resistance to Single-Dose Nevirapine for Perinatal HIV Prevention: a Randomized Pilot Study

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Key words: pharmacokinetics, prevention of mother-to-child transmission of HIV, Africa, phenytoin, nevirapine

Abstract

Objectives

To confirm whether 7-days phenytoin, an enzyme inducer, decreases the elimination half-life of single-dose nevirapine and to investigate its effect on nevirapine resistance development in pregnant, HIV-infected women.

Methods

In a pharmacokinetic pilot trial(NCT01187719), Zambian/Tanzanian HIV-infected, antiretroviral (ARV)-naive pregnant women ≥18 years with CD4 >350 cells/mm^3 were randomized 1:1 to control (zidovudine pre-delivery, single-dose nevirapine/zidovudine/lamivudine at delivery, zidovudine/lamivudine for 7 days post-delivery) or intervention (control plus 184mg phenytoin once-daily for 7 days post-delivery) groups. Primary endpoints were nevirapine pharmacokinetics and resistance.

Results
35/37 women were allocated to control/intervention groups with median (IQR) age of 27 (23-31) and 27 (23-33) years, respectively. 23/23 had detectable nevirapine levels at delivery and subsequent samples in control/intervention groups, respectively. Geometric mean (95%CI) nevirapine plasma levels at delivery were 1.02 (0.58-1.78) mg/L and 1.14 (0.70-1.86) mg/L in control/intervention groups (p=0.76). One week after delivery, 0/23 (0%) and 15/22 (68%) control/intervention mothers had undetectable levels (<0.05 mg/L; p<0.001). One week later, this was 10/21 (48%) and 18/19 (95%), respectively (p=0.002). GM(95%CI) nevirapine half-lives were 63.2 (52.8-75.7) versus 25.5 (21.6-30.1) hours in control versus intervention groups (p<0.001). New nevirapine mutations were found in 0/20 (0%) intervention vs. 1/21 (5%) control mothers. There was no difference in adverse events (p>0.28).

Conclusions
Adding 7-days of an enzyme inducer to single-dose nevirapine to prevent mother-to-child transmission significantly reduced subtherapeutic nevirapine levels by shortening nevirapine half-life. As prolonged subtherapeutic nevirapine leads to resistance emergence, a single-dose nevirapine could be used with phenytoin as an alternative if other ARVs are unavailable.

Introduction

While the risk of HIV mother-to-child transmission (MTCT) is 20-40% without treatment,[1, 2] a simple cheap intervention, single-dose nevirapine at labour onset, reduces MTCT by ~50%.[1, 3] Its major disadvantage is development of nevirapine resistance in mothers (1-69%) and infants,[4] most likely due to its long elimination half-life (61 hours),[5] leading to several days to weeks of subtherapeutic plasma concentrations, coupled with its low genetic barrier to resistance[6]. Newly emergent

resistant HIV may be transmitted to the infant or to others, limiting their treatment options, and may also reduce future combination antiretroviral therapy (cART) efficacy in the mother.[7]

Given its simplicity and efficacy, single-dose nevirapine is nevertheless still endorsed by the WHO as part of the regimen for preventing MTCT (pMTCT) in resource-limited settings, when cART (WHO Option B/B+) is not feasible or available. To cover the prolonged presence of subtherapeutic nevirapine plasma concentrations after single-dose nevirapine at labour onset, Option A of the WHO (2012) guidelines recommend adding zidovudine/lamivudine for seven days postpartum.[8, 9] This approach reduces resistance development to 4-16%,[4] but does not fully eliminate it.

Nevirapine is extensively metabolized in the liver by cytochrome P450 (CYP) isoenzymes *3A4* and *2B6.*[10] A pharmacological, rather than antiretroviral, approach of adding a CYP*3A4* enzyme inducer has been shown to decrease nevirapine elimination half-life in healthy women,[11] with greatest reductions from carbamazepine and phenytoin. In our previous trial (VITA-1), addition of single-dose carbamazepine to single-dose nevirapine at labour onset also significantly reduced nevirapine plasma concentrations one week after delivery in HIV-infected women, with a trend towards fewer resistance mutations.[12]

The CYP*3A4* enzyme inducer phenytoin is a low cost, widely available anticonvulsant and anti-arrhythmic drug, which is not secreted into breast milk in clinically important amounts (in contrast to carbamazepine) and can therefore be safely given to breastfeeding mothers.[13] In

this pilot study we investigated the impact of seven days phenytoin on nevirapine pharmacokinetics and nevirapine resistance development after single-dose nevirapine as a component of antiretroviral (ARV) prophylaxis for pMTCT (VITA-2 trial).

Methods

Study participants

Participants were recruited from the Pasua and Majengo antenatal clinics (ANCs) in Moshi, Tanzania and University Teaching Hospital (UTH) in Lusaka, Zambia. Eligible, HIV-infected, pregnant women were aged ≥18 years, ARV-naive, starting ARV prophylaxis for pMTCT, not intending to relocate during study participation, and willing to attend follow-up visits. Exclusion criteria were serious illness requiring systemic treatment/hospitalization, use of concomitant medication which may interfere with ARVs or phenytoin, or CD4 <350 cells/mm^3 (eligible for cART). All women gave written informed consent; illiterate patients gave oral consent documented by their own thumbprint and a witness. The study was approved by institutional review boards of Kilimanjaro Christian Medical University College (KCMU-Co), Moshi, Tanzania, the National Institute of Medical Research in Dar es Salaam, Tanzania, and UTH, Lusaka, Zambia. The study is registered with ClinicalTrials.gov (NCT01187719).

Eligible women all received pMTCT ARV prophylaxis recommended by national Tanzanian/Zambian guidelines. Subjects started zidovudine 300mg twice daily from 28/14 weeks of gestation in Tanzania/Zambia, respectively, or as soon as possible thereafter pre-delivery. At labour onset, women received 200mg single-dose nevirapine plus oral 300mg zidovudine every three hours and lamivudine 150mg every 12 hours until delivery (Tanzania) or oral zidovudine 600mg and lamivudine 300mg every 12 hours until delivery (Zambia). Post-delivery zidovudine 300mg and lamivudine 150mg was taken twice daily for seven days. Newborns were given 2mg/kg single-dose nevirapine suspension within 24-72 hours after birth then zidovudine syrup 4mg/kg (Tanzania) or nevirapine suspension 2mg/kg (Zambia) twice daily for seven days. All women in the trial breastfed their children for six months and then weaned rapidly.

Women were randomized 1:1 to either the national standard of care or the national standard of care plus 184mg phenytoin (2x92mg tablets) once daily from labour onset for seven days. Participant codes and allocations were held in secure envelopes stored by the project manager at each site. At enrolment (pre-delivery), women were randomized by the study doctor at the clinic opening the next envelope. When the woman presented in labour at the clinic, a study nurse confirmed and recorded time of study drug(s) ingestion by direct observation of intake or by asking the woman if she had already taken the study drug(s) at home.

Objectives, outcomes and follow-up

The primary objectives of the pilot study were to determine the effect of seven days phenytoin on the elimination half-life of nevirapine and the development of nevirapine resistance in HIV-infected pregnant women receiving a single-dose nevirapine as part of perinatal HIV prevention. The primary outcomes were nevirapine pharmacokinetic parameters (elimination half-life, time to achieve an undetectable plasma concentration) and nevirapine resistance (primary nevirapine mutations L100I, K101P, K103N/S, V106A/M, V108I, Y181C/I, Y188C/L/H, G190A)[14] at weeks 4-6. Secondary outcomes were all adverse events (AEs) possibly/probably related to pMTCT ARV prophylaxis or phenytoin, and HIV infection of the infant.

Haematology and biochemistry tests were performed at enrolment and one week post-partum. CD4 cell counts and viral load (VL) were assayed at delivery. Infants were tested just

after birth (<30 minutes) and at week 4-6 by DNA PCR assays. Blood was stored from the women at delivery and days 1,3,5,7 and 14 postpartum, and from the children at delivery and day 7 post-delivery for retrospective determination of nevirapine (and phenytoin) plasma concentrations at the Department of Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Nevirapine assay used high performance liquid chromatography with a lower limit of quantification (LLOQ) of 0.05 mg/L [15] and phenytoin was determined by a validated immunoassay with an LLOQ of 0.4 mg/L. Nevirapine resistance was assayed in plasma stored from samples at baseline and week 4/6 at the Department of Virology of the University Medical Centre Utrecht, The Netherlands.

The sample size of 50 subjects (25 per arm) delivering in the study clinic provided >80% power to detect a decrease of at least 27% in nevirapine elimination half-life associated with seven days phenytoin allowing 20% drop-out (without follow-up samples, based on VITA-1).[12]

Safety analyses included all women who were observed or reported taking study medication (safety population). Analyses of pharmacokinetics and resistance included the safety population who did not receive a second nevirapine dose, who delivered vaginally (not by caesarean section (C/S)) and had pharmacokinetic evidence of nevirapine, and phenytoin if allocated (detectable plasma concentration one day post-delivery) (protocol-specified primary PK population). Analyses were also done including mothers who delivered by C/S, as no difference in pharmacokinetic parameters was observed between C/S and vaginal deliveries in VITA-1. Women in the control group with phenytoin detected in any sample were excluded from PK/resistance analyses. Pharmacokinetic analysis was performed using Phoenix 64, WinNonlin 6.3 (Pharsight

Page 2611 -->

Corporation, CA, USA) and statistical analysis using SPSS, version 18.0 (SPSS Inc.).

Results

Study participants

We screened 335 HIV-infected, pregnant women from July 2010 to June 2011; most of the 262 women not randomized were already on cART (n=94), had CD4 <350 cells/mm^3 (n=82) or did not return after screening (n=56) (Figure 1). Seventy-three (22%) women were randomized: 35 and 37 were allocated to control and intervention groups, respectively. One woman was randomized twice; the second randomization was excluded and the woman followed the first randomization. Demographic characteristics at enrolment and delivery were generally reasonably balanced between the two groups (Table 1; Supplementary Table 1); the main difference was significantly shorter time from nevirapine ingestion to delivery in the intervention group, which must

Page 2612 -->

have occurred by chance. The difference is not expected to have impact on our pharmacokinetic data, as the elimination half-life of nevirapine is long. Also, no differences were observed between laboratory values at enrolment and delivery within either group (Supplementary Table 1).

Pharmacokinetics

At delivery, restricting to women who delivered vaginally (not by C/S), there was no significant difference in nevirapine plasma concentrations in the two groups (geometric mean (GM) (95%CI) was 1.08 (0.63-1.84) mg/L in control patients versus 1.14 (0.70-1.86) mg/L in

intervention patients, GM ratio (GMR) (90%CI) 1.05 (0.58-1.92), t-test p=0.82). Subsequently nevirapine plasma concentrations decreased significantly faster (Figure 2) and undetectable levels were reached significantly earlier (Table 2) in the intervention group compared to the control group. All results were similar including women who delivered by C/S, and so those from the (larger) group including women who delivered by C/S are presented subsequently (see Supplementary Table 2 for pharmacokinetic results of the (smaller) group excluding women who delivered by C/S).

One week post-delivery, nevirapine plasma concentrations were reduced in both groups, but to a significantly lesser extent in the control group (GMR (1-week:delivery) (90%CI) 0.22 (0.18-0.28), 20 matched pairs) than the intervention group (GMR (1-week:delivery) (90%CI) 0.031 (0.026-0.038), 22 matched pairs). Overall, levels were 85% lower in intervention than control groups (GMR (intervention:control) (90%CI) 0.15 (0.11-0.20), t-test p<0.001). The GM (95%CI) nevirapine elimination half-life was 63.2 (52.8-75.7) versus 25.5 (21.6-30.1) hours in the control versus intervention groups respectively (p<0.001; t-test), a 60% reduction (GMR (90%CI) 0.40 (0.33-0.49)). The GM (95%CI) time to achieving an undetectable nevirapine plasma concentration was 16.3 (13.8-19.3) versus 6.7 (5.7-7.8) days in the control versus intervention groups respectively (p<0.001; t-test). Consequently a significantly greater proportion of control women had detectable nevirapine plasma levels at one and two weeks post-delivery (Table 2). All 23 (100%) women in the control group versus 7/22 (32%) women in the intervention group had a detectable nevirapine plasma concentration at one week post-delivery (p<0.001; exact); and 11/21 (52%) women in the control group versus 1/19 (5%) women in the intervention group at two weeks post-delivery (p=0.002; exact).

The median (range) phenytoin plasma concentration in all samples taken from delivery to one week post-delivery in the intervention group was 1.5 (<0.4-24.7) mg/L. Twenty-one of 22 (95%) mothers had only subtherapeutic phenytoin levels (therapeutic range defined as 10-20 mg/L).[16] One (5%) mother had an undetectable plasma concentration at delivery, but her plasma level was detectable on day 1 and increased to 24.7 mg/L one week post-delivery. The median phenytoin plasma level in the infants was <0.4 (range <0.4-1.9 mg/L).

Resistance

Samples 4-6 weeks post-delivery were available from 21 control women (1 missed week 6 visit; for 1

sample amplification failed due to low VL) and 20 intervention women (3 missed week 4/6 visit). One (5%) of the 21 women in the control group had one nevirapine-associated resistance mutation (elimination half-life 123.5 hours), which was not present at baseline, versus 1/20 (5%) with one nevirapine-associated resistance mutation in the intervention group. However, the mutation in the patient from the intervention group was already present in a sample stored at delivery. Both mutations were detected as mixtures with wild-type nucleotide sequence (V106MV control; K103KN intervention).

Safety

The 28 control and 26 intervention mothers gave birth to 30 (two pairs of twins) and 28 babies (two pairs of twins), respectively. Twenty-one (one pair of twins) and 19 (one pair of twins) infants respectively were tested just after birth and at weeks 4-6 post-delivery. The overall transmission rate was 0/21 (0%) in the control group and 1/19 (5%) in the intervention group. However, the infected child tested positive at birth and must therefore have been infected during the intrauterine period. In the infants, ten clinical AEs were reported: four in the control group (n=1 grade 1, n=1 grade 2, n=2 grade 4) and six in the intervention group

(n=1 grade 1, n=1 grade 2, n=1 grade 3, n=3 grade 4). The grade 4 AEs were a hospitalization for overweight after birth and a death just after birth due to congenital malformation in the control group and three still births (two fresh, one macerated) in the intervention group.

Both platelets ($p<0.001$) and alanine transaminase (ALT) ($p<0.001$) increased significantly between enrolment and one week post-delivery in each randomized group, but there was no difference between randomized groups in any laboratory safety parameter one week post-delivery ($p>0.05$). In total, 29 laboratory AEs were reported: n=14 in the control group versus n=15 in the intervention group ($p=1.0$; exact). Most were grade 1 (n=11 in each group), four (n=2 in each group) grade 2, one grade 3 (intervention) and two were grade 4 (haemoglobin <6.5 g/dL; one in each group). Eight clinical AEs were reported: three in the control group (n=1 grade 2, n=1 grade 3, n=1 grade 4 (an emergency C/S)) and five in the intervention group (n=1 grade 1, n=3 grade 2, n=1 grade 3). None of the laboratory and clinical AEs in the mothers or infants were judged possibly/probably related to study medication.

Discussion

Here we demonstrate that adding a seven-day course of phenytoin, as enzyme inducer, from labour onset produces a large and significant reduction in the elimination half-life of nevirapine in HIV-infected, pregnant women using single-dose nevirapine as part of pMTCT ARV prophylaxis. Seven days phenytoin was safe and effective with no new nevirapine resistance mutations observed.

Importantly, nevirapine plasma concentrations at delivery were similar in those receiving and not receiving phenytoin, and also compared with previous studies,[5, 11] similarly to our previous study evaluating a single-dose of carbamazepine as enzyme inducer.[12] The time lag in enzyme inducer effect

reflects the time required for transcription of CYP enzymes and protein synthesis. The delay in enzyme induction and the knowledge that phenytoin minimally penetrates into breast milk therefore ensures the protective perinatal effect of single-dose nevirapine is maintained. The absence of HIV-transmission during the postpartum period, similar or even lower than rates in previous studies,[8] confirms also the efficacy of the pMTCT regime.

Post-delivery, nevirapine pharmacokinetic parameters were substantially affected by enzyme induction. Adding phenytoin to single-dose nevirapine reduced nevirapine plasma levels by 85% and produced a significantly larger proportion of women with undetectable nevirapine levels one and two weeks post-delivery. Both effects are a consequence of the 60% reduction in the nevirapine elimination half-life, an absolute difference of -35.8 hours. This is the largest decline in nevirapine elimination half-life ever reported, especially in the target population of HIV-infected, pregnant women. For example, the pilot study of L'homme *et al* found a median elimination half-life reduction of 38% (-19.4 hours) in four healthy, Dutch women receiving single-dose nevirapine with seven days phenytoin,[11] and a single-dose of carbamazepine reduced nevirapine levels by 36% in HIV-infected, pregnant women receiving single-dose nevirapine.[12] Not surprisingly, a seven-day course of an enzyme inducer has a greater effect than a single-dose alone on the elimination half-life of nevirapine in HIV-infected, pregnant women.

The mechanisms by which the CYP enzymes are induced involves the transcription factors pregnane X receptor and the constitutive androstane receptor. The enzyme inducer binds to these receptors, thereby stimulating the activation of the CYP enzyme.[17] Studies have shown

that CYP enzyme induction is correlated with dose and drug level [18] with higher doses and a higher plasma level of the enzyme inducer resulting in lower serum levels of the test drug. This likely explains why induction of the CYP enzyme has a greater effect with a long-course of an enzyme inducer than only the single-dose used in our previous study.

Although current guidelines, including zidovudine monotherapy pre-delivery and seven days zidovudine/lamivudine post-delivery are complex, they have reduced emergence of nevirapine resistance substantially by protecting the subtherapeutic nevirapine 'tail', since the lengthy duration of low and subtherapeutic levels of nevirapine in blood are plausibly associated with increases in nevirapine resistance. A meta-analysis estimated that 4.5% of women using single-dose nevirapine and additional ARVs postpartum had nevirapine resistance 4-8 weeks post-partum.[4] In our study, overall new nevirapine resistance prevalence was 2.4% (1 out of 41 samples); although we observed no nevirapine resistance mutations after single-dose nevirapine in combination with seven days phenytoin, clearly numbers are too few to make any conclusions about nevirapine resistance on the basis of this study alone. However, it raises the prospect that full elimination of nevirapine resistance could be possible. In the VITA-1 trial we found that women with undetectable nevirapine plasma concentrations one week post-delivery were less likely to develop nevirapine resistance mutations, and that the elimination half-life in women with new nevirapine mutation(s) was almost two and five times longer than the median half-lives in the control and intervention groups, respectively. Thus it is plausible that adding a seven-day course of phenytoin at labour onset might have significant additional benefits in reducing selection of nevirapine resistance mutations, even on top of the current ARV prophylaxis "tail".

The trial was designed as a pilot powered to detect a difference between intervention and control groups in the nevirapine elimination half-life. A much larger sample size (~200; 100 per arm) would be needed to detect significant differences in the development of nevirapine resistance between the two groups. However, this group of women is extremely challenging to recruit and retain (Figure 1): only 23% of those assessed for eligibility pre-delivery were randomized, a further 26% of those randomized dropped out before delivery, and a further 23% of those who delivered in the study clinic did not provide samples 4-6 weeks post-delivery. We would therefore have needed to screen ~1,600 women to achieve 200 women with weeks 4-6 samples. Whilst ideally a larger phase III trial should confirm the efficacy of 7-days phenytoin on resistance as a primary outcome, the substantial significant reductions in nevirapine half-life, coupled with previous data demonstrating a trend towards a causal association between nevirapine half-life and emergence of resistance, suggests it is highly likely to be effective. Another limitation was the standard HIV genotyping assay used which only detects mutations present in >20% of the viral population, and not subpopulations of mutants. Deep sequencing of these samples is planned.

It is estimated that 18-64% of the women living in Sub-Saharan Africa with HIV are receiving cART for pMTCT, as now recommended by WHO (Option B+). However, this means that thousands of women are still receiving single-dose nevirapine,[19] and demonstrates the challenge of widespread implementation of the current guidelines. Phenytoin can be used safely during pregnancy and breastfeeding [20] and side-effects are expected to be infrequent using such a low dose for only a short period. Where it is not possible to provide cART, phenytoin is cheap and available in almost every clinic. Phenytoin may also be a useful intervention when women stop nevirapine at the end of breastfeeding within Option B+. We have demonstrated that implementation of this intervention would substantially and significantly reduce nevirapine half-life; previous data demonstrating a trend towards a causal relationship between nevirapine half-life and resistance emergence suggest that implementation would not only facilitate the reduction of nevirapine resistance, but also enable further increases in coverage for pregnant women in need of perinatal HIV

prophylaxis, whilst likely retaining the benefits of single-dose nevirapine in reducing transmission. This strategy might therefore support the overarching goal of the technical consultation of the WHO to reduce the overall HIV transmission rate from pMTCT to <5% at the population level by the end of 2015.

In summary, addition of an enzyme inducer for seven days to single-dose nevirapine for pMTCT greatly reduced the presence of subtherapeutic nevirapine levels by significantly shortening the nevirapine elimination half-life, with no new nevirapine resistance mutations observed. Since prolonged subtherapeutic nevirapine exposure is known to lead to nevirapine resistance emergence,[5, 6, 12] and since phenytoin is safely and widely used in women,[13] to minimise HIV transmission from mother-to-child, single-dose nevirapine could be used with phenytoin as an alternative if other ARV drugs are unavailable.

Page 2615 -->

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Transparency declarations
None of the authors has any conflict of interest.

Table 1: Demographic characteristics of the women and infants in the VITA-2 trial

	Control (n=28)	**Intervention (7 days phenytoin; n=26)**	**p-value**
At enrolment			
Age (years)	27 (23-31)	27 (23-33)	0.74
Weight (kg)	62 (55-73)	66 (56-81)	0.11
BMI (kg/m^2)	24.1 (21.7-27.9)	26.2 (23.3-30.9)	0.10
At delivery			
Gestational age at delivery (weeks)	39 (38-42)	39 (38-41)	0.78
CD4 cell count (cells/µl)	366 (318-522)	412 (317-518)	0.76
HIV-1 RNA (copies/ml)	2832 (1000-26518)	2420 (1542-11261)	0.99
Birth weight (kg)	3.0 (2.7-3.2)	3.0 (2.7-3.4)	0.87
Time from nevirapine ingestion to delivery (hours)	9.1 (2.5-12.6)	2.1 (1.0-4.9)	0.003

Data are presented as median (IQR) and were tested with Rank-sum.

Table 2: Maternal nevirapine plasma concentrations at delivery, week 1 and week 2 of women in the VITA-2 trial, including women who delivered by C/S.

	Women who delivered, including women who delivered by C/S			
	Control	**Intervention**	**p-value**	**GMR (90% CI)**
At delivery				
Samples taken (n)	20	22		
nevirapine plasma conc. (mg/L; GM (95% CI))	1.02 (0.58-1.78)	1.14 (0.70-1.86)	0.76 †	1.12 (0.61-2.03)
<0.05 mg/L nevirapine (n (%))	1 (5%)	1 (5%)		
1 week after delivery				
Samples taken (n)	23	22		
nevirapine plasma conc. (mg/L; GM (95% CI))	0.23 (0.18 - 0.31)	0.035 (0.027-0.046)	<0.001†	0.15 (0.11-0.20)
<0.05 mg/L nevirapine (n(%))	0 (0%)	15 (68%)	<0.001*	
Women with 1 week and delivery samples (n)	20	22		
nevirapine plasma concentration 1 week : delivery (GMR (90% CI))	0.22 (0.18-0.28)	0.031 (0.026-0.038)		
2 weeks after delivery				
Samples taken (n)	21	19		
nevirapine plasma conc. (mg/L; GM (95% CI))	0.044 (0.031-0.062)	0.026 (0.024-0.029)	0.006†	0.59 (0.44-0.80)
<0.05 mg/L nevirapine (n(%))	10 (48%)	18 (95%)	0.002*	
Women with week 2 and delivery samples (n)	18	18		
nevirapine plasma conc 2 weeks : delivery (GMR (90% CI))	0.030 (0.026 - 0.034)	0.022 (0.015 - 0.031)		

† T-test, * Exact test.
Median nevirapine plasma concentrations are similar to GM and are therefore not shown in Table 2.

Figure legends

Figure 1: Profile of the VITA-2 trial
Figure 2: Geometric mean nevirapine plasma concentrations over time post-delivery (all women who delivered including those who delivered by C/S)

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