Clinical pharmacology of nevirapine in HIV-infected patients in Tanzania

Studies on prevention of mother-to-child transmission and therapeutic drug monitoring

Eva Prosper Muro

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Thesis, Radboud University Medical Centre, the Netherlands

Design and Lay-out by: Offpage, Amsterdam, the Netherlands
Printed by: Offpage, Amsterdam, the Netherlands
ISBN/EAN: 978-94-6182-404-2

Part of this work was supported by the European and Developing Countries Clinical Trials Partnership (EDCTP) (number CT.2006.33020.006), and by PRIOR (Poverty Related Infection Oriented Research), a research network sponsored by The Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO) and The Netherlands Foundation for Health Research and Development (ZonMw).

Printing and dissemination of this thesis was financially supported by the Radboud University Medical Centre.

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Proefschrift
ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op woensdag 26 maart 2014
om 11.00 uur precies

door

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Doctoral thesis

to obtain the degree of doctor

from Radboud University Nijmegen

on the authority of the Rector Magnificus, prof. dr. S.C.J.J. Kortmann

according to the decision of the Council of Deans

to be defended on Wednesday, 26 March 2014

at 11:00 hours

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CONTENTS

Dedication

To my Late Father Honourable Justice Augustine Saidi
To my Mother Elizabeth
To my Loving Husband Prosper and children Sharoon, Brenda and Jan
To my niece Mary
To my grand children Daniella, Dereck and Natasha

1

INTRODUCTION AND OUTLINE OF THESIS

MOTHER TO CHILD TRANSMISSION

Mother-to-child transmission (MTCT) of HIV refers to the transmission of HIV from HIV-infected mothers to their infants. MTCT can occur during pregnancy, labour and delivery and breast-feeding.[1] Without the use of preventive measures, the risk of MTCT of HIV-1 is estimated to vary between 35 and 40%.[2-3] The risk of MTCT can be reduced to less than 2% by interventions that include: antiretroviral (ARV) prophylaxis given to women during pregnancy and labour; ARV prophylaxis to the infant in the first weeks of life; Obstetrical interventions including elective caesarean section; and complete avoidance of breast-feeding.[4]

PREVENTION OF MOTHER TO CHILD TRANSMISSION (PMTCT)

ARV drugs reduce MTCT of HIV by inhibiting viral replication and thus lowering plasma viral load in pregnant women, both when given during pregnancy and when given during the breast feeding period. In newborns receiving post-exposure prophylaxis after delivery, inhibition of viral replication contributes to prevent HIV infection.[5-6] Several preventive strategies have been evaluated, but most of them are too expensive for implementation in resource-limited countries. The regimen of a single dose of nevirapine (SD NVP) to the mother just before delivery and SD NVP to the newborn within 24-72 hours after birth reduces the risk of MTCT by 50%, is affordable in many situations[7] and was therefore until recently the standard of care in many Africans countries, like Tanzania. The low genetic barrier[8] and the long elimination half-life of NVP leads to persistence of sub-therapeutic NVP plasma concentrations which leads to primary NVP resistance[9-11], detected by standard genotyping in 15-75% women. In view of such high percentage of NVP resistance, the new 2007 PMTCT Tanzania guideline was followed[12] The recommended combination regimen for HIV-infected women, who present during pregnancy at the antenatal clinic (ANC) is AZT 300 mg BD from 28 weeks of gestation or anytime thereafter, combined with SD NVP 200 mg at onset of labour, and AZT 300 mg and 3TC 150 mg given every 12 hours until delivery. During the postpartum period, AZT 300 mg BD and 3TC 150 mg BD is continued for 7 days. This combined regimen has reduced the MTCT rates to less than 2% when breast-feeding is avoided.[13-14] For HIV infected pregnant women who were receiving effective ARV treatment continued throughout labor and delivery, as well as the postpartum period the regimen on schedule as much as possible to provide maximal virologic effect and to minimize the chance of development of drug resistance.[15] All infants born to HIV-infected women should receive daily NVP as soon as possible after delivery regardless of whether the mother has received or not received ARV treatment or prophylaxis. For breastfed infants, daily NVP should be given as soon as possible and continued until one week after complete cessation of breast-feeding. For formula fed infants and breast fed infant whose mother is on ARV therapy; daily NVP should continue for six weeks.[16]

NEVIRAPINE AND NEVIRAPINE RESISTANCE

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI). It binds to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent polymerase activities

by causing disruption of the enzyme's catalytic site. NVP is used as a single ARV prophylaxis during labour because of its potent antiretroviral effect (IC_{50} of 10ng/ml), rapid and almost complete absorption after oral intake, its long elimination half-life and its ability to cross the placenta.[17] Even when using antepartum, intrapartum and postpartum combination ARV therapy for PMTCT still NVP resistance of 4-16% occurs.[18] Resistance to NVP remains therefore a serious concern. NVP resistance has three major implications. Firstly, the efficacy of NVP or other NNRTIs in combination ARV therapy may be diminished when a patient harbours resistant virus,[19] secondly NVP-resistant strains may be transmitted to others, limiting treatment options for newly infected patients. Thirdly, NVP resistance could reduce the efficacy of SD NVP in subsequent pregnancies.[20-21] The risk of development of resistance is increased when sub-therapeutic NVP concentrations are present in blood for a long time.

CLINICAL PHARMACOLOGY OF NEVIRAPINE IN PMTCT

Small pharmacokinetic studies demonstrated that the elimination half-life after SD NVP is approximately 60 hours.[22] This implies that sub-therapeutic plasma concentrations of NVP will be detectable in mothers for several days after delivery. These plasma levels present the perfect environment for the occurrence of resistance as the concentrations are sub-inhibitory for several days.

A pilot study in healthy volunteers demonstrated that the elimination half-life of SD NVP could be reduced by adding the enzyme inducer carbamazepine.[23] This was a phase-I single-centre, open-label, two period, nine-group, PK study. A single 200 mg dose of NVP was administered to 36 HIV negative non-pregnant women in both period 1 and 2, blood samples were taken twice weekly for 21 days. In period 2 additional interventions (single dose carbamazepine, phenobarbital or phenytoin; phenytoin for 3 or 7 days; St Johns Wort, vitamin A or cholecalciferol for 14 days) were administered to all participants except for the control group. Three of the interventions resulted in the half-life of NVP being significantly reduced. These included a single 400mg dose of carbamazepine (p=0.002), once a day 184mg phenytoin for three days (p=0.001) and once a day 184mg phenytoin for seven days (p=0.002). The half-life of NVP was reduced by 35.3%, 38.2% and 35.9%.[23]

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is used to optimize drug regimens for the individual patient by increasing efficacy and avoiding drug-related toxicity Patient taking NNRTI are at a greater risk for developing resistance and treatment limiting toxicity. Virological suppression can only be reached when taking at least 95% of the prescribed doses. Patients at risk for virological failure are those with sub-therapeutic plasma concentrations of nevirapine.[24] TDM is a well known tool for the optimization of NVP dosing in the developed world, but not often performed in resource limited settings due to lack of simple and affordable assays to determine the exposure to NVP. TDM of ARVs aims to improve ART efficacy and safety by maintaining individual patient ARV concentrations within a therapeutic range. Many review articles suggest that ARV TDM

may be a useful tool for improving outcomes for HIV-infected individuals. They suggest that TDM of ARVs can potentially identify patients with sub-therapeutic, toxic or appropriate drug concentrations. In patients with advanced HIV disease, a retrospective analysis found an association between subtherapeutic drug concentrations at the commencement of ART and poorer immunological outcomes and failure to achieve virologic suppression in the first year of treatment.[25] ARV TDM is therefore potentially a rational tool to optimise efficacy and minimise toxicity of ARV therapy.

OUTLINE OF THE THESIS

In **Chapter 1**, an introduction is given on mother-to-child transmission of HIV and PMTCT, after which the outline of the thesis is presented.

Part I of the thesis focuses on the effects of enzyme inducers on the pharmacokinetics and the development of resistance to a SD NVP in perinatal HIV prevention.

In **Chapter 2**, a study is presented investigating whether NVP plasma concentrations are still detectable more than 2 weeks after administration. The study was conducted in the Netherlands in non pregnant healthy Dutch women.

Chapter 3 presents the results of a phase II trial, investigating whether the addition of single-dose carbamazepine would diminish NVP resistance development by reducing elimination half-life after exposure to SD NVP at onset of labour in HIV-infected women. As reported in **Chapter 4**, we also investigated whether a longer course of an enzyme inducer as opposed to only a single-dose might have greater impact on the reduction of NVP elimination half-life and resistance development. As reported in **Chapter 5** a systematic review and meta-analysis was done which compared the different drug interventions used to reduce NVP resistance after SD NVP as part of antiretroviral prophylaxis to prevent HIV mother-to-child transmission.

Part II of the thesis focuses on TDM in HIV– infected adults using a simple and inexpensive TLC assay for semi-quantitative measurement of NVP saliva concentrations.

Chapter 6 describes the validation of a simple and economical thin layer chromatography method for semi-synthetic detection of NVP in saliva of HIV- infected adults. In **Chapter 7**, we compare saliva concentrations of NVP with self-reported adherence, in patients on NVP-containing ARV treatment.

Finally, in **Chapter 8**, our research findings are discussed and put into the perspective of related research data. In addition, avenues for future research in this area are presented.

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CLINICAL PHARMACOLOGY OF NEVIRAPINE IN PMTCT

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2

NEVIRAPINE PLASMA CONCENTRATIONS ARE STILL DETECTABLE AFTER MORE THAN 2 WEEKS IN THE MAJORITY OF WOMEN RECEIVING SINGLE-DOSE NEVIRAPINE IMPLICATIONS FOR INTERVENTION STUDIES

J Acquir Immune Defic Syndr 2005;39:419-421

ABSTRACT

Background

Single-dose nevirapine is a highly cost-effective strategy to reduce perinatal HIV-1 transmission. Its major disadvantage is the selection of nevirapine resistance in 20% to 30% of women, probably attributable to the long elimination half-life of nevirapine. To develop intervention strategies, it is important to know the interpatient variability in nevirapine half-life in women receiving a single dose of nevirapine

Methods

HIV-negative, healthy, nonpregnant Dutch women were eligible for this study. After administration of a single 200-mg dose of nevirapine to the subjects, blood was sampled for measurement of nevirapine twice a week for a total of 21 days. Nevirapine plasma levels were determined by a validated high-performance liquid chromatography method with a lower limit of quantification of 0.15 mg/L. The primary end point was the first sample with an undetectable nevirapine concentration.

Results

Forty-four subjects participated. The median age, height, and body weight (interquartile range) were 26 (21–33) years, 1.72 (1.68–1.75) m, and 64 (59–75) kg, respectively. The median elimination half-life of nevirapine was 56.7 hours, with a range of 25.6 to 164 hours. The time to the first undetectable nevirapine plasma concentration was 10 days in 4 subjects, 14 days in 12 subjects, 17 days in 12 subjects, and 21 days in 9 subjects. In the remaining 7 subjects, nevirapine was still detectable on day 21, the last day of sampling. Time to an undetectable nevirapine plasma concentration was influenced by oral contraceptive use but not by age, height, body weight, body surface area, alcohol use, or smoking.

Conclusions

Most women who received a single 200-mg nevirapine dose still had detectable plasma concentrations of nevirapine after more than 2 weeks. This information is valuable for designing intervention studies to prevent the development of nevirapine resistance.

INTRODUCTION

Without the use of preventive measures, the risk of mother-to-child transmission (MTCT) of HIV-1 is estimated to vary between 25% and 48%. Several preventive strategies have been evaluated, but most of them are too expensive to implement in resource-limited countries. The regimen of a single dose of nevirapine to the mother just before delivery and a single dose of nevirapine to the newborn between 24 and 72 hours after birth reduces the risk of MTCT by 50% and is affordable in many situations.[1] Recent studies, however, have shown that this single dose to the mother can induce nevirapine resistance in 20% to 30% of the mothers.[2] The development of this resistance may have major implications. First, it is uncertain whether a subsequent course of nevirapine is still effective for the prevention of MTCT when these women become pregnant again. Second, the efficacy of subsequent treatment with a nevirapine-based highly active antiretroviral therapy (HAART) regimen is diminished when the patient is harboring a resistant virus.[3] Finally, nevirapine-resistant strains may be transmitted to other people. The mechanism of the occurrence of nevirapine resistance after a single dose is most likely related to the long elimination half-life of nevirapine and the low genetic barrier to resistance. Small pharmacokinetic studies have demonstrated that the elimination half-life after a single dose of nevirapine is approximately 60 hours.[4] This implies that plasma concentrations of nevirapine are detectable in mothers for several days after delivery. The subtherapeutic but detectable plasma levels present the perfect environment for the occurrence of resistance, because the concentrations may be subinhibitory for several days. The primary objective of this study was to investigate the intersubject variability and potential influencing factors in the decay of plasma nevirapine concentrations after a single 200-mg dose. A secondary objective was the evaluation of the use of saliva as an alternative to blood sampling for measurement of nevirapine concentrations. The study was conducted in The Netherlands as a prelude to a similar study in Tanzania.

METHODS

The present study was a single-center, open-label, single-dose, single-period pharmacokinetic study. Nonpregnant healthy women aged 18 to 40 years were eligible for enrolment after pre-entry and laboratory evaluation. Women who tested positive for HIV and/or hepatitis B or C virus were excluded. The study protocol was reviewed and approved by Ethics Committee of the Radboud University Medical Centre, Nijmegen, The Netherlands. Informed consent was obtained from all women before enrolment. All study subjects received a single oral dose of 200 mg of nevirapine on day 0, and the Principal Investigator directly observed medication ingestion. Sampling of blood and saliva was done just before and 3, 7, 10, 14, 17, and 21 days after sampling. Stimulated saliva was obtained by a salivette (Sarstedt, Etten-Leur, The Netherlands) using a dental cotton roll impregnated with citric acid (20 mg), which stimulates the salivary flow. Study subjects were asked to chew on the cotton roll for approximately 1 minute. Saliva was obtained by centrifugation of the cotton roll. The plasma and saliva samples were stored at - 40^{0}C until analysis. Plasma and saliva concentrations of nevirapine were determined by a validated high-performance liquid chromatography (HPLC) assay with ultraviolet (UV) detection.[5] The lower and upper limits of quantification were 0.15 and 15 mg/L, respectively. The intra-and interday precision ranged from 1.3% to 3.9% and from 1.9%

to 3.0%, respectively. The accuracy of the assay ranged from 91.5% to 102.6%. The typical median inhibitory concentration (IC50) value of nevirapine is 0.1 mg/L; corrected for 60% protein binding, this corresponds to a plasma level of approximately 0.2 mg/L. It is currently unknown, however, at what plasma level nevirapine selects for resistance. Clinical studies have determined that effective concentrations of nevirapine are greater than 3 to 4 mg/L, whereas levels less than 0.1 to 0.2 mg/L do not have selective pressure. Therefore, any plasma level between 0.2 and 3.0 mg/L has been defined by us as subtherapeutic. The following patient factors were tested for an association with the time to undetectable nevirapine plasma concentration: age, height, weight, body surface area, alcohol use, smoking habits, and oral contraceptive use.

RESULTS

Forty-four nonpregnant healthy women were enrolled in the protocol. The median age, height, and body weight (interquartile range) were 26 (21–33) years, 1.72 (1.68–1.75) m, and 64 (59–75) kg, respectively. Other than 1 Asian woman and 1 woman of mixed background, the remaining 42 women were white. The pharmacokinetic parameters of the study subjects are presented in Table 1. The median elimination half-life (t½) for nevirapine in plasma was 56.7 hours, with a range of 25.6 to 164 hours. Maximum nevirapine plasma levels on day 3 (first postdose measurement) ranged from 0.36 to 1.59 mg/L, with a median value of 0.71 mg/L. The median time to the first undetectable nevirapine plasma concentration was 17 days. There were 7 subjects in whom nevirapine was still detectable on day 21, however, the last day of sampling (Fig. 1).

Except for oral contraceptive use, none of the other patient characteristics seemed to be related to the time to an undetectable nevirapine concentration in plasma. There were 17 women who reported taking oral contraceptives, and they had a median time to the first undetectable nevirapine plasma level of 21 days. This was significantly longer than for the remaining 27 women who reported not taking oral contraceptives (14 days; $P<0.001$). The difference in the median plasma half-life of nevirapine in oral contraceptive users versus nonusers was not significant (69.7 vs. 52.8 hours, respectively; $P = 0.053$). Saliva nevirapine concentrations were approximately half of the values observed in plasma. Nevirapine levels in saliva were significantly correlated with nevirapine levels in plasma at the first day of sampling: $[NVP]_{saliva} = 0.002 + 0.495 \times [NVP]_{plasma}$ ($R^2 = 0.531$, $F = 47.496$, $P < 0.001$). Time to an undetectable nevirapine concentration was shorter in saliva than in plasma: median values were 14 and 17 days, respectively (see Fig. 1).

Table 1. Pharmacokinetic Parameters of Nevirapine after a 200-mg Single Oral Dose (median values + range)

	Plasma	Saliva
CL/F (L/h . kg)	0.04 (0.02-0.10)	
T ½ (h)	56.7 (25.6-164.1)	77.1 (35.8-264.7)
Vd/F (L/kg)	2.8 (0.8-5.3)	
Cmax (mg/L)	0.71 (0.36-1.59)	0.35 (0.03-0.77)
Time to undetectable concentration (days)	17 (10 to >21)	14 (7-21)

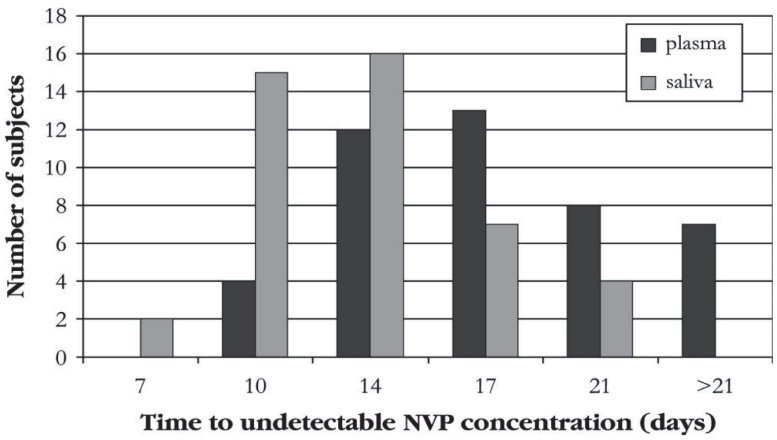

Figure 1. Distribution of time to undetectable NVP concentrations (in days) for 44 included subjects.

DISCUSSION

In this study of 44 healthy, non-pregnant, HIV-1- uninfected Dutch women, a single dose of 200 mg of nevirapine had an average half-life of 56.7 hours (or 52.8 hours in women who did not use contraceptives). Nevirapine levels remained detectable in plasma for a median of 17 days (range: 10 to >21 days). In 16% of women, nevirapine was detectable at the last measured time point, 21 days after the single dose. Thus, a single dose of nevirapine was associated with persistent measurable drug levels beyond 3 weeks after administration. It is clear that our study population of healthy nonpregnant Dutch women of childbearing age is not similar to the setting in Tanzania (or other sub-Saharan African countries), where HIV-infected pregnant women are black and have different dietary habits, body weights, and comedications, for example. Nevertheless, the median nevirapine half-life that we observed in our group of 44 subjects (56.7 hours) is not too different from the average value as reported by Musoke et al[4] in a smaller group of pregnant HIV-infected Ugandan women receiving single-dose nevirapine (61.3 hours). We were not able to identify any significant patient factor (other than oral contraceptive use) that was associated with an influence on nevirapine half-life. Oral contraceptives may be able to inhibit hepatic metabolism of nevirapine, although this effect was previously not observed (and thus not expected by us) in a formal drug–drug interaction study.[6] It must be noted, however, that our study was not designed to address causality between oral contraceptive use and nevirapine half-life.

Most importantly, our data describe the window of opportunity for the virus to select for nevirapine-resistant mutations. The longer the time to an undetectable nevirapine level in plasma, the longer the virus has time to replicate. In 2000, Jackson et al[7] reported that among the 15 women in the HIVNET 006 trial in whom virus was tested for the K103N mutation, the 3 women who developed the mutation had a significantly longer elimination half-life of nevirapine than the 12 women in whom no resistance was detected (74.8 vs. 51.8 hours; P = 0.01). Thus, one of the most rational interventions is the addition of other antiretroviral agents after

delivery to cover this window of opportunity for the virus to select for nevirapine resistance. Recently, preliminary data were presented that short courses (4–7 days) of zidovudine plus lamivudine (Combivir) added to single-dose nevirapine in the prevention of MTCT significantly reduced the development of nevirapine resistance when compared with no intervention.[8] One could speculate that this may not be sufficient to prevent the development of all nevirapine mutations. Indeed, nevirapine resistance was not fully absent in the intervention arms. Extending the duration of administering additional antiretroviral agents after delivery may also increase the development of resistance to these drugs, however. It may be attractive to use methods other than collecting blood samples for measurement of exposure to nevirapine. As reported earlier, nevirapine can be detected in saliva.[9] In our study, we observed a strong correlation between nevirapine levels in saliva versus plasma, and in almost all subjects, an undetectable nevirapine level was detected 3 days earlier in saliva than in plasma (see Fig. 1). Collecting saliva samples for measurement of nevirapine levels has the advantage of taking samples at home (by the patient herself, with no skilled personnel needed), less discomfort for the patient, and less infection risk for health care workers who draw the sample. In conclusion, most women who received a single nevirapine dose of 200 mg still had detectable plasma concentrations of nevirapine after more than 2 weeks. This information is valuable for designing intervention studies to prevent the development of nevirapine resistance.

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3

INTRAPARTUM SINGLE-DOSE CARBAMAZEPINE REDUCES NEVIRAPINE LEVELS FASTER AND MAY DECREASE RESISTANCE AFTER A SINGLE DOSE OF NEVIRAPINE FOR PERINATAL HIV PREVENTION

J Acquir Immune Defic Syndr 2012, 59:266-273.

ABSTRACT

Background

World Health Organization guidelines recommend zidovudine + lamivudine for 7 days from labor onset in HIV-infected women receiving single-dose nevirapine (sdNVP) to cover prolonged subtherapeutic nevirapine concentrations. Although effective, this is complicated and does not eliminate resistance; alternative strategies could add benefit.

Methods

Antiretroviral-naive HIV-infected pregnant women aged 18–40 years, with CD4 >200 cells per cubic millimeter, able to regularly attend the antenatal clinics in Moshi, Tanzania, were enrolled 1:1 by alternate allocation to receive 200 mg sdNVP alone or in combination with open-label 400-mg single-dose carbamazepine (sdNVP/CBZ) at delivery (ClinicalTrials.gov NCT00294892). The coprimary outcomes were nevirapine plasma concentrations 1 week and nevirapine resistance mutations 6 weeks postpartum. Analyses were based on those still eligible at delivery.

Results

Ninety-seven women were assigned to sdNVP and 95 to sdNVP/CBZ during pregnancy, of whom 75 sdNVP and 83 sdNVP/CBZ were still eligible at delivery at study sites. The median (interquartile range) nevirapine plasma concentration was 1.55 (0.88–1.84) mg/L in sdNVP (n = 61) and 1.40 (0.93–1.97) mg/L in sdNVP/CBZ (n = 72) at delivery (P = 0.91), but 1 week later was significantly lower in sdNVP/CBZ [n = 63; 0.09 (0.05–0.20) mg/L] than in sdNVP [n = 52; 0.20 (0.09–0.31) mg/L; rank-sum: P = 0.004] (geometric mean ratio: 0.64, 95% confidence interval: 0.43 to 0.96; P = 0.03). Six weeks postpartum, nevirapine mutations were observed in 11 of 52 (21%) in sdNVP and 6 of 55 (11%) in sdNVP/CBZ (odds ratio = 0.46, 95% confidence interval: 0.16 to 1.34; P = 0.15).

Conclusions

Addition of single-dose carbamazepine to sdNVP at labor onset in HIV-infected, pregnant women did not affect nevirapine plasma concentration at delivery, but significantly reduced it 1 week postpartum, with a trend toward fewer nevirapine resistance mutations.

INTRODUCTION

Without intervention, the risk of mother-to-child HIV transmission in the breastfed population is 35%–40%.[1,2] Single-dose nevirapine (sdNVP) to the mother at onset of labor and sdNVP to the newborn within 24–72 hours after birth remains part of treatment for prevention of mother-to-child HIV transmission (pMTCT) in most resource-limited countries.[2,3] This simple and affordable intervention reduces mother-to-child HIV transmission by 40% and has benefited thousands of babies since its introduction in 2000. Nevirapine is a nonnucleoside reverse transcriptase inhibitor (NNRTI) with potent antiviral activity, but a low genetic barrier, with one mutation causing high-level resistance. It is rapidly absorbed when given orally and passes quickly through the placenta. The primary elimination pathway for nevirapine is oxidative metabolism by cytochrome P-450 3A4 and 2B6 enzymes.[4] The drug has a long elimination half-life in pregnant women using a single 200-mg dose at labor (median 61–66 hr). This leads to persisting subtherapeutic plasma concentrations, which, given its low genetic barrier to resistance,[5,6] means a single dose can lead to primary nevirapine resistance in 15%–75% mothers.[7–9] Clinically, nevirapine resistance has 3 major implications. First, the efficacy of nevirapine or other NNRTIs in combination antiretroviral therapy (ART) may be diminished when a patient harbors resistant virus. Indeed, studies in sub-Saharan Africa suggest that recent exposure to sdNVP (<6 months) is associated with increased risk for virological and clinical failure.[7,10–13] Second, nevirapine-resistant strains may be transmitted to others, limiting their treatment options. Third, nevirapine resistance could reduce the efficacy of sdNVP in subsequent pregnancies,[14,15] although data suggest nevirapine resistance fades in these women over time and sdNVP remains effective for prophylaxis in subsequent pregnancies.[16,17]

Updated World Health Organization (WHO) guidelines therefore now recommend adding zidovudine + lamivudine for 7 days postpartum to cover the prolonged presence of subtherapeutic nevirapine concentrations after sdNVP at labor onset.[18–20] Recent studies show adding single-dose tenofovir + emtricitabine to sdNVP and short-course zidovudine further reduces NNRTI resistance.[21–23] Of note, none of these interventions fully eradicated nevirapine resistance,[19] which therefore remains a serious and growing concern. A different approach to limit nevirapine resistance development would be a pharmacological intervention to reduce nevirapine elimination half-life. Carbamazepine is a low cost widely available anticonvulsant, an inducer of cytochrome P-450 3A4 enzymes, which passes into breast milk and might decrease nevirapine elimination half-life and resistance development in the mother. A pilot study comparing 8 different CYP3A induction strategies with different drugs demonstrated that elimination half-life of sdNVP was reduced most (by 35%) by adding single-dose carbamazepine in healthy volunteers.[4] In this phase II trial, we therefore investigated whether the addition of single-dose carbamazepine, as CYP3A4 enzyme inducer, would diminish nevirapine resistance development by reducing elimination half-life after exposure to sdNVP at onset of labor in HIV-infected, pregnant, Tanzanian women.

METHODS

Study Participants HIV-1–infected, pregnant women attending antenatal clinics in Moshi, Tanzania, were recruited from Kilimanjaro Christian Medical Centre (KCMC) as the consultant

hospital, 3 primary and 1 tertiary antenatal care units. Counseling and voluntary HIV testing (by the dual rapid-test algorithm) was offered routinely to all pregnant women attending antenatal services in these clinics as part of the national pMTCT program. Women testing positive received posttest counseling and were informed about the trial. Eligible HIV-infected women were as follows: aged 18–40 years, CD4 count >200 cells per cubic millimeter (ie, did not qualify for ART at the time), antiretroviral (ARV) naive, living within the catchment area, not intending to relocate during study participation, willing to adhere to the follow-up schedule, able and willing to give informed consent, and to regularly attend the antenatal clinic. Exclusion criteria were: previously treated with ARVs including sdNVP in a previous pregnancy and serious illness that required systemic treatment/hospitalization. Women who qualified for ART (ie, CD4 <200 cells/mm^3) were referred for care and not enrolled. Written consent was obtained where possible; for eligible women who could not read, consent was obtained orally and documented by a witness. The study was approved by institutional review boards of KCMC, Moshi, Tanzania, and Radboud University Nijmegen Medical Centre, The Netherlands. The study is registered with ClinicalTrials. gov, number NCT00294892. After 28 weeks gestation, eligible women who had provided informed consent were allocated 1:1 to receive either the national standard care at the time of sdNVP (200 mg) or sdNVP plus single-dose carbamazepine (sdNVP/CBZ, 400 mg) orally at onset of labor (open-label). Nevirapine (and carbamazepine if allocated) was handed to the woman at enrolment following standard antenatal policy, ideally at 28 weeks of pregnancy at her routine checkup visit and at the latest just before delivery following standard antenatal policy. In both groups, sdNVP suspension was administered to the baby within 24–72 hours after birth. The randomization list was generated as alternate allocations by the study pharmacist (ie, was quasi randomized) and was held centrally at the KCMC pharmacy. Allocations were made by local medical officers at antenatal clinics phoning the pharmacist: as there were 4 local antenatal clinics, the next allocation was concealed to study teams until it was made. Participants were asked to take study drugs at onset of labor pain and to come to the labor ward for delivery. If the woman vomited within 30 minutes of study drug(s) administration or did not deliver within 48 hours, a second single dose of nevirapine (not carbamazepine) was given. When the women presented in labor, a study nurse confirmed study drug(s) ingestion by direct observation of intake at the clinic or by asking the women when they had taken study drug(s) already at home. Replacement drugs were provided for those who had not taken them. The time of ingestion of study drugs was recorded in the case report form. As the national guidelines recommended, all women (100%) in the trial opted to exclusively breastfeed for 6 months and then wean rapidly.

Objectives, Outcomes, and Follow-up

The primary objectives were to determine the effect of adding single-dose carbamazepine on sdNVP pharmacokinetics and nevirapine resistance development in the women. The first coprimary outcome was therefore nevirapine plasma concentration 1 week postpartum. Blood samples were taken 1 week postpartum (and at delivery) for pharmacokinetic evaluation. The second coprimary outcome was the proportion of women with any of the following major nevirapine resistance mutations L100I, K101P, K103N, V106A/M, V108I, Y181C/I, Y188C/L/H,

G190A[9,24] on majority sequencing 6 weeks postpartum. Secondary outcomes were any positive HIV RNA polymerase chain reaction test of the newborn and adverse events (including any grade of toxicity in laboratory safety tests taken 1-week postpartum) possibly/probably/definitely related to study drugs. Hematology and biochemistry tests were determined at enrollment and 1 week postpartum. CD4 cell counts and quantitative plasma HIV-1 RNA were assayed just after delivery. Infants were tested just after birth, week 6, and month 4 by HIV-1 RNA polymerase chain reaction assays.

Blood was taken at delivery and 1 week postpartum, and plasma stored for bioanalysis of nevirapine plasma concentrations, done in the Department of Pharmacy, Radboud

University Nijmegen Medical Centre, Nijmegen, The Netherlands, using high-performance liquid chromatography (HPLC) (lower limit of quantification 0.05 mg/L).[25] Resistance was assayed in plasma stored from samples with >500 copies per milliliter at delivery and the 6-week antenatal visit at the Department of Virology of the University

Medical Centre Utrecht, The Netherlands (samples with <500 copies/mL, the lower limit of quantification of the resistance assay, were assumed to have no important resistance mutations).

The intended sample size of 100 women per group provided 80% power to detect a 2-fold reduction in the proportion with resistance (from 36% to 18%) with 2-sided alpha = 0.05. Statistical analysis of nevirapine plasma concentrations and mutations was undertaken using SPSS, version 13.0 (SPSS Inc). Categorical variables were analyzed with χ^2 tests (or exact tests for expected cell frequency, 5) and continuous variables with rank-sum tests. Analyses were based on women still eligible at delivery (Fig. 1). As the primary outcome measures were based on batched laboratory measures, analyses also excluded women who had evidence of noncompliance (carbamazepine detected by HPLC in sdNVP group or no carbamazepine detected by HPLC in sdNVP/CBZ group).

RESULTS

We screened 354 HIV-infected ARV-naive pregnant women between February 2006 and April 2009 (Fig. 1). One-hundred ninety-two (54%) were eligible; the remainder were not eligible (mostly CD4 <200 cells/mm^3) or declined to participate. The study was terminated in April 2009 after 192 patients had been enrolled when the new WHO pMTCT regimen (with zidovudine + lamivudine tail) was introduced in Moshi. Groups were reasonably balanced for age, body mass index, weight, CD4 count, hematology and biochemistry parameters at enrollment and delivery (Table 1). Thirty-four women were randomized but did not take study drugs at delivery. Four (all sdNVP) had CD4 declines to <200 cells per cubic millimeter and were referred for ART.

Twenty-one (12 sdNVP, 9 sdNVP/CBZ) did not return after enrolment for subsequent antenatal visits or delivery, 7 (5 sdNVP, 2 sdNVP/CBZ) delivered at home or at nonstudy sites, and 2 (1 sdNVP, 1 sdNVP/CBZ) withdrew or did not want study participation disclosed before or at delivery. Thus 75 sdNVP and 83 sdNVP/CBZ women remained in the study at delivery. One hundred thirty-three of 158 (84%) delivery samples were available [62/75 (83%) sdNVP, 72/83 (87%) sdNVP/CBZ]. Missing samples were not taken or had insufficient volume or clotted before analysis. One woman in the control group had detectable carbamazepine levels and was excluded from analyses.

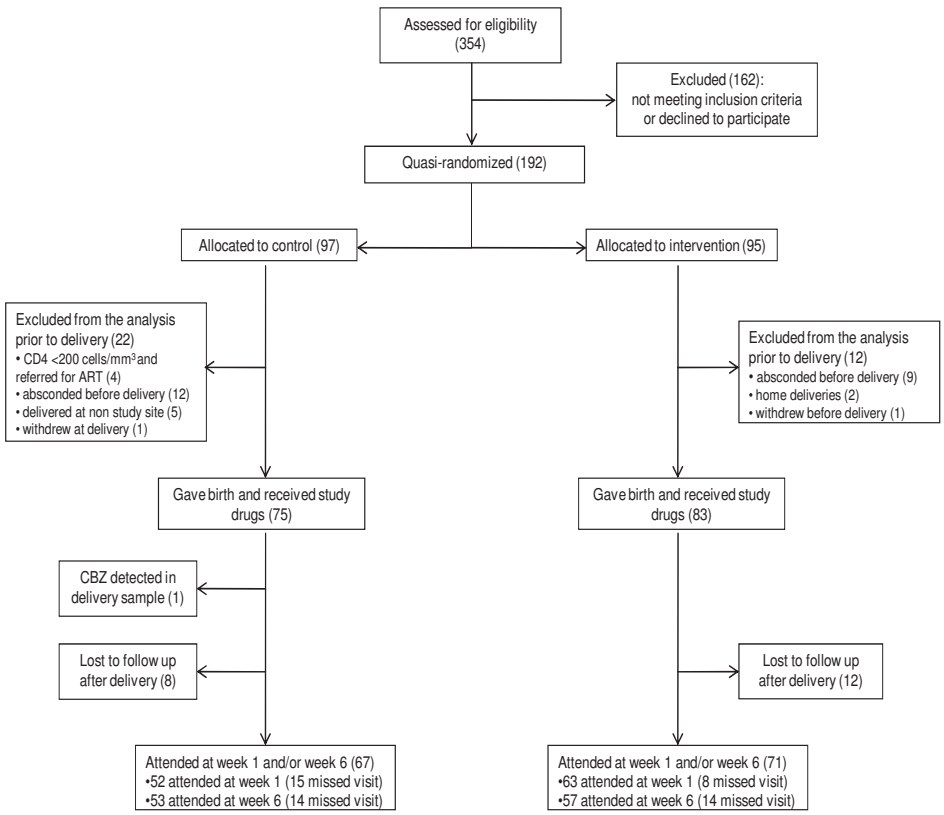

Figure 1. Trial profile.

Pharmacokinetics

The median (interquartile range) nevirapine plasma concentrations immediately after delivery were 1.55 (0.88–1.84) mg/L for the sdNVP group and 1.40 (0.93–1.97) mg/L for the sdNVP/CBZ group (Table 2) (rank-sum P = 0.91) [geometric mean ratio: 1.17, 95% confidence interval (CI): 0.76 to 1.81; P = 0.48]. As expected, nevirapine plasma concentrations were significantly reduced in both groups 1 week after delivery [based on matched pairs of measurements, sdNVP geometric mean ratio (1 week:delivery, n = 44) = 0.18, 95% CI: 0.12 to 0.26; sdNVP/CBZ geometric mean ratio (1 week:delivery, n = 54) = 0.10, 95% CI: 0.07 to 0.14): results similar using unmatched data]. In the sdNVP/CBZ group, week 1 nevirapine plasma concentrations were significantly lower compared with the sdNVP group (geometric mean ratio: 0.64, 95% CI: 0.43 to 0.96; P = 0.03; Table 2) with median (interquartile range) nevirapine concentration 0.09 (0.05–0.20) mg/L in the sdNVP/CBZ group versus 0.20 (0.09–0.31) mg/L in the sdNVP group (rank-sum: P = 0.004). Moreover, there was a trend to a greater proportion with undetectable nevirapine levels (<0.05 mg/L) in sdNVP/CBZ than sdNVP groups; 15 of 63 (24%) versus 6 of 52 (12%), respectively (χ^2 P = 0.09).

Table 1. Characteristics of Women at Enrollment, Delivery, and 1 Week After Delivery

	sdNVP	sdNVP/CBZ
At enrolment	n=97	n=95
Age (years)	29 (25-32)	29 (25-31)
Weight (kg)	63 (57-73)	65 (56-71)
BMI (kg/m²)	25.7 (23.4-28.5)	26.0 (23.1-29.6)
Alkaline phosphatase (U/L)	117 (76-180)	140 (88-185)
Bilirubin (µmol/L)	8.6 (<8.6-12.6)	<8.6 (<8.6-12.1)
Creatinine (µmol/L)	51.7 (<44.2- 62.1)	51.9 (<44.2-58.9)
AST (U/L)	9.4 (7.9-12.1)	9.5 (8.3-11.2)
ALT (U/L)	4.4 (3.4-7.5)	4.7 (3.5-6.4)
Haemoglobin (g/L)	98 (57-109)	97 (53-105)
Platelets(U/L)	261 (220-347)	234 (180-311)
Leukocytes (U/L)	6.7 (5.2-8.0)	6.3 (5.1-7.4)
Neutrophils (U/L)	4.1 (3.3-5.1)	3.6 (2.8-4.4)
At delivery	n=75	n=83
CD4 (cells/µl)	353 (212-466)	296 (200-531)
HIV-1 RNA (copies/ml)	8533 (3290-48768)	6339 (1311-48865)
Birth weight (kg)	3.01 (2.80-3.30)	3.20 (2.90-3.40)
Hours from nevirapine ingestion to delivery	5.02 (3.08-15.50)	3.58 (2.00-8.67)
1 week after delivery	n=52	n=63
Alkaline phosphatase (U/L)	134 (112-173)	125 (100-185)
Bilirubin (µmol/L)	9.1 (<8.6-13.6)	8.8 (<8.6-13.4)
Creatinine (µmol/L)	62.8 (46.5-75.0)	56.3 (47.1-69.3)
AST (U/L)	11.5 (9.6-14.6)	10.8 (8.3-17.4)
ALT (U/L)	9.7 (6.6-12.3)	9.8 (6.7-14.7)
Haemoglobin (g/L)	111 (89-122)	108 (96-122)
Platelets(U/L)	401 (277-534)	382 (300-422)
Leukocytes (U/L)	7.2 (6.3-10.1)	6.4 (5.5-7.8)
Neutrophils (U/L)	4.5 (3.6-6.4)	3.9 (3.1-5.2)

Values are median (IQR).
BMI, body mass index; IQR, interquartile range.

Nevirapine Resistance

At week 6, 110 (53 sdNVP, 57 sdNVP/CBZ) samples were available for HIV genotyping. For 3 women, plasma volumes were too limited, leaving 107 with valid genotypes. Eleven (21%) of the 52 women in the sdNVP group had 1 or more nevirapine-associated resistance mutations, compared with 6 of 55 (11%) in the sdNVP/CBZ group (odds ratio =0.46, 95% CI: 0.16-1.34, χ^2 P = 0.15). Eight women in the sdNVP group had 1 nevirapine mutation and 3 had 2 mutations (Fig. 2), compared with 5 and 1, respectively, in the sdNVP/CBZ group [poisson incidence rate

ratio for number of mutations in sdNVP/CBZ vs. sdNVP = 0.47 (95% CI:0.19 to 1.17) P = 0.11]. All but 2 of the mutations were detected as mixtures with wild-type nucleotide sequence. Only 1 of 2 mutations in 1 woman in the sdNVP group was already present in a sample stored at the enrolment visit. None of the other nevirapine mutations were already present in enrollment samples. Figure 3 shows the distribution of the nevirapine mutations 6 weeks after delivery.

Table 2. Maternal NVP Plasma Concentrations at Delivery and 1 Week After Delivery

	sdNVP	sdNVP/CBZ	P	Geometric Mean Ratio (95%CI)
At delivery				
Samples taken (n)	61	72	—	
NVP plasma concentration [mg/L; median (IQR)]	1.55 (0.88–1.84)	1.40 (0.93–1.97)	0.91	
NVP plasma conc. [mg/L; geometric mean (95% CI)]	0.89 (0.63 - 1.26)	1.04 (0.79 - 1.38)	0.48	1.17 (0.76 - 1.81)
<0.05 mg/L NVP [n (%)]	6 (10%)	4 (6%)	0.51	
1 week after delivery				
Samples taken (n)	52	63	—	
NVP plasma concentration [mg/L; median (IQR)]	0.20 (0.09–0.31)	0.09 (0.05–0.20)	0.004	
NVP plasma conc. [mg/L; geometric mean (95% CI)]	0.16 (0.12 - 0.21)	0.10 (0.08 -0.14)	0.03	0.64 (0.43 -0.96)
<0.05 mg/L NVP [n (%)]	6 (12%)	15 (24%)	0.09	
NVP plasma concentration 1 week: delivery [number of paired measurements; GMR (95%CI)]	n=44; 0.18 (0.12-0.26)	n=54; 0.10 (0.07-0.14)	—	—

The P values from rank-sum tests for plasma concentration, exact, and χ^2 tests for <0.05 mg/L at delivery and week 1, respectively.
CI, confidence interval; GMR, geometric mean ratio; IQR, interquartile range.

K103N, Y181C/I, and G190A were the most frequently observed nevirapine mutations. The most common mutation was K103N, present in 9 women, alone in 8 women and with G190A in 1.

COMBINED PHARMACOKINETIC

Resistance Analysis

Nevirapine plasma concentrations 1-week postpartum were available from 14 of 17 (82%) women with mutations versus 71 of 90 (79%) without mutations. Median week-1 nevirapine plasma concentrations were similar in women with [median (interquartile range) 0.12 (0.09–0.29) mg/L] and without [0.11 (0.06–0.22) mg/L] mutations (P = 0.36). However, none of the 15 women with undetectable nevirapine levels at week 1 developed nevirapine resistance compared with 14 of 70 (20%) of those with detectable nevirapine at week 1 (exact P = 0.07).

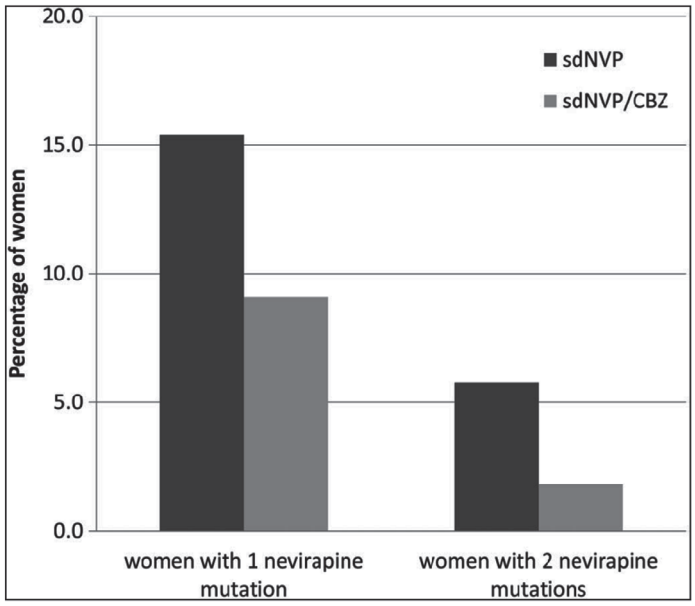

Figure 2. Percentage of women with nevirapine mutations.

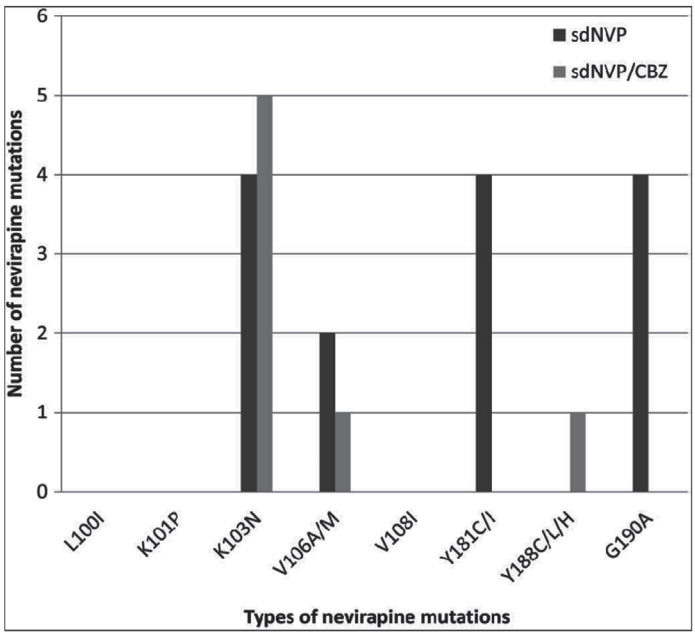

Figure 3. Number of mutation related to nevirapine resistance.

Thus, irrespective of group assignment, women with undetectable plasma concentrations at week 1 were less likely to develop nevirapine-associated mutations after sdNVP for pMTCT.

Safety

Samples were available for viral load testing from 102 newborns (93%), of which, 3 were of insufficient quantity or clotted. The overall HIV-1 transmission rate was 6 of 50 (12%) in the sdNVP/CBZ group and 3 of 49 (6%) in sdNVP group (exact P = 0.49). Although not all infants were tested at delivery, 1 of 6 (17%) and 0 of 3 (0%) infected babies in the sdNVP/CBZ and sdNVP groups, respectively, were known to have been infected during the intrauterine period. Three babies (1 sdNVP, 2 sdNVP/CBZ) died at delivery, 1(sdNVP/CBZ) died in the first 6 weeks after delivery and 1(sdNVP/CBZ) after 4 months of delivery. There were no adverse events in the infants possibly/probably/definitely related to study medication(s). Women were similar in all laboratory safety parameters at enrollment (P = 0.06 to P = 0.80) (Table 1). Forty-two adverse events were reported in the sdNVP group and 40 in the sdNVP/CBZ group between enrollment and delivery (ie, before trial intervention), mostly grade 1 (31 sdNVP, 29 sdNVP/CBZ). Only 7 were grade 4 (3 sdNVP, 4 sdNVP/ CBZ); 6 women had grade 4 anemia (Hb <6.5 g/dL) and 1 had AST >10.0 x upper limit of normal. One week after delivery, women were also comparable in hematology and biochemistry parameters (P >0.26) except for leucocytes (P = 0.002) and neutrophils (P = 0.009, Table 1). However, nearly all values [27/27 (100%) sdNVP; 38/39 (97%) sdNVP/CBZ) (>2.5 x10^9/L). After delivery, 6 adverse events were reported in the sdNVP group and 21 in the sdNVP/CBZ group. Most events were grade 1 (2 sdNVP; 13 sdNVP/CBZ); of the remaining events, 7 were grade 2 (3 sdNVP; 4 sdNVP/CBZ), 3 grade 3 (1sdNVP; 2 sdNVP/CBZ), and only 2 were grade 4 (2 sdNVP/CBZ), both anemia (Hb <6.5 g/dL). None of the adverse events (including the grade-4 anemias) were considered possibly/probably/definitely drug related.

DISCUSSION

This trial provides the first data on adding a single-dose of an enzyme inducer (carbamazepine) to sdNVP for pMTCT in HIV-infected pregnant women. Single-dose carbamazepine significantly reduced nevirapine plasma concentrations 1 week after delivery, with a trend toward fewer resistance mutations. We also found a trend toward lower rates of nevirapine resistance in women with undetectable nevirapine concentrations across the whole population, supporting the validity of this approach. Nevirapine plasma concentrations were determined in samples obtained immediately after delivery and 1 week postpartum. Overall, these plasma concentrations were comparable with previous studies.[6,26,27] Adding single-dose carbamazepine did not affect nevirapine plasma concentrations immediately after delivery, similarly to L'homme et al[4] in healthy women. A delay in effect on enzyme induction by single-dose carbamazepine would be expected because increased protein synthesis is required, which takes a few days for maximum impact.[4] This is important as the addition of an enzyme inducer should not negatively influence the protective effect of sdNVP on HIV mother-to-child transmission. The delay in enzyme induction and the low concentrations of carbamazepine in breast-milk mean that reductions in infant nevirapine levels are unlikely.[28] Indeed, perinatal

HIV- 1 transmission rates were similar to rates reported in previous studies of women using sdNVP for the first time.[2,15,29–31]

One week after delivery, nevirapine concentrations were 36% lower in the sdNVP/CBZ group, with a trend toward a higher proportion with undetectable plasma concentrations in this group. Plasma concentrations 1-week postpartum are determined by nevirapine elimination halflife, and, therefore, significantly lower concentrations in the sdNVP/CBZ group directly translate into significantly higher elimination half-life. In a previous pilot study[4] in healthy Dutch female volunteers, the nevirapine elimination half-life declined by 35% with additional single-dose carbamazepine, and the median time to undetectable levels was reduced by 23%. Our study differed in study design (single sample only) and subject population, but single-dose carbamazepine seems to reduce nevirapine elimination half-life at least as effectively in HIV-infected pregnant Tanzanian women. As our sample size was determined by the (binary) nevirapine resistance coprimary endpoint, our power to detect changes in nevirapine concentrations was higher, with even 50 patients per group providing at least 80% power to detect a 50% relative decrease in 1-week nevirapine concentration based on standard deviations observed in the pilot study.[4] To reduce the emergence of nevirapine resistance after sdNVP, several studies have investigated the addition of other ARVs after delivery. A meta-analysis reported 36% average nevirapine resistance prevalence for women using sdNVP (with or without other ARVs).[32] For those women receiving ARVs postpartum, this fell to 4.5%. Although nevirapine resistance drops dramatically with these new strategies including combination "tail" ARVs, the major disadvantages are their complexity and the emergence of lamivudine resistance (due to long intracellular elimination half-life), which is associated with virological and clinical failure in women starting lamivudine-containing ART for their own health.[11,33] To preserve its simplicity, Chi et al[22] administered a single dose of emtricitabine + tenofovir with sdNVP in 400 women, which also halved nevirapine resistance; 25% versus 12%. As it targets a different mechanism, the pharmacologic approach described here might have an additive effect and also maintains simplicity.

Our study has several limitations. First, we had high loss to follow-up. This is unfortunately not uncommon in pMTCT programs in sub-Saharan Africa.[34,35] Traditions, stigmatization, and fear of harassment and rejection accounted for most of our loss to follow-up. More intensive counseling for women using pMTCT prophylaxis and more aggressive methods to identify and locate those not returning for appointments should reduce loss to follow-up rates in future studies. Second, the randomization list was generated as alternate allocations (ie, was only quasi-randomized) by the study pharmacist, who assigned groups centrally and did not include patients herself; previous allocations were concealed from the local medical officers enrolling patients. This together with the open-label allocation could have led to bias, although not in the direct assessment of coprimary outcomes (pharmacokinetics and resistance) which were measured on batched stored samples blinded to allocation. The third limitation was the far smaller sample size for resistance testing than designed, which limited our power to detect differences in resistance between the groups. Finally, the standard HIV genotyping assays could only detect viral mutations found in>20% of the overall HIV population and not subpopulations of mutants. Deep sequencing is planned for future projects. The original pilot study in healthy Dutch

female volunteers was performed to identify enzyme inducers that could reduce nevirapine half-life. The mechanisms by which the CYP3A4 enzyme are induced involves the activation of the transcription factors pregnane X receptor and the constitutive androstane receptor.[36] Nevirapine is a drug that binds to those receptors, where after activation of the CYP3A4 enzyme starts. From the 8 interventions single-dose carbamazepine and phenytoin for 3 or 7 days were shown to decrease the nevirapine half-life significantly.[5] We, therefore, chose single-dose carbamazepine as our study intervention because of its simplicity. Single-dose carbamazepine could lead to greater adherence compared with the current national and WHO guidelines and is highly likely to reduce emergence of NNRTI resistance even further. Moreover, single-dose carbamazepine is a very low-cost intervention and even when NRTIs turn out not to be available, carbamazepine is in almost every clinic in resource-limited countries. The current national and WHO guidelines for pMTCT recommend new and more effective but also more complex and expensive regimens than sdNVP alone; their widespread implementation is challenging. A second pharmacokinetic trial (VITA-2;ClinicalTrial.gov ID: NCT01187719) has consequently started to evaluate the effect of 7 days of phenytoin, a different course of an enzyme inducer, also shown to be effective in the previous pilot study, on the pharmacokinetics and resistance of sdNVP in HIV-infected pregnant women in combination with the new recommended WHO and national guidelines. Studies, as the VITA-2 trial, with different enzyme inducers given for an extended period will be in addition to the new WHO pMTCT guidelines combining sdNVP at delivery with zidovudine from the second trimester of gestation and zidovudine + lamivudine labor for 7 days postpartum. The fact that nevirapine resistance is still observed using these approaches suggests pharmacologic interventions such as evaluated in this study may still add benefit.

In conclusion, our study demonstrates that a simple intervention of single-dose carbamazepine in addition to sdNVP alone for antiretroviral prophylaxis for pMTCT considerably reduces the nevirapine plasma concentrations and likely reduces emergence of nevirapine resistance mutations. Enzyme inducers, such as carbamazepine, show new possibilities for pMTCT programs to reduce the development of nevirapine resistance in settings where other options are limited.

ACKNOWLEDGMENTS

We thank all the patients and staff from all the clinics participating in the VITA1 trial. VITA Trial Team: Kilimanjaro Christian Medical College, Moshi, Tanzania:J. Shao, F. Mosha, E. R. Kisanga, E. P. Muro, J. Mushi. Pasua Antenatal Clinic: G. Mariki, Shirima, Salma. Bondeni Antenatal Clinic: M. Kitiwi, M. Hassan. Mawenzi Antenatal Clinic: S. Masawe, A. Mchaki, R. Mushi, M. Chuwa. Majengo Antenatal Clinic: Mandari, Fimbo. Labor ward Kilimanjaro Christian Medical Centre: P. Mlay, Kiwia, Herini, Maleo, Nyalu, Mushi. Department of Virology, University of Utrecht, The Netherlands: R. Schuurman, S. Aitken. MRC Clinical Trials Unit, London, United Kingdom: D. M. Gibb, A. S. Walker, M. J. Thomason. Department of Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands: D. M. Burger, R. L'homme, Q. Fillekes. Biotechnology Laboratory of Kilimanjaro Christian Medical Centre, Moshi, Tanzania: L. Wolters, A. Ndaro, S. Matondo, E. Msuya. Laboratory of Department of Pharmacy Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands: M. Teulen, K. Asouit.

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4

EFFECT OF SEVEN DAYS OF PHENYTOIN ON THE PHARMACOKINETICS OF AND THE DEVELOPMENT OF RESISTANCE TO SINGLE-DOSE NEVIRAPINE FOR PERINATAL HIV PREVENTION: A RANDOMIZED PILOT TRIAL

Journal of Antimicrobial Chemotherapy 2013;68(11):2609-15.

ABSTRACT

Objectives

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

Methods

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

Results

Thirty-five and 37 women were allocated to the control and intervention groups, with median (IQR) ages of 27 (23–31) and 27 (23–33) years, respectively. Twenty-three and 23 had detectable nevirapine levels at delivery and subsequent samples in the control/intervention groups, respectively. Geometric mean (GM) (95% CI) plasma levels of nevirapine at delivery were 1.02 (0.58–1.78) mg/L and 1.14 (0.70–1.86) mg/L in control and intervention groups, respectively (P=0.76). One week after delivery, 0/23 (0%) and 15/22 (68%) control and intervention mothers, respectively, had undetectable levels of nevirapine (<0.05 mg/L; P <0.001). One week later, the figures were 10/21 (48%) and 18/19 (95%) mothers, respectively (P=0.002). The GM (95% CI) half-life of nevirapine was 63.2 (52.8–75.7) versus 25.5 (21.6–30.1) h in the control versus the intervention group (P <0.001). New nevirapine mutations were found in 0/20 (0%) intervention group versus 1/21 (5%) control group mothers. Overall, there was no difference in the number of adverse events reported between the control and intervention arms (P >0.28).

Conclusions

Adding 7 days of an enzyme inducer to single-dose nevirapine to prevent mother-to-child transmission of HIV significantly reduced subtherapeutic nevirapine levels by shortening the half-life of nevirapine. As prolonged subtherapeutic nevirapine dosage leads to the emergence of resistance, single-dose nevirapine could be used with phenytoin as an alternative if other ARVs were 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 the onset of labour—reduces MTCT by ~50%.[1,3] Its major disadvantage is the development of nevirapine resistance in both mothers (1%–69%) and infants,[4] most probably due to its long elimination half-life (61 h),[5–7] which leads to several days to weeks of subtherapeutic plasma concentrations, coupled with its low genetic barrier to resistance.[8] Newly emergent resistant HIV may be transmitted to the infant or to others, limiting their treatment options, and may also reduce the efficacy of future combination antiretroviral therapy (cART) in the mother.[9] Given its simplicity and efficacy, single-dose nevirapine is nevertheless still endorsed by the WHO as part of the regimen for prevention of MTCT(pMTCT) in resource-limited settings, when cART(WHO Option B/B+) is not feasible or not available. To cover the prolonged presence of subtherapeutic plasma concentrations of nevirapine after single-dose nevirapine at the onset of labour, Option A of the WHO (2012) guidelines recommend adding zidovudine/lamivudine for 7 days post-partum.[10,11] This approach reduces the development of resistance 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.[12] A pharmacological, rather than antiretroviral (ARV), approach of adding a CYP3A4 enzyme inducer has been shown to decrease the elimination half-life of nevirapine in healthy women,[13] the greatest reductions being seen with carbamazepine and phenytoin. In our previous trial (VITA-1), the addition of single-dose carbamazepine to single-dose nevirapine at the onset of labour also significantly reduced plasma concentrations of nevirapine 1 week after delivery in HIV-infected women, with a trend towards fewer resistance mutations.[14] The CYP3A4 enzyme inducer phenytoin is a low-cost, widely available anticonvulsant and antiarrhythmic drug, which is not secreted into breast milk in clinically important amounts (in contrast to carbamazepine) and can therefore be safely given to breast-feeding mothers.[15] In this pilot study, we investigated the impact of 7 days of phenytoin on the pharmacokinetics of and development of resistance to nevirapine after single-dose nevirapine as a component of ARV prophylaxis forpMTCT (the VITA-2 trial). The hypothesis was that 7 days of phenytoin would reduce the elimination half-life of nevirapine and hence the emergence of nevirapine resistance mutations.

METHODS

Study participants

Participants were recruited from the Pasua and Majengo antenatal clinics in Moshi, Tanzania, and the University Teaching Hospital 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 or hospitalization, the use of concomitant medication that might interfere with ARVs or phenytoin, or a CD4 cell count <350 cells/mm^3 (making the women eligible for cART). All the women gave written informed consent; illiterate patients gave oral consent documented by their own thumbprint and a witness. The study was approved

by the institutional review boards of the Kilimanjaro Christian Medical University College, Moshi, Tanzania, the National Institute of Medical Research in Dar es Salaam, Tanzania, and the University Teaching Hospital, Lusaka, Zambia. The study is registered with http://ClinicalTrials.gov (NCT01187719). Eligible women received pMTCT ARV prophylaxis as recommended by national Tanzanian or Zambian guidelines. Subjects started 300 mg of zidovudine twice daily from 28 and 14 weeks of gestation in Tanzania and Zambia, respectively, or as soon as possible thereafter pre-delivery. At the onset of labour, women received 200 mg of single-dose nevirapine plus 300 mg of oral zidovudine every 3 h and 150 mg of lamivudine every 12 h until delivery (Tanzania), or 600 mg of oral zidovudine and 300 mg of lamivudine every 12 h until delivery (Zambia). Post-delivery, 300 mg of zidovudine and 150 mg of lamivudine were taken twice daily for 7 days. Newborns were given 2 mg/kg of single-dose nevirapine suspension within 24–72 h after birth and then 4 mg/kg of zidovudine syrup (Tanzania) or 2 mg/kg of nevirapine suspension (Zambia) twice daily for 7 days. All the women in the trial breastfed their children for 6 months and then weaned them rapidly. Women were randomized 1:1 in a parallel group design to either the national standard of care or the national standard of care plus 184 mg of phenytoin (2×92 mg tablets) once daily from the onset of labour for 7 days. The randomization sequence was generated by a trial statistician from the MRC using simple randomization blocks. 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 the time of ingestion of the study drug(s) 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 7 days of phenytoin administration 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 out comes were the pharmacokinetic parameters of nevirapine (elimination half-life and 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 and G190A)[16] at week 4–6. Secondary outcomes were all adverse events (AEs) possibly or probably related to pMTCT ARV prophylaxis or phenytoin, and HIV infection of the infant.

Haematology and biochemistry tests were performed at enrolment and 1 week post-partum. CD4cell counts and viral loads (VL) were assayed at delivery. Infants were tested just after birth (<30 min) and at week 4–6 by DNA PCR assays. Blood was taken from the women and stored at delivery and days 1, 3, 5, 7 and 14 post-partum, and from the children at delivery and on day 7 post-delivery for the retrospective determination of plasma concentrations of nevirapine (and phenytoin) at the Department of Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Nevirapine assay used HPLC with a lower limit of quantification (LLOQ) of 0.05 mg/L,[17] 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 at week 4/6 at the Department of Virology of the University Medical Centre Utrecht, The Netherlands. Pharmacokinetic and resistance assays were performed blinded to randomized allocation.

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 the elimination half-life of nevirapine associated with 7 days of phenytoin, allowing a 20% drop-out rate (lack of follow-up samples, based on VITA-1).[14] Safety analyses included all women who were observed or reported taking study medication (the safety population). Analyses of pharmacokinetics and resistance included the safety population who did not receive a second dose of nevirapine, who delivered vaginally [rather than by caesarean section (C/S)] and who had pharmacokinetic evidence (a detectable plasma concentration 1 day post-delivery) of taking nevirapine, and phenytoin if this had been allocated (the protocol-specified primary pharmacokinetic population). Analyses were also carried out that included mothers who delivered by C/S, as no difference in pharmacokinetic parameters had been observed between C/S and vaginal deliveries in the VITA-1 trial. Women in the control group with phenytoin detected in any sample were excluded from the pharmacokinetic and resistance analyses. Pharmacokinetic analysis was performed using Phoenix 64 WinNonlin 6.3 (Pharsight Corporation, CA, USA) and statistical analysis using SPSS version 18.0 (SPSS Inc.). Randomized groups were compared using t-tests for continuous pharmacokinetic variables [after transformation to the log scale, i.e. comparing geometric means (GMs)], rank-sum tests for all other continuous variables and exact tests for categorical variables.

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 a CD4 cell count <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 the control and intervention groups, respectively. One woman had been randomized twice, so the second randomization was excluded and the woman followed the first randomization. The demographic characteristics at enrolment and delivery were generally reasonably balanced between the two groups [Table 1 and Table S1 (available as Supplementary data at JAC Online)], the main difference being the significantly shorter time from nevirapine ingestion to delivery in the intervention group, which must have occurred by chance. This difference is, however, not expected to have had an impact on our pharmacokinetic data, as the elimination half-life of nevirapine is long. In addition, no differences were observed between the laboratory values at enrolment and delivery within either group (Table S1). The study finished once the recruitment target had been met.

Pharmacokinetics

At delivery, considering only the women who delivered vaginally (and not by C/S), there was no significant difference in plasma concentration of nevirapine between the two groups [GM (95% CI) was 1.08 (0.63–1.84) mg/L in the control patients versus 1.14 (0.70–1.86) mg/L in the intervention patients; GM ratio (GMR) (90%CI) 1.05 (0.58–1.92), P=0.82; t-test].

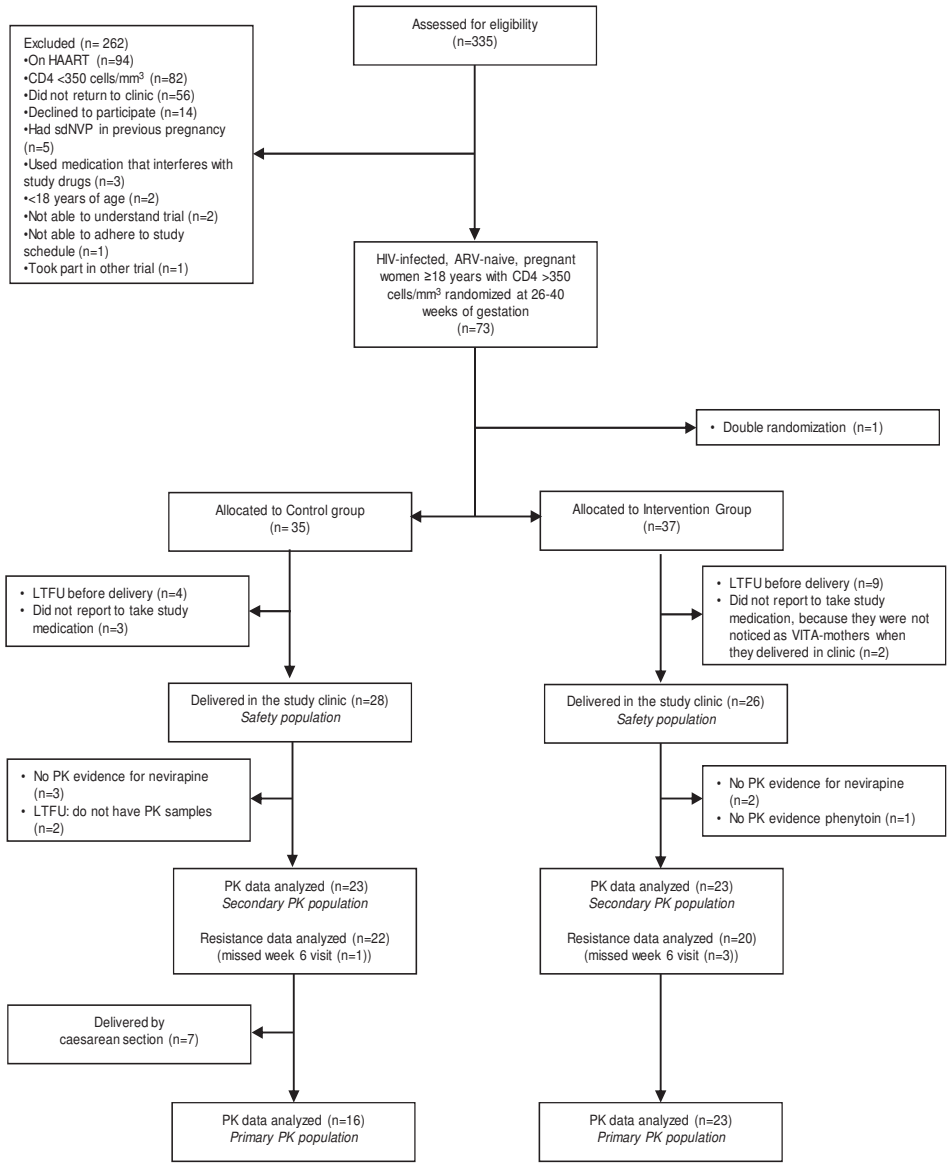

Figure 1. Profile of the VITA-2 trial (CONSORT diagram). sdNVP, single-dose nevirapine; LTFU, lost to follow up; PK, pharmacokinetic.

Plasma concentrations of nevirapine subsequently decreased significantly more quickly (Figure 2), and undetectable levels were reached significantly earlier (Table 2), in the intervention group compared with the control group. All results were similar, including for those women

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

	Control (n=28)	Intervention (7 days of 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/mm^3)	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 (h)	9.1 (2.5-12.6)	2.1 (1.0-4.9)	0.003

Data are presented as median (IQR) and were tested using rank-sum

who delivered by C/S, so results from the (larger) group including women who delivered by C/S are subsequently presented [see Table S2 (available as Supplementary data at JAC Online) for pharmacokinetic results for the (smaller) group excluding women who delivered by C/S].

One week post-delivery, plasma concentrations of nevirapine 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 the intervention than the control group [GMR (intervention: control) (90% CI) 0.15 (0.11–0.20), P <0.001; t-test]. The GM (95% CI) elimination half-life of nevirapine was 63.2 (52.8–75.7) versus 25.5 (21.6–30.1) h in the control and the intervention group, respectively (P <0.001; t-test), representing a 60% reduction [GMR (90% CI) 0.40 (0.33–0.49)]. The GM (95% CI) time to achieving undetectable nevirapine plasma concentration was 16.3 (13.8–19.3) versus 6.7 (5.7–7.8) days in the control versus the intervention group (P<0.001; t-test). Consequently, a significantly greater proportion of control group women had detectable plasma levels of nevirapine at 1 week and 2 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 plasma concentration of nevirapine at 1 week post-delivery (P <0.001; exact text) as did 11/21 (52%) women in the control group versus 1/19 (5%) women in the intervention group at 2 weeks post-delivery (P= 0.002; exact test). The median (range) plasma concentration of phenytoin in all the samples taken from delivery to 1 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 (the therapeutic range being defined as 10–20 mg/L).[18] 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 1 week post-delivery. The median plasma level of phenytoin in the infants was <0.4 (range <0.4–1.9) mg/L).

Resistance

Samples taken 4–6 weeks post-delivery were available from 21 control women (as one missed the week 6 visit, and for one woman sample amplification failed due to a low VL) and 20 intervention women (as three missed the 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 h) that had not been 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 the wild-type nucleotide sequence (V106MV in the control group, and K103KN in the intervention group).

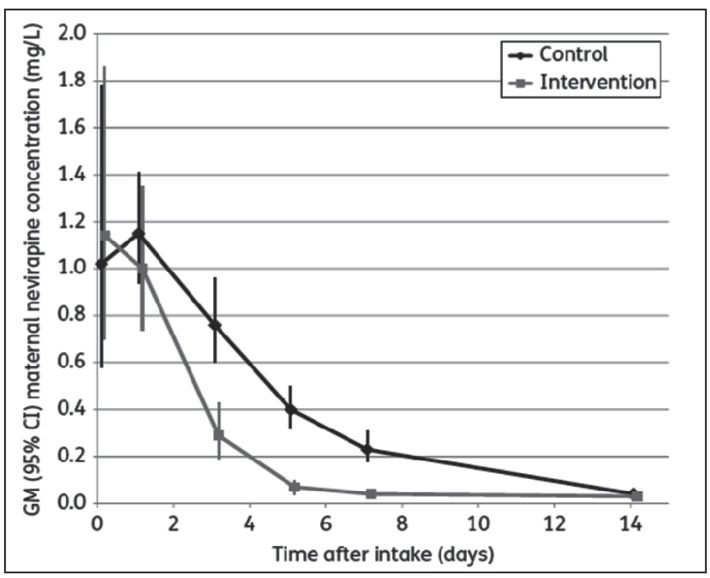

Figure 2. GM plasma concentrations of nevirapine over time post-delivery (all women who delivered including those who delivered by C/S).

Safety

The 28 control and 26 intervention mothers gave birth to 30 (two pairs of twins) and 28 (two pairs of twins) babies, respectively. Twenty-one (one pair of twins) and 19 (one pair of twins) infants, respectively, were tested just after birth and at week 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, 10 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).

Table 2. Maternal nevirapine plasma concentrations at delivery, week 1 and week 2 for 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)**
samples taken(n)	20	22		
nevirapine plasma concentration (mg/L), GM (95% CI),	1.02 (0.58–1.78)	1.14 (0.70–1.86)	0.76[a]	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 concentration (mg/L), GM (95% CI)	0.23 (0.18–0.31)	0.035 (0.027-0.046)	0.001[a]	0.15 (0.11–0.20)
<0.05 mg/L nevirapine, n (%)	0 (0)	15 (68%)	0.001[b]	
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 concentration (mg/L), GM (95% CI)	0.044 (0.031–0.062)	0.026 (0.024–0.029)	0.006[a]	0.59 (0.44–0.80)
<0.05 mg/L nevirapine (n (%))	10 (48)	18 (95)	0.002[b]	
women with week 2 and delivery samples(n)	18	18		
nevirapine plasma concentration 2 weeks: delivery, (GMR (90% CI))	0.030 (0.026–0.034)	0.022 (0.015–0.031)		

[a]t-test, [b]Exact test. Median nevirapine plasma concentrations are similar to the GM and are therefore not shown in Table 2.

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 stillbirths (two fresh and one macerated) in the intervention group. Both platelet (P <0.001) and alanine transaminase; P <0.001) levels increased significantly between enrolment and 1 week post- delivery in each randomized group, but there was no difference between randomized groups in any laboratory safety parameter 1 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 test). Most were Grade 1 (n=11 in each group), but four (n =2 in each group) were Grade 2, one was 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=1Grade 1, n= 3 Grade 2, n=1Grade 3). None of the laboratory and clinical AEs in the mothers or infants was judged possibly or probably related to the study medication.

DISCUSSION

Here we demonstrate that adding a 7 day course of phenytoin, as an enzyme inducer, from the onset of labour 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 their pMTCT ARV prophylaxis. Seven days of phenytoin administration was safe and effective, with no new nevirapine resistance mutations observed. Importantly, plasma concentrations of nevirapine at delivery were similar in those receiving and not receiving phenytoin, and also comparable to those in reported previous studies,[5,13] similar to our previous study evaluating a single dose of carbamazepine as an enzyme inducer.[14] 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 that the protective perinatal effect of single-dose nevirapine is maintained. The absence of HIV transmission during the postpartum period, similar to or even lower than rates in previous studies, [10] also confirms the efficacy of the pMTCT regimen.

Post-delivery, the pharmacokinetic parameters of nevirapine were substantially affected by enzyme induction. Adding phenytoin to single-dose nevirapine reduced plasma levels of nevirapine by 85% and produced a significantly larger proportion of women with undetectable nevirapine levels 1 week and 2 weeks post-delivery. Both effects are a consequence of the 60% reduction in the elimination half- life of nevirapine, an absolute difference of 235.8 h. This is the largest decline in elimination half-life of nevirapine ever reported, especially in the target population of HIV-infected, pregnant women. For example, the pilot study of L'Homme et al.[13] found a median elimination half-life reduction of 38% (-19.4 h) in four healthy Dutch women receiving single-dose nevirapine with 7 days of phenytoin administration, and a single-dose of carbamazepine reduced nevirapine levels by 36% in HIV-infected, pregnant women receiving single-dose nevirapine.[14] Not surprisingly, a 7 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 involve the transcription factors pregnane X receptor and the constitutive androstane receptor. The enzyme inducer binds to these receptors, thereby stimulating activation of the CYP enzyme.[19]

Studies have shown that CYP enzyme induction is correlated with dose and drug level,[20] with higher doses and a higher plasma level of the enzyme inducer resulting in lower serum levels of the test drug. This probably explains why induction of the CYP enzyme has a greater effect with a long course of an enzyme inducer than with only the single dose used in our previous study. Although current guidelines, including zidovudine monotherapy pre-delivery and 7 days of zidovudine/lamivudine post-delivery are complex, they have substantially reduced the emergence of nevirapine resistance by protecting the subtherapeutic nevirapine 'tail', since the lengthy duration of low and subtherapeutic levels of nevirapine in the blood is plausibly associated with increases in nevirapine resistance. A meta-analysis estimated that 4.5% of women using single-dose nevirapine and additional ARVs postpartum showed nevirapine resistance 4 – 8 weeks post-partum.[4] In our study, the overall prevalence of new nevirapine resistance was 2.4%(1 out of 41 samples); although we observed no nevirapine resistance mutations after single-dose nevirapine in combination with 7 days of phenytoin, the numbers

are clearly too low 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 1 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 7 day course of phenytoin at the onset of labour might have significant additional benefits in reducing the selection of nevirapine resistance mutations, even on top of the current ARV prophylaxis 'tail'. The main limitation of the study was its relatively small size. The trial was designed as a pilot powered to detect a difference between the intervention and control groups in the elimination half-life of nevirapine. A much larger sample size (approximately 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 (see Figure 1): only 22% of those assessed for eligibility pre-delivery were randomized, a further 26% of those randomized dropped out before delivery, and a further 24% of those who delivered in the study clinic did not provide samples 4 – 6 weeks post-delivery. We would therefore have needed to screen approximately 1600 women to achieve 200 women with week 4 – 6 samples. Although a larger Phase III trial should ideally confirm the efficacy of 7 days of phenytoin treatment on resistance as a primary outcome, the substantial significant reductions in nevirapine half-life, coupled with previous clinical and sophisticated modelling studies[6,7] demonstrating a causal association between the half-life of nevirapine and the emergence of resistance, suggests that 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 the WHO (Option B+). However, this means that thousands of women are still receiving single-dose nevirapine[21] and demonstrates the challenge of widespread implementation of the current guidelines. Phenytoin can be used safely during pregnancy and breast-feeding[22] 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 breast-feeding within Option B+. We have demonstrated that the implementation of this intervention would substantially and significantly reduce the half-life of nevirapine; previous data demonstrating a causal relationship between nevirapine half-life and emergence of resistance [6,7,14] 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, while probably 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, the addition of an enzyme inducer for 7 days to single-dose nevirapine for pMTCT greatly

reduced the presence of subtherapeutic nevirapine levels by significantly shortening the elimination half-life of nevirapine, with no new nevirapine resistance mutations observed. Since prolonged subtherapeutic nevirapine exposure is known to lead to the emergence of nevirapine resistance,[6,7,14] and since phenytoin is safely and widely used in women[15] to minimize HIV transmission from mother to child, single-dose nevirapine could be used with phenytoin as an alternative if other ARV drugs were unavailable.

ACKNOWLEDGEMENTS

We thank all the patients and staff from all the clinics participating in the VITA-2 trial. University Teaching Hospital: C. Mkandawire, C. Simbao, E. Chama, D. Rutagwera. Kilimanjaro Christian Medical College, Moshi, Tanzania: R. Malya, A. Mchaki, E. Msoka, J. Mushi, R. Kavishe, A. Ndaro, S. Matondo, S. Mkumbaye. Pasua Antenatal Clinic: G. Mariki, S. Mgange, C. Shirima. Majengo Antenatal Clinic: R. Mandari, S. Katutu, F. Mjaka. Labour Ward Kilimanjaro Christian Medical Centre: P. Mlay, J. Maleo, R. Herini. Department of Virology, University of Utrecht, The Netherlands: R. Schuurman. Laboratory of Department of Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands: N. van Ewijk-Beneken Kolmer, M. Meuleman, E. van den Hombergh. Monitors: S. Mulambo, M. Waweru, G. Ogetii. Data Safety Monitoring Board: M. Siwale, J. Todd, Y. Hekster.

FUNDING

This work was supported by the European and Developing Countries Clinical Trials Partnership (EDCTP) (number CT.2006.33020.006).

TRANSPARENCY DECLARATIONS

None to declare.

SUPPLEMENTARY DATA

Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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EFFECTIVENESS OF DRUG INTERVENTIONS TO REDUCE NEVIRAPINE RESISTANCE AFTER SINGLE-DOSE NEVIRAPINE AS PART OF ANTIRETROVIRAL PROPHYLAXIS TO PREVENT HIV MOTHER-TO-CHILD TRANSMISSION - A SYSTEMATIC REVIEW AND META-ANALYSIS

Manuscript in preparation

ABSTRACT

Objectives

To assess the effectiveness of different drug interventions on nevirapine resistance development after use of single-dose nevirapine as part of antiretroviral prophylaxis for prevention of HIV mother-to-child transmission (pMTCT).

Design

A systematic review including meta-analyses

Methods

Systematic search of electronic databases (MEDLINE, EMBASE and Cochrane) was performed. Studies included HIV-infected, pregnant women, who were administered single-dose nevirapine for pMTCT and who were receiving a drug intervention to reduce nevirapine resistance. Primary outcome was the proportion of nevirapine resistance detected in plasma samples collected ≤3 months postpartum. The reducing effect of drug interventions on nevirapine resistance was assessed in meta-analyses using random effects models and the GRADE approach for quality of evidence.

Results

In a total of 18 eligible studies included, the estimated pooled proportion for the detection of nevirapine resistance mutations upon single-dose nevirapine at labor was 31% (95%CI 7.6-54); this was reduced to 21% (95%CI 8.6-33) with addition of antepartum zidovudine. A combination of antepartum zidovudine, single-dose nevirapine and a short (<8 days) postpartum regimen resulted in a major reduction in nevirapine resistance to 0.011% (95%CI -0.11-0.13). The summary effect of 20-30 days of postpartum drug regimens combined with antepartum zidovudine and single-dose nevirapine was associated with a slightly lower incidence of nevirapine resistance, namely 0.003% (95%CI -0.054-0.060).

Conclusions

Antepartum zidovudine plus antiretroviral drugs postpartum have shown to nearly eliminate nevirapine resistance. Although 20-30 days post partum regimens might be slightly more effective compared to a short (<8 days) postpartum regimen, longer term antiretroviral therapy is more complex and more challenging to implement in daily practice. The WHO guideline option A (antepartum zidovudine, single-dose nevirapine and one week of lamivudine/zidovudine postpartum) should be followed to achieve a feasible minimum risk of nevirapine resistance in regions where single-dose nevirapine is still being used.

INTRODUCTION

Without antiretroviral drugs, the overall cumulative risk of mother-to-child transmission (MTCT) of HIV-1 is 30-45% in the breast-feeding population [1]. Nearly half of the risk is around the time of delivery [1] and hence, it has become a major focus in the prevention of MTCT (pMTCT). In 2000, a single-dose of nevirapine administered to the mother at onset of labor was introduced as a simple and affordable strategy for prevention of perinatal HIV transmission in resource-limited settings. It reduces MTCT by ~50% and has benefited thousands of babies.

However, a major disadvantage of single-dose nevirapine is the development of nevirapine resistance in mothers and infants [2]. This is clearly due to its long elimination half-life (61 hours [3]) and low genetic barrier to resistance leading to several days to weeks of subtherapeutic plasma concentrations [4]. Newly emergent resistant HIV may be transmitted to the infant or to others, limiting their future treatment options, and may also reduce future combination antiretroviral therapy (cART) efficacy in the mother [5]. In a previous meta-analysis, published in 2007, the prevalence of nevirapine resistance using a single-dose alone at labour onset with or without antepartum antiretroviral drugs was estimated to be 36%[2]. Given that since then more effective options have become available, this simple intervention has therefore in international guidelines turned into a fading concept, although single-dose nevirapine alone is still widely used by thousands of women in resource-constrained settings, particularly in more rural areas where access to cART is more limited.

In order to reduce the emergence of nevirapine resistance, co-interventions, such as short-course antiretroviral regimens in combination with single-dose nevirapine, have been evaluated. Addition of a short (4-7 days) postpartum course of lamivudine/zidovudine to single-dose nevirapine and antepartum zidovudine reduced viral resistance mutations tremendously[6] and is currently recommended by WHO (Option A), when long-term cART (WHO Option B/B+[7, 8]) is not available or unfeasible. Many other approaches, such as extension of the duration of the abovementioned course or use of other drugs, have been studied since the introduction of single-dose nevirapine. Until now, an overview of all evaluated co-interventions and their effectiveness aiming to reduce nevirapine resistance has not been conducted. We therefore performed a systematic review and meta-analyses to assess the effect of different drug interventions and duration of intake on nevirapine resistance development after use of a single-dose nevirapine as part of antiretroviral prophylaxis for pMTCT.

METHODS

Search strategy

A literature search for this review was performed in the MEDLINE database, the Cochrane Library and the EMBASE database until March 20, 2013. Combinations of the following free keywords and medical subject heading terms and variations on these words were used in the search strategy: 'pregnancy', 'prevention-of-mother-to-child-transmission', 'nevirapine', 'viral drug resistance' (detailed search strategy can be found in Supplementary Materials S1). For additional studies, the ISI Web of Knowledge citation index and the reference lists of included studies were searched.

Inclusion criteria

Titles and abstracts of all included studies identified by the search strategy were independently screened for eligibility by two reviewers (QF, BB). Subsequently, a second screening session by two reviewers (QF, BB) was assessed on full texts of the primarily eligible studies. Discrepancies were resolved by consensus. Studies were eligible for inclusion if they met the following selection criteria: HIV-infected, pregnant women, who were administered nevirapine as a single-dose for pMTCT of HIV-1 were enrolled; a drug intervention reducing nevirapine resistance development; and a proportion of resistance was determined <3 months after intake of single-dose nevirapine. No design, place or language restrictions were set, but full-texts were required to be available. Conference abstracts were excluded.

Data extraction

Data for each study were extracted by a single author (QF) on: authors' name, title, journal, year of publication, inclusion criteria, study location, study design, study population, study intervention, time of genotypic assessment after intake of single-dose nevirapine and type of genotypic assessment (consensus or sensitive), number of samples with nevirapine resistance as per intervention arm, total number of measured samples as per intervention arm. Data duplicated in different papers were only extracted once. A second reviewer (BB) randomly checked the data extraction sheet on appropriate data entry by the first reviewer (QF).

Primary and secondary outcomes

The primary outcome of our interest was the proportion of nevirapine resistant viral mutations (primary nevirapine mutations L100I, K101P, K103N/S, V106A/M, V108I, Y181C/I, Y188C/L/H, G190A[9]) per drug intervention detected in plasma samples collected <3 months postpartum using population sequencing.

Secondary outcomes were: 1) the addition of antepartum zidovudine on the proportion of nevirapine resistant viral mutations after single-dose nevirapine alone, 2) the addition of antepartum zidovudine on the reducing effect of postpartum interventions on nevirapine resistance after single-dose exposure, 3) the effect of short-course postpartum interventions on the proportion of nevirapine resistance after single-dose exposure, 4) the effect of the duration of postpartum interventions on the proportion of resistance after single-dose nevirapine, measured using population sequencing.

Data analysis

The effect of drug interventions in the reduction of nevirapine resistance was assessed in a meta-analysis using random effects models. The proportion of nevirapine resistance and its 95% confidence interval (CI) of each eligible study arm was graphically presented in a forest plot. The summary effect per type of intervention to the development of nevirapine resistance and its 95% CI were calculated using Microsoft Excel (Microsoft, 2007. Computer Software; Redmond, Washington[10]). The presence of statistically significant heterogeneity among studies was assessed by Q (significant when p-value <0.05) and the extent of the observed heterogeneity was assessed by I^2 (ranging from 0-100%).

Quality of evidence

The GRADE approach was used as a transparent and structured system of rating quality of evidence of research questions and grading strength of recommendations in our systematic review. The quality of evidence was considered high (three points), moderate (two points), low (one point) or very low (no points) based on five predefined GRADE criteria[11-15], including risk of bias, inconsistency, imprecision, indirectness and publication bias. An overview of the five predefined GRADE criteria and the system used to establish rating of the quality of evidence for these criteria are shown in Table 1. For each outcome measure, all criteria were scored independently by two reviewers (QF and BB). Discrepancies were resolved by consensus.

For quality assessment of randomised clinical trials (RCTs) the guidelines for systematic reviews of the Cochrane Collaboration Back Review Group were used[16, 17]. The criteria according to the STROBE guidelines were used to assess the quality of observational studies (Table 2)[11-15, 18, 19]. The initial quality of evidence for RCTs was considered as high (i.e. 3 points) and for observational studies as low (i.e. 1 point)[20].

RESULTS

Literature search

Through our literature search strategy 823 articles were identified. Two-hundred and forty-nine duplicates were removed. Based on the inclusion and exclusion criteria a total of 556 (501+51+4) studies were excluded resulting in 18 eligible studies for our systematic review (Flowchart, see Figure 1). Nine were RCTs, one was a random subset of an RCT and eight were observational studies. Eleven (24 study arms) of the 18 studies contained nevirapine resistance data measured by a population drug resistance assay and eight (18 study arms) by more sensitive drug genotypic techniques.

Eleven studies were conducted in Sub-Saharan Africa, one in West Africa, four in Asia and two studies had sites in both Asia and Africa. Of the 18 included studies, five had one eligible arm, seven studies had two, three studies had three, two had four eligible arms and one study had six eligible arms available for analysis. In total, 3,726 patients from 42 study arms were included in our analyses (Table 3).

Antepartum zidovudine

Since only studies with a drug intervention reducing nevirapine resistance were included in our systematic review, no more than three study arms provided a single-dose nevirapine alone. The summary proportion of the emergence of nevirapine resistance mutations was 31% (95%CI: 7.6-54); Figure 2), but statistically significant heterogeneity was observed between these three study arms ($p<0.005$, $I^2=88\%$).

1,218 HIV-infected pregnant women from 11 different study arms (six populations and five sensitive sequencing) were on antepartum zidovudine from 28-36 weeks of gestation combined with single-dose nevirapine. Hundred sixty-seven of 698 women (summary effect 21% (95% CI: 8.6-33)) had nevirapine resistance mutations (Figure 2) measured by population genotypic assessments. However, also here a significant difference ($p<0.005$, $I^2=97\%$) was found on the effect of antepartum zidovudine on nevirapine resistance.

Table 1. Schematic overview for scoring GRADE criterions per outcome measure

GRADE-criterion	Method	Quality indicator	Cut off quality indicator for each individual study	Score
Risk of bias	Quality assessment of individual studies (Table 2)	Inappropriate selection of groups Failure to control for confounding (observational studies only) Selective outcome reporting Lack of blinding (RCTs only) High loss to follow up Lack of allocation concealment (RCTs only) Intention-to-treat principle violated (RCTs only)	-1: >75% of the prioritized items are not adequately described and >50% of the non-prioritized items are not adequately described (Table 2)	-1: >50% of the study participants has scored -1
Inconsistency	Forest plot	Extent of CI overlap		-1: p<0.05 (and CIs of the different studies have little or no overlap)
	Test for heterogeneity (Q)	p-value		
Imprecision	Quality assessment of individual studies (Table 2)	Description of power analysis	-1: no power analysis described	-1: no power analysis described in >25% of the study participants or >50% of study participants in studies with a non-acceptable drop-out rate (i.e. >30%) or if upper/lower range of the CI of any study represents the risk difference
		Drop-out rate	-1: non-acceptable drop-out rate (i.e. >30%)	
	Forest plot	Width of CI		
Indirectness	Quality assessment individual studies (Table 2)	Description of setting, patient selection, outcome measures	-1: >50% of the scoring items are not adequately described	-1: majority of study participants is a 'between' studies comparison (and <50% of the scoring items are adequately described)
	Comparison within or between studies	Comparison within or between studies		
Publication bias	Quality assessment individual studies (Table 2)	Funding	-1: Industry sponsoring described	-1: >75% of studies sponsored and/or (if ≥5 studies) asymmetrical distribution of funnel plot
	Funnel plot	Symmetry of the plot		

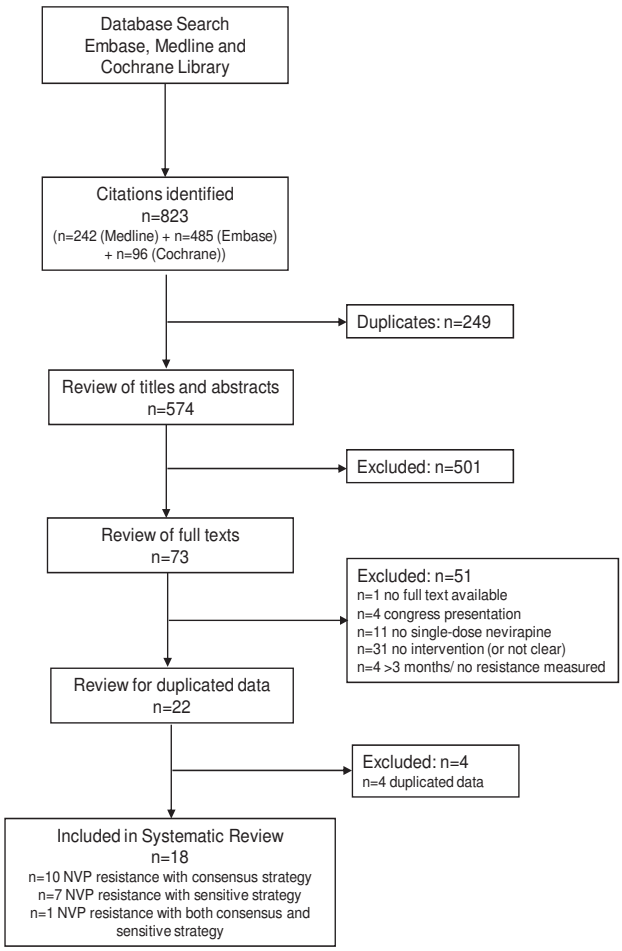

Figure 1. Flow chart of literature search and selection procedure.

Hence, antepartum zidovudine combined with a single-dose of nevirapine shows a relative decrease of 32% in the proportion of nevirapine resistance compared to single-dose nevirapine alone. The quality of evidence for this research question was, however, very low (0 points in the GRADE range of 0-3).

Antepartum zidovudine on the effect of postpartum interventions

In total, there were eleven short-course interventions postpartum, including two single-dose drug interventions; four were without antepartum zidovudine and seven interventions were with the addition of antepartum zidovudine (Figure 2). Evaluating the effect of antepartum zidovudine on nevirapine resistance, five study arms were selected with the same postpartum interventions: three intervention arms without antepartum zidovudine and two with

Table 2. Overview of quality assessment per individual study

Item quality assessment	GRADE criterion	Study type	
		RCT	Observational study
Setting/generalizability	Indirectness	+	+
Patient selection			
Eligibility criteria specified	Indirectness	**+**	**+**
Methods of inclusion/selection	Risk of bias	+	+
Treatment allocation and concealment	Risk of bias	+	-
Matched controls, matching criteria	Risk of bias	-	+
Similarity of groups at baseline	Risk of bias	**+**	**+**
Potential confounders	Risk of bias	**-**	**+**
Report number of individuals at each stage of study	Risk of bias	+	+
Reasons for non-participation	Risk of bias	+	+
intervention			
Explicit description of exposure	Indirectness	**+**	**+**
Blinding of care provider	Risk of bias	+	-
Avoidance of co-interventions	Risk of bias, indirectness	**+**	**+**
Define effect modifiers	Risk of bias	-	+
Acceptable compliance	Risk of bias	**+**	**+**
Blinding of patient	Risk of bias	+	-
Outcome measurement			
Blinding of outcome assessor	Risk of bias	+	-
Relevance of outcome measures	Indirectness	+	+
Description of adverse effects	Risk of bias	+	+
Description of withdrawal/drop-out rate	Risk of bias	**+**	**+**
Acceptable drop-out rate	Risk of bias	**+**	**+**
Timing follow-up measurement correct	Indirectness	**+**	**+**
Timing of outcome measurement in both groups comparable	Indirectness	**+**	**+**
Statistics			
Power analysis	Imprecision	**+**	**+**
Intention-to-treat analysis included	Risk of bias	+	-
Presentation of point estimated and measures of variability	Imprecision	**+**	**+**
Loss to follow up	Risk of bias	**+**	**+**
Sensitivity analysis	Risk of bias	-	+
Effort to address potential sources of bias	Risk of bias	-	+
Funding	Publication bias	+	+

Note 1: '+' item is required to be scored for the type of study, '-' item is not applicable for type of study.
*Note 2: **Bold** values are the prioritized items for the quality assessment of GRADE criteria (Table 1).*

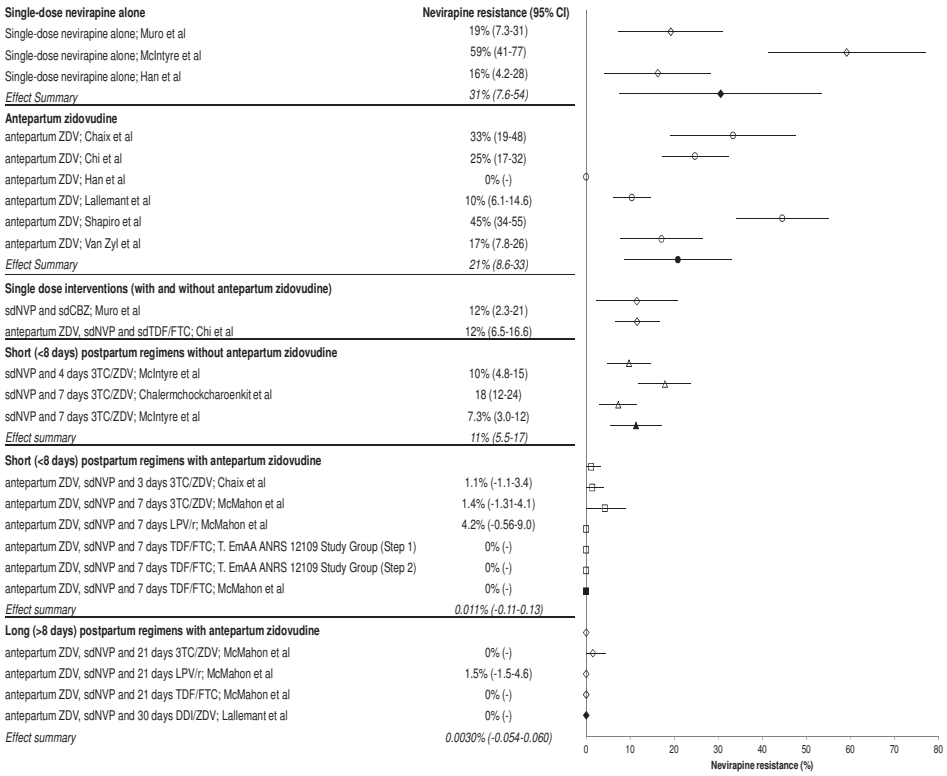

Figure 2. Forest plot of nevirapine resistance in each intervention arm.

antepartum zidovudine as part of their pMTCT prophylaxis. All women in these selected study arms received a short (<8 days) postpartum course of lamivudine/zidovudine. The summary effect of the proportion of nevirapine resistance without antepartum zidovudine was 11% (95% CI: 5.5-17). However, the effect of no antepartum zidovudine on nevirapine resistance differed between these three studies ($p<0.005$, $I^2=75\%$). The effect summary of the proportion of nevirapine resistance of the two studies using antepartum zidovudine was 1.2% (95% CI: -0.48-2.9) with no clear statistical presence of heterogeneity ($p>0.05$, $I^2=0\%$). Therefore, antepartum zidovudine shows a ten times lower prevalence of nevirapine resistance compared to postpartum interventions where antepartum zidovudine was not provided. However, quality of evidence for this question was low (1 point in the GRADE range of 0-3).

Effects short-course postpartum drug interventions

The effectiveness of three different short (<8 days) postpartum drug interventions on the occurrence of nevirapine resistance mutations was tested in women who received single-dose of nevirapine combined with antepartum zidovudine (Figure 2). Three of the six study arms used emtricitabine/tenofovir for 7 days, one used 7 days of ritonavir-boosted lopinavir and two arms used lamivudine/zidovudine either 3 or 7 days. The overall estimated proportion

Table 3. Characteristics of studies included in this systematic review

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Number of women with new resistance	Total number of women	Percentage of resistance
Muro et al[32]	2012	J Acquir Immune Defic Syndr	RCT	VITA-1	Tanzania	6 weeks postpartum	Sequencing	sdNVP and sdCBZ	6	52	11%
T. EmAA ANRS 12109 Study Group[34]	2010	AIDS	observational study		Cambodia, Côte d'Ivoire, South Africa	4 weeks postpartum	consensus technique of the AC11 ANRS Resistance group	**step 1:** antepartum ZDV and sdNVP at labor and 7 days TDF/FTC postpartum	0	37	0.0%
								step 2: antepartum ZDV and sdNVP at labor and TDF/FTC for 7 days postpartum	1	34	2.9%
Lallemant et al[35]	2010	Clin Infect Dis	observational study	PHPT-4	Thailand	7-120 days postpartum	population sequencing (HIV-1 ViroSeq Genotyping System, version 2.6)	antepartum ZDV and sdNVP at labor	23	222	10%
								antepartum ZDV and sdNVP at labor and ZDV/DDi during labor and 30 days postpartum	0	222	0.0%

Table 3. Characteristics of studies included in this systematic review (*continued*)

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Number of women with new resistance	Total number of women	Percentage of resistance
McIntyre et al[6]	2009	PLoS Med	RCT	TOPS	South Africa	2/6 weeks or both postpartum	(TruGEne HIV-1 genotyping kit and OpenGene DNA sequencing system, Bayer)	sdNVP and ZDV/3TC during labor and 4 days ZDV/3TC postpartum	15	154	9.7%
								sdNVP and ZDV/3TC during labor and 7 days ZDV/3TC postpartum	11	151	7.3%
Chalermchock-charoenkit et al[36]	2009	Clin Infect Dis	observational study	-	Thailand	1 month postpartum	TruGene HIV-1 genotyping assay (Bayer Healthcare)	sdNVP and ZDV/3TC during labor and 7 days ZDV/3TC postpartum	34	190	18%
J. Han et al[37]	2009	Int J STD AIDS	observational study	-	China	3 months postpartum	ABI 3730x1 DNA sequencer (applied Biosystems)	antepartum ZDV and sdNVP at labor	0	16	0.0%
Van Zyl et al[38]	2008	J Med Virol	observational study	-	South Africa	4-8 weeks postpartum	ABI 3130 DNA Sequencer	antepartum ZDV and sdNVP at labor	13	76	17%
Chi et al[39]	2007	Lancet	RCT	-	Zambia	6 weeks (and 2 weeks) postpartum	Viro Seq HIV-1 Genotypinh, Celera Diangnostics, Alameda, CA, USA	antepartum ZDV and sdNVP at labor	41	166	25%

Table 3. Characteristics of studies included in this systematic review (*continued*)

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Number of women with new resistance	Total number of women	Percentage of resistance
Shapiro et al[40]								antepartum ZDV and sdNVP plus sdTDF/FTC at labor	20	173	12%
	2006	AIDS	RCT	-	Botswana	1 month postpartum	ViroSeq HIV-1 Genotyping System (Applied Biosystems, Foster City, CA, USA)	antepartum ZDV and sdNVP at labor	69	155	45%
Chaix et al[41]	2006	Journal of Infectious Diseases	observational study	SIDA DITRAME Plus Study Group	Cote d'Ivoire	week 4 postpartum	ANRS consensus technique	antepartum ZDV and sdNVP at labor	21	63	33.3%
								anterpartum 3TC/ZDV and sdNVP plus 3TC/ZDV at labor and 3 days 3TC/ZDV postpartum	1	88	1.1%
McMahon et al[27]	2013	Clin Infect Dis	RCT	-	Sub-Saharan Africa, India, Haiti	6 weeks after completion of study treatment	ViroSeq HIV-1 Genotyping System (Aversion 2.0, Celera Diagnostics, Alameda, CA, USA)	antepartum ZDV and sdNVP at labor and 7 days 3TC/ZDV postpartum	1	73	1.4%

Table 3. Characteristics of studies included in this systematic review (*continued*)

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Number of women with new resistance	Total number of women	Percentage of resistance
								antepartum ZDV and sdNVP at labor and 21 days 3TC/ ZDV postpartum	0	67	0.0%
								antepartum ZDV and sdNVP at labor and 7 days TDF/ FTC postpartum	0	71	0.0%
								antepartum ZDV and sdNVP at labor and 21 days TDF/ FTC postpartum	0	65	0.0%
								antepartum ZDV and sdNVP at labor and 7 days LPV/r postpartum	3	71	4.2%
								antepartum ZDV and sdNVP at labor and 721days LPV/r postpartum	1	65	1.5%
Micek et al[42]	2012	J Infect Dis	observational prospective cohort study	-	Mozambique	2 specimens 2-8 weeks postpartum	Oligonucleotide ligation assay (OLA)	antepartum ZDV and sdNVP at labor	4	10	40%
								antepartum ZDV and sdNVP at labor and 7 days ZDV postpartum	7	21	33%

Table 3. Characteristics of studies included in this systematic review (*continued*)

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Number of women with new resistance	Total number of women	Percentage of resistance
Hauser et al[43]	2012	PLoS One	observational study	-	Tanzania	Week 1-2, Week 4-6 and Week 12-16 postpartum	highly sensitive allele-specific PCR (ASPCR) assay	antepartum ZDV and sdNVP at labor and 7 days 3TC/ ZDV postpartum	6	50	12%
Palmer et al[44]	2012	Antivir Ther	Random selection of samples	TOPS	South Africa	6 weeks postpartum	allele specific real-time PCR (ASP)	sdNVP at labor and 4 days 3TC/ZDV postpartum	7	24	29%
								sdNVP at labor and 7 days 3TC/ZDV postpartum	8	24	33%
Van Dyke et al[45]	2012	Clin Infect Dis	RCT	IMPAACT P1032	Thailand	At day 10 or week 6 postpartum	detects mutation 20-50% of the viral population and if negative by optimized for HIV subtype AE >5 % of the viral population	antepartum ZDV and sdNVP at labor	15	112	13%
								antepartum ZDV and sdNVP at labor and ZDV/DDi/LPV/r during labor and 7 days postpartum	0	56	0.0%
								antepartum ZDV and sdNVP at labor and ZDV/DDi during labor and 30 days postpartum	0	56	0.0%

Table 3. Characteristics of studies included in this systematic review (*continued*)

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Number of women with new resistance	Total number of women	Percentage of resistance
								antepartum ZDV and sdNVP at labor and ZDV/ DDi/LPV/r during labor and 30 days postpartum	0	57	0.0%
Farr et al[46]	2010	J Acquir Immune Defic Syndr	observational study	BAN	Malawi	6 weeks post partum	population and sensitive real-time PCR	sdNVP and 7 days ZDV/3TC postpartum	4	40	10%
Lallemant et al[35]	2010	Clin Infect Dis	observational study	PHPT-4	Thailand	7-120 days postpartum	OLA	antepartum and plus sdNVP at labor	42	222	19%
								antepartum ZDV and sdNVP at labor and ZDV/ DDi during labor and 30 days postpartum	4	222	1.8%
Chi et al[47]	2009	AIDS Res Hum Retroviruses	RCT	-	Zambia	6 weeks (and 2 weeks) postpartum	OLA	antepartum ZDV and sdNVP at labor	66	160	41%
								antepartum ZDV and sdNVP plus sdTDF/FTC at labor	29	155	19%
Lehman et al[48]	2009	J Acquir Immune Defic Syndr	RCT	-	Kenya	3 months postpartum	allele specific real-time PCR (ASP)	antepartum ZDV and sdNVP at labor	12	16	75%

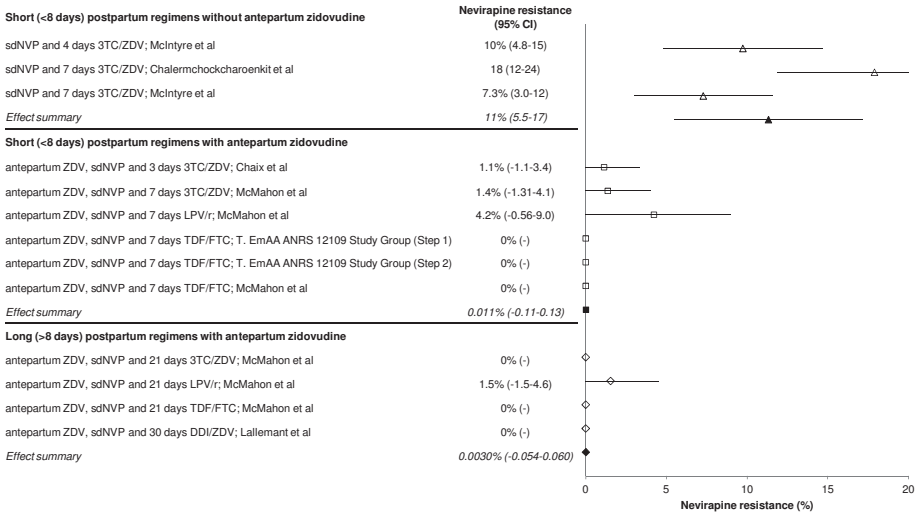

Figure 3. Forest plot of nevirapine resistance in short and long postpartum antiretroviral strategies.

of nevirapine resistance in patients who received a short postpartum drug intervention using the random effects model was 0.011% (95% CI: -0.11-0.13) with no significant difference ($p>0.05$, $I^2=0\%$) between the five studies, although the GRADE score for quality of evidence for these studies was very low (0 points in the range of 0-3).

Duration of long-course postpartum drug interventions

In four study arms postpartum drugs were provided for a longer time period (Figure 2): one study arm provided 21 days of lamivudine/zidovudine, or ritonavir/boosted lopinavir, or tenofovir/emtricitabine and one study provided didanosine/zidovudine to the women for 30 days. The estimated pooled proportion of nevirapine resistance was 0.0030% (95% CI: -0.054-0.060) with no clear evidence ($p>0.05$, $I^2=0\%$) that these four studies differed. Comparing these data to short postpartum drug interventions, nevirapine resistance of longer postpartum drug regimen appeared to be slightly lower with moderate quality of evidence (2 points in the GRADE range of 0-3). Ritonavir-boosted lopinavir appeared to be less effective, since it has shown higher nevirapine resistance in both short and long postpartum courses compared to other regimens, but this could not be statistically confirmed.

DISCUSSION

The present systematic review shows that the summary effect of nevirapine resistance through postpartum drug regimens combined with antepartum zidovudine and intrapartum nevirapine has been estimated to be <1%. A postpartum regimen of 20-30 days might be slightly more effective compared to a short (<8 days) postpartum regimen with a prevalence of nevirapine resistance of 0.003% versus 0.011%, respectively.

Nevirapine resistance was estimated to be 31% after single-dose exposure of nevirapine alone. However, the number of study arms for this pooled estimate was small, as we included only studies containing drug interventions to reduce nevirapine resistance after single-dose exposure in our systematic review. Nevirapine resistance of single-dose nevirapine combined with antepartum zidovudine was ~30% lower to 21%. Despite of small numbers, these values are consistent with previous studies showing 20-69% nevirapine resistance and an estimated pooled value of 36% in mothers exposed to single-dose with or without antepartum zidovudine[2, 21-26]. Addition of only a seven-day course of ART to antepartum zidovudine and single-dose of nevirapine has shown an extensive effect in decreasing nevirapine resistance from 21% to 0.011%, probably due to the effect in suppressing viral replication of the seven-day postpartum antiretroviral course. Almost all strategies evaluated in this systematic review were supplemental postpartum antiretroviral therapies intending to suppress viral replication until nevirapine concentrations are no longer sufficient to select for resistant strains, since the lengthy duration of low and subtherapeutic levels of nevirapine in blood are plausibly associated with increases in nevirapine resistance. The estimated pooled effect was nearly 0% and most effective by protecting the subtherapeutic nevirapine 'tail' when single-dose nevirapine was combined with antepartum zidovudine and a long course postpartum regimen. This is consistent with a recent study of McMahon *et al* evaluating long versus short course antiretroviral strategies. Noteworthy, they contributed largely in the research question of our review, their findings showed that 21-day regimens were significantly better than seven-day regimens[27].

The estimated pooled effects in many of the evaluated interventions of our systematic review have shown high heterogeneity. An important reason for this could be a difference in baseline plasma HIV-1 RNA viral load. Previous studies have shown that higher baseline plasma HIV-1 RNA viral load was associated with increase in the proportion of nevirapine resistance [2, 28], probably due to higher viral load when single-dose nevirapine is used. Also study location, which is associated with the presence of difference in viral subtypes, could have played an important role in the high heterogeneity we found in our analyses [2].

A limitation in our systematic review is that the number of studies was small, especially for the quantification of effect summaries. We also did not calculate proportions related to single-dose nevirapine alone, but our data were consistent compared with previous reviews [1, 2]. We also did not statistically assess potential sources of between-study heterogeneity; variability between studies was high and factors of heterogeneity have been evaluated in an earlier study [2]. Strength of our study was our validated analysis using the GRADE approach to determine the quality of evidence, which was generally low, at evaluating our research questions.

Simple and standardized pMTCT regimens (Option B/B+) need widespread implementation in resource limited settings. Due to the challenges in the scale-up of pMTCT programs, thousands of women are still using a single-dose nevirapine alone in pMTCT of HIV[29]. Ideally, based on our findings, each HIV-infected pregnant woman living in a resource-limited region on single-dose nevirapine at labor onset should combine this with antepartum zidovudine plus a postpartum antiretroviral strategy of preferably two nucleoside reverse transcriptase inhibitors, which have shown to nearly eliminate nevirapine resistance. A major disadvantage of these long-course

drug regimens, however, is its complexity and consequently risk to non-adherence, but also the emergence of lamivudine resistance (due to long intracellular elimination half-life), which is associated with virological and clinical failure in women starting lamivudine-containing ART for their own health[30, 31]. The use of shorter postpartum regimens (WHO option A[8]) will therefore still be advocated, but might need to be intensified by adding other drugs to achieve the smallest possible effect (i.e. elimination) on nevirapine resistance. A single-dose[32] or a seven-day course of an enzyme inducer[33] at labour onset might have significant additional benefits in reducing selection of nevirapine resistance mutations, even on top of the current antiretroviral prophylaxis "tail". These and other regimens need urgent evaluation.

Our findings have shown that the optimal management to nearly eliminate nevirapine resistance after single-dose exposure is the addition of antepartum zidovudine plus a 20-30 days course of antiretroviral drugs postpartum, while a seven-day postpartum course already have shown a low prevalence of nevirapine resistance <1%. Given its complexity and the current challenge in the scale-up of pMTCT programs, the WHO guideline of option A should be followed to reach the overarching goal of the WHO to reduce eliminate HIV transmission from pMTCT. Intensification of the current postpartum courses or alternative regimens needs to be evaluated to achieve the best feasible minimum risk of nevirapine resistance in regions where single-dose nevirapine is still being used.

SUPPLEMENTARY MATERIALS S1

Search strategies for the MEDLINE (PubMed) database, the Embase database and the Cochrane library:

Search strategy for the MEDLINE (PubMed) database:

("Pregnancy"[Mesh] OR "Pregnant Women"[Mesh] OR pregnancy[tiab] OR pregnant[tiab] OR labor[tiab] OR labour[tiab] OR intrapartum[tiab] OR delivery[tiab] OR "Infectious Disease Transmission, Vertical"[Mesh] OR Vertical Infectious Disease Transmission[tiab] OR Vertical Infection Transmission[tiab] OR Vertical Transmission of Infectious Disease[tiab] OR Maternal-Fetal Infection Transmission[tiab] OR Maternal Fetal Infection Transmission[tiab] OR mother-to-child transmission[tiab] OR mother-to-child-transmission[tiab] OR PMTCT[tiab] OR mtct[tiab] OR Mother To Child Transmission[tiab] OR prevention-of-mother-to-child-transmission[tiab]) AND ("Nevirapine"[Mesh] OR Nevirapine[tiab] OR viramune[tiab] OR nvp[tiab] OR sdnvp[tiab]) AND ("Drug Resistance, Viral"[Mesh] OR Resistance[tiab] OR resistant[tiab])

Search strategy for the Embase database:

exp pregnancy/ or labor onset/ or labor onset/or (pregnancy or pregnant or labor or labour or intrapartum).ti,ab. or delivery.ti,ab. Or exp vertical transmission/or(Vertical Infectious Disease Transmission or Vertical Infection Transmission or Vertical Transmission of Infectious Disease or Maternal-Fetal Infection Transmission or Maternal Fetal Infection Transmission or mother-to-child transmission or mother-to-child-transmission or PMTCT or mtct or Mother To Child Transmission or prevention-of-mother-to-child-transmission).ti,ab. Or vertical transmission.ti,ab. And exp nevirapine/ or (Nevirapine or viramune or nvp or sdnvp).ti,ab. And exp antiviral resistance/ resistance.ti,ab. resistant.ti,ab.

Search strategy for the Cochrane library:

MeSH descriptor: [Nevirapine] explode all trees or nevirapine or viramune:ti,ab,kw (Word variations have been searched) NVP:ti,ab,kw (Word variations have been searched or sdnvp:ti,ab,kw (Word variations have been searched) and MeSH descriptor: [Drug Resistance, Viral] explode all trees or resistance or resistant:ti,ab,kw (Word variations have been searched)

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THERAPEUTIC DRUG MONITORING OF NEVIRAPINE IN DEVELOPING COUNTRIES

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6

THERAPEUTIC DRUG MONITORING OF NEVIRAPINE IN RESOURCE-LIMITED SETTINGS

Clinical Infectious Diseases 2008; 47:1339–44

ABSTRACT

Background

We developed a simple and inexpensive thin-layer chromatography (TLC) assay for semiquantitative detection of saliva concentrations of nevirapine in resource-limited settings. The method was validated in an African target population.

Methods

Paired plasma and saliva nevirapine concentrations were assayed by high-performance liquid chromatography (HPLC); saliva concentrations of nevirapine were also assayed by TLC. The rate of false-positive results was the proportion of subtherapeutic nevirapine saliva and plasma concentrations determined by HPLC that were judged to be therapeutic in saliva specimens by TLC. The rate of false-negative results was the proportion of therapeutic nevirapine saliva and plasma concentrations determined by HPLC that were judged to be subtherapeutic in saliva specimens by TLC. The extent of agreement in TLC readings between 5 technicians and 2 batches of TLC sheets was evaluated.

Results

Twenty-five (9%) of 286 African adults had a subtherapeutic plasma nevirapine concentration. The median ratio of nevirapine concentrations in saliva to those in plasma was 0.51:1. The rate of false-positive results for TLC was 0% (0 of 23 specimens) when TLC results were compared with HPLC results for saliva specimens and 8% (2 of 25 specimens) when TLC results were compared with HPLC results for plasma specimens. The rate of false-negative results for TLC was 1% (3 of 263 specimens) when TLC results were compared with HPLC results for saliva specimens and 1% (3 of 261 specimens) when TLC results were compared with HPLC results for plasma specimens. The extent of agreement of TLC results was substantial for the 5 technicians (Fleiss's kp0.77) and for the 2 batches of sheets (Cohen's kp0.80).

Conclusions

The TLC assay was found to be sensitive, specific, and robust in the detection of subtherapeutic nevirapine concentrations in saliva specimens obtained from African HIV-infected adults. It is an attractive alternative to HPLC for therapeutic drug monitoring of nevirapine in resource-limited settings.

INTRODUCTION

Nevirapine is widely prescribed in combination with nucleoside reverse-transcriptase inhibitors for the treatment of HIV infection in resource-limited countries. Adequate plasma concentrations of nevirapine are required to achieve a successful response, whereas subtherapeutic concentrations (defined as concentrations <3.0 mg/L) are related to development of mutations and virological failure [1–3]. Even in the case of perfect adherence to regimens at standard doses, some patients will remain at risk for underdosing because of interpatient variability in nevirapine exposure or drug-drug interactions. In the developed world, therapeutic drug monitoring (TDM) [3] is a well-known tool for the optimization of nevirapine dosing in HIV-infected patients. As a result of a lack of simple and affordable methods to determine the level of exposure to nevirapine, TDM is hardly ever performed in resource-limited settings. Previous studies have suggested that saliva specimens may be used as an alternative body fluid sample for TDM of nevirapine [4, 5]. Saliva concentrations of nevirapine in HIV-infected [4] and healthy [5] white persons are approximately one-half of the values observed in plasma specimens obtained from such persons. We recently developed a thin-layer chromatography (TLC) method to provide a simple and economical tool for semiquantitative measurement of the saliva concentration of nevirapine. The primary objective of this study was to validate our newly developed TLC method for TDM of nevirapine concentrations in saliva samples obtained from HIV-infected Africans. Secondary objectives were to determine the relationship between saliva and plasma nevirapine concentrations and the proportion of subtherapeutic nevirapine concentrations in an African population.

METHODS

Study population

Three hundred HIV-infected adults who had been receiving a regimen that contained nevirapine for at least 4 weeks were eligible for enrollment at a routine visit to the adult HIV clinic of the Kilimanjaro Christian Medical Centre (Moshi, Tanzania). Patients who had oral lesions or ulcers and those who were unable to self-report the date and time of the most recent ingestion of nevirapine were excluded from the study. The protocol was reviewed and approved by the Ethics Committee of Tumaini University (Moshi). Written informed consent was obtained from all subjects before enrollment.

Sample collection and drug concentration assays

At a routine visit to the clinic, unannounced paired blood and saliva samples were collected within 5 min of each other. Stimulated saliva specimens were obtained with a salivette (Sartstedt; Etten- Leur) using a dental cotton roll impregnated with citric acid (20 mg), which stimulates the salivary flow. Study subjects were asked to chew on the roll for ~ 1 min. Blood and saliva samples were stored at 2°C–8°C for a maximum of 8 h. Plasma was separated and stored at -80°C until transportation to The Netherlands on dry ice. Saliva was obtained by centrifugation of the cotton roll at 800 *g* for 10 min. Two aliquots were stored at - 80°C: one for analysis by TLC in Tanzania, and one for transportation to The Netherlands and analysis by high-

performance liquid chromatography (HPLC). Saliva and plasma concentrations of nevirapine were determined at the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) using validated HPLC assays with UV detection, as modified from a method described by Hollanders et al. [6]. Samples were pretreated as described by Hollanders and colleagues, and preparation of saliva samples did not differ from preparation of plasma samples. In brief, 150 µL mL of saliva or plasma was mixed with 150 µL mL of perchloric acid, vortexed for 20 s, and centrifuged for 5 min at 12,175 *g*. Subsequently, 200 mL of the clear supernatant was transferred to insert vials and placed in the auto sampler. The lower and upper limits of quantification of the modified assays were 0.167 and 16.7 mg/L, respectively, for plasma and 0.158 and 15.8 mg/L, respectively, for saliva. The intraday precision of the assays ranged from 0.4% to 3.2% for plasma and from 1.3% to 4.1% for saliva. Additional variation as a result of performing the assays on different days ranged from 0.0% to 0.4% for plasma and from 0.8% to 2.2% for saliva. The accuracy of the assays ranged from 102% to 105% for plasma and from 99% to 102% for saliva. Ratios of saliva nevirapine concentration to plasma nevirapine concentration and the proportion of subjects with a subtherapeutic nevirapine plasma concentration (i.e., <3.0 mg/L) [3] were determined.

In addition, saliva concentrations of nevirapine were semi-quantitatively analyzed at the Biotechnology Laboratory of Kilimanjaro Christian Medical Centre using a newly developed TLC method.

Experimental TLC method

A reference solution of nevirapine (Viramune; Boehringer Ingelheim), 1.75 mg/L, was obtained by dilution of a stock solution (nevirapine, 0.875 mg/mL, in dimethylsulfoxide [DMSO]; Merck) in blank saliva (i.e., saliva without nevirapine or any other drug). Both were kept at -80°C. The reference solution and saliva samples were thawed at room temperature, mixed for 1 min, and centrifuged at 9000*g* for 1 min. Of the reference and samples, 1.0 mL was transferred into a 10-mL glass test tube. After addition of 0.5 mL of 0.2 M ammonia (diluted from 25% ammonia; Labopharma) and 5 mL tert-butylmethylether (Riedel–de Haën), tubes were closed, mixed for 1 min, centrifuged at 1150 *g* for 5 min, and stored at -80°C until the lower layer was frozen completely (20 min). The organic layer was poured into a 10-mL glass test tube and was dried in 2 days. An eluent (or mobile phase) was prepared (ratio of toluene to ethyl acetate, 1:1; ACME Chemicals and Unilab, respectively) and poured into a TLC container. The container was closed and left for 1 h. After addition of 50 mL of methanol to the dried test tubes, they were closed and mixed for 1 min. One mL of the reference and of the samples was slowly pipetted in small, dense dots at least 1.5 cm from the side and 2.0 cm from the bottom of a silica gel TLC sheet (height, 10.0 cm; width, 20.0 cm; Merck), as shown in figure 1. After drying, spots were checked for similarity of size and shape under UV light (254 nm; CAMAG 022.9120). The TLC sheet was placed in the container with eluent for 12 min (figure 2).

It was marked for the distance that the eluent had moved and was dried in the air. The intensity of the spots was determined under UV light and compared with the reference spot (figure 3). A spot that was less intense than the reference was considered to be subtherapeutic. A spot that was comparable to or more intense than the reference spot was considered to be therapeutic.

Figure 1. Preparation of thin-layer chromatography sheet with saliva samples.

Technical validation of TLC method

All saliva samples were used to determine the technical sensitivity and specificity of the TLC method. The readings were performed by 1 technician. Saliva nevirapine concentrations determined by HPLC were defined as subtherapeutic if they were <1.5 mg/L, which is one-half of the cutoff value for plasma specimens (<3.0 mg/L) [3]. The rate of false-positive results was defined as the proportion of subtherapeutic saliva concentrations of nevirapine (i.e., < 1.5 mg/L), as determined by HPLC [6], that were reported to be therapeutic on the basis of TLC of saliva specimens.

The rate of false-negative results was defined as the proportion of therapeutic saliva concentrations of nevirapine (≥1.5 mg/L), as determined by HPLC [6], that were reported to be subtherapeutic on the basis of TLC of saliva specimens. Twenty-five saliva samples with

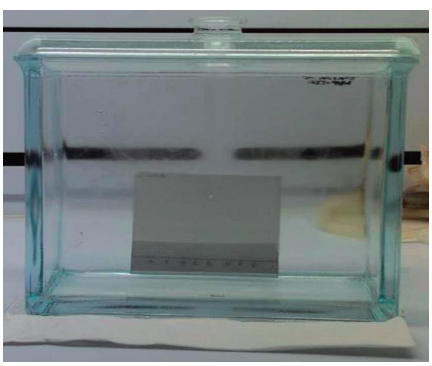

Figure 2. Thin-layer chromatography sheet in container with eluent.

Figure 3. Determination of spot intensity under UV light.

nevirapine concentrations determined by HPLC [6] closest to the TLC reference value of 1.75 mg/L were selected to test the robustness of the TLC method. The extent of agreement in TLC results between 5 different technicians (Fleiss's k [7]) and 2 different batches of TLC sheets (Cohen's k [8]) was evaluated in these 25 saliva samples. The stability of the stock solution (nevirapine in DMSO) was tested after storage at -40°C for 50 months. The stability of the reference solution (nevirapine in blank saliva) was tested after storage at room temperature for 17 h, after storage at - 40°C for 2 months, and after freezing and thawing twice. Interference of possible concurrently used medications was evaluated by comparison of the retention factor (i.e., the distance travelled by compound divided by distance travelled by eluent front) of extractable and detectable (UV light; 254 nm) compounds with the retention factor value of nevirapine. In addition, blank saliva samples obtained from at least 6 subjects who had not been taking nevirapine were tested for interference by endogenous substances.

Biological validation of TLC method

All plasma and saliva samples were used to determine the biological sensitivity and specificity of the TLC method. The rate of false-positive results was defined as the proportion of subtherapeutic nevirapine plasma concentrations (<3.0 mg/L [3]) determined by HPLC [6] that were reported to be therapeutic on the basis of TLC of saliva specimens. The rate of false-negative results was defined as the proportion of therapeutic nevirapine plasma concentrations (≥3.0 mg/L [3]) determined by HPLC [6] that were reported to be subtherapeutic on the basis of TLC of saliva specimens.

RESULTS

Of the 300 subjects enrolled in the study, 14 were excluded because of problems with labelling, because the saliva volume was too small, or because the saliva specimen was lost. The mean age of the remaining 286 African subjects (200 of whom were women) was 41 years (range, 17–71 years). All subjects were treated with a nevirapine dosage of 200 mg twice per day, in combination with lamivudine and either stavudine or zidovudine. The mean plasma concentration of nevirapine, as determined by HPLC, was 7.35 mg/L (range, <0.167 to 28.59 mg/L). Twenty-five (9%) of 286 subjects had a sub-therapeutic plasma nevirapine concentration (i.e. <3.0 mg/L); 18 of 25 had a concentration that was less than the detection limit of the HPLC assay (i.e. <0.15 mg/L). The median ratio of saliva nevirapine concentration to plasma nevirapine concentration, as determined by HPLC, was 0.51:1 (interquartile range, 0.47:1–0.54-:1), and the median time from the last ingestion of medication and to sample collection was 3.40 h (interquartile range, 2.52– 4.37 h). Six subjects had a ratio of saliva nevirapine concentration to plasma nevirapine concentration 11.00 (range, 1.25– 15.68); the median time from last ingestion of medication to sampling for these 6 subjects was 1.19 h (interquartile range, 0.85–1.74 h). None of the 23 saliva specimens that were found to contain subtherapeutic concentrations of nevirapine (i.e.,<1.5 mg/L) by HPLC were reported to have therapeutic values by TLC (rate of false-positive results, 0%); the technical sensitivity of the TLC method was 100% (table 1). Three of the 263 saliva specimens that were found to have therapeutic concentrations of nevirapine (i.e., ≥1.5 mg/L; 1.62, 1.74, and 1.96 mg/L) by HPLC were reported to have subtherapeutic values by TLC (rate of false-negative results, 1%); the technical specificity of the TLC method was 99% (table 1).

Table 1. Number of patients (with (sub) therapeutic nevirapine concentrations according to thin-layer chromatography (TLD), compared with according to high-performance liquid chromatography (HPLC), and sensitivity and specificity of the TLC assay

	TLC of saliva specimens No. of results			
Specimen, concentration determined by HPLC	Subtherapeutic concentration	Therapeutic concentration	Sensitivity, %	Specificity, %
Saliva				
Subtherapeutic concentration	23	0	100	...
Therapeutic concentration	3	260	...	99
Plasma				
Subtherapeutic concentration	23	2	92	...
Therapeutic concentration	3	258	...	99

NOTE. Subtherapeutic concentrations of nevirapine were defined as <1.75 mg/L for TLC of saliva specimens, <1.5 mg/L for HPLC of saliva specimens, and <3.0 mg/L for HPLC of plasma specimens. Therapeutic concentrations were defined as values greater than these cutoff values

The extent of agreement among TLC results (i.e., subtherapeutic or therapeutic nevirapine saliva concentrations) for 25 selected samples with a concentration closest to the reference sample was substantial for the 5 technicians (Fleiss's k, 0.77) and 2 batches of TLC sheets (Cohen's k, 0.80).

The stock solution for the TLC method (nevirapine in DMSO) was stable at - 40°C for at least 50 months (mean rate of recovery ± SD, 99.7% ± 0.3%). The reference solution (nevirapine in blank saliva) was stable at room temperature for at least 17 h (mean rate of recovery ± SD, 107.6% ± 5.8%) and at - 40° C for at least 2 months (mean rate of recovery ± SD, 94.3% ± 3.2%). Freezing and thawing twice had no effect on the stability of nevirapine in blank saliva (mean rate of recovery ± SD, 95.1% ± 1.1%). The mean nevirapine retention factor value was 0.29; none of the possible comedications that were extractable and detectable had a similar retention factor value. Also, no interference by endogenous substances was observed in the blank saliva samples obtained from subjects not taking nevirapine. Two of the 25 plasma samples found to have subtherapeutic nevirapine concentrations (<3.0 mg/L; 2.61 and 2.91 mg/L) by HPLC were reported to have therapeutic concentrations byTLC (rate of false-positive results, 8%); the biological sensitivity of the TLC method was 92% (table 1). Three of the 261 plasma samples found to have therapeutic nevirapine concentrations (≥ 3.0 mg/L; 3.41, 3.52, and 4.28 mg/L) by HPLC were reported to have subtherapeutic concentrations by TLC (rate of false negative results, 1%); the biological specificity of the TLC method was 99% (table 1).

DISCUSSION

Twenty-five (9%) of 286 HIV-infected African adults had a subtherapeutic plasma concentration of nevirapine (i.e., <3.0 mg/L) [3]. Saliva nevirapine concentrations were approximately one-half of the values observed in plasma specimens. A TLC method for semiquantitative detection of

nevirapine in saliva specimens was found to be sensitive, specific, and robust. This simple and economical tool is a good option for TDM of nevirapine in resource-limited settings. Use of saliva specimens instead of plasma specimens for TDM of nevirapine implies painless and noninvasive sampling, a diminished risk of HIV transmission to health care workers, and a lower cost [4]. TLC is a relatively inexpensive assay technique, compared with HPLC [9], which is commonly used in the developed world for TDM of nevirapine in plasma samples. An HPLC system for TDM of nevirapine costs approximately €40,000, and the costs for consumables per sample are estimated to be €15. In contrast, the estimated initial setup cost of a new TLC method for semiquantitative measurement of nevirapine in saliva is €800, and the cost of consumables per sample is approximately €1.60. Because this simple and inexpensive method was developed to perform TDM of nevirapine in resource-limited settings, it was validated in an African target population in our study. The percentage of HIV-infected adults in our Tanzanian population (9%) with a subtherapeutic plasma concentration of nevirapine [3], as determined by HPLC [6], was lower than the percentage in a previous report from Malawi (16%) [10]. This may be associated with the unreliable drug supply during the time of the Malawian study (2003), which explained more than one-third of the total reported nonadherence to treatment [10]. The fact that 18 of the 25 subtherapeutic nevirapine plasma concentrations in our Tanzanian population were undetectable indicates nonadherence to treatment, because nevirapine is generally detected up to weeks after termination of drug intake because of its long elimination half-life [11]. The median ratio of saliva nevirapine concentration to plasma nevirapine concentration of 0.51:1, as determined by HPLC [6], in our population of HIV-infected Africans was comparable to the ratios observed in HIV-infected [4] and healthy [5] white persons. It has been suggested that thorough rinsing of the mouth is required before saliva sampling, because remnants of orally administered medicines may contaminate saliva specimens and yield spuriously high values [12]. Indeed, 6 of 286 subjects in our study had an unexpectedly high ratio of saliva to plasma nevirapine concentration of >1:1 [4,12]; this may be because the subjects had chewed tablets without rinsing the mouth before samples were obtained. This explanation is supported by our observation that the time between last ingestion and sampling was shorter for subjects with unexpectedly high nevirapine concentrations in saliva specimens, compared with concentrations in plasma specimens. Because the saliva nevirapine concentrations determined by HPLC were approximately one-half of the values observed in plasma specimens, they were defined as subtherapeutic if they were <1.5 mg/L rather than if they were less than the cutoff value for plasma (i.e., 3.0 mg/L) [3]. The main requirement of our TLC method for semiquantitative detection of nevirapine in saliva specimens was appropriate sensitivity, meaning detection of nevirapine concentrations that were found to be subtherapeutic by HPLC, to pick out subjects at risk for nevirapine resistance. During the development of our TLC method, subtherapeutic saliva concentrations slightly less than 1.5 mg/L were sufficiently distinguishable from a TLC reference value of 1.75 mg/L but not from a reference value of 1.5 mg/L. Therefore, for the validation of our TLC method, we chose a reference value of 1.75 mg/L, which led to excellent technical sensitivity (100%) and specificity (99%). None of the subtherapeutic nevirapine saliva concentrations found by HPLC were reported to be therapeutic by TLC, and the few therapeutic saliva concentrations that were reported to be subtherapeutic by TLC were all close to the cutoff

value for saliva (1.5 mg/L). Biological validation compared saliva concentrations of nevirapine determined by TLC with plasma concentrations determined by HPLC (either subtherapeutic or therapeutic levels), finding acceptable sensitivity (92%) and excellent specificity (99%). False-positive and false-negative results were all close to the cutoff value for plasma (3.0 mg/L). The somewhat lower biological sensitivity can be explained by the fact that some subjects with a subtherapeutic plasma concentration determined by HPLC had a therapeutic saliva concentration according to both HPLC and TLC. Although these subjects had a higher ratio of saliva to plasma nevirapine concentration than average, none of them had a ratio >1:1. As expected, it was found to be most difficult to interpret nevirapine saliva concentrations that were closest to the TLC reference value. To test the worst-case scenario robustness of the TLC method, we selected 25 samples closest to the reference value. The fact that the extent of agreement in TLC results between 5 technicians and 2 batches of TLC sheets was substantial provides an indication that the outcome of our TLC method is reliable during normal use.

We are planning to roll out the TLC method in various African countries and in Indonesia. This will make it possible to continue to evaluate the method's robustness and its practical application to adherence monitoring. Potentially, the assay could be further developed commercially.

In conclusion, we developed a simple and economical TLC method for semiquantitative determination of saliva concentrations of nevirapine. The assay was found to be sensitive, specific, and robust for detection of subtherapeutic nevirapine concentrations in an African population. It is an attractive alternative to HPLC for TDM of nevirapine in resource-limited settings.

ACKNOWLEDGMENTS

We thank the patients for their participation in this study. Laizer Leshoki Saitory of the Kilimanjaro Christian Medical Centre is kindly acknowledged for the screening of study subjects. The study nurses at Kilimanjaro Christian Medical Centre are thanked for the collection of study samples.

FINANCIAL SUPPORT

Poverty Related Infection Oriented Research, a research network sponsored by The Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO) and The Netherlands Foundation for Health Research and Development (ZonMw).

POTENTIAL CONFLICTS OF INTEREST

All authors: no conflicts.

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7

NEVIRAPINE CONCENTRATIONS IN SALIVA MEASURED BY THIN LAYER CHROMATOGRAPHY, AND SELF-REPORTED ADHERENCE IN PATIENTS ON ANTI-RETROVIRAL THERAPY AT KILIMANJARO CHRISTIAN MEDICAL CENTRE, TANZANIA

Therapeutic Drug Monitoring. 2013 Dec 3. [Epub ahead of print]

ABSTRACT

Background

Thin layer chromatography (TLC) can be used to perform therapeutic drug monitoring in resource-limited settings, where more expensive analytical methods such as high-performance liquid chromatography (HPLC) or liquid chromatography mass-spectrometry (LC-MS) are not feasible.

Objectives

In this cross-sectional study, we aimed at comparing saliva concentrations of nevirapine (NVP) with self-reported adherence, in patients on NVP-containing anti-retroviral (ARV) treatment at Kilimanjaro Christian Medical Centre, Moshi, Tanzania.

Methods

We included HIV-infected patients using a combination of zidovudine + lamivudine + NVP, or stavudine + lamivudine + NVP, for more than four weeks. Saliva samples were collected using dental cotton rolls impregnated with citric acid (20 mg). Saliva NVP concentrations were analyzed using TLC. Adherence to ARV medication was assessed by self-reporting using the Morisky scale.

Results

Seventy nine (86.8%) of 91 study participants had therapeutic saliva NVP concentrations (i.e. >1.75 mg/L) and 12 (13.2%) had sub-therapeutic concentrations. Self-reported adherence among the study participants was high in 62 (68.1%), moderate in 24 (26.4%), and low in 5 (5.5%). Fifty seven (91.9%) of the study participants with high self-reported adherence had therapeutic saliva NVP concentrations. Of the 5 participants with low self-reported adherence, three had therapeutic NVP concentrations.

Conclusions

A high proportion of patients had therapeutic NVP saliva concentrations as measured by TLC, which showed good agreement with self-reported adherence.

INTRODUCTION

In Tanzania, antiretroviral (ARV) drugs are given free of charge to eligible HIV-infected patients. By the end of 2010 about 42% of HIV infected patients were receiving ARV treatment.[1] Nevirapine (NVP) is one of the ARV drugs belonging to the class of non-nucleoside reverse transcriptase inhibitors (NNRTI). According to Tanzania National Guidelines for the management of HIV and AIDS (2009)[2], NVP is used as a first-line drug combined with two nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine (AZT) and lamivudine (3TC), or stavudine (d4T) and 3TC, or stavudine (d4T) with tenofovir and emtricitabine.

Adequate plasma concentrations of NVP are required to achieve a successful response, whereas sub-therapeutic concentrations, defined as concentrations below 3.0 mg/L, are related to development of mutations and virological failure.[3-7]

Studies have shown that among patients who are highly adherent to regimens at standard doses, some will still remain at risk of under exposure because of inter-patient variability in NVP concentrations, or due to drug-drug interactions.[8] Studies from both resource-rich and resource-limited settings have repeatedly demonstrated that high levels of ARV adherence are associated with better immunological and virological outcomes, decreased risk of developing AIDS-defining illnesses, and improved survival.[9] A study in Malawi found that 148 (84%) of study patients had NVP plasma concentrations in the therapeutic range or higher, and that those with sub-therapeutic concentrations (16%) had a six fold increased risk of developing virological failure as compared with those with therapeutic concentrations.[10] A study conducted at Kilimanjaro Christian Medical Centre (Moshi, Tanzania) found that 25 (9%) of 286 African adults had sub-therapeutic plasma NVP concentrations.[11]

Saliva can provide a rapid and non-invasive sample for NVP compliance testing, direct measurement of adherence, and for pharmacokinetic comparisons.[12] A simple and inexpensive thin layer chromatography (TLC) assay for semi-quantitative measurement of NVP saliva concentrations in resource-limited settings has been validated by our group.[11] It was observed that the median ratio of saliva-to-plasma NVP concentrations using high-performance liquid chromatography (HPLC) was 0.51, i.e. the concentration of NVP in saliva was almost 50% of that in plasma.[11] Sub-therapeutic saliva concentrations of NVP were defined as <1.75 mg/L using TLC, and <1.5 mg/L using HPLC, whereas therapeutic saliva NVP concentrations were defined as values greater than 1.75 mg/L and 1.5 mg/L, using TLC and HPLC methods, respectively.[11]

Several studies have investigated the relationship between NVP concentrations in saliva and plasma using non-invasive procedures (saliva stimulation) and observed that NVP plasma concentrations in patients taking 200 mg of NVP twice daily were strongly associated with saliva concentrations. The saliva concentration of NVP was independent of the time after ingestion.[11-15]

Therapeutic drug monitoring of NVP by HPLC in resource-limited settings such as Tanzania is expensive and can hardly be used in routine care. Therefore the use of relatively inexpensive methods such as TLC is highly recommended.

The rational use of drugs comprises taking the right drugs at the correct doses and times of administration, so as to achieve adherence levels >95%. In studies on self-reported adherence to ARV drugs, >95% adherence levels were found.[16-17]

The primary objectives of this study were to measure saliva NVP concentrations, classify them as therapeutic or sub-therapeutic, and to assess self-reported adherence, in patients using NVP-containing ARV regimens.

Secondary objectives were to compare the proportion having therapeutic or sub-therapeutic saliva NVP concentrations among participants using different NVP containing regimens, to compare self-reported adherence with NVP saliva concentrations, and to determine reasons for missed doses.

METHODS

Study design

This cross-sectional study enrolled HIV-infected patients who attended the adult Care and Treatment Clinic (CTC) at Kilimanjaro Christian Medical Centre (KCMC), a tertiary care hospital and medical training centre in Moshi, Tanzania between January and April 2012. We selected participants who had been on the following NVP-containing ARV regimens; zidovudine + lamivudine + NVP, or stavudine + lamivudine + NVP, for more than four weeks.

Study population

101 HIV-infected adults were recruited during routine visits to the CTC. Patients who had eaten a major meal, smoked, used dental flossing, tooth brushing, or drunk anything 60 minutes prior to saliva collection were excluded. Those who had taken alcohol less than 12 hours before saliva sample collection, and those unable to self-report the date and time of the most recent ingestion of NVP were also excluded. The protocol was reviewed and approved by the Research and Ethics Review Committee of the Kilimanjaro Christian Medical University College, Moshi. Written informed consent was obtained from all subjects before enrolment.

Sample collection and drug concentration assays

Cotton gauze was used to remove colour from study participants who had painted their lips. Any removable dental prosthesis was also removed. Active smokers and drinkers who had refrained from their habit for more than 12 hours before saliva collection were eligible for the study. Hands were washed and mouths were rinsed twice with cold water just before saliva collection. Stimulated saliva specimens were obtained with a Salivette - a dental cotton roll impregnated with 20 mg citric acid - (Sartstedt, Etten-Leur, The Netherlands), which stimulates saliva flow. Study participants were asked to chew on the roll for approximately 1 min. Saliva samples were stored at ambient temperature for a maximum of 2-3 hrs at the study site. NVP in saliva is stable for 17 hours at ambient temperature (23°C). Saliva was obtained by centrifugation of the cotton roll at 800g for 10 min on the same day and the aliquots were stored at -80^{0}C for analysis at the Biotechnology Laboratory of KCMC. The centrifugation process removed particulate matter from saliva samples. On average, more than 2 mL of saliva was collected at once from each patient.

Experimental TLC method

The TLC method applied was as described and validated previously.[11] Briefly, one microlitre (µL) of the reference solution of 1.75 mg/L NVP, and saliva samples 1 µL were pipetted in small dense

dots 1.5 cm from the side and 2.0 cm from the bottom of a silica gel TLC sheet, which was then placed in a container with eluent (Toluene: Ethyl acetate=1:1) for approximately 12 minutes. When the solvent front reached its maximum distance, the TLC plate was removed and marked for the distance the eluent had moved**.** After drying, the spots were checked for similarity of size and shape under UV light (254 nm, CAMAG 022.9120) and compared with the reference spot. A spot less intense than the reference was considered to be sub-therapeutic. A spot comparable to or more intense than the reference spot was considered to be therapeutic.

Adherence measurement by self-reporting

Adherence to intake of a NVP-containing regimen was measured using a self-reported questionnaire, which consisted of 4 closed questions (Morisky scale).[18] The questions were in the native language and measured both intentional and non-intentional non-adherence, based on forgetting, carelessness and stopping medication when feeling better or worse.

Statistical analysis

Data were entered in SPSS version 16 and analysed by SPSS and STATA version 12. Categorical variables were expressed in proportions and percentages. Comparison between groups was performed by using Fisher's exact test.

Logistic regression analysis was performed and an odds ratio (OR) with 95% confidence interval (CI) determined to find the association between saliva NVP concentration (sub-therapeutic level) and factors such as body mass index (BMI), reasons for missed doses, adherence level, and sex. A p-value of <0.05 (2-tailed) was used to indicate statistical significance.

RESULTS

We included 101 HIV- infected adults, 15 to 49 years of age taking NVP-containing ARV regimens for more than four weeks. Ten patients (9.9%) were excluded as the volume of saliva obtained after centrifugation was insufficient for the assay (<0.5 mL), leaving saliva from 91 patients for analysis.

Of the study participants 78% were women**.** A higher proportion of the study participants (80.2%) was on a fixed combination comprising AZT 200mg, 3TC 150mg, and NVP 200mg; 19.8% was on a d4T 30 mg, 3TC 150mg and NVP 200mg regimen (**Table 1**). The enrolled study participants had been on the ARV combination medications for a period of between one month and more than five years. The average time from last intake of NVP to saliva collection was 3.06 hrs (SD ± 2.52). Thirteen participants were using other drugs apart from ARV's for the management of opportunistic infections, hypertension, epilepsy and / or peripheral neuropathy. The majority of participants were not smoking (98.9%) nor drinking alcohol (89%. Self-reported adherence was high, moderate or low in 68.1%, 26.4% and 5.5% of participants, respectively (**Table 1**). Reasons for missed doses mentioned by participants were forgetting (33%) work (4.4%) and side effects (5.5%). The remaining could not give a particular reason.

Seventy nine (86.8%) of participants had saliva NVP concentrations at the therapeutic level (1.75 mg/L) or higher, and 12 (13.2%) had sub-therapeutic concentrations (**Table 2**). Of the patients on the NVP + AZT + 3TC regimen, 86.3% had saliva NVP concentrations at therapeutic

Table 1. Demographic characteristics and self-reported adherence of participants (N=91)

Characteristics	Particulars	N (%)
Age (years)	15-29	10 (11)
	30-39	26 (28.6)
	40-49	55 (60.4)
Gender	Female	71 (78)
Standard ART regimen	d4T+3TC+NVP	18 (19.8)
	AZT+3TC+NVP	73 (80.2)
Co-medication	Yes	13 (14.3)
	No	78 (85.7)
Body mass index (kg/m^2)	<18.5	4 (4.4)
	18.5-24.9	47 (51.7)
	>24.9	27 (29.7)
	>30	13 (14.3)
Smoking	Yes >12hrs	1 (1.1)
	No	99 (98.9)
Alcohol intake	Yes >12hrs	10 (11)
	No	81 (89)
Duration on ART	>1Month-1Year	12 (13.2)
	>1Year-2Years	9 (9.9)
	>2years-5Years	26 (28.6)
	>5Years	44 (48.4)
Self-reported adherence (*)		
	Low	5 (5.5)
	Moderate	24 (26.4)
	High	62 (68.1)

N (%), number and percent; d4T,stavudine 30mg; 3TC, lamivudine 150mg; NVP, nevirapine 200mg; the fixed dose combination is called Triomune 30; AZT, zidovudine 300mg; 3TC, lamivudine 150mg; NVP, nevirapine 200mg; the fixed dose combination is called Duovir N.
(*) Classified by Morisky scale.[18]

level or higher, a proportion which was not statistically significant different from those on the NVP + 3TC + d4T regimen (88.9%; p=0.56).

The association between self-reported adherence and saliva NVP concentration is summarized in **Table 2**. In 57 of 62 participants (91.9%) with high self-reported adherence, saliva NVP concentrations were at the therapeutic concentration or higher (>1.75 mg/L), while 5 (8.1%) had sub-therapeutic concentrations. Three of 5 participants with low self-reported adherence, had saliva NVP concentrations at therapeutic level. The association between self-reported adherence and saliva NVP concentrations was close to statistical significance (p=0.057).

The majority of participants who were using ARV alone had saliva NVP concentrations at the therapeutic level or higher (91%).

Table 2. Association between self-reported adherence and saliva nevirapine concentrations

Saliva NVP concentration	>1.75 mg/L N=79	<1.75mg/L N=12	Total N=91	P value
Adherence (*) N (%)				
Low adherence	3 (60.0)	2 (40.0)	5 (100)	
Moderate adherence	19 (79.2)	5 (20.8)	24 (100)	0.057†
High adherence	57 (91.9)	5 (8.1)	62 (100)	

† Fisher's exact test (significance level less than or equal to 5%);
(*) Self-reported adherence classified using Morisky scale.[18]

Logistic regression was performed to determine the association between saliva NVP concentrations (sub-therapeutic concentration) and various factors. Participants with high self-reported adherence were 87% less likely to have a sub-therapeutic saliva NVP concentration compared to those with low self-reported adherence (OR 0.13 (95%CI 0.02-0.98, p=0.05). BMI and gender were not associated with sub-therapeutic saliva NVP concentrations.

DISCUSSION

This study demonstrated high adherence among the participants. Therapeutic saliva NVP concentrations (>1.75 mg/L or higher) were observed in 86.8% of participants and sub-therapeutic concentrations were observed in 13.2% of the participants. Our findings were comparable to those obtained by L'homme et al.[11] The proportion of participants with therapeutic NVP concentrations on the two fixed-dose NVP-containing regimens, i.e. d4T+3TC+NVP, and AZT+3TC+NVP was comparable (88.9% and 86.3%, respectively).

There is scarce information regarding the NVP saliva or plasma concentration relationships between the two NVP-containing fixed-combination regimens. Dubuisson et al.[19] found that concomitant AZT use may confound the TLC assay results because AZT has a retardation factor (RF) value similar to the NVP reference standard. Jean-Baptiste R,[20] observed that adherence rates were much higher in combination regimens containing d4T compared to patients on AZT containing combinations, suggesting that combination type as opposed to pill burden was associated with adherence. This can be explained by the different toxicity profiles of the medications, because those who experience higher rate of adverse events are mostly likely to skip or stop taking medications.

Self-reported adherence observed in our study was comparable to that observed in Rwanda (73%)[13] where pill count and a questionnaire were used to measure adherence, and in Botswana (81.3%)[16] where self-reported adherence was assessed using the Morisky scale. We observed that 91.9% of participants with self-reported high adherence also had therapeutic or higher saliva NVP concentrations, while 8.1% the same group had sub-therapeutic NVP concentrations.

HIV-infected patients taking ARVs often use non-ARV co-medication for other indications such as opportunistic infections. This increases the probability of drug-drug interactions which may affect ARV treatment by affecting drug concentrations or adherence. Because the

number of patients using co medications was small (3 on antihypertensives, 6 on drugs against opportunistic infection, and 4 on anti-epileptics), a larger study powered to address the impact of pharmacokinetic interactions is warranted.

HIV patients are committed to take their ARV medications for life, but due to various reasons they may skip some doses. In our study, 57.1% reported no clear reasons for not taking their medications and 33% reported forgetting as the main reason. Our data are in line with other studies which reported "simply forgetting" as the main reason behind missed doses in Malawi.[10]

Our study has some limitations. The TLC assay only measures the amount of NVP in saliva semi-quantitatively and the exact concentrations of NVP were not calculated. Self-reported adherence may be biased by over or under reporting, as the Morisky scale is only a reflection of the true ARV intake. Other adherence measurement tools such as the Medication Event Monitoring System (MEMS), may be biased as well, because patients may change their medication behaviour because they knew they were being monitored, and because opening or closing the bottles does not confirm drug intake.[21]

Also, in the present study adherence was measured mostly in female patients (71/91 of study subjects). C. Ortego et al [22] found higher rates of adherence in Africa, Asia, and South America, amongst women, when the sample included more widows.

CONCLUSIONS

A high proportion of patients in this study had therapeutic NVP saliva concentrations as measured by TLC, and saliva concentrations generally were in agreement with self-reported adherence.

ACKNOWLEDGMENTS

We thank all the patients and staff from the Care and Treatment Clinic at Kilimanjaro Christian Medical Centre. We appreciate the assistance given by T.B. Sonda, Kilimanjaro Clinical Research Institute, Moshi, Tanzania.

Supported by the European and Developing Countries Clinical Trials Partnership (EDCTP) [CT.2006.33020.006].

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SUMMARY

MUHTASARI

SAMENVATTING

LIST OF PUBLICATIONS

ACKNOWLEDGEMENTS

CURRICULUM VITAE

SUMMARY

Introduction

This thesis presents the first output in clinical pharmacological research on adding an enzyme inducer to the prophylaxis regimen used for prevention of mother-to-child transmission (pMTCT) and the use of thin layer chromatography for therapeutic drug monitoring in HIV-infected adult patients. In this thesis, collaboration between partners from The Netherlands, United Kingdom, Zambia and Tanzania has led to clinical pharmacological research on HIV-infected patients that are specifically relevant for resource-limited countries. **Part I** deals with clinical pharmacological aspects of nevirapine (NVP) use in pMTCT. In particular, the effects are presented of adding a short course of different enzyme inducers to the pMTCT prophylaxis regimen on the development of resistance[1] by reducing the elimination half life of NVP in HIV-infected pregnant women. **Part II** focuses on validating and using the newly developed Thin Layer Chromatography for Therapeutic Drug Monitoring of NVP in saliva of HIV- infected individuals at the adult care and treatment clinic at Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania.

In 2010, an estimated 3.4 million children under 15 years were living with HIV/AIDS worldwide and at least 250 000 died of AIDS. Transmission around the time of delivery accounted for nearly half of overall MTCT in breast-feeding populations,[2;3] and this peri-partum period has become the major focus of prevention of MTCT (pMTCT) strategies, especially with antiretroviral drugs (ARVs). The regimen is cheap and relatively easy to administer. Since 2000, Sd-NVP has been endorsed by the World Health Organization (WHO) as one of several recommended ARV regimens for pMTCT in resource-limited settings.[6] Studies in the early 2000s, however, have shown that Sd-NVP to the mother can induce the occurrence of NVP resistance in mothers and infants. The estimated resistance prevalence ranging from 1 to 69% for women and from 0 to 87% for neonates.[7] Studies have suggested that NVP induced resistance after exposure for pMTCT may impair virological response to the subsequently used non nucleoside reverse transcriptase inhibitors (NNRTIs) in mothers and infants,[8;9] The most frequently detected mutations were the K103N and Y181C. The two drugs most likely to select for resistance in these regimens are NVP and lamivudine (3TC), both of which require only one point mutation in the viral codon to confer resistance. And with the introduction of antepartum, intrapartum and postpartum combination therapy for pMTCT to reduce NVP resistance there is still found a prevalence of NVP resistance of 4-16%.[7] The long term implications of the emergence of resistant mutations following the use of these short course regimens require further study.

In developed-country settings, MTCT of HIV is a rare event, given the wide availability of a comprehensive package of MTCT prevention interventions. In contrast, in many resource-limited settings many pregnant, HIV-infected women cannot access even basic pMTCT interventions, such as counselling and testing and ARV prophylaxis. Data from developed-country settings suggest that the effectiveness of pMTCT regimens that use three ARVs is superior to regimens that use only one or two.

In **chapter 2** we have shown that most of the healthy HIV-uninfected Dutch women who received a single 200-mg nevirapine dose had detectable NVP plasma concentrations after more than 2 weeks.[10] The median NVP half-life observed in the 44 subjects was 56.7h, a finding

which is similar to Musoke et al.[11] In resource limited settings, where many women present late for the antenatal clinic and too few are screened for CD4 cell count, Sd-NVP will most likely remain an important intervention for the prevention of mother to child transmission. Also, in Tanzania the antenatal HIV screening and ARV prophylaxis coverage is still low, with one third of women still on Sd-NVP for pMTCT according to UNAIDS report on the Global AIDS Epidemic,2010.[12] The sub-therapeutic detectable NVP plasma concentrations present the perfect environment for the occurrence of resistance, because the concentrations may be sub-inhibitory for several days, a finding which has been reported by Jackson et al.[13] However, some recent studies suggest that the use of Sd-NVP given to the delivering woman and to the neonate within 72 h of birth has been proven to be a safe and effective intervention to prevent MTCT.[4;5] This information is valuable for designing intervention studies to prevent the development of resistance.

In **chapter 3** describes an innovative study to investigate whether the use of single dose of carbamazepine, a CYP3A4 enzyme inducer, added to the pMTCT regimen, diminishes NVP resistance by accelerating elimination of NVP after exposure to Sd-NVP[14] at onset of labor in HIV-infected, pregnant women. We found that addition of single-dose carbamazepine did not affect NVP plasma concentration at delivery and these plasma concentrations were comparable with previous studies[11;15-17], but significantly reduced NVP plasma concentrations one week postpartum. At week 6 after delivery HIV genotyping was done and observed NVP mutations K103N, present in 9 women, alone in 8 women and with G190A in 1. The standard HIV genotyping assays could only detect viral mutations found in 20% of the overall HIV population and not subpopulations of mutants. Deep sequencing should be planned for future projects. We also found a trend towards lower rates of NVP resistance in women with undetectable NVP concentrations across the whole population, supporting the validity of this approach. The infants NVP concentrations were not likely reduced due to the delayed enzyme induction and the low concentrations of carbamazepine in breast-milk.[18] Indeed, perinatal HIV- 1 transmission rates were similar to rates reported in previous studies of women using Sd-NVP for the first time.[4;19] Other findings used short course interventions and significantly reduced the development of NVP resistance[14;20-22] although the prevalence of resistance mutations decreased with time after exposure to Sd-NVP, there is some evidence that women with pre-existing mutations as well as those exposed to Sd-NVP but without mutations respond less well to antiretroviral treatment.[23] The major disadvantages are their complexity and the emergence of 3TC resistance which is associated with virological and clinical failure in women starting 3TC-containing ART for their own health leading to recommendations that this dual therapy AZT/3TC regimens should be used with caution.[24;25] To preserve its simplicity, another finding by Chi et al[26] administered a single dose of emtricitabine + tenofovir with Sd-NVP in 400 women, which also halved NVP resistance; 25% versus 12%. A recent study in Malawi[27] has shown that WHO option B+ has rapidly increased access to efficacious ART for HIV$^+$ pregnant women. However, each intervention has its advantages and disadvantages and currently there are raised concerns about HIV drug resistance with long term use of ART when initiated in early HIV disease, safety of increased ARV exposure for the fetus/infant, acceptability and equity. This study shows possibilities for pMTCT programs of adding an enzyme inducer to

reduce the development of NVP resistance in settings where other options are limited. In addition to the initiatives of WHO, these findings apart from being new in the field of pMTCT also showed that the pharmacological approach described might have an additive effect and also maintains simplicity and effectiveness when used for pMTCT in subsequent pregnancies in resource limited setting where access to care and treatment is not accessible thus Sd-NVP will still remain the first option for pMTCT.

Chapter 4 describes a study on the use of a short (seven days) postpartum course of an enzyme inducer, phenytoin to accelerate elimination of NVP, with the aim of decreasing the frequency of development of NVP resistance postpartum. Nevirapine plasma concentrations at delivery were similar in those receiving and not receiving phenytoin, and also comparable with previous studies.[11;17] Post-delivery, nevirapine pharmacokinetic parameters were substantially affected by enzyme induction. Adding phenytoin to Sd-NVP reduced nevirapine plasma levels by 85% and produced a significantly larger proportion of women with undetectable NVP levels one and two weeks post-delivery. These effects reduced the NVP elimination half-life by 60%, an absolute difference of -35.8 hours. This is the largest decline in NVP elimination half-life ever reported, especially in the target population of HIV-infected, pregnant women. Currently a one week course of AZT/3TC is recommended to reduce NVP resistance in HIV-infected women who receive single dose NVP during labor as part of pMTCT ARV prophylaxis. [6;28-30] We found that adding a seven-day course of phenytoin at onset of labor lead to a significant reduction in the elimination half-life of NVP. Furthermore, seven days postpartum phenytoin was safe with no new NVP resistance mutations observed. We recommend however this to be confirmed in a prospective larger study powered to demonstrate that phenytoin significantly accelerates NVP elimination and hastens its disappearance postpartum. However, thousands of women are still receiving Sd-NVP[31], and demonstrates the challenge of implementation of the current guidelines. Phenytoin can be used safely during pregnancy and breast-feeding[32] and side-effects are expected to be infrequent with such a low dose for a short period. The addition of an enzyme inducer for seven days to Sd-NVP for pMTCT reduced the presence of subtherapeutic NVP concentrations by significantly shortening the NVP elimination half-life, with no new nevirapine resistance mutations observed. Since prolonged subtherapeutic NVP exposure is known to lead to emergence of NVP resistance, and since phenytoin is safe, single dose NVP could be used with phenytoin if other ARV drugs are unavailable and should be investigated further in a larger phase III trial.

In **chapter 5** we performed a systematic review and meta-analysis to assess the effect of different drug interventions and duration of intake on NVP resistance development after use of a single-dose NVP as part of antiretroviral prophylaxis for pMTCT. Studies have shown that antepartum AZT plus short course (<8 days) antiretroviral drugs postpartum have been shown to nearly eliminate NVP resistance (<0.02%). Furthermore, 20-30 days[33;34] post partum regimens were slightly more effective compared to a (<8 days postpartum regimen (0.003%)[30;34], however, longer term antiretroviral therapy is more complex and challenging to implement in daily practice in resource limited settings. The WHO guideline option A (antepartum AZT, Sd-NVP and one week of 3TC/AZT postpartum)[28] should be followed to achieve a feasible minimum risk of NVP resistance in regions where Sd-NVP is still being used.

In **chapter 6** describes a study on therapeutic drug monitoring (TDM) of NVP. Most of the generic antiretroviral regimens that are available in developing countries combine the NNRTI nevirapine with two NRTIs. A disadvantage of NVP is the low genetic barrier for the development of resistance.[35] Resistant strains against NVP- based regimens provided in national programmes of developing countries could be spread through the population. This is a concern as there are few second line options for antiretroviral treatment in resource-limited countries. From a pharmacological point of view, TDM would be an important tool to identify patients with an inappropriate exposure to drugs such as NVP and thus at risk for virological failure and resistance development. To facilitate the implementation of TDM in developing countries, alternative options for sampling and assay technique were investigated. Previous studies have shown that saliva may be used as an alternative body fluid to plasma for TDM of NVP, implying painless and non-invasive sampling with diminished risk of HIV-transmission to health care workers, and at a low cost.[36] As an alternative to HPLC, a relatively inexpensive thin layer chromatography (TLC) technique has been investigated for the detection of NVP in plasma.[37] We developed a cheap and simple TLC method for semi-quantitative detection of NVP in saliva. The assay was validated in HIV-infected Tanzanian adults on NVP containing regimens at KCMC and was found to be sensitive, specific and robust in the detection of sub-therapeutic NVP concentrations. Global provision of HIV care and treatment would benefit from the transfer of this technique to other developing countries. We recommend this cheap and simple TLC technique for detecting NVP saliva concentration to be used as a tool for TDM in resource limited countries.

In **chapter 7** we compared saliva concentrations of NVP with self-reported adherence in patients on NVP-containing anti-retroviral (ARV) treatment at KCMC, Tanzania. Rational use of drugs comprises taking the right drugs at right doses, right quantities and right time so as to achieve adherence concentrations >95%. In studies on self-reported adherence to ARV drugs, >95% adherence concentrations were found.[38;39] Self-reported adherence observed in our study was comparable to that observed in Rwanda[40] where pill count and a questionnaire were used to measure adherence, and in Botswana where self-reported adherence was assessed using the Morisky scale.[38] Furthermore the proportion of participants with therapeutic NVP concentrations on the two fixed-dose NVP-containing regimens, i.e. d4T+3TC+NVP, and AZT+3TC+NVP were comparable in our study. A disadvantage of drug-drug interaction in HIV-infected patients is the risk of development of subtherapeutic saliva concentrations of antiretroviral drugs. As the number of patients using co-medications was small (3 on anti-hypertensives, 6 on drugs against opportunistic infection, and 4 on anti-epileptics), it was difficult to draw conclusions. We recommend a larger prospective study powered to address the pharmacokinetics interactions. Therapeutic drug monitoring of NVP by HPLC in resource-limited settings like Tanzania is expensive, and can hardly be used in routine care. Resistance to ART and treatment failure, as consequences of sub-optimal adherence, are often difficult to diagnose and manage in routine clinical care in resource-limited settings. Since second-line ART is expensive and not often readily available in these settings, effective treatment of HIV becomes a challenge when first-line drug failure is detected. TDM of plasma drug concentrations can be a valuable tool to directly measure adherence, though results can vary between patients based on rates of absorption and drug interactions. Cost constraints have also

prevented TDM from being incorporated into routine patient management even in resource-rich settings. The use of relatively inexpensive, non-invasive and less expensive methods like TLC is highly recommended in a resource limited setting.

Chapter 8 summarizes our main findings in part I &II of our thesis.

FUTURE PERSPECTIVES

Our results on the use of enzyme inducers stimulate further research on pharmacogenetics. Genetic differences in metabolic pathways can affect individual responses to drugs, both in terms of therapeutic effect as well as adverse effects**.** NVP has been demonstrated to be dominantly metabolized by CYP3A and CYP2B6.[41] These enzymes exhibit inter-individual differences in the expression or activity levels which likely contribute to the variability of NVP pharmacokinetics. The CYP2B6 gene is highly polymorphic with numerous single nucleotide polymorphisms (SNPs) and associated haplotypes. This variability is largely due to the genetic polymorphisms in the cytochrome enzymes which metabolize NVP.[42;43]

We recommend the following for future research in Tanzania: Genotyping of gene variants associated with altered expression of CYP2B6 and CYP3A5. Stratification of patients according to the gene variant predicted CYP2B6 and CYP3A5 expression levels and IL-6 may help to detect the effect of enzyme induction on NVP disposition.[42-44]

Our findings in part II of the thesis showed that the validated method using TLC for the TDM of HIV treatment has bridged the gap between laboratory and the clinics.

We recommend the following:

- The quality control of NVP extracted from saliva using TLC for TDM should be encouraged in all care and treatment clinic in developing countries, including Tanzania, on a regular basis to assess adherence to therapy, improve efficacy and preserve treatment options.
- Furthermore, research is needed to find out whether approved antiretroviral drug other than NVP can be extracted from saliva and could also be measured using TLC.

Use of Sd-NVP for pMTCT, which reduces mother-to-child transmission to 11-13%, remains an option for resource limited settings, although wider use of more complicated methods based on combination ARV's in pregnancy after week 14 and after delivery (reducing mother-to-child transmission to 1-2%) should be considered. In this exercise, not only the extra expenses for the more complex approach (material costs and manpower) should be weighted, but also the extra number of HIV infections in neonates prevented, as less HIV infections reduce health care expenses, this apart from reducing human suffering.

- Therefore, we recommend as well that such cost-benefit analysis for the best approach to pMTCT should be performed for resource limited settings, including Tanzania.

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MUHTASARI

Repoti hii inatoa taarifa ya kwanza ya utafiti wa kifamacologia kwa wajawazito inayohusu kuongezwa kwa dawa inayosababisha uzalishaji wa kimeng'enya (enzyme inducer) kwenye dawa inayotumika kuzuia maambukizo kutoka kwa mama kwenda kwa mtoto (PMTCT). Pia kueleza matumizi ya teknolojia ya TLC (Thin Layer Chromatography) kufuatilia kwa ukaribu kiasi cha dawa mwilini zinazotumika kupunguza makali ya virusi (HIV) visababishavyo ukimwi kwa watu wazima. Ripoti hii, kwa ushirikiano wa Uholanzi, Uingereza, Zambia na Tanzania imeonesha umhimu wa utafiti wa kifamisia hasa kwa watu wanaoishi na virusi (HIV) visababishavyo ukimwi katika nchi zisizo na rasimali za kutosha.

Sehemu ya kwanza ya ripoti ya utafiti huu imejikita katika kueleza matumizi ya dawa ya Nevirapine (NVP) inayotumika kuzia maambukizo kutoka kwa mjamzito kwenda kwa mtoto (PMTCT). Hasa hasa, kueleza matokeo ya kuongezwa kwa dawa inayosababisha uzalishaji wa kimeng'enya (enzyme inducer) ili kuzuia ongezeko la usugu[1] (development of resistance) wa virusi (HIV) kwa kupunguza muda wa kuondoa (elimination half life) Nevirapine (NVP) mwilini mwa wajawazito wanaoishi na virusi (HIV) visababishavyo ukimwi.

Sehemu ya pili ya ripoti hii inathibitisha na kuonesha matumizi ya teknolojia ya TLC (Thin Layer Chromatography) namna inavyoweza kutumiwa kufuatilia kwa ukaribu kiasi cha dawa ya Nevirapine (NVP) katika mate ya wagonjwa waiishio na virusi (HIV) visababishavyo ukimwi wanaopata huduma ya CTC (care and treatment clinic) katika hospitali ya rufaa KCMC iliyopo mjiini Moshi mkoani Kilimanjaro nchini Tanzania.

Mwaka 2010, duniani kote ilikadiriwa kuwa watoto milioni 3.4 walio na umri chini ya miaka 15 walikuwa wanaishi na virusi (HIV) na kwa uchache watoto 250,000 walikufa kwa UKIMWI. Maambukizi ya virusi (HIV) wakati wa kujifungua yakichangia karibu nusu ya maambukizi yote toka kwa mama kwenda kwa mtoto (MTCT) katika jamii zenye tabia ya kunyonyesha watoto [2;3] (breast-feeding populations) na kipindi hiki ndicho kimekuwa kikitiliwa mkazo zaidi miongoni mwa mikakati ya kuzuia maambukizi toka kwa mama kwenda kwa mtoto (pMTCT) hasa kwa kutumia dawa za ARVs ili kupunguza makali ya virusi (HIV). Dozi hii ni ya gharama nafuu na rahisi kuitoa ukilinganisha na zingine. Tangu mwaka 2000, Sd-NVP imeruhusiwa ili kutumika na shirika la afya la kimataifa (WHO) kama mojawapo ya dozi za ARV dhidi ya pMTCT katika nchi zisizo na rasimali za kutosha[6]. Hata hivyo tafiti nyingi mwanzoni mwa miaka ya 2000, zimeonesha kwamba matumizi ya Sd-NVP kwa akina mama inaweza sababisha ongezeko la usugu wa NVP (resistance) kwa akina mama na vichanga vyao.

Kiasi cha ongezeko la usugu wa NVP (resistance) kinakadiriwa kuwa ni kuanzia 1 hadi 69% kwa akina mama na kuanzia 0 hadi 87% kwa vichanga.[7] Tafiti zimeonesha kwamba usugu usababishwao na NVP baada ya matumizi yake dhidi ya ya pMTCT unaweza kupunguza nguvu ya dawa aina ya non nucleoside reverse transcriptase inhibitors (NNRTIs) inayotumiwa baadaye na akina mama na vichanga dhidi ya virusi (HIV)[8;9] Mara nyingi mabadiliko ya vinasaba yasababishayo usugu huu yamegundulika kuwa katika K103N na Y181C.

Dawa mbili ambazo angalau mara zote ni chaguo la usugu katika matibabu ni NVP na lamivudine (3TC), ambapo dawa zote mbili huhitaji badiliko kwenye nafasi moja pekee katika vinasaba vya kirusi (HIV) ili kusababisha usugu. Pamoja na uanzishaji wa matumizi mseto ya matibabu kabla, wakati na baada ya kujifungua dhidi ya pMTCT ili kupunguza usugu wa NVP

bado kiwango cha usugu wa NVP ni kati ya 4-16%.[7] Madhara ya muda mrefu yatokanayo na kuibuka kwa vinasaba sugu kufuatia matumizi ya muda mfupi wa matibabu haya yanahitaji utafiti wa kina zaidi.

Katika nchi zilizoendelea, MTCT ni tukio la nadra sana, ikizingatia kwamba kuna upatikanaji bora wa huduma kama kinga dhidi ya MTCT. Kinyume chake katika nchi zisizo na rasimali za kutosha wajawazito wengi waiishio na virusi (HIV) hawawezi kupata hata huduma za msingi za pMTCT kama vile ushauri, kupima na kuanza matumizi ya dawa za kinga (ARV). Takwimu kutoka nchi zisizo na rasimali za kutosha zinaonesha kwamba matibabu dhidi ya pMTCT yanayotumia dawa tatu za ARVs ni bora zaidi ukilinganisha na matumizi ya dawa moja au mbili.

Katika sura ya pili (2) ya ripoti hii tumeonesha kuwa wanawake wa kiholanzi wasiokuwa na maambukizi ya virusi (HIV) waliotumia dozi moja ya nevirapine ya miligramu 200 (200mg Sd-NVP) wana kiasi kikubwa cha NVP mwilini hata baada ya zaidi ya majuma mawili kupita tangu walipotumia dawa hii.[10] Kwa wastani muda (half life) wa kuondoa dawa ya Nevirapine (NVP) mwilini katika watu 44 waliofanyiwa utafiti ulikuwa saa 56.7, kiasi ambacho pia kilionekana katika utafiti uliofanywa na Musoke na wenzake.[11]

Katika nchi zisizo na rasimali za kutosha ambapo wanawake wengi wanachelewa kuja ili kupata huduma za ANC (antenatal clinic), hata wakija kwenye kliniki pia wachache sana ndio wanaopata huduma ya kipimo cha CD4, hivyo basi Sd-NVP itabaki kuwa ndiyo kinga muhimu dhidi ya maambukizi ya virusi (HIV) yatokayo kwa mama kwenda kwa mtoto. Nchini Tanzania huduma za ANC za kupima virusi (HIV) na ugawaji wa ARVs kama kinga dhidi ya maambukizi toka kwa mama kwenda kwa mototo bado iko chini, ikiwa theluthi moja (1/3)ya wanawake ndiyo wanapata matibabu ya Sd-NVP kwa ajili ya pMTCT, hii ni kulingana na ripoti ya shirika la kimaifa linalopambana na UKIMWI duniani kote[12] (UNAIDS report on the Global AIDS Epidemic, 2010). Kiasi cha dawa ya NVP chini ya kiwango kinachotakiwa mwilini (sub-therapeutic) ndicho kinachotengeneza mazingira mazuri kwa usugu wa dawa kujitokeza; kwa sababu kiwango hiki cha dawa kinakuwa na uwezo ulio chini ya kiwango (sub-inhibitory) kinachotakiwa ili kupambana na virusi (HIV) kwa muda mrefu sana, matokeo hayo yameonekana katika utafiti uliofanywa na Jackson na wenzake.[13]

Ingawaje, tafiti za hivi karibuni zimeonesha kwamba matumizi ya dawa ya Sd-NVP kwa wanawake wanapojifungua na vichanga vyao ndani ya saa 72 tangu kuzaliwa ni kinga salama na madhibuti dhidi ya MTCT.[4;5] Tafiti hizi zimetoa taarifa muhimu na yenye thamani sana itakayosaidia kuandaa na kufanya tafiti ambazo zitatoa taarifa na njia bora dhidi ya ongezeko la usugu wa dawa ya NVP.

Katika sura ya tatu (3) ya ripoti hii ya uatfiti tumeeleza na kuonesha matumizi ya dozi moja ya carbamazepine (CYP3A4 enzyme inducer) inayosababisha uzalishaji wa kimeng'enya atumiapo kinga dhidi ya pMTCT ili kuona kama usugu wa dawa ya NVP unapunguzwa kwa kuongeza utoaji wa NVP mwilini mara itumikapo dawa ya Sd-NVP[14] mara tu uchungu wa uzazi unapoanza kwa mjamzito aishiye na virusi (HIV). Tumegundua kuwa matumizi ya dozi moja ya carbamazepine (CYP3A4 enzyme inducer) haukubadili kiwango cha dawa ya NVP mwilini wakati wa kujifungua na kiwango hicho cha dawa kinalingana na kile kilichoonekana katika tafiti zilizopita[11;15-17], lakini imeonekana kupunguza kwa kiwango kikubwa dawa ya NVP mwilini juma moja baada ya kujifungua. Katika juma la sita baada ya kujifungua kipimo cha utambuzi wa

mabaliko ya vinasaba ya kirusi (HIV) kutokana na NVP katika K103N ulifanyika na ikagundulika kuwa upo kwa wanawake 9 ambapo 8 pekee kwenye Y181C/I na 1 kwenye G190A. Kwa kawaida kipimo cha utambuzi wa mabadiliko ya vinasaba ya virusi (HIV) iliweza kugundua mabadiliko hayo kwa 20% miongoni mwa wote waishio na virusi (HIV) na siyo kwenye makundi madogo madogo yenye mabadiliko hayo. Kipimo kingine (deep sequencing) ambacho kina uwezo wa kutambua hata mabadiliko madogo ya vinasaba ni muhimu itumike kwa tafiti za usoni. Pia tumeona kiasi kikipungua cha usugu wa dawa ya NVP miongoni mwa wanawake wenye kiwango kidogo sana (undetectable) cha NVP katika jamii nzima, hii ikithibitisha uwezo na usahihi wa technolojia tuliotumia katika utafiti wetu.

Kiwango cha NVP kwa vichanga haikuonekana kupungua kwa sababu ya kiwango kidogo cha carbamazepine kwenye maziwa ya mama hivyo kupelekea ucheleweshwaji wa kuzalisha kimeng'enya (delayed enzyme induction) mwilini.[18] Hata hivyo kiwango cha maambukizi ya virusi (HIV- 1) mara baada ya kuzaliwa kichanga kinaonekana kufanana na tafiti zilizopita wakati wajawazito walipotumia Sd-NVP kwa mara ya kwanza.[4;19]

Matokeo ya tafiti zingine kuhusu kinga za muda mfupi yameonesha kupunguza ongezeko la usugu wa dawa ya NVP[14;20-22] kwa kiasi kikubwa, ingawaje kiasi cha mabadiliko ya vinasaba yasababishayo usugu wa dawa ya Sd-NVP yamepungua baada ya muda fulani, lakini bado kuna ushahidi unaonekana kwa baadhi ya wanawake kuwa tayari na mabadiliko hayo hata kabla ya matumizi ya Sd-NVP na hata baadhi ya wasio na mabadiliko ya vinasaba miili yao huwa ina mwitikio mdogo sana kwa dawa za ARVs zitumikazo kupunguza makali ya virusi ya HIV.[23] Tatizo la dawa za ARV zenye 3TC kwa wanawake wanapoanza nazo ni ugumu na kujitokeza kwa usugu mwilini ambalo linapelekewa na kushindwa kwa dawa kupambana na virusi hivyo nao mwili kushindwa kutengeneza kinga baada ya kuzidiwa na virusi, kwa kuzingatia hayo tunashauri kwamba matumizi ya hizi dawa mbili za AZT/3TC yafanyike kwa uangalifu zaidi.[24 25] Ili kuendelea kutunza urahisi wa matumizi ya dozi moja ya NVP, matokeo mengine ya utafiti wa Chi na wenzake[26] aliyetoa dozi moja yenye Sd-NVP, emtricitabine na tenofovir kwa wanawake 400, ulipunguza usugu wa dawa ya NVP kwa nusu toka 25% hadi 12%. Utafiti wa hivi karibuni nchini Malawi[27] umeonesha kwamba chaguo la B+ la WHO umeongeza kwa kiasi kikubwa utumiaji wa dawa madhubuti kwa wanawake wajawazito waishio na virusi vya HIV. Ingawaje, kila dawa ina uzuri na ubaya wake na kwa sasa changamoto iliyojitokeza ni kuhusu usugu wa dawa za virusi kufuatia matumizi mrefu ya ARV kwa watumiaji, usalama wa matumizi ya ARV kwa vichanga, mapokeo na usawa wa dawa. Utafiti huu umeonesha uwezekano kwa programu za pMTCT kuongeza dawa inayosababisha uzalishaji wa kimeng'enya (enzyme inducer) ili kupunguza ongezeko la usugu wa dawa za NVP katika jamii zenye dawa chache za kuchagua. Pamoja na jitihada za WHO, matokeo ya utafiti huu mbali na kuwa mapya katika nyanja za pMTCT pia yameonesha kwamba utafiti huu wa kifamacologia una faida ya ziada pamoja na urahisi na kuwa kinga bora itumikapo dhidi ya pMTCT katika mimba zinazofuata kwenye nchi zisizo na rasilimali za kutosha ambapo upatikanaji wa huduma za CTC haupo na hivyo dawa ya Sd-NVP itabaki kuwa ni chaguo la kwanza dhidi ya pMTCT.

Sura ya nne (4) inaonesha matokeo ya utafiti juu ya matumizi ya muda mfupi (seven days) baada ya kujifungua ya dawa ya phenytoin inayoongeza utoaji mwilini wa NVP, kwa lengo la kupunguza kiwango cha ongezeko la usugu wa NVP baada ya kujifungua. Kiwango

cha Nevirapine mwilini wakati wa kujifungua umeonekana kufanana na wale wasiotumia phenytoin, na matokeo haya pia yakilingana na matokeo ya tafiti zingine zilizopita[11;17] Baada ya kujifungua, vipimo vingine vya kifamacologia ya dawa ya nevirapine vilionekana kuathiriwa kwa kiasi kikubwa na kemikali (enzyme inducer). Baada ya kuongeza phenytoin kwenye dawa ya Sd-NVP ilipunguza kiwango cha nevirapine mwilini kwa 85% na kuonesha kwamba kiasi kikubwa cha wanawake kuwa na kiwango kidogo sana (undetectable) mwilini cha NVP juma la kwanza na la pili baada ya kujifungua. Hii imepunguza muda (half-life) wa kuondoa NVP kwa 60%, ikiwa ni pungufu ya masaa 35.8. Huu ni upungufu mkubwa wa muda (half-life) kuliko yote kuwahi kuripotiwa hasa kwa wanawake wajawazito waishio na virusi (HIV). Kwa sasa matumizi ya juma moja ya dawa ya AZT/3TC inashauriwa kupunguza usugu wa NVP kwa wajawazito wenye virusi wanaotumia Sd-NVP wakati wa kujifungua ikiwa ni kama sehemu ya kinga dhidi ya pMTCT[6;28-30] Tumegungua kwamba dawa ya phenytoin ikiongezwa kwenye dozi ya siku 7 pindi uchungu wa kujifungua unapoanza inaweza kupunguza kwa kiasi kikubwa muda wa uondoshaji wa dawa ya NVP mwilini. Aidha dozi ya phenytoin ya siku 7 baada ya kujifungua imeonekana kuwa salama tena bila kuonesha mabadiliko mapya ya vinasaba yasababishayo usugu dhidi ya dawa ya NVP. Hata hivyo tunashauri tafiti kubwa zaidi kufanyika ili kuweza kuonesha na kuthibitisha kwamba dawa ya phenytoin kwa kiasi kikubwa inaongeza utoaji wa dawa ya NVP mwilini na kuharakisha upoteaji wake mara baada ya kujifungua. Ingawa, maelfu ya wajawazito bado wanatumia dawa ya Sd-NVP[31], hii inaonesha changamoto ya utekelekezaji wa miongozo ya sasa. Phenytoin ni salama kuweza kutumika wakati wa ujauzito na unyonyeshaji[32] na madhara kuwa machache sana kwa vile dozi ni ndogo na inatumika kwa muda mfupi sana. Uongezaji wa dawa inayosababisha uzalishaji wa kimeng'enya (an enzyme inducer) kwa siku 7 kwenye dawa ya Sd-NVP dhidi ya pMTCT imepunguza uwepo wa kiwango chini ya kinachitakiwa (sub therapeutic) mwilini cha NVP kwa kupunguza muda wa uondoaji wa NVP mwilini kwa kiasi kikubwa bila kuonesha mabadiliko mapya ya vinasaba yasababishayo usugu wa virusi. Kwa kuwa uwepo kwa kiwango chini (sub therapeutic) mwilini cha NVP kwa muda mrefu unajulikana kupelekea kutokea kwa usugu dhidi ya NVP, na kwa kuwa phenytoin ni salama, Sd-NVP ingeweza kutumiwa ikiwa pamoja na phenytoin itokeapo kuwa dawa zingine za ARV hazipatikani na utumizi wake ukichunguzwa kwanza kwa kina katika tafiti kubwa zaidi (phase III trial).

Katika sura ya tano (5) tumefanya upembuzi na marejeo ya kina ya tafiti zingine ili kuchunguza madhara ya dawa kadha wa kadha na kipindi cha matumiazi ya NVP na kujitokeza kwa usugu dhidi ya Sd-NVP kama sehemu ya kinga dhidii ya pMTCT. Tafiti zimeonesha kwamba utumiaji wa AZT kabla ya kujifungua ikijumlishwa na utumiaji wa ARV kwa muda mfupi (chini ya siku 8) baada ya kujifungua zimeonesha kwamba zimeondosha usugu dhidi ya NVP chini ya 0.02%. Aidha dawa zilizotumika kwa siku, 20-30[33;34] baada ya kujifungua zimeonesha uwezo mkubwa zaidi ikilinganishwa na zile za muda mfupi (chini ya siku 8) kwa kiasi cha 0.03%[30;34], ingawaje matumizi ya ARV kwa muda mrefu ni magumu na yana changamoto kubwa zaidi kiutekelezaji katika nchi zisizo na rasilimali za kutosha. Miongozo ya WHO ya utumiaji wa chaguo A (kabla ya kujifungua AZT, Sd-NVP na juma moja baada ya kujifungua 3TC/AZT)[28] lazima ifuatwe ili kupunguza kwa kiasi kikubwa madhara yaletayo usugu dhidi ya NVP kwenye maeneo ambapo dawa ya Sd-NVP bado inatumika.

Katika sura ya sita (6) tumeeleza matokeo ya utafiti kuhusu namna ya ufuatialiji wa kiwango cha dawa ya NVP kinachotakiwa mwilini (therapeutic drug monitoring (TDM)). Dawa nyingi za ARV zinazopatikana katika nchi nyingi zinazoendelea ni mseto wa NNRTI nevirapine na zingine mbili aina ya NRTIs. Tatizo kubwa la dawa ya NVP kuwa na uwezo mdogo sana wa vinasaba wa kujikinga dhidi ya ongezeko la usugu wa virusi[35] Aina sugu ya virusi dhidi ya NVP iliyotokeza na kusambaa kwenye jamii imetokana na program zinazoruhusu matumizi hayo katika nchi zinazoendelea. Hii imekuwa tatizo sana katika nchi zisizo na rasilimali za kutosha kwa vile kuna dawa chache sana za ARV ambazo hutumika kama chaguo la pili. Kwa upande wa kifamacologia, TDM inaweza kuwa ni chombo muhimu sana kitakacho tumika kuwatambua wagonjwa wasio na kiwango kinachotakiwa cha dawa kama vile NVP mwilini ambao wako katika hatari ya kushindwa kuondoa virusi mwilini hivyo kusababisha ongezeko la usugu dhidi ya dawa. Ili kuwezesha utumiaji wa TDM katika nchi zinazoendelea, njia mbadala za kuchukua sampuli na kufanya vipimo tulizitumia na kuzichunguza. Tafiti zilizopita zimeonesha kwamba mate yanaweza kutumika kama sampuli mbadala wa sampuli za damu kwa ajili ya TDM ili kuangalia NVP mwilini, hii njia haiumizi ni salama, haina gharama na inapunguza hatari ya kuambukiza virusi wafanyakazi wa afya wanapochukua sampuli kutoka kwa wagonjwa wa HIV [36] Kama teknolojia mbadala wa HPLC, tumetumia teknolojia rahisi yenye gharama ndogo ya TLC ili kufuatilia kiasi cha dawa za NVP mwilini[37] Tulitengeneza TLC rahisi yenye uwezo wa kutambua kiwango (semi-quantitative) cha NVP iliyopo kwenye mate. Njia hii tuliijaribu na kuitumia kwa watu wazima wenye virusi vya HIV Tanzania wanaotumia dawa za NVP katika hospitali ya KCMC na iligundulika kuwa na uwezo mkubwa sana wa kutambua na kugundua kiwango hata kile kidogo cha dawa za NVP mwilini (sub-therapeutic). Utoaji wa huduma za kitabibu kwa wagonjwa wa HIV duniani kote watafaidika na teknolojia hii kama itahamishiwa hasa kwenye nchi zingine zinazoendelea. Tunashauri hii teknolojia ya TLC ambayo ni rahisi na yenye gharama nafuu itumike kufuatilia kiwango (TDM) cha NVP mwilini kupitia mate katika nchi zisizo na rasilimali za kutosha.

Katika sura ya saba (7) tulilinganisha kiwango cha NVP kwenye mate kwa wagonjwa wanaotumia ARV zenye NVP katika hospitali ya KCMC nchini Tanzania. Matumizi bora ya dawa ni pamoja na utumiaji wa dawa sahihi, dozi sahihi, kiwango sahihi na kwa muda sahihi ili kiwango cha dawa mwilini cha >95%. Katika tafiti kuhusu wagonjwa walioripoti wenyewe uaminifu kwenye matumizi sahihi ya ARV, kiwango cha dawa mwilini kilikuwa >95%[38;39] Taarifa za uaminifu juu ya matumizi ya sahihi katika utafiti wetu zimeonekana kulingana zile zilizopatikana nchini Rwanda[40] ambapo uhesabuji wa vidonge na maswali kwa mgonjwa vilitumika kupima uaminifu juu ya matumizi sahihi ya dawa na zile za nchini Botswana ambako uaminifu juu ya matumizi sahihi ulipimwa kutumia kipimo cha Morisky (Morisky scale).[38] Aidha kiasi cha wagonjwa wenye kiwango cha kutosha cha dawa mwilini kwa waliotumia dozi hizi mbili za NVP i.e. d4T+3TC+NVP, and AZT+3TC+NVP walionekana kutotofautiana kwa utafiti wetu. Tatizo kubwa la mpambano wa dawa na dawa kwa wagonjwa wa HIV ni hatari ya ongezeko la kiwango cha dawa za ARV kilicho chini ya kinachotakiwa kwenye mate. Kwa kuwa kiasi cha wagonjwa waliokuwa wanatumia dawa zaidi ya moja kuwa ni ndogo sana (3 walitumia dawa za shinikizo, 6 dawa za magonjwa nyemelezi na 4 dawa za kifafa), ilituwia vigumu sana kutoa hitimisho juu ya mpambano wa dawa na dawa. Tunashauri zifanyike tafiti kubwa ambazo zinauwezo wa kufuatilia ama kuchunguza na kutoa taarifa juu ya mpambano wa dawa na dawa mwilini

(pharmacokinetics interactions). TDM ya kiwango cha dawa za NVP mwilini kwa teknolojia ya HPLC kwa nchi zisizo na rasilimali za kutosha kama Tanzania ni ghali, na ni ngumu kuitumia kwa matumizi ya kawaida kwa wagonjwa kila siku. Usugu wa virusi dhidi ya ARV na kushindwa kufanya kazi kwa matibabu kwa sababu ya kutozingatia masharti ya matumizi ya dawa, mara nyingi ni vigumu sana kupima na kukabiliana nayo katika utoaji wa matibabu ya kila siku katika nchi zisizo na rasilimali za kutosha. Kwa kuwa chaguo la pili la dawa za ARV ni ghali na hazipatikani kwa urahisi katika nchi hizi, tiba madhubuti kwa wagonjwa wa HIV yanakuwa ni changamoto hasa pale dawa za chagua la kwanza zinaposhindwa kufanya kazi bila kugundulika kama zimeshindwa. TDM ya kiwango cha dawa mwilini itakuwa ni kifaa chenye thamani kwani kitakuwa na uwezo wa kupima moja kwa moja kiwango cha uzingativu wa masharti ya matumizi ya dawa, ingawa kiwango na kasi ya kuchukua dawa toka tumboni na mpambano wa dawa na dawa unatofautiana kati ya mgonjwa mmoja na mwingine. Pia matatizo ya gharama za vipimo yamepelekea kipimo cha TDM kutojumuishwa miongoni mwa vipimo vinavyohitajika katika utoaji wa matibabu ya kila siku hata nchi zilizo na rasilimali za kutosha. Utumiaji wa teknolojia kama TLC ambayo haina gharama, isiyosababishia maumivu kwa mgonjwa inashauriwa itumike katika nchi zisizo na rasilimali za kutosha.

Sura ya nane (8) inatoa majumuisho ya matokeo yote muhimu ya utafiti wetu kwenye sehemu ya kwanza na ya pili ya ripoti hii.

MITAZAMO YA BAADAYE

Matokeo ya utafiti wetu kuhusu uongezaji wa dawa isababishayo uzalishaji wa kimeng'enya (enzyme inducer) uchochee utafiti zaidi katika nyanja za vinasaba (pharmacogenetics). Utofauti wa vinasaba katika mifumo ya kimmeng'enyo (metabolic pathways) inaweza sababisha miitikio tofauti kwa dawa, hii ikiwa ni pamoja na matokeo tarajiwa na madhara mengine yaletwayo na matumizi ya dawa. Dawa ya NVP imeoneshwa katika tafiti nyingi kwamba kwa kiasi kikubwa huvunjwa vunjwa na mifumo ya metaboliki ya CYP3A na CYP2B6.[41] Vimeng'nya hivi hutofautiana sana kati ya mtu mmoja na mwingine katika utendaji kazi wake na hii kwa kiasi kikubwa inapelekea kutokea kwa utofauti wa kiwango cha dawa ya NVP mwilini. Vinasaba vya CYP2B6 hutofautiana kwa kiasi kikubwa sana na utofauti wake wa herufi moja (single nucleotide polymorphisms, SNPs) tu unatosha kuleta mabadilko makubwa katika mfumo mzima. Utofauti huu kwa kiasi kikubwa unasababishwa na kutofautiana kwa vinasaba katika cytochrome zinazotengeneza vimeng'enya vitumikavyo kumeng'enya dawa za NVP.[42;43]

Tunashauri kufanya yafuatayo kwa tafiti zitakazofanyika hapo baadaye nchini Tanzania:

Kufanyika kipimo kitachoweza kutambua tofauti ya vinasaba (Genotyping of gene variants) visababishavyo mabadiliko katika utendaji kazi wa CYP2B6 na CYP3A5. Kutofautisha kati ya wagonjwa wenye tofauti za vinasaba visababishavyo mabadiliko ya CYP2B6 na CYP3A5 katika kiwango cha uzalishaji wa vimeng'nya na IL-6 inaweza kusaidia kutambua madhara ya uzalishaji huo dhidi ya dawa za NVP.[42-44]

Matokeo ya utafiti wetu kwenye sehemu ya pili ya ripoti hii tumeonesha kwamba teknolojia ya TLC kwa ajili ya kufuatilia kiwango cha dawa mwilini (TDM) kwa matibabu ya wagonjwa wa HIV yamesaidia kuziba pengo lililokuwepo kati ya maabara na kliniki.

Hivyo tunashauri yafuatayo:

- Udhibiti ubora wa kiasi cha dawa za NVP kwenye mate kwa teknolojia ya TLC dhidi ya TDM ni lazima ipewe msisitizo kwenye huduma zote za CTC kwa nchi zote zinazoendelea ikiwemo Tanzania katika matumizi ya kila siku ili kufuatilia uzingativu na matumizi bora ya dawa ili kuboresha umadhubuti wa tiba na kutunza chaguzi za dawa.
- Zaidi sana, tafiti zinatakiwa kuchunguza endapo dawa zaingine za ARV zilizoruhusiwa kutumika mbali na NVP kama zinaweza kupatikana kwenye mate na pia kuweza kupimwa kwa teknolojia ya TLC.

Matumizi ya dawa ya Sd-NVP dhidi ya pMTCT, ambazo hupunguza maambukizi toka kwa mama kwenda kwa mtoto kwa kiwango cha 11-13%, itabaki ndiyo chaguo pekee kwa nchi zisizo na rasilimali za kutosha, ingawa matumizi makubwa zaidi ya njia ngumu za dawa mseto za ARV baada ya juma la 14 la ujauzito na baada ya kujifungua (zikipunguza maambukizi kwa 1-2%) lazima pia zijumuishwe. Katika zoezi hili, siyo tu gharama za ziada kwa tafiti ngumu (gharama za vifaa na rasilimali watu) ndiyo za kupimwa, lakini pia idadi kubwa ya maabukizi ya HIV dhidi ya vichanga inayozuiliwa, kwa kuwa kama maambukizi ya HIV yanakuwa chini na gharama za huduma za tiba zinapungua mbali na kupunguza idadi ya wahudumu wa afya.

- Hivyo tunashauri pia uchambuzi wa kina wa gharama (cost-benefit analysis) uwe ni sehemu katika utoaji wa huduma za pMTCT kwa nchi zisizo na rasilimali za kutosha ikiwemo Tanzania.

SAMENVATTING

Introductie

In dit proefschrift worden de eerste resultaten gepresenteerd van klinisch farmacologisch onderzoek naar het effect van toevoeging van een enzym inducer aan de geneesmiddelencombinatie voor preventie van moeder-op-kind HIV transmissie (pMTCT) en van de toepassing van dunne laag chromatografie (TLC) voor het monitoren van behandeling met geneesmiddelen (therapeutic drug monitoring - TDM) bij HIV-geïnfecteerde volwassenen. Bij dit onderzoek heeft samenwerking tussen partners uit Nederland, het Verenigd Koningrijk, Zambia en Tanzania geleid tot klinisch farmacologisch onderzoek bij HIV-geïnfecteerde patiënten dat met name relevant is voor ontwikkelingslanden. **Deel I** gaat over klinisch farmacologische aspecten van het gebruik van nevirapine (NVP) bij pMTCT. Met name wordt het effect gepresenteerd van toevoeging van een korte kuur van verschillende enzym inducers aan de pMTCT prophylaxe op de ontwikkeling van resistentie[1] door verkorting van de eliminatiehalfwaardetijd van NVP bij HIV-geïnfecteerde zwangere vrouwen. **Deel II** focust op de validatie en de toepassing van de nieuw ontwikkelde TLC-techniek voor TDM in speeksel van HIV-geïnfecteerde patiënten van de Adult Care and Treatment Clinic in Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania.

In 2010 leefden naar schatting 3,4 miljoen kinderen onder de 15 jaar met HIV/AIDS wereldwijd en tenminste 250.000 stierven aan AIDS. HIV transmissie rond de bevalling maakt bijna de helft uit van de transmissie van moeder naar kind (MTCT) bij bevolkingen waar borstvoeding gebruikelijk is,[2;3] en deze tijd rond de bevalling is daarom het voornaamste aandachtsgebied geworden voor pMTCT strategieën, met name door gebruik van antiretrovirale geneesmiddelen (ARV's). Van single-dose nevirapine (sd-NVP) toegediend aan de vrouw tijdens de bevalling en aan de neonaat binnen 72 uur na de geboorte is aangetoond dat het veilig is en effectief voor pMTCT.[4;5] Het regiem is goedkoop en vrij gemakkelijk toe te dienen. Sinds 2000 wordt sd-NVP door de Wereld Gezondheidsorganisatie (WHO) aanbevolen als een van de ARV strategieën voor pMTCT in ontwikkelingslanden.[6] Onderzoek in het begin van de 21ste eeuw heeft echter aangetoond dat sd-NVP toegediend aan de moeder NVP resistentie kan doen ontstaan zowel bij moeder als bij haar kind. NVP resistentie komt in die omstandigheden naar schatting voor bij tot maximaal 69% van de vrouwen en maximaal 87% van de nieuwgeborenen.[7] Ook gaven studies aan dat NVP resistentie na blootstelling aan NVP in het kader van pMTCT de virologische respons op later toegediende non-nucleoside reverse transcriptase inhibitors (NNRTIs) bij moeders en kinderen verminderde.[8;9] De meest waargenomen mutaties waren K103N en Y181C. De twee ARV's met de grootste kans op resistentieontwikkeling bij gebruik in het kader van pMTCT zijn NVP en lamivudine (3TC), dit omdat hiervoor bij deze middelen slechts een enkele punt-mutatie in het virale codon nodig is. Ook bij antepartum, intrapartum en postpartum combinatietherapie met NVP voor pMTCT wordt nog steeds een prevalentie van NVP resistentie van 4-16% gevonden.[7] De implicaties voor de lange termijn van het optreden van resistentie-mutaties na gebruik van korte therapie-schema's voor pMTCT moeten nader onderzocht worden.

In meer ontwikkelde landen komt HIV transmissie van moeder naar kind tijdens de zwangerschap en rond de bevalling weinig voor omdat er een veelomvattend pakket van

pMTCT maatregelen beschikbaar is. In ontwikkelingslanden daarentegen hebben vele met HIV geïnfecteerde vrouwen geen toegang tot zelfs de meest basale vormen van pMTCT, zoals counselling, een HIV test en ARV prophylaxe. Data uit ontwikkelde landen suggereren dat de effectiviteit van pMTCT regiems waarbij drie ARV's worden toegepast groter is dan bij gebruik van slechts een of twee middelen.

In **hoofdstuk 2** laten wij zien dat de meeste gezonde en niet met HIV geïnfecteerde Nederlandse vrouwen aan wie een enkele dosis van 200 mg nevirapine (sd-NVP) werd toegediend nog aantoonbare concentraties van NVP in hun bloed hadden na meer dan 2 weken.[10] De mediane NVP halfwaardetijd, gevonden in de 44 onderzochte personen, was 56,7 uur. Een vergelijkbare waarde werd gevonden door Musoke et al.[11] In omstandigheden met beperkte financiële middelen, waar veel vrouwen pas laat in de zwangerschap voor het eerst een zwangerschapspolikliniek bezoeken, en slechts bij weinigen een onderzoek naar het aantal CD4 cellen in het bloed wordt verricht, zal sd-NVP waarschijnlijk nog lange tijd een belangrijke interventie blijven voor pMTCT. Ook in Tanzania heeft nog slechts een beperkt deel van de zwangere vrouwen toegang tot onderzoek op HIV in de zwangerschap en prophylaxe met ARV's. Een op de drie vrouwen krijgt nog sd-NVP voor pMTCT volgens het Global AIDS Epidemic Report van UNAIDS, 2010.[12] Door de sub-therapeutische NVP plasma concentraties die hierbij voorkomen, ontstaat makkelijk NVP resistentie, omdat de concentraties gedurende meerdere dagen lager zijn dat benodigd voor het remmen van de HIV replicatie, zoals gerapporteerd werd door Jackson et al.[13] Dit is van belang voor de opzet van interventie-studies om de ontwikkeling van NVP resistentie te voorkomen.

In **hoofdstuk 3** beschrijven wij een innovatief onderzoek naar de toepassing van een enkele dosis carbamazepine, een CYP3A4 enzym inducer, die werd toegevoegd aan de pMTCT medicatie. Wij vonden dat hierdoor NVP resistentie minder vaak optrad doordat NVP sneller werd geëlimineerd na toediening van sd-NVP[14] bij het begin van de bevalling bij HIV-geïnfecteerde vrouwen. We vonden dat toevoeging van één dosis carbamazepine de NVP plasma concentraties tijdens de bevalling niet beïnvloedde en dat deze plasma concentraties vergelijkbaar waren met die welke in eerdere studies waren gevonden[11;15-17]. Echter de NVP plasma concentraties een week na de bevalling waren significant lager. Zes weken na de bevalling werd HIV genotypering gedaan. De meest voorkomende mutaties waren K103N, Y181C/I en G190A. Van deze kwam K103N het meest voor (bij 9 vrouwen), en wel als de enige mutatie bij 8 van de 9 vrouwen, en in combinatie met G190A bij 1 vrouw. Met standaard HIV genotypering assays werden slechts bij 20% van de HIV populatie virus mutaties gevonden, en geen subpopulaties van mutanten. Verdergaande genotypering (deep sequencing) moet bij toekomstig onderzoek worden overwogen. We vonden ook een aanwijzing dat bij vrouwen met ondetecteerbare NVP concentraties NVP resistentie minder voorkwam, hetgeen het belang van deze benadering ondersteunt. De NVP concentraties bij de pasgeborenen waren waarschijnlijk niet gereduceerd vanwege de vertraagde enzym inductie en de lage concentraties van carbamazepine in de moedermelk.[18] In overeenstemming hiermee was de perinatale HIV-1 transmissie vergelijkbaar met die welke was gerapporteerd uit eerdere studies bij vrouwen die sd-NVP voor de eerste maal gebruikten.[4;19] Anderen gebruikten kortdurende interventies en reduceerden het optreden van NVP resistentie in belangrijke mate[14;20-22] ofschoon de prevalentie van resistentie mutaties afnam

naarmate de expositie aan sd-NVP langer geleden was. Toch bestaan er enkele aanwijzingen dat vrouwen met pre-existente mutaties alsook vrouwen blootgesteld aan sd-NVP maar zonder mutaties minder goed op ARV behandeling reageren.[23] Het grootste problem is de complexiteit van deze behandelingsmethoden, alsook het optreden van lamivudine (3TC) resistentie, welke gepaard gaat met virologisch en klinisch falen van de behandeling bij vrouwen die beginnen met 3TC-bevattende ARV combinatietherapie voor hun eigen gezondheid. Dit heeft geleid tot de aanbeveling terughoudend te zijn met de toepassing van AZT/3TC bevattende ARV behandelingsschema's.[24;25] Om de eenvoud van de behandeling te behouden, pasten Chi et al.[26] een enkele dosis emtricitabine + tenofovir toe samen met sd-NVP bij 400 vrouwen. Dit halveerde eveneens het optreden van NVP resistentie (25% versus 12%). Een recent onderzoek in Malawi[27] toonde aan dat de WHO optie B+ van pMTCT de toegang tot effectieve ARV behandeling voor HIV- geïnfecteerde zwangere vrouwen snel heeft doen toenemen. Echter elke interventie heeft zijn voor- en nadelen en momenteel zijn er toenemende zorgen over het ontstaan van resistentie tegen HIV geneesmiddelen bij langdurig gebruik als deze vroeg in het beloop van een HIV infectie worden toegepast. Ook de veiligheid van toepassing van ARV's voor de foetus, resp. de baby, en acceptatie en gelijke toegang tot deze middelen, blijven punten van aandacht. Ons onderzoek laat zien dat het mogelijk is om in pMTCT programma's een enzym inducer toe te voegen ter vermindering van de ontwikkeling van NVP resistentie in omstandigheden waar andere uitgebreidere behandelingsmogelijkheden (nog) niet haalbaar zijn. Als aanvulling op de initiatieven van de WHO tonen onze bevindingen, die nieuw zijn op het gebied van de pMTCT, aan dat de farmacologische benadering zoals door ons beschreven, mogelijk een extra effect heeft, terwijl toch de eenvoud van de behandeling behouden blijft. Ook blijft bij deze sd-NVP vorm van pMTCT de effectiviteit behouden als het middel opnieuw in volgende zwangerschappen wordt toegepast. Dit alles geldt voor omstandigheden waar toegang tot het volledige programma van ARV combinatietherapie nog niet mogelijk is.

In **hoofdstuk 4** wordt een onderzoek beschreven naar het effect van toediening van fenytoine gedurende 7 dagen na de bevalling bij vrouwen die sd-NVP gebruiken in het kader van pMTCT. Fenytoine is een enzym inducer die daardoor de eliminatie van NVP versnelt, met als gevolg dat ontwikkeling van NVP resistentie wordt tegengegaan. Wij vonden dat de NVP plasma concentraties bij de bevalling op hetzelfde niveau lagen bij de vrouwen die fenytoine kregen als bij degenen die het niet kregen, en de waarden die wij vonden waren goed vergelijkbaar met die gevonden bij eerder onderzoek.[11;17] Na de bevalling waren de NVP farmacokinetische parameters substantieel veranderd door de enzym inductie. Door toevoeging van fenytoine aan sd-NVP waren de NVP concentraties in het plasma 85% lager, en een significant groter deel van de vrouwen had ondetecteerbare NVP concentraties één en twee weken na de bevalling. Hierdoor was de NVP eliminatie halfwaardetijd 60% korter (in absolute waarden 35,8 uren korter). Dit is de sterkste verkorting van de NVP eliminatie halfwaardetijd die ooit werd gerapporteerd, met name in de doelgroep van HIV-geïnfecteerde zwangere vrouwen. Momenteel wordt een kuur van een week zidovudine/lamivudine aanbevolen om het ontstaan van NVP resistentie te voorkomen bij HIV-geïnfecteerde vrouwen die sd-NVP ontvangen tijdens de bevalling als onderdeel van pMTCT.[6;28-30] Wij vonden dat toevoeging van een korte kuur van 7 dagen fenytoine aan het begin van de bevalling een significante verkorting van de

eliminatie halfwaardetijd van NVP gaf. Zeven dagen fenytoine toedienen na de bevalling leidde niet tot nieuwe NVP resistentie mutataties. Wij bevelen echter aan dat dit wordt bevestigd in een groter prospectief onderzoek met voldoende power om aan te tonen dat fenytoine de verdwijning van NVP uit het bloed significant versnelt na de bevalling. Duizenden vrouwen krijgen nog steeds sd-NVP toegediend[31], hetgeen laat zien dat de implementatie van de nieuwe richtlijnen niet eenvoudig is. Fenytoine kan veilig in de zwangerschap worden toegepast, en ook tijdens borstvoeding[32] en er zijn bij zo'n kortdurend gebruik met zo'n lage dosis weining bijwerkingen te verwachten. De toevoeging van een enzym inducer aan sd-NVP voor pMTCT gedurende zeven dagen reduceerde het optreden van subtherapeutische NVP concentraties door een significante verkorting van de NVP eliminatie halfwaardetijd, terwijl geen nieuwe NVP resistentie mutaties werden gevonden. Omdat blootstelling aan subtherapeutische NVP concentraties kan leiden tot het ontstaan van NVP resistentie, en omdat fenytoine veilig is, kan sd-NVP met fenytoine worden toegepast als andere ARV middelen niet beschikbaar zijn. Deze toepassing dient in een groter fase III onderzoek nader te worden onderzocht.

In **hoofdstuk 5** worden de resultaten gepresenteerd van een systematisch overzicht en een meta-analyse betreffende de effecten van interventies met verschillende geneesmiddelen, en de duur van de inname ervan, op het ontstaan van NVP resistentie na gebruik van sd-NVP als onderdeel van ARV prophylaxe in het kader van pMTCT. In verschillende studies werd aangetoond dat inname van zidovudine vóór de bevalling plus een korte kuur (<8 dagen) met ARV's ná de bevalling het ontstaan van NVP resistentie bijna elimineerde (<0.02%). Bovendien werd aangetoond dat een kuur met ARV's gedurende 20-30 dagen na de bevalling[33;34] iets meer effectief was vergeleken met gebruik gedurende <8 dagen na de bevalling (0.003%)[30;34], echter een langduriger ARV behandeling is meer complex en moeilijker te implementeren in de dagelijkse praktijk in ontwikkelingslanden. De WHO richtlijn optie A (antepartum zidovudine, sd-NVP en een week zidovudine/lamivudine postpartum)[28] moet gevolgd worden om een aanvaardbaar minimaal risico van NVP resistentie te hebben in gebieden waar sd-NVP nog wordt toegepast.

In **hoofdstuk 6** beschrijven wij een onderzoek naar therapie monitoring van geneesmiddelen (TDM) van NVP. In de meeste generieke ARV combinaties die beschikbaar zijn in ontwikkelingslanden wordt het NNRTI nevirapine gecombineerd met twee nucleoside reverse transcriptase inhibitors (NRTIs). Een nadeel van NVP is dat het een lage genetische barriere heft tegen de ontwikkeling van resistentie.[35] HIV stammen die resistent zijn tegen NVP bevattende ARV combinatietherapie die verstrekt wordt in het kader van nationale AIDS bestrijdingsprogramma's in ontwikkelingslanden, zouden zich kunnen verspreiden in de algemene bevolking. Dit is zorgwekkend omdat er maar weinig tweedelijns geneesmiddelenopties zijn in ontwikkelingslanden. Vanuit farmacologisch perspectief zou TDM een belangrijk middel kunnen zijn om patiënten met een onjuiste expositie aan geneesmiddelen zoals NVP te identificeren, en daarmee ook het risico op virologisch therapiefalen en de ontwikkeling van resistentie op te sporen. Om de implementatie van TDM in ontwikkelingslanden te faciliteren, onderzochten wij alternatieve mogelijkheden voor het afnemen van materiaal bij de patiënt, en werd een andere techniek voor de bepaling onderzocht. Bij eerder onderzoek was aangetoond dat speeksel kan gebruikt worden als alternatief materiaal in plaats van plasma voor TDM van NVP. Dit is een

pijnloze en niet-invasieve methode van monsterafname met bovendien een lager risico van HIV-transmissie naar de gezondheidswerkers, en het kost weinig geld.[36] Als alternatief voor HPLC werd de relatief goedkope dunne laag chromatografie (thin layer chromatography -TLC) techniek onderzocht voor het meten van NVP in plasma.[37] Wij ontwikkelden een goedkope en eenvoudige TLC methode voor het semi-kwantitatief meten van NVP in speeksel. De methode werd gevalideerd in HIV-geïnfecteerde Tanzaniaanse volwassenen die met NVP bevattende ARV combinaties werden behandeld in KCMC. We vonden dat de methode sensitief, specifiek en betrouwbaar was voor het aantonen van sub-therapeutische NVP concentraties. Als deze techniek ook naar andere ontwikkelingslanden zou kunnen worden overgebracht, zou dit de verstrekking van HIV zorg en behandeling wereldwijd ten goede komen. Wij bevelen daarom aan dat deze goedkope en eenvoudige TLC techniek toegepast gaat worden voor het meten van NVP in speeksel in ontwikkelingslanden.

In de studie beschreven in **hoofdstuk 7** vergeleken wij NVP concentraties gemeten in speeksel met therapietrouw door de patiënten zelf gerapporteerd, bij patienten die met NVP-bevattende ARV's werden behandeld in KCMC, Tanzania. Rationeel gebruik van geneesmiddelen omvat het nemen van het geneesmiddel in de juiste dosering, de juiste hoeveelheid en op de juiste tijd zodanig dat de juiste concentraties worden bereikt bij >95% van de patiënten. In studies naar zelf gerapporteerde therapietrouw bij patiënten die ARV's gebruikten, werd bij > 95% adherentieconcentraties gevonden.[38;39] De zelf gerapporteerde therapietrouw die wij in onze studie vonden, was vergelijkbaar met die gevonden in Rwanda[40] waar het tellen van de pillen en een vragenlijst werden gebruikt om de adherentie te meten, en in Botswana waar zelf-gerapporteerde therapietrouw werd gemeten op de schaal van Morisky.[38] Bovendien was in onze studie onder de deelnemers die één van de twee NVP bevattende ARV combinaties gebruikten (d4T+3TC+NVP, respectievelijk AZT+3TC+NVP) de proportie met therapeutische NVP concentraties vrijwel gelijk. Door geneesmiddeleninteracties die kunnen voorkomen bij HIV-geïnfecteerde patiënten kunnen sub-therapeutische speekselconcentraties van ARV's ontstaan. Omdat het aantal patiënten dat niet-ARV co-medicatie gebruikte klein was, (3 patiënten gebruikten anti-hypertensiva, 6 geneesmiddelen tegen opportunistische infecties, en 4 anti-epileptica), was het moeilijk hierover conclusies te trekken in ons onderzoek. We bevelen daarom aan een grotere prospectieve studie te doen met voldoende power om farmacokinetische interacties vast te stellen. TDM van NVP via HPLC is erg kostbaar in ontwikkelingslanden zoals Tanzania, en is nauwelijks toepasbaar bij routine medische zorg. ARV resistentie en falen van de behandeling als consequentie van sub-optimale therapietrouw, zijn vaak moeilijk vast te stellen en niet gemakkelijk goed aan te pakken tijdens routinematige klinische patiëntenzorg in ontwikkelingslanden. Omdat behandeling met tweedelijns ARV's duur is en de medicijnen daarvoor niet altijd makkelijk beschikbaar, is effectieve behandeling van HIV infectie moeilijk als de eerstelijnsmedicatie niet werkt. TDM door het meten van geneesmiddelenconcentraties in het plasma kan nuttig zijn om op directe wijze de therapietrouw vast te stellen, echter de concentraties variëren tussen patiënten ten gevolge van wisselende absorptie, en door geneesmiddeleninteracties. Door de hoge kosten is het niet gelukt om TDM op te nemen in de routine patiëntenzorg, zelfs in ontwikkelde landen met een

ruimer budget voor medische zorg. Daarom bevelen wij voor ontwikkelingslanden aan gebruik te maken van de relatief goedkope en niet-invasieve TLC methode.

Hoofdstuk 8 bevat een samenvatting van de voornaamste bevindingen in deel I & deel II van het proefschrift.

TOEKOMSTPERSPECTIEVEN

Onze resultaten op het gebied van enzym inducers vormen een stimulans tot nader farmacogenetisch onderzoek. Genetische verschillen in metabolisme kunnen de individuele respons op geneesmiddelen beïnvloeden, zowel wat betreft het therapeutisch effect, als wat bijwerkingen betreft. NVP wordt voornamelijk gemetaboliseerd door CYP3A en CYP2B6.[41] Deze enzymen vertonen inter-individuele verschillen in expressie of in mate van activiteit, die waarschijnlijk bijdragen aan de farmacokinetische variabiliteit van NVP. Het CYP2B6 gen is zeer polymorf met talloze single nucleotide polymorfismen (SNPs) en daarmee geassocieerde haplotypen. Deze variabiliteit is grotendeels het gevolg van genetische polymorfismen in de cytochroom enzymen die NVP metaboliseren.[42;43]

Wij bevelen daarom het volgende wetenschappelijk onderzoek aan in Tanzania:

- GGenotypering van genetische varianten die zijn geassocieerd met een veranderde expressive van CYP2B6 en CYP3A5. Stratificatie van de patiënten op basis van de volgens de genetische variant voorspelde CYP2B6 en CYP3A5 expressie niveau's kan helpen het effect van enzym inductie op de NVP dispositie te ontdekken.[42-44]

Onze resultaten gepresenteerd in deel II van het proefschrift, laten zien dat de gevalideerde methode van TLC voor TDM tijdens behandeling van patiënten met HIV infectie de kloof tussen het laboratorium en de klinische praktijk kan overbruggen.

Wij bevelen daarom het volgende aan:

- Kwaliteitscontrole in de vorm van TDM tijdens behandeling door het meten van NVP in speeksel met behulp van TLC moet worden aangemoedigd in alle centra in ontwikkelingslanden waar medische HIV zorg verleend wordt, ook in Tanzania, en wel routinematig, met als doel de therapietrouw te meten, de effectiviteit van behandeling te verbeteren en behandelinsmogelijkheden te behouden voor de toekomst.
- Daarnaast is onderzoek nodig om vast te stellen of andere toegepaste ARV's dan NVP in speeksel kunnen worden gemeten met behulp van TLC.

De toepassing van sd-NVP voor pMTCT, waardoor de moeder-kind transmissie van HIV teruggebracht wordt tot 11-13%, blijft een optie voor ontwikkelingslanden, ofschoon een bredere toepassing van meer complexe schema's van ARV combinaties vanaf de 14e zwangerschapsweek en na de bevalling dient te worden overwogen. Hierdoor wordt de HIV-transmissie van moeder op kind teruggebracht tot 1-2%. Hierbij dienen niet alleen de extra uitgaven voor de meer complexe benadering (materiaalkosten en personeelskosten) in acht te worden genomen, maar ook het aantal additioneel voorkomen HIV infecties bij pasgeborenen. Immers, door minder HIV infecties verminderen de uitgaven voor de gezondheidszorg, dit nog ongeacht de vermindering van menselijk lijden die het teweeg brengt.

- Wij bevelen daarom aan een kosten-baten analyse uit te voeren teneinde de best haalbare benadering voor pMTCT te vinden voor toepassing in ontwikkelingslanden, inclusief Tanzania.

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ACKNOWLEDGEMENTS

Praise and Glory to the almighty God that his will, blessings and guidance has made this work possible. Many people have contributed to the success of this work and I wish to acknowledge and appreciate everything I got from them.

Supervisors/Promotors and advisors

I send my sincere thanks to Prof. David Burger, Prof. Wil Dolmans, my supervisors and promotors. Thanks for your academic guidance, encouragement, patience and critical review for several publications included in this thesis. You had much confidence on me from the beginning to the end. I feel privileged to have worked with you and I look forward to future collaborations.

Dr. Elton Kisanga, my co-promotor thank you for your advice and scientific inputs.

To the manuscript committee, Professor F.G.M. Russel, Professor J. Van der Velden and Dr. Richter Thanks for your quick and positive judgement on my manuscript.

I would also like to thank various people from my institution, KCMC. First of all to the KCMC administration and particular *Prof. John Shao* and *Dr. Mark Swai* for their commitment to develop the institution and personnel, and it is through this commitment that I was able to do my PhD. Thank you too *Prof. F. W Mosha* for your advice, support and you always inquired about my progress.

I am also deeply indebted to the Pharmacy department members of KCMC for taking all my responsibilities during the time I was abroad and when I was busy in the field while in Tanzania. Thank you *Lucas Chagula*, *Faustin Sasi,* and *Abraham Makundi*. *Mama Martha Shayo* for your assistance at the study sites.

I wish to mention and appreciate the support from *Siphanesta Richard*. Thank you *Faraja King'ori, Tupokigwe, Anita Zeramula, T.B Sonda, Elizatbeth Msoka and Prisca Njau* and all other members from KCRI.

All the fieldwork would not have been successful without great contribution, support and inputs from more people than I can mention on a page, these are the research team members. Rose Mallya (the trial manager). Our driver John Mushi (you always took us to the field and back safely). Thank you Lisselotte Wolter, Arnold Ndaro, Emma Msuya for the laboratory work and timely ordering for supplies.

I would like to express my sincere appreciation to all the mother/infant pair and staff from all the antenatal clinics participating in the trial. (Pasua, Majengo, Bondeni and Mawenzi antenal clinics).

Further thanks to all colleagues from the VITA team at UTH (Lusaka, Zambia) and MRC CTU (London, UK). It has been absolutely great to work with you in the VITA study.

Mirjam Oudijk & Minke Huibers for working with VITA 1 studies. I hope you enjoyed your stay in Tanzania.

Special thanks to Rafaella L'homme and Quirine Fillekes for the pleasant cooperation and for the hard work collecting our study samples. I hope you enjoyed your stay in Tanzania.

In the Netherlands I would like to thank all the personnel in the Laboratorium Klinische Farmacie for doing all the analysis for the pharmacokinetic studies.

Special thanks to Prof. Andre van der Ven who brought the APRIORI project to KCMC which enabled part of the studies presented in this thesis.

In a special way I wish to express my sincere appreciation to my family for encouragement and advice extended to me all the time when I was struggling with my studies.

Lastly my husband: For sure I owe the greatest gratitude to my husband and children. Your love support and understanding has helped me through this journey. To my children Sharoon, Brenda and Jan, this book will remind you of what your mummy was doing when you missed her at home. Thank you for your love.

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