JOINING FORCES IN THE FIGHT AGAINST HIV/AIDS IN AFRICA

Clinical Pharmacology Studies in Pregnant Women and Children

Quirine Fillekes

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"From the people in need to the sun and the seas
We're in this together"

SIMPLY RED

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CHAPTER 01

Introduction

GLOBAL EPIDEMIC OF HIV

In 1981, the first cases of the acquired immunodeficiency syndrome (AIDS) were observed in San Francisco, US[1]. At that time AIDS had not yet been recognized as a result of a viral infection. Two years later, it was found that a new retrovirus, now known as the human immunodeficiency virus (HIV), was the causative agent of AIDS[2].

Since the early 1980s, the pandemic of HIV has had an enormous impact. Until now, more than 30 million people have died from HIV/AIDS and currently 34 million people are estimated to be living with HIV[3]. The southern part of Africa is the region most severely affected, and it accounts for 68% of the global total living with HIV. In contrast to most other regions, where particular groups, such as men who have sex with men or injectable drug users, are most at risk, the epidemic in Sub-Saharan Africa has substantially affected women in the general population. Since the prevalence is high in women of reproductive age, transmission of HIV from mother to child (MTCT) is also common in the region. In 2011, 330.000 children acquired HIV infection (90% due to MTCT) and more than 90% of those live in Sub-Saharan Africa[3].

The total number of people living with HIV still continues to rise, but the overall growth appears to have stabilized, as the number of people newly infected globally each year is continuing to fall and fewer people are dying from AIDS-related causes worldwide, since the introduction of combination antiretroviral therapy (cART). Despite the progress made in the fight against HIV/AIDS during the last decade, the HIV pandemic remains one of the most serious challenges to global health.

ANTIRETROVIRAL TREATMENT

Prior to 1987, no antiretroviral drugs were available and therapy consisted of treating complications from HIV/AIDS. Zidovudine was the first approved drug for HIV treatment and is still used in current regimens. However, it took almost 10 years before viral replication could be adequately suppressed. In 1996, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) became available, resulting in major advances. Rather than monotherapy, more potent, so called combination ART, which consists of a combination of three or more antiretroviral drugs from at least two different classes, was introduced leading to a significant decrease of the AIDS mortality rates and a dramatic increase in the life-expectancy of HIV-infected people[4]. HIV has become eventually more of a chronic disease rather than a fatal one – at least for those able to access drugs.

At present, six different classes containing a total of 24 drugs with varied mechanisms to inhibit HIV replication are approved by the Food and Drug Authorization (FDA) for HIV treatment. An overview of those is presented in Table 1.

Table 1 ▶ Antiretroviral drugs in HIV treatment

Class	Mechanism	Drugs
Nucleoside reverse transcriptase inhibitors (NRTIs)	Inhibition of the viral enzyme reverse transcriptase. NRTIs are prodrugs that require intra-cellular phophorylation to the active tri-phosphate metabolites[27].	Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, Tenofovir, Zidovudine
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Induction of allosteric changes in the viral enzyme reverse transcriptase[28].	Efavirenz, Etravirine, Nevirapine, Rilpivirine
Protease inhibitors (PIs)	Inhibition of HIV reproduction by inhibition of the viral enzyme protease[29].	Atazanavir, Darunavir , Fosamprenavir, Indinavir, Lopinavir Nelfinavir, Ritonavir, Saquinavir , Tipranavir
Integrase inhibitors	Inhibiting of viral DNA strand transfer to the host genome in the nucleus[30].	Elvitegravir, Raltegravir
CCR5 receptors antagonists	Inhibition of HIV entry into the cell by inhibition of binding of HIV to the CCR5 co-receptor on the CD4-cell[31].	Maraviroc
Fusion inhibitors	Prevention of fusion of the viral envelope with the host cell[32].	Enfuvirtide

ART IN AFRICA

Unfortunately, ART is still not universally accessible. Since the turn of the millennium, a massive scale-up of access to ART in low-income countries, especially in Africa, has been realized with political commitments[5-7] and extensive international financial support. To further scale-up access to ART in resource-limited countries, the Western approach of individualized patient care using advanced laboratory monitoring, such as viral load testing, resistance genotyping and routine high-technology therapeutic drug monitoring, and the full range of antiretroviral drugs, is not feasible[8]. In resource-limited settings, doctors are scarce, laboratory infrastructure is lacking and not all antiretroviral drugs are available. Therefore, the World Health Organisation's (WHO) approach to rapid scale-up of universal ART access is a population-based strategy using standardized antiretroviral treatment protocols, such as simplified drug regimens and appropriate fixed-dose combination (FDC) drug formulations.

Major gains have been achieved in scaling-up ART. By the end of 2011, 8 million HIV-infected adults and children were taking cART, a 20-fold increase since 2003[3]. For the first time the majority (54%) of people eligible for cART in low- and middle-income countries were receiving it. Despite these remarkable achievements, the global coverage for the most vulnerable population in need for ART is still low to moderately low; only 28% of children 0-14 years of age in need for ART and 50% of pregnant women and infant pairs in need of antiretroviral prophylaxis for the prevention of MTCT (pMTCT) are currently receiving the life-saving medicines[3].

ART in the prevention of MTCT in Africa

Since 2010, HIV-infected pregnant women with CD4 counts ≤350 cells/mm^3 or WHO stage 3 or 4 disease are recommended to start on combination therapy for life for their own health as well as for the prevention of infant HIV infection.

For pregnant women with CD4 counts >350 cells/mm^3, who do not require treatment according to current criteria, pMTCT guidelines recommend starting antiretroviral prophylaxis early in pregnancy and the use of antiretroviral drugs by either the mother or child during the peripartum period. Since 2000, WHO recommended a simple, affordable and effective intervention of a single-dose of nevirapine at labour onset for pMTCT of HIV[9]. This has reduced MTCT by 40% and has saved thousands of babies from HIV infection. However, a major disadvantage is the development of nevirapine resistance in mothers (1-69%) and infants[10]. Single-dose nevirapine alone is therefore no longer endorsed as a preferred option, but is recommended only as part of a longer prophylactic regimen (zidovudine pre-delivery, single-dose nevirapine and zidovudine/lamivudine at delivery, zidovudine/lamivudine for 7 days post-delivery). This (option A) and other more complex regimens (option B/B+) have been shown to be more effective in preventing MTCT with less occurrence of nevirapine resistance[11].

It is estimated that 18-64% of women living in Sub-Saharan Africa with HIV are receiving cART for pMTCT, as now recommended by WHO (Option B+). The simple single-dose nevirapine at labour is a fading concept, but in practice it is still widely used by thousands of women in resource-limited settings, particularly in more rural areas where access to cART is more limited.

ART in children in resource-limited settings

Over the past years, uniform and simple first-line regimens, generally comprising two NRTIs and one NNRTI (nevirapine or efavirenz), have been developed for children in line with WHO's population-based strategy for the scale-up of ART access in resource-limited settings[12,13]. Also access to second-line therapy, which includes two NRTIs and one ritonavir-boosted PI (lopinavir), has been promoted. cART has become more and more available as generic inexpensive fixed-dose combination (FDC) formulations in low-income countries. However, many of these formulations have been developed for adults. Appropriate and affordable antiretroviral formulations for children are still lacking. Scarcity has restricted roll-out in resource-limited settings (that is why coverage is only 28%), but a number of FDC tablets with dose ratios correct for children have and are being developed in order to simplify first- and second-line treatment and increase the number of children on cART[14]. Generic pharmaceutical manufacturing companies are leading many of these initiatives and trying to fill the gap in resources for the unmet need of ART in the paediatric population in Africa.

AIM OF THE THESIS

The overall aim of this thesis was to optimize HIV treatment in HIV-infected pregnant women and children in Africa by studying clinical pharmacology of antiretroviral drugs in the most vulnerable populations in resource-limited settings.

The research objectives of this thesis were:

- To investigate simple, accessible and affordable strategies for the prevention and the reduction of MTCT of HIV in Sub-Saharan Africa using enzyme inducers for the elimination of nevirapine resistance after single-dose nevirapine as part of the anti-retroviral prophylaxis for MTCT.
- To optimize paediatric antiretroviral regimens for the scale-up of HIV treatment in resource-limited settings, where new appropriate antiretroviral drug formulations were evaluated and newly developed WHO weight-band based dosing recommendations were validated for the use in resource constraint settings.

CLINICAL PHARMACOLOGY STUDIES

Clinical pharmacology emphasizes the pharmacokinetic and pharmacodynamics of drugs in humans. Pharmacokinetics describes the effect of the human body on the drug, which includes absorption, distribution, metabolism and excretion. Pharmacodynamics is the study of the relationship of drug concentration and effect on the human body.

Part I: Clinical pharmacology studies in HIV-infected pregnant women

The first part of this thesis deals with clinical pharmacology studies in pregnant women using single-dose nevirapine as part of antiretroviral prophylaxis for pMTCT.

Notably, single-dose nevirapine is still being used globally in pMTCT, with a risk of 1-76% of development resistance against nevirapine from just this single dose[10]. Development of nevirapine resistance is most likely due to its long elimination half-life (61 hours[15]), leading to several days to weeks of subtherapeutic plasma concentrations, coupled with its low genetic barrier to resistance. Newly emergent resistant HIV may be transmitted to the infant or to others, limiting their treatment options, and may also reduce future combination antiretroviral therapy (cART) efficacy in the mother[16]. **Chapter 2** of this thesis includes a systematic review of data on the different interventions, which have been investigated until now, for reducing nevirapine resistance. Most interventions are antiretroviral-based, but a totally different approach is a pharmacological strategy of adding a CYP*3A4* enzyme inducer to the single-dose of nevirapine. Nevirapine is extensively metabolized in the liver by cytochrome P450 (CYP) isoenzymes *3A4* and *2B6*[17] and other drugs which affect these enzymes have been shown to decrease nevirapine elimination half-life in healthy women[18], with greatest reductions from carbamazepine and phenytoin. In this thesis a clinical trial investigating the effect of a single-dose of carbamazepine (**Chapter 3**; VITA-1 trial) and a pilot study investigating the effect of seven days phenytoin (**Chapter 4**; VITA-2 trial) on nevirapine pharmacokinetics and the development of nevirapine resistance in HIV-infected pregnant women in Tanzania and Zambia are presented.

Part II: Clinical pharmacology studies in HIV-infected children

The second part of this thesis focuses on the clinical pharmacology of cART in HIV-infected children in Africa. Adequate drug exposure is essential in HIV treatment of children, who generally start treatment earlier and will need to take it for far longer than adults: it is therefore a major goal in the development of appropriate paediatric FDCs. However, obtaining optimal drug exposure in children is challenging, as they cannot simply be regarded as small adults[19]. Pharmacokinetics of antiretroviral drugs are highly variable in the paediatric population as children mature and grow rapidly[20]. In an open randomized trial, called CHAPAS-1 (Children with HIV in Africa-Pharmacokinetics and Adherence of Simple antiretroviral regimens), appropriate dosing of FDC tablets, approved based on early pharmacokinetic data generated within the trial, was assessed. The tablets have higher nevirapine to lamivudine/stavudine dose ratio compared to adults FDCs. It is known that children, particularly the youngest, metabolize nevirapine more rapidly than adults[21, 22]. **Chapter 5** (substudy of CHAPAS-1) reports the exposure to nevira-

pine in African, HIV-infected infants weighing 3-6 kg taking these paediatric FDC tablets. WHO guidelines recommend nevirapine dose escalation (half dose for the first 14 days) when initiating ART to avoid toxicity from high initial nevirapine levels. Since young children metabolize nevirapine more rapidly, there is the potential for inadequate nevirapine plasma levels during dose escalation, which could result in slower viral load suppression or increased risk of later virological failure. In the subsequent chapter (**Chapter 6**; also a substudy of CHAPAS-1) the pharmacokinetics of nevirapine at week 2 after nevirapine initiation with and without dose escalation strategy was evaluated in Zambian HIV-infected infants/children and its relationship with safety and efficacy was assessed.

Furthermore, paediatric dosing has not only been standardized with FDC formulations, but also by using simplified weight-bands based dosing recommendations. However, these have typically been determined using extrapolation from licensed mg/kg or mg/m^2 doses, and require validation for optimal HIV treatment. **Chapter 7** describes actual efavirenz plasma exposure in children treated following the 2006 efavirenz weight-band based dosing recommendations of WHO using full pharmacokinetic information. This was a substudy of the ARROW trial, an open-label randomized trial comparing routine laboratory (toxicity, CD4) versus clinically driven monitoring strategies, and also comparing three different first-line ART strategies in HIV-infected infants and children in Uganda and Zimbabwe (www.arrowtrial.org)[23].

There are numerous factors which may lead to interindividual variability in pharmacokinetics, such as gender, age, body weight, non-compliance, and genetic factors[24, 25]. **Chapter 8** evaluates mid-dosing interval efavirenz plasma concentrations and the influence of CYP*2B6* polymorphisms in relation to efficacy, tolerability, and adherence in Rwandan HIV-infected children.

In **Chapter 9** (again a pharmacokinetic substudy of ARROW) we evaluated a different weight-band based dosing recommendation from WHO, for twice-daily zidovudine, which has not been studied before, to investigate whether this provides optimal exposure in Ugandan, HIV-infected children.

The final chapter before the General Discussion expands on the scale-up of second-line ART access. ART formulations suitable for children should ideally be pleasant-tasting, with no food restrictions, be heat-stable and require administration no more than twice-daily. The present accessible antiretroviral drug for second-line in resource-limited settings is ritonavir-boosted lopinavir. This drug is currently only available as syrup, which tastes bad, requires a stable cold chain and has a relatively short shelf-life, or as tablets, which are very hard, relatively large, unscored and cannot be cut into pieces (as this significantly reduces bioavailability). tablets cannot typically therefore be taken by children younger than 5-6 years old – in contrast to many of the FDCs discussed

above which can be taken by young children, and are generally preferred by carers[26]. A randomised pharmacokinetic bio-equivalence phase Ib trial (CHAPAS-2) was designed (**Chapter 10**) to assess the pharmacokinetics and acceptability of ritonavir-boosted lopinavir in sprinkle and tablet formulations for treatment of HIV infected children in Africa.

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PART I

CLINICAL PHARMACOLOGY STUDIES IN HIV-INFECTED PREGNANT WOMEN

CHAPTER 02

Effectiveness of drug interventions reducing nevirapine resistance after single-dose exposure for perinatal HIV prevention: a systematic review

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In preparation

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ABSTRACT

Objectives

To assess the effectiveness of different drug interventions to prevent 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 the emergence of nevirapine resistance conferring mutations 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 not different with a 0.003% (95% CI -0.054-0.060) incidence of nevirapine resistance conferring mutations.

Conclusions

Addition of antepartum zidovudine plus antiretroviral drugs postpartum to single-dose nevirapine for pMTCT has shown to nearly eliminate the emergence mutations associated with reduced susceptibility to nevirapine. The effect of longer (20-30 days) post partum regimens was similar compared with a short (<8 days) postpartum regimen. The WHO guideline option A (antepartum zidovudine, single-dose nevirapine and one week of lamivudine/zidovudine postpartum) seems to be a rational approach to effectively reduce the risk of emerging 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 breastfeeding population[1]. Nearly half of the risk of MTCT occurs 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.

A major disadvantage of single-dose nevirapine is the rapid emergence of mutations in the HIV-1 reverse transcriptase gene (RT) that confer reduced susceptibility to nevirapine. These mutations may be selected in both the mothers and the infants[2]. A major factor contribution to the rapid selection of these variants is the long elimination half-life of nevirapine (61 hours[3]) resulting in several days to weeks of subtherapeutic nevirapine plasma concentrations[4]. Secondly, the genetic barrier to resistance for nevirapine is considered to be low, which means that only one or two mutations in HIV-1 RT are sufficient to render the virus resistant to antiviral treatment with nevirapine. HIV-1 variants harbouring nevirapine resistance mutations may be transmitted to the infant or to other individuals, limiting their future treatment options. It may also reduce efficacy in the mother with future combination antiretroviral therapy (cART) containing non-nucleoside reverse transcriptase in-hibitors, such as nevirapine[5]. In a previous meta-analysis, published in 2007, the prevalence of nevirapine resistance mutations upon a single-dose nevirapine at labour onset with or without antepartum antiretroviral drugs was estimated to be 36% between four to eight weeks postpartum[2]. Given that since then more effective options have become available, this simple and single time point intervention has faded from current state of the ART clinical practice in developed countries, but single-dose nevirapine in the absence of additional antiretroviral agents is still widely administered to thousands of women in resource-constrained settings, particularly in more rural areas where access to cART is 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) for situations where 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 the use of other drugs, have been studied since the introduction of single-dose nevirapine. Until now, evaluation studies on the efficacy of co-interventions to reduce the emergence of nevirapine drug resistance mutations (DRMs) has not yet been conducted. We therefore performed a systematic review and meta-analyses to assess the effect of different drug interventions and duration, on nevirapine resistance development after use of a single-dose nevirapine as part of antiretroviral prophylaxis for pMTCT.

METHODS

Search strategy

A literature search 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 received a single-dose of nevirapine for pMTCT were enrolled; a drug intervention aiming at reducing nevirapine resistance development and resistance incidence was determined <3 months after intake of single-dose nevirapine. No design, place or language restrictions were set, but full-text publications 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 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 effect of adding antepartum zidovudine on the proportion of nevirapine resistant viral mutations after single-dose nevirapine alone, 2) the effect of adding antepartum zidovudine on the reducing effect of postpartum interventions to reduce nevirapine resistance after single-dose nevirapine, 3) the effect of short-course

postpartum interventions on the proportion of nevirapine resistance after single-dose nevirapine, 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 on 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%)[11].

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[12-16], 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[17, 18]. The criteria according to the STROBE guidelines were used to assess the quality of observational studies (Table 2)[12-16, 19, 20]. 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)[21].

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
In-consistency	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 ana-lysis 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
	Forest plot	Drop-out rate	-1: non-acceptable drop-out rate (i.e. >30%)	
		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		

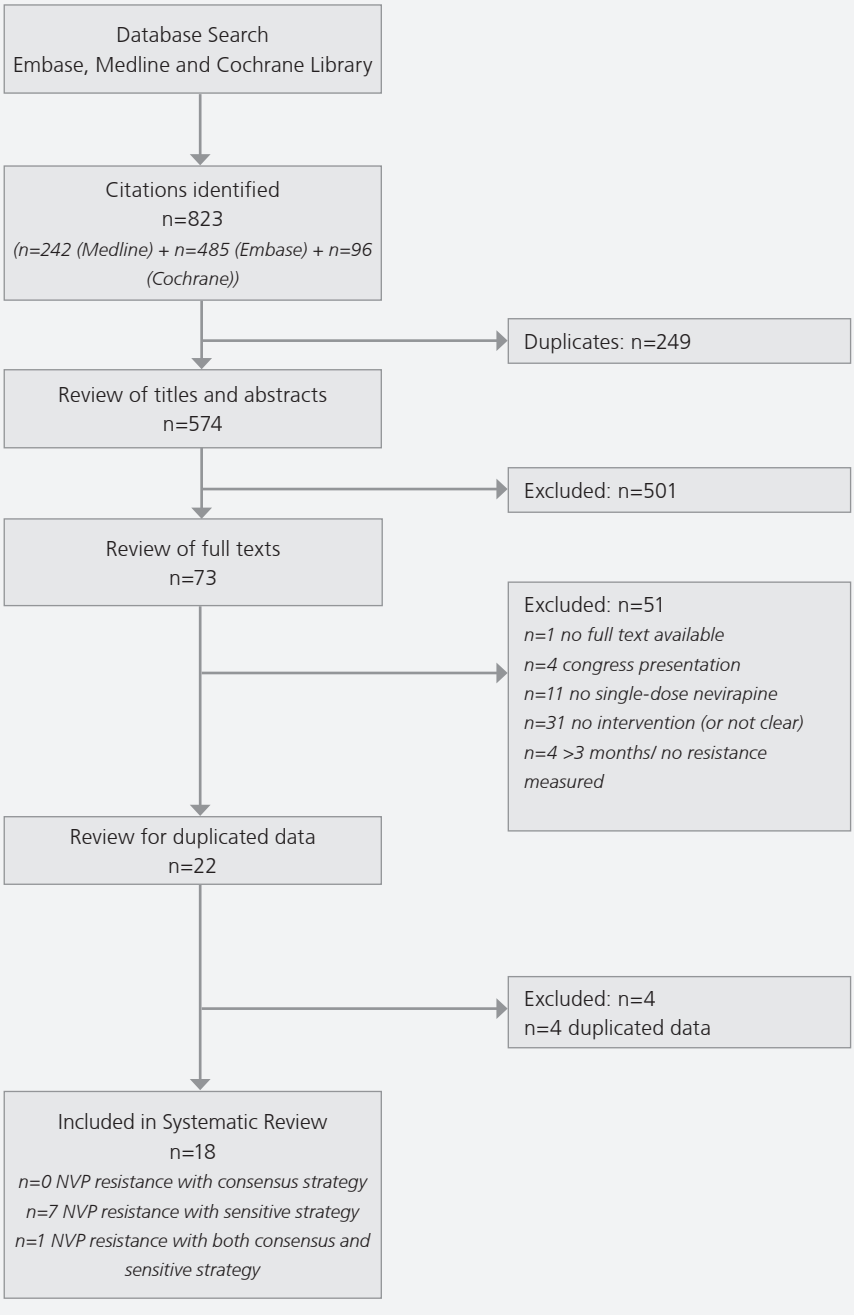
TABLE 2 Schematic overview for scoring GRADE criterions per outcome measure

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 1 ▸ Flow chart of literature search and selection procedure



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.

One study presented nevirapine resistance data measured both by a population sequencing based drug resistance mutation detection assay and by more sensitive drug genotypic techniques. Eleven (24 study arms) of the 18 studies contained nevirapine resistance data measured by a population sequencing based drug resistance mutation detection 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).

Effect of antepartum zidovudine

Since only studies with a drug intervention aiming at 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 population and five sensitive sequencing) were on antepartum zidovudine from 28-36 weeks of gestation combined with single-dose nevirapine at delivery. 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. 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).

Effect of 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 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 of 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 and 3). 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 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). This shows a >2800 times lower incidence compared to women receiving a single-dose nevirapine alone.

Duration of long-course postpartum drug interventions

In four study arms postpartum drugs were provided for a longer time period (Figure 2 and 3): 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 (0.011%), 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 upon both short and long postpartum courses compared to other regimens, but this could not be statistically confirmed.

FIGURE 2 Forest plot of nevirapine resistance in each intervention arm

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	Nr. of women with new resistance	Total nr 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%

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%
Chalermchockcharoenkit 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%

TABLE 3 ▸ Continued

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Nr. of women with new resistance	Total nr. of women	Percentage of resistance
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%
Shapiro et al[40]	2006	AIDS	RCT	-	Botswana	1 month postpartum	ViroSeq HIV-1 Genotyping System (Applied Biosystems, Foster City, CA, USA)	antepartum ZDV and sdNVP plus sdTDF/FTC at labor	20	173	12%
								antepartum ZDV and sdNVP at labor	69	155	45%
Chaix et al[41]	2006	Journal of Infectious Diseases	observational study	SIDA DIT-RAME 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%

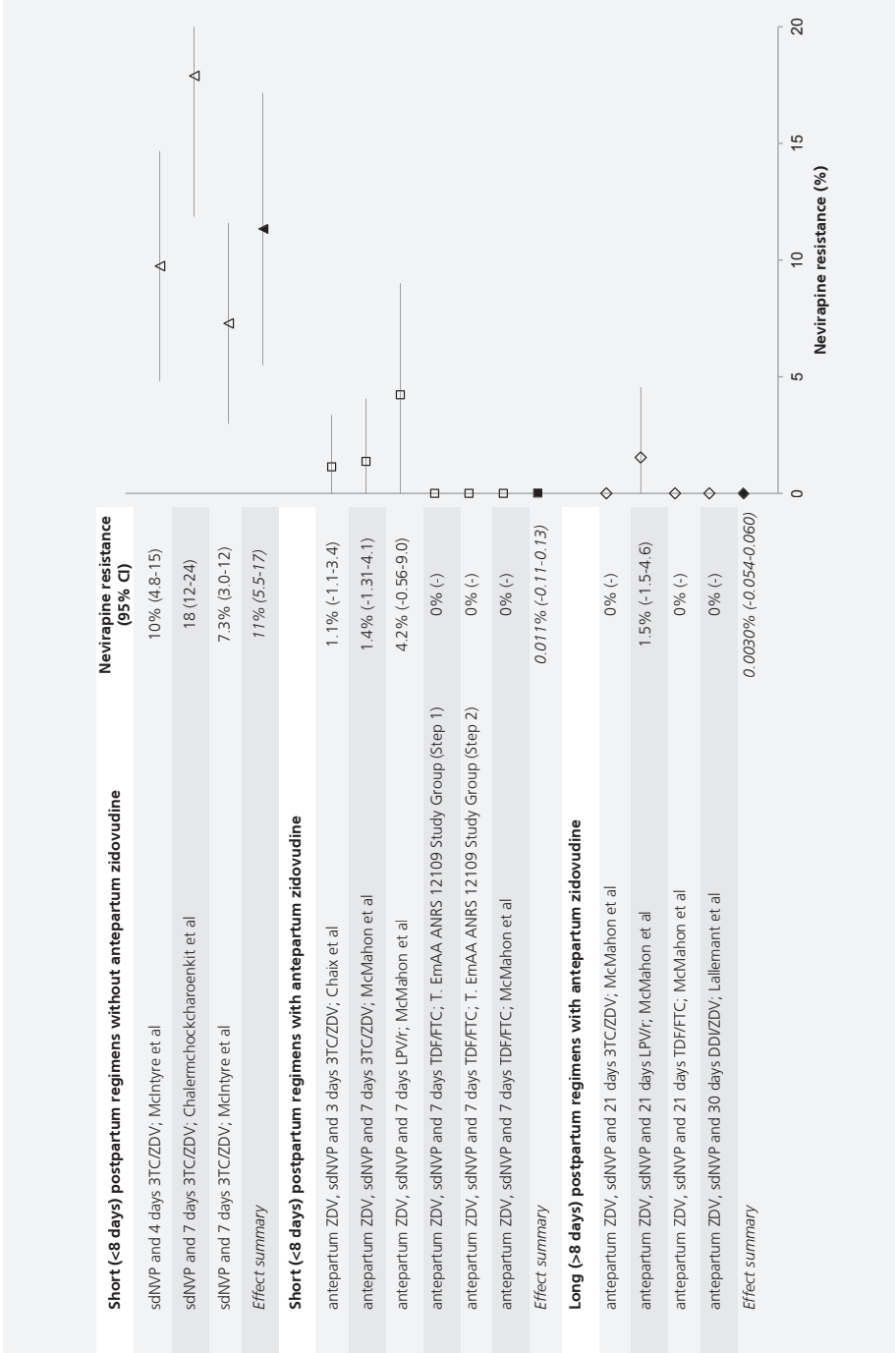
McMahon et al[27]	2013	Clin Infect Dis	RCT	-	Sub-Saharan Africa, India, Haiti	6 weeks after completion of study treatment	ViroSeq HIV-1 Genotyping System (Aversion 2.0, Celera Diagnostics, Alameda, CA, USA)	antepartum ZDV and sdNVP at labor and 7 days 3TC/ZDV postpartum	1	73	1.4%
								antepartum ZDV and sdNVP at labor and 21 days 3TC/ZDV post-partum	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	observatio-nal pros-pective co-hort 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%
Hauser et al[43]	2012	PLoS One	obser-vational 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%

TABLE 3 ▸ Continued

Author	Year	Journal	Study design	Study name	Country	Time of genotypic assessment	Type of genotypic assessment	Arm	Nr. of women with new resistance	Total nr. of women	Percentage of resistance
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	IM-PAACT 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%
								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	obser-vational 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	obser-vational 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



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 at 21% compared to the incidence in women receiving single-dose nevirapine alone. Despite 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, 22-27]. Addition of only a seven-day course of antiretroviral drugs 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 anti-retroviral 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 the findings of McMahon *et al* evaluating long versus short course antiretroviral strategies. Noteworthy, their findings showed that 21-day regimens were significantly better than seven-day regimens[28].

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, 29], probably due to higher level of viral replication when single-dose nevirapine is used.

A limitation in our systematic review is that the number of studies was small, especially for the quantification of effect summaries, 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]. A 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[30]. 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 cART for their own health[31, 32]. 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 maximal effect on preventing the emergence of nevirapine resistance (i.e. elimination). A single-dose[33] or a seven-day course of an enzyme inducer[34] 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 for pMTCT 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 strongly reduce or eliminate MTCT. Intensification of the current postpartum courses or alternative regimens need to be evaluated to achieve the best feasible minimum risk of nevirapine resistance to emerge in regions where single-dose nevirapine is still being used.

SUPPLEMENTARY MATERIAL 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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CHAPTER 03

Intrapartum single-dose carbamazepine reduces nevirapine levels faster and may decrease resistance after a single-dose of nevirapine for perinatal HIV prevention

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ABSTRACT

Background

WHO guidelines recommend zidovudine+lamivudine for seven days from labor onset in HIV-infected women receiving single-dose nevirapine 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/mm^3, able to regularly attend the antenatal clinics in Moshi, Tanzania were enrolled 1:1 by alternate allocation to receive 200 mg single-dose nevirapine alone (sdNVP), or in combination with open-label 400 mg single-dose carbamazepine (sdNVP/CBZ) at delivery (ClinicalTrials.gov NCT00294892). The co-primary outcomes were nevirapine plasma concentrations one week and nevirapine resistance mutations six weeks post-partum. 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 (IQR) 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) (GMR: 0.64, 95% CI: 0.43-0.96; p=0.03). Six weeks postpartum, nevirapine mutations were observed in 11/52 (21%) in sdNVP and 6/55 (11%) in sdNVP/CBZ (odds ratio=0.46, 95% CI: 0.16-1.34; p=0.15).

Conclusions

Addition of single-dose carbamazepine to single-dose nevirapine at labor onset in HIV-infected, pregnant women did not affect nevirapine plasma concentration at delivery, but significantly reduced it one week postpartum, with a trend towards 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 to the mother at onset of labour and single-dose nevirapine 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 MTCT by 40% and has benefited thousands of babies since its introduction in 2000.

Nevirapine is a non-nucleoside 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 61-66h). 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 three major implications. Firstly, the efficacy of nevirapine or other NNRTIs in combination antiretroviral therapy (ART) may be diminished when a patient harbours resistant virus. Indeed, studies in sub-Saharan Africa suggest that recent exposure to single-dose nevirapine (<6 months) is associated with increased risk for virological and clinical failure[7,10-13]. Secondly, nevirapine-resistant strains may be transmitted to others, limiting their treatment options. Thirdly, nevirapine resistance could reduce the efficacy of single-dose nevirapine in subsequent pregnancies[14,15], although data suggest nevirapine resistance fades in these women over time and single-dose nevirapine remains effective for prophylaxis in subsequent pregnancies[16,17].

Updated World Health Organisation (WHO) guidelines therefore now recommend adding zidovudine+lamivudine for seven days postpartum to cover the prolonged presence of subtherapeutic nevirapine concentrations after single-dose nevirapine at labor onset[18-20]. Recent studies show adding single-dose tenofovir+emtricitabine to single-dose nevirapine 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 eight different CYP*3A* induction strategies with different drugs demonstrated that elimination half-life of single-dose nevirapine 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 CYP*3A4* enzyme inducer, would diminish nevirapine resistance development by reducing elimination half-life after exposure to single-dose nevirapine 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, three primary and one 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 post-test counseling and were informed about the trial. Eligible HIV-infected women were: aged 18-40 years, CD4 count >200 cells/mm^3 (i.e. did not qualify for ART at the time), antiretroviral (ARV) naïve, 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 single-dose nevirapine in a previous pregnancy and serious illness that required systemic treatment/hospitalization. Women who qualified for ART (i.e. 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 (RUNMC), 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 single-dose nevirapine (200 mg) or single-dose nevirapine plus single-dose carbamazepine (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 single-dose nevirapine suspension was administered to the baby within 24-72 hours after birth. The randomization list was generated as alternate allocations by the study pharmacist (i.e. 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 four 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 six months and then wean rapidly.

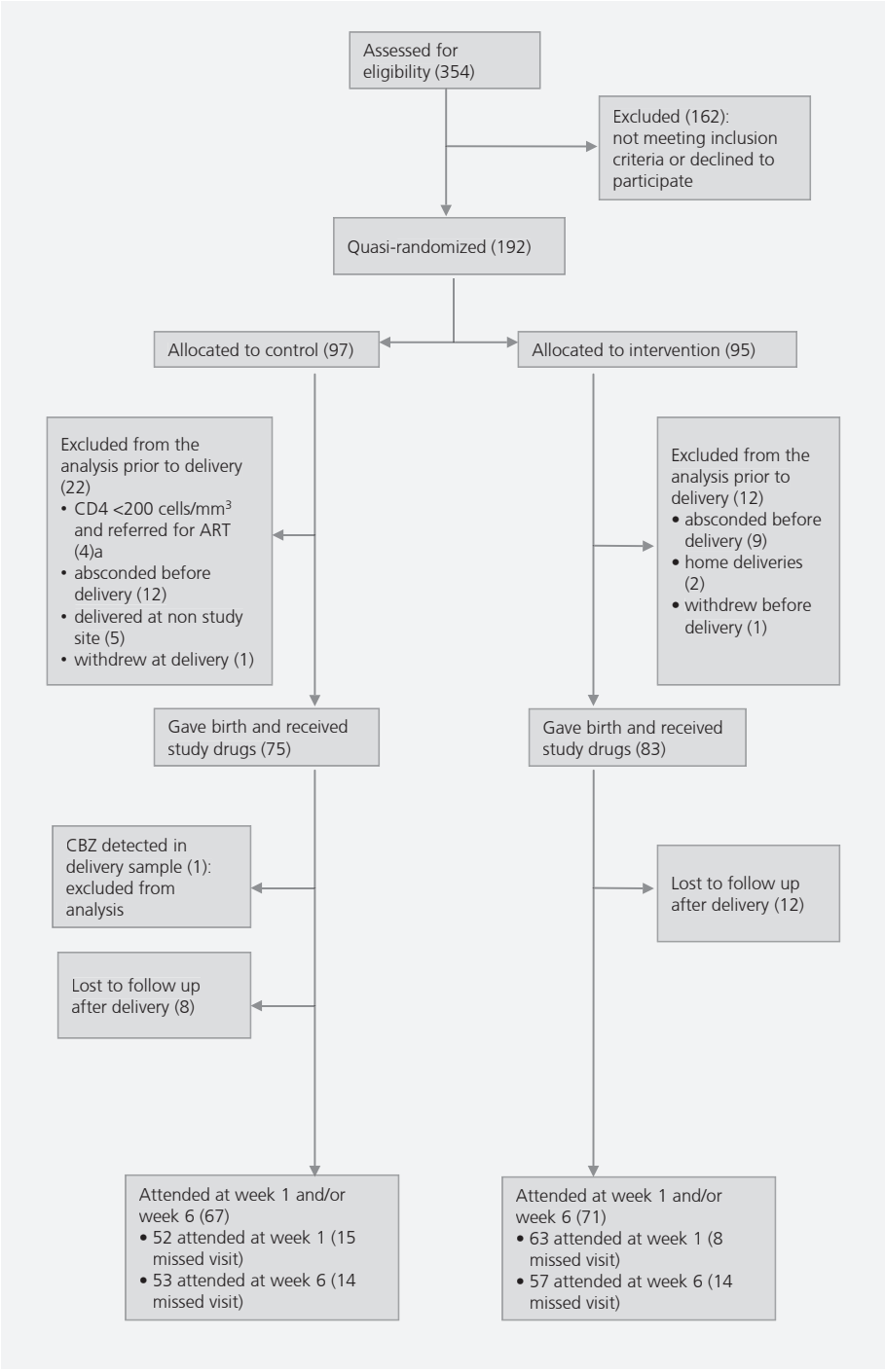
Objectives, outcomes and follow-up

The primary objectives were to determine the effect of adding single dose carbamazepine on single-dose nevirapine pharmacokinetics and nevirapine resistance development in the women. The first co-primary outcome was therefore nevirapine plasma concentration one week postpartum. Blood samples were taken one week post-partum (and at delivery) for pharmacokinetic evaluation. The second co-primary 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 six weeks post-partum. Secondary outcomes were any positive HIV RNA PCR test of the newborn and adverse events (including any grade of toxicity in laboratory safety tests taken one week post-partum) possibly/probably/definitely related to study drugs.

Hematology and biochemistry tests were determined at enrolment and one week post-partum. 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 PCR assays. Blood was taken at delivery and one week post-partum, and plasma stored for bioanalysis of nevirapine plasma concentrations, done in the Department of Clinical Pharmacy, RUNMC, Nijmegen, The Netherlands using high performance liquid chromatography (lower limit of quantification 0.05 mg/L)[25]. Resistance was assayed in plasma stored from samples with >500 copies/ml at delivery and the 6 week antenatal visit at the Department of Virology of the University Medical Centre Utrecht, The Netherlands (samples <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 two-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 chi-squared 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 (Figure 1). As the primary outcome measures were based on batched laboratory measures, analyses also excluded women who had evidence of non-compliance (carbamazepine detected by HPLC in sdNVP group or no carbamazepine detected by HPLC in sdNVP/CBZ group).

FIGURE 1 ▸ Trial profile



RESULTS

We screened 354 HIV-infected, ARV-naïve, pregnant women between February 2006 and April 2009 (Figure 1). 192 (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, BMI, weight, CD4 count, hematology and biochemistry parameters at enrolment 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/mm3 and were referred for ART. 21 (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 non-study 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. 133/158 (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 prior to analysis. One woman in the control group had detectable carbamazepine levels, and was excluded from analyses

Pharmacokinetics

The median (IQR) 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) (ranksum p=0.91) (GMR: 1.17, 95% CI 0.76-1.81; p=0.48). As expected nevirapine plasma concentrations were significantly reduced in both groups one week after delivery (based on matched pairs of measurements, sdNVP GMR (1 week:delivery, n=44)=0.18, 95% CI: 0.12-0.26; sdNVP/CBZ GMR (1 week:delivery, n=54)=0.10, 95% CI 0.07-0.14): results similar using unmatched data). In the sdNVP/CBZ group, week 1 nevirapine plasma concentrations were significantly lower compared to the sdNVP group (GMR: 0.64, 95% CI 0.43-0.96; p=0.03; Table 2) with median (IQR) nevirapine concentration 0.09 (0.05-0.20) mg/L in the sdNVP/CBZ group vs. 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; 15/63 (24%) vs. 6/52 (12%), respectively (chi-squared p=0.09).

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 one or more nevirapine-associated resistance mutations, compared to 6/55 (11%) in the sdNVP/CBZ group (odds ratio=0.46, 95% CI 0.16-1.34, chi-squared p=0.15). Eight women in the sdNVP group had one nevirapine mutation and three had two mutations (Figure 2), compared to five and one, respectively in the sdNVP/CBZ group (poisson incidence rate ratio for number of

TABLE 1 ▸ Characteristics of women at enrolment, delivery and one 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)
Hemoglobin (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)
One 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)
Hemoglobin (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)

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

TABLE 2 Maternal nevirapine plasma concentrations at delivery and one week after delivery

	sdNVP	sdNVP/CBZ	p	Geometric Mean Ratio (95% CI)
At delivery				
Samples taken (n)	61	72		
NVP plasma conc. (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	
One week after delivery				
Samples taken (n)	52	63		
NVP plasma conc. (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 conc. 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)		

Note: p-values from rank-sum tests for plasma concentration, exact and chi-squared tests for <0.05 mg/L at delivery and week 1, respectively.

mutations in sdNVP/CBZ vs sdNVP=0.47 (95% CI 0.19-1.17) p=0.11). All but two of the mutations were detected as mixtures with wild-type nucleotide sequence. Only one of two mutations in one 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 enrolment samples. Figure 3 shows the distribution of the nevirapine mutations six weeks after delivery. K103N, Y181C/I and G190A were the most frequently observed nevirapine mutations. The most common mutation was K103N, present in nine women, alone in eight women and with G190A in one.

Combined pharmacokinetic–resistance analysis

Nevirapine plasma concentrations one week postpartum were available from 14/17 (82%) women with mutations vs. 71/90 (79%) without mutations. Median week 1 nevirapine plasma concentrations were similar in women with (median (IQR) 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 to 14/70 (20%) of those with detectable nevirapine at week 1 (exact p=0.07). Thus, irrespective of group assignment, women with undetectable plasma concentrations at week 1 were less likely to develop nevirapine-associated mutations after single-dose nevirapine 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 6/50 (12%) in the sdNVP/CBZ group and 3/49 (6%) in sdNVP group (exact p=0.49). Although not all infants were tested at delivery, 1/6 (17%) and 0/3 (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, one (sdNVP/CBZ) died in the first 6 weeks after delivery and one (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 enrolment (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 enrolment and delivery (i.e. before trial intervention), mostly grade 1 (31 sdNVP, 29 sdNVP/CBZ). Only seven were grade 4 (3 sdNVP, 4 sdNVP/CBZ); six women had grade 4 anemia (Hb<6.5 g/dl) and one had AST>10.0*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, 37/38 (97%) sdNVP/CBZ) were in the normal range for neutrophils (>1.3*10^9/L) and for leucocytes (27/27 (100%) sdNVP; 38/39 (97%) sdNVP/CBZ) (>2.5*10^9/L). After delivery, six 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 seven were grade 2 (3 sdNVP; 4 sdNVP/CBZ), three grade 3 (1 sdNVP; 2 sdNVP/CBZ) and only two 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.

FIGURE 2 Percentage of women with nevirapine mutations. Tested for nevirapine resistance at week 6: 107 women (52 sdNVP and 55 sdNVP/CBZ).

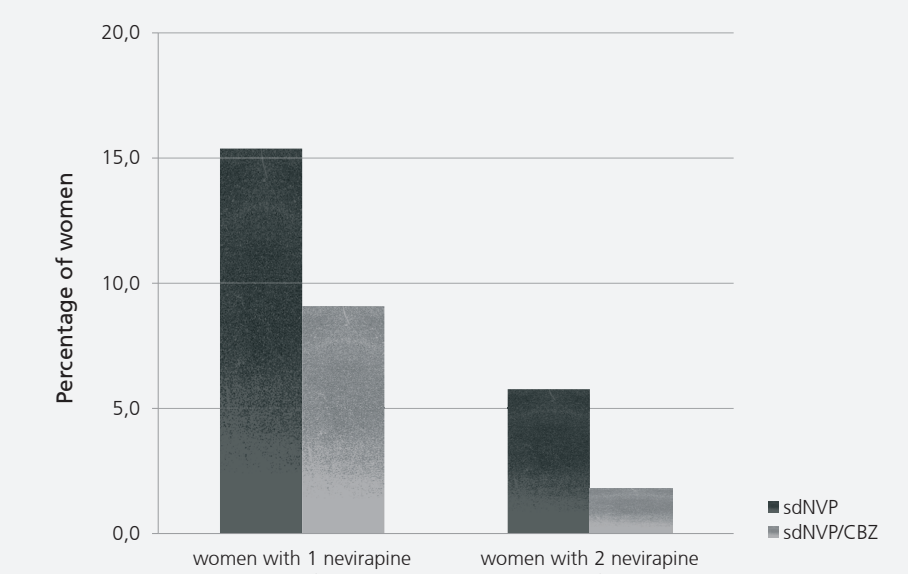
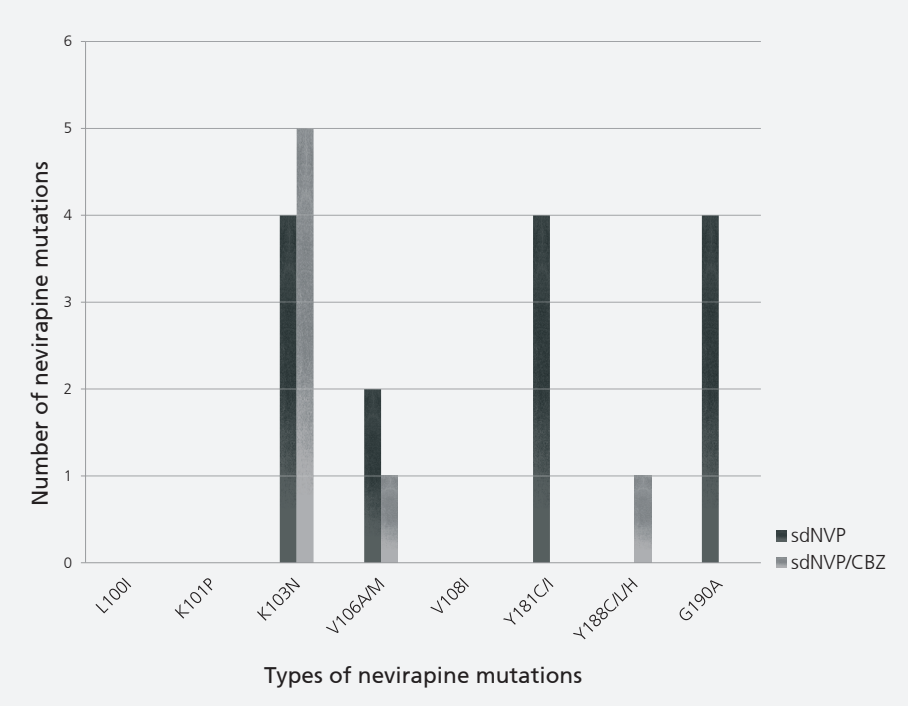


FIGURE 3 Number of mutations related to nevirapine resistance.



DISCUSSION

This trial provides the first data on adding a single-dose of an enzyme inducer (carbamazepine) to single-dose nevirapine for pMTCT in HIV-infected, pregnant women. Single dose carbamazepine significantly reduced nevirapine plasma concentrations one week after delivery, with a trend towards fewer resistance mutations. We also found a trend towards 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 one week postpartum. Overall, these plasma concentrations were com-parable to previous studies[6,26,27]. Adding single-dose carbamazepine did not affect nevirapine plasma concentrations immediately after delivery, similarly to to the study of L'homme et al. in healthy women[4]. 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 single-dose nevira-pine on HIV MTCT. 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 single-dose nevirapine 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 towards a higher proportion with undetectable plasma concentrations in this group. Plasma concentrations one week post-partum are determined by nevirapine elimination half-life, 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 co-primary 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 single-dose nevirapine, several studies have investigated the addition of other ARVs after delivery. A meta-analysis reported 36% average nevirapine resistance prevalence for women using single-dose nevirapine (with or without other ARVs)[32]. For those women receiving ARVs postpartum, this fell to 4.5%. Whilst 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 single dose nevirapine in 400 women, which also halved nevirapine resistance; 25% vs. 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. Firstly, 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. Secondly, the randomization list was generated as alternate allocations (i.e. 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 co-primary 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 sub-populations 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 CYP*3A4* enzyme are induced involves the activation of the transcription factors pregnane X receptor (PXR) and the constitutive androstane receptor (CAR)[36]. Nevirapine is a drug that binds to those receptors, whereafter activation of the CYP*3A4* enzyme starts. From the eight 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 to 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 single-dose nevirapine alone; their widespread implementation is challenging. A second pharmacokinetic trial (VITA-2; ClinicalTrial.gov ID: NCT01187719) has consequently started to evaluate the effect of seven 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 single-dose nevirapine at delivery with zidovudine from the second trimester of gestation and zidovudine+lamivudine labor for seven days post-partum. 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 single-dose nevirapine 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.

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03

CHAPTER 04

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

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ABSTRACT

Objectives

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

Methods

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

Results

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

Conclusions

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

INTRODUCTION

While the risk of HIV mother-to-child transmission (MTCT) is 20-40% without treatment,[1, 2] a simple cheap intervention, single-dose nevirapine at labour onset, reduces MTCT by ~50%.[1, 3] Its major disadvantage is development of nevirapine resistance in mothers (1-69%) and infants,[4] most likely due to its long elimination half-life (61 hours),[5-7] leading 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 future combination antiretroviral therapy (cART) efficacy 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 preventing MTCT (pMTCT) in resource-limited settings, when cART (WHO Option B/B+) is not feasible or available. To cover the prolonged presence of subtherapeutic nevirapine plasma concentrations after single-dose nevirapine at labour onset, Option A of the WHO (2012) guidelines recommend adding zidovudine/lamivudine for seven days postpartum.[10, 11] This approach reduces resistance development to 4-16%,[4] but does not fully eliminate it.

Nevirapine is extensively metabolized in the liver by cytochrome P450 (CYP) isoenzymes *3A4* and *2B6*.[12] A pharmacological, rather than antiretroviral, approach of adding a *CYP3A4* enzyme inducer has been shown to decrease nevirapine elimination half-life in healthy women,[13] with greatest reductions from carbamazepine and phenytoin. In our previous trial (VITA-1), addition of single-dose carbamazepine to single-dose nevirapine at labour onset also significantly reduced nevirapine plasma concentrations one week after delivery in HIV-infected women, with a trend towards fewer resistance mutations.[14]

The CYP*3A4* enzyme inducer phenytoin is a low cost, widely available anticonvulsant and anti-arrhythmic drug, which is not secreted into breast milk in clinically important amounts (in contrast to carbamazepine) and can therefore be safely given to breastfeeding mothers.[15] In this pilot study we investigated the impact of seven days phenytoin on nevirapine pharmacokinetics and nevirapine resistance development after single-dose nevirapine as a component of antiretroviral (ARV) prophylaxis for pMTCT (VITA-2 trial). The hypothesis was that seven days phenytoin would reduce nevirapine elimination half-life and hence emergence of nevirapine resistance mutations.

METHODS

Study participants

Participants were recruited from the Pasua and Majengo antenatal clinics (ANCs) in Moshi, Tanzania and University Teaching Hospital (UTH) in Lusaka, Zambia. Eligible, HIV-infected, pregnant women were aged ≥18 years, ARV-naive, starting ARV prophylaxis for pMTCT, not intending to relocate during study participation, and willing to attend follow-up visits.

Exclusion criteria were serious illness requiring systemic treatment/hospitalization, use of concomitant medication which may interfere with ARVs or phenytoin, or CD4 <350 cells/mm^3 (eligible for cART). All women gave written informed consent; illiterate patients gave oral consent documented by their own thumbprint and a witness. The study was approved by institutional review boards of Kilimanjaro Christian Medical University College (KCMU-Co), Moshi, Tanzania, the National Institute of Medical Research in Dar es Salaam, Tanzania, and UTH, Lusaka, Zambia. The study is registered with ClinicalTrials.gov (NCT01187719).

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

Women were randomized 1:1 in a parallel group design to either the national standard of care or the national standard of care plus 184 mg phenytoin (2x 92 mg tablets) once daily from labour onset for seven days. The randomization sequence was generated by a trial statistician of 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 time of study drug(s) ingestion by direct observation of intake or by asking the woman if she had already taken the study drug(s) at home.

Objectives, outcomes and follow-up

The primary objectives of the pilot study were to determine the effect of seven days phenytoin on the elimination half-life of nevirapine and the development of nevirapine resistance in HIV-infected pregnant women receiving a single-dose nevirapine as part of perinatal HIV prevention. The primary outcomes were nevirapine pharmacokinetic parameters (elimination half-life, time to achieve an undetectable plasma concentration) and nevirapine resistance (primary nevirapine mutations L100I, K101P, K103N/S, V106A/M, V108I, Y181C/I, Y188C/L/H, G190A)[16] at weeks 4-6. Secondary outcomes were all adverse events (AEs) possibly/probably related to pMTCT ARV prophylaxis or phenytoin, and HIV infection of the infant.

Haematology and biochemistry tests were performed at enrolment and one week postpartum. CD4 cell counts and viral load (VL) were assayed at delivery. Infants were tested just after birth (<30 minutes) and at week 4-6 by DNA PCR assays. Blood was stored from the women at delivery and days 1,3,5,7 and 14 postpartum, and from the children at delivery and day 7 post-delivery for retrospective determination of nevirapine (and phenytoin) plasma concentrations at the Department of Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Nevirapine assay used high performance liquid chromatography with a lower limit of quantification (LLOQ) of 0.05 mg/L[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 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 nevirapine elimination half-life associated with seven days phenytoin allowing 20% drop-out (without follow-up samples, based on VITA-1).[14]

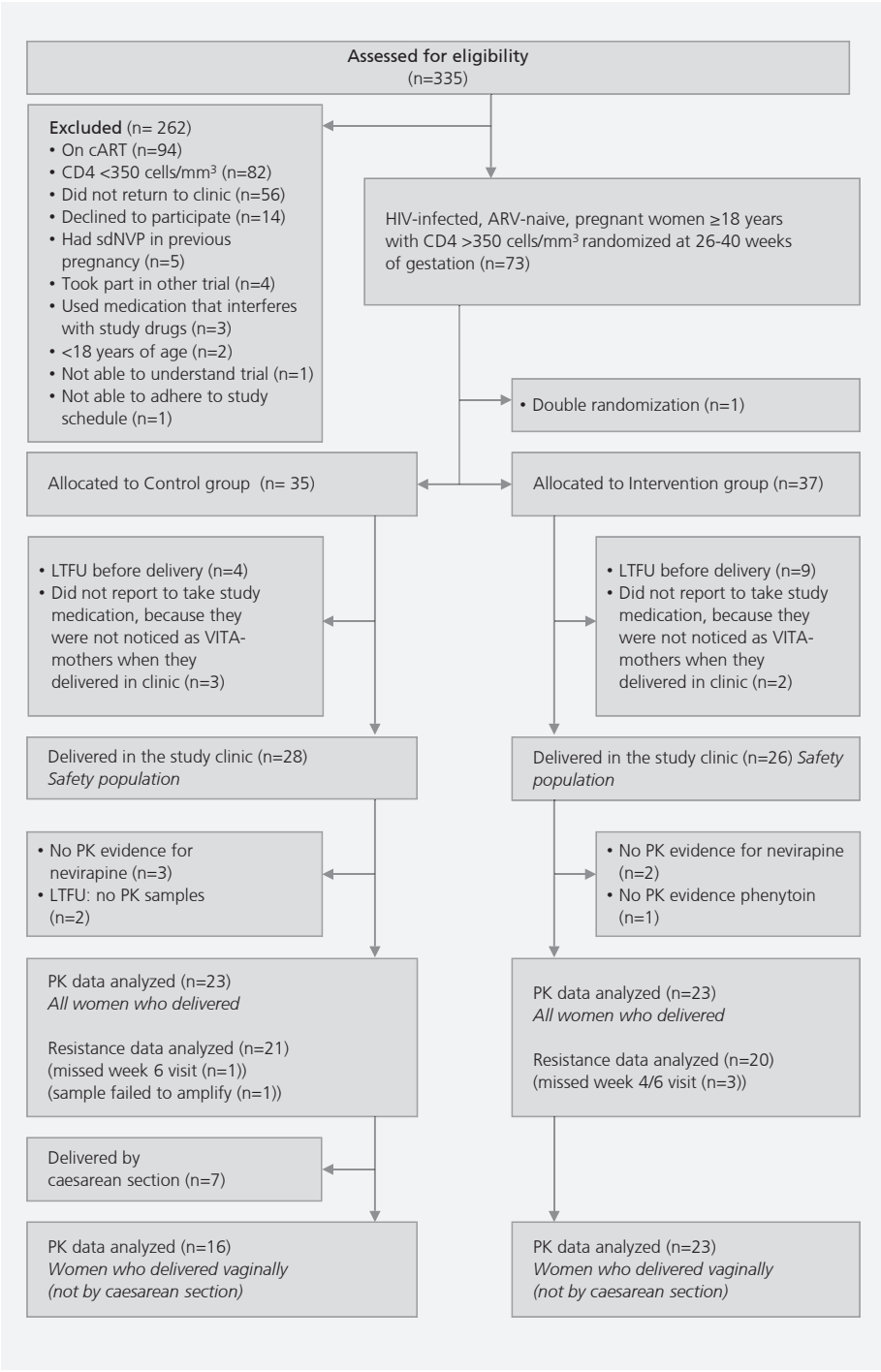
Safety analyses included all women who were observed or reported taking study medication (safety population). Analyses of pharmacokinetics and resistance included the safety population who did not receive a second nevirapine dose, who delivered vaginally (not by caesarean section (C/S)) and had pharmacokinetic evidence of nevirapine, and phenytoin if allocated (detectable plasma concentration one day post-delivery) (protocol-specified primary PK population). Analyses were also done including mothers who delivered by C/S, as no difference in pharmacokinetic parameters was observed between C/S and vaginal deliveries in VITA-1. Women in the control group with phenytoin detected in any sample were excluded from PK/resistance analyses. Pharmacokinetic analysis was performed using Phoenix 64, WinNonlin 6.3 (Pharsight 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, ie comparing geometric means), ranksum 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 CD4 <350 cells/mm^3 (n=82) or did not return after screening (n=56) (Figure 1). Seventy-three (22%) women were randomized: 35 and 37 were allocated to control and intervention groups, respectively. One woman was randomized twice; the second randomization was excluded and the woman followed the first randomization. Demographic characteristics at enrolment and delivery were generally reasonably balanced between the two groups (Table 1; Sup-

FIGURE 1 ▸ Profile of the VITA-2 trial (CONSORT diagram)



plementary Table 1); the main difference was significantly shorter time from nevirapine ingestion to delivery in the intervention group, which must have occurred by chance. The difference is not expected to have impact on our pharmacokinetic data, as the elimination half-life of nevirapine is long. Also, no differences were observed between laboratory values at enrolment and delivery within either group (Supplementary Table 1). The study finished once the recruitment target had been met.

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

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

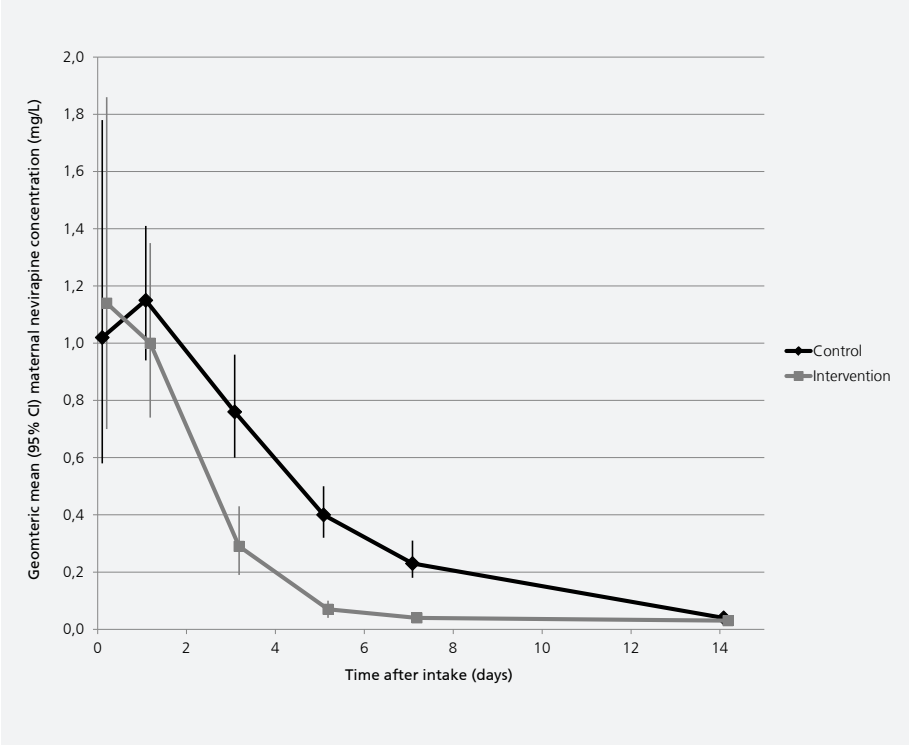
Data are presented as median (IQR) and were tested with Rank-sum.

Pharmacokinetics

At delivery, restricting to women who delivered vaginally (not by C/S), there was no significant difference in nevirapine plasma concentrations in the two groups (geometric mean (GM) (95% CI) was 1.08 (0.63-1.84) mg/L in control patients versus 1.14 (0.70-1.86) mg/L in intervention patients, GM ratio (GMR) (90% CI) 1.05 (0.58-1.92), t-test p=0.82). Subsequently nevirapine plasma concentrations decreased significantly faster (Figure 2) and undetectable levels were reached significantly earlier (Table 2) in the intervention group compared to the control group. All results were similar including women who delivered by C/S, and so those from the (larger) group including women who delivered by C/S are presented subsequently (see Supplementary Table 2 for pharmacokinetic results of the (smaller) group excluding women who delivered by C/S).

One week post-delivery, nevirapine plasma concentrations were reduced in both groups, but to a significantly lesser extent in the control group (GMR (1-week:delivery) (90% CI) 0.22 (0.18-0.28), 20 matched pairs) than the intervention group (GMR (1-week:delivery) (90% CI) 0.031 (0.026-0.038), 22 matched pairs). Overall, levels were 85% lower in intervention than control groups (GMR (intervention:control) (90% CI) 0.15 (0.11-0.20), t-test p<0.001). The GM (95% CI) nevirapine elimination half-life was 63.2 (52.8-75.7)

FIGURE 2 ▸ Geometric mean nevirapine plasma concentrations over time post-delivery (all women who delivered including those who delivered by C/S)



versus 25.5 (21.6-30.1) hours in the control versus intervention groups respectively (p<0.001; t-test), a 60% reduction (GMR (90% CI) 0.40 (0.33-0.49)). The GM (95% CI) time to achieving an undetectable nevirapine plasma concentration was 16.3 (13.8-19.3) versus 6.7 (5.7-7.8) days in the control versus intervention groups respectively (p<0.001; t-test). Consequently a significantly greater proportion of control women had detectable nevirapine plasma levels at one and two weeks post-delivery (Table 2). All 23 (100%) women in the control group versus 7/22 (32%) women in the intervention group had a detectable nevirapine plasma concentration at one week post-delivery (p<0.001; exact); and 11/21 (52%) women in the control group versus 1/19 (5%) women in the intervention group at two weeks post-delivery (p=0.002; exact).

The median (range) phenytoin plasma concentration in all samples taken from delivery to one week post-delivery in the intervention group was 1.5 (<0.4-24.7) mg/L. Twenty-one of 22 (95%) mothers had only subtherapeutic phenytoin levels (therapeutic range defined as 10-20 mg/L).[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 one week post-delivery. The median phenytoin plasma level in the infants was <0.4 (range <0.4-1.9 mg/L).

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

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

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

Resistance

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

Safety

The 28 control and 26 intervention mothers gave birth to 30 (two pairs of twins) and 28 babies (two pairs of twins), respectively. Twenty-one (one pair of twins) and 19 (one pair of twins) infants respectively were tested just after birth and at weeks 4-6 post-delivery. The overall transmission rate was 0/21 (0%) in the control group and 1/19 (5%) in the intervention group. However, the infected child tested positive at birth and must therefore have been infected during the intrauterine period. In the infants, ten clinical AEs were reported: four in the control group (n=1 grade 1, n=1 grade 2, n=2 grade 4) and six in the intervention group (n=1 grade 1, n=1 grade 2, n=1 grade 3, n=3 grade 4). The grade 4 AEs were a hospitalization for overweight after birth and a death just after birth due to congenital malformation in the control group and three still births (two fresh, one macerated) in the intervention group.

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

DISCUSSION

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

Importantly, nevirapine plasma concentrations at delivery were similar in those receiving and not receiving phenytoin, and also compared with previous studies,[5, 13] similarly to our previous study evaluating a single-dose of carbamazepine as 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 the protective perinatal effect of single-dose nevirapine is maintained. The absence of HIV-transmission during the postpartum period, similar or even lower than rates in previous studies,[10] confirms also the efficacy of the pMTCT regime.

Post-delivery, nevirapine pharmacokinetic parameters were substantially affected by enzyme induction. Adding phenytoin to single-dose nevirapine reduced nevirapine plasma levels by 85% and produced a significantly larger proportion of women with undetectable nevirapine levels one and two weeks post-delivery. Both effects are a consequence of the 60% reduction in the nevirapine elimination half-life, an absolute difference of -35.8 hours. This is the largest decline in nevirapine elimination half-life ever reported, especially in the target population of HIV-infected, pregnant women. For example, the pilot study of L'homme ***et al*** found a median elimination half-life reduction of 38% (-19.4 hours) in four healthy, Dutch women receiving single-dose nevirapine with seven days phenytoin,[13] and a single-dose of carbamazepine reduced nevirapine levels by 36% in HIV-infected, pregnant women receiving single-dose nevirapine.[14] Not surprisingly, a seven-day course of an enzyme inducer has a greater effect than a single-dose alone on the elimination half-life of nevirapine in HIV-infected, pregnant women.

The mechanisms by which the CYP enzymes are induced involves the transcription factors pregnane X receptor and the constitutive androstane receptor. The enzyme inducer binds to these receptors, thereby stimulating the activation of the CYP enzyme.[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 likely explains why induction of the CYP enzyme has a greater effect with a long-course of an enzyme inducer than only the single-dose used in our previous study.
Although current guidelines, including zidovudine monotherapy pre-delivery and seven days zidovudine/lamivudine post-delivery are complex, they have reduced emergence of nevirapine resistance substantially by protecting the subtherapeutic nevirapine 'tail', since the lengthy duration of low and subtherapeutic levels of nevirapine in blood are plausibly

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

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

It is estimated that 18-64% of the women living in Sub-Saharan Africa with HIV are receiving cART for pMTCT, as now recommended by WHO (Option B+). However, this means that thousands of women are still receiving single-dose nevirapine,[21] and demonstrates the challenge of widespread implementation of the current guidelines. Phenytoin can be used safely during pregnancy and breastfeeding [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 breastfeeding within Option B+. We have demonstrated that implementation of this intervention would

substantially and significantly reduce nevirapine half-life; previous data demonstrating a causal relationship between nevirapine half-life and resistance emergence[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, whilst likely retaining the benefits of single-dose nevirapine in reducing transmission. This strategy might therefore support the overarching goal of the technical consultation of the WHO to reduce the overall HIV transmission rate from pMTCT to <5% at the population level by the end of 2015.

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

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SUPPL. TABLE 1 ▸ Laboratory values of the women in the VITA-2 trial

	Control (n=28)	Intervention (7 days phenytoin; n=26)	p-value	Control (week 1 vs enrolment) p-value	Intervention (week 1 vs enrolment) p-value
At enrolment					
Alkaline phosphatase (U/L)	108 (82-163)	124 (105-149)	0.28	0.17	0.16
Bilirubin (µmol/L)	<8.6 (<8.6-<8.6)	<8.6 (<8.6-<8.6)	0.24	0.14	0.49
Creatinine (µmol/L)	62.3 (51.4-65.9)	59.4 (49.1-69.9)	0.82	0.83	0.50
AST (U/L)	21 (17-27)	21 (18-26)	0.98	0.21	0.50
ALT (U/L)	10 (9-13)	11 (9-14)	0.92	<0.001	<0.001
Haemoglobin (g/L)	102 (88-111)	102 (90-109)	0.94	0.40	0.30
Platelets(U/L)	272 (217-295)	256 (201-300)	0.85	<0.001	<0.001
Leucocytes (U/L)	6 (5-8)	6 (5-7)	0.36	0.37	0.97
Neutrophils (%)	62 (55-66)	54 (50-62)	0.03	0.05	0.68
1 week after delivery					
Alkaline phosphatase (U/L)	139 (104-161)	151 (114-202)	0.39		
Bilirubin (µmol/L)	<8.6 (<8.6-9.2)	<8.6 (<8.6-<8.6)	0.05		
Creatinine (µmol/L)	60.9 (55.6-65.0)	62.9 (55.4-73.3)	0.63		
AST (U/L)	24 (19-32)	23 (19-28)	0.59		
ALT (U/L)	18 (14-213)	19 (13-24)	0.54		
Haemoglobin (g/L)	105 (94-115)	108 (91-124)	0.89		
Platelets (U/L)	418 (331-475)	432 (358-459)	0.79		
Leucocytes (U/L)	6 (4-8)	6 (5-8)	0.59		
Neutrophils (U/L)	53 (41-61)	54 (49-60)	0.87		

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

SUPPL. TABLE 2 Maternal nevirapine plasma concentrations at delivery, week 1 and week 2 of women in the VITA-2 study, who delivered vaginally (not by C/S)

	Women who delivered vaginally (not by C/S)			
	Control	**Intervention**	**p**	**GMR (90% CI)**
At delivery				
Samples taken (n)	13	22		
nevirapine plasma conc. (mg/L; GM (95% CI))	1.08 (0.63-1.84)	1.14 (0.70-1.86)	0.82†	1.05 (0.58-1.92)
<0.05 mg/L nevirapine (n (%))	0 (0)	1 (5)		
1 week after delivery				
Samples taken (n)	16	22		
nevirapine plasma conc. (mg/L; GM (95% CI))	0.25 (0.18-0.34)	0.035 (0.027-0.046)	<0.001†	(0.14 (0.10-0.20)
<0.05 mg/L nevirapine (n(%))	0 (0)	15 (68)	<0.001*	
nevirapine plasma conc 1 week : delivery (n of pairs)	13	22		
nevirapine plasma conc 1 week : delivery (GMR (90% CI))	0.23 (0.20-0.26)	0.031 (0.026-0.038)		
2 weeks after delivery				
Samples taken (n)	15	19		
nevirapine plasma conc. (mg/L; GM (95% CI))	0.045 (0.029-0.074)	0.026 (0.024-0.029)	0.005†	0.58 (0.42-0.80)
<0.05 mg/L nevirapine (n(%))	7 (47)	18 (95)	0.004*	
nevirapine plasma conc 2 weeks : delivery (n of pairs)	12	18		
nevirapine plasma conc 2 weeks : delivery (GMR (90% CI))	0.036 (0.029 - 0.044)	0.022 (0.015 - 0.031)		

*† T-test, * Exact test. Median nevirapine plasma concentrations are similar to GM and are therefore not shown in Table 1.*

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PART II

CLINICAL PHARMACOLOGY STUDIES IN HIV-INFECTED CHILDREN

CHAPTER 05

Pharmacokinetics of nevirapine in HIV-infected 3-<6 kg infants taking paediatric fixed dose combination tablets

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ABSTRACT

Objectives

To evaluate pharmacokinetics of nevirapine, lamivudine and stavudine in HIV-infected Zambian infants receiving fixed dose combination (FDC) antiretroviral tablets (Triomune Baby).

Design

Phase I/II study.

Methods

16 HIV-infected children ≥1 month, weighing 3-<6kg were enrolled. Blood was sampled at t=0, 2, 6 and 12 hours after observed intake of one FDC tablet (50 mg nevirapine, 6 mg stavudine, 30 mg lamivudine) 4 weeks after starting treatment. Safety and viral load response over 48 weeks were determined.

Results

The median (interquartile range, IQR) age, body weight and daily nevirapine dose in 15 included children (8 girls) were 4.8 (4.2,8.4) months, 5.3 (4.3,5.5) kg and 348 (326,385) mg/m^2, respectively. The median (IQR) nevirapine area under the concentration-time curve ($AUC_{0\text{-}12h}$), C_{max} and C_{12h} were 70 (56,104) h.mg/L, 7.5 (6.2,10) mg/L, and 4.3 (2.9,6.9) mg/L, respectively. Values were on average higher than reported in adults, but ~20% lower than previously reported in children >6kg. Four of 15 (27%) children had a subtherapeutic nevirapine C_{12h} (defined as <3.0 mg/L) compared to only 3/63 (5%) children >6kg (p=0.02), whilst children aged <5 months (3/6 (50%)) may have the highest risk for subtherapeutic nevirapine C_{12h} (p=0.24). No association was found between viral load values and nevirapine plasma pharmacokinetic parameters (p>0.3). Stavudine/lamivudine pharmacokinetic parameters were broadly comparable to heavier children.

Conclusion

Exposure to nevirapine in African, HIV-infected infants with low body weight taking FDC tablets appears on average to be adequate, but due to large inter-subject variability a relatively high proportion had subtherapeutic nevirapine C_{12} levels, particularly those aged <5 months.

INTRODUCTION

Given high morbidity/mortality risks, WHO 2010 guidelines[1] recommend HIV-infected infants initiate antiretroviral therapy (ART) immediately at diagnosis, regardless of CD4 or WHO clinical stage. Small, scored, dispersible fixed-dose-combination tablets (FDCs) have been developed for HIV-infected infants/children: Triomune Baby (50 mg nevirapine, 6 mg stavudine, 30 mg lamivudine) and Triomune Junior (double Baby dose). They have higher nevirapine to nucleoside reverse transcriptase inhibitor (NRTI) dose ratios compared to adult FDCs, consistent with paediatric dose recommendations[1, 2]. Using the same ratios as adult FDCs would underdose nevirapine[3-7], because children metabolize nevirapine more rapidly than adults. They have somewhat lower stavudine and higher lamivudine doses than licensed paediatric mg/kg doses, considering toxicity profiles.

A previous 12-hour pharmacokinetic (PK) study in Zambian, HIV-infected children taking Triomune Baby/Junior[4] found higher but more variable nevirapine levels than in adults[8]. However, only two children weighing 3-<6kg were included. We therefore undertook a new phase I/II study of PK, safety and viral load (VL) responses with Triomune Baby in infants weighing 3-<6kg.

METHODS

CHAPAS-1 (Children with HIV in Africa-Pharmacokinetics and Adherence of Simple antiretroviral regimens) was an open, randomised trial to assess appropriate dosing of FDC tablets of nevirapine/lamivudine/stavudine[9]. This PK substudy included 16 HIV-infected infants aged ≥1 month, weighing 3-<6 kg and fulfilling WHO criteria for initiating ART, enrolled in CHAPAS-1 June 2006-June 2008[9] and followed until trial closure (October 2008).. Exclusion criteria for the main trial[9] were previous ART exposure (including ART to prevent mother-to-child transmission); and for the PK substudy were abnormal laboratory values (week 2), reported non-adherence, active opportunistic infections and treatment with medication known to interact with any ART. All caretakers gave written informed consent. Since children were identified prospectively after birth due to acute illness, and mothers were referred to adult facilities for treatment/care, no mother was receiving ART at enrolment/PK day. The study was approved by the Biomedical Research Ethical Committee in Lusaka, Zambia and Ethics Committee of UCL London, United Kingdom.

Daily nevirapine, stavudine and lamivudine doses targeted 300-400 mg/m^2, 2 mg/kg and 8 mg/kg respectively, in an FDC (Triomune Baby: 50 mg nevirapine, 6 mg stavudine, 30 mg lamivudine) taken as one tablet twice-daily for the 3-<6 kg weight-band, following WHO recommendations[10].

At least four weeks after initiating Triomune Baby at enrolment, when steady state nevirapine plasma concentrations were achieved, a 12-hour PK limited sampling session was performed[11], with 2.0mL samples at t=0, 2, 6 and 12 hours after observed intake of one

tablet. $AUC_{0\text{-}12h}$ was calculated by WinNonlin (version 5.2; Pharsight, CA) . Two children had full PK curves (at t=0,1,2,4,6,8 and 12 hours after observed intake) in the original PK study[4]. HIV-1 RNA viral load (VL) was assayed on available plasma stored at baseline, weeks 4, 12, and then 12-weekly until trial closure in October 2008. Small sample volumes necessitated dilution, so lowest cut-offs ranged from <80 to <1600 copies/mL. (See[4, 12, 13] for details on plasma drug concentration measurement and pharmacokinetic analysis). PK parameters for nevirapine, lamivudine and stavudine were compared with those from children ≥ 6kg using ranksum tests. Subsequent analysis focussed on nevirapine given previous data relating plasma concentrations, and VL response[2] assessed here using Spearman correlations and virologic failure defined as VL>5000 copies/mL after 24 weeks on ART, following WHO[10, 14].

RESULTS

Sixteen children were enrolled; one was excluded due to evidence of non-adherence ($C_0 < C_{12h}/3$). In the 15 included children (eight girls) median (interquartile range, IQR) age and body weight were 4.8 (4.2,8.4) months and 5.3 (4.3,5.5) kg, respectively. All 15 children were breastfed. Fourteen were moderately-to-severely stunted (height-for-age z-score<-2: median (IQR) -3.30 (-5.18,-2.81)) and wasted (weight-for-age z-score <-2 median (IQR): -3.23 (-4.52,-2.64)). The median (IQR) daily prescribed nevirapine dose at enrolment and time from ART initiation to PK day was 348 (324,386) mg/m^2 and 29 (28,29) days, respectively.

The median (IQR) $AUC_{0\text{-}12h}$, C_{max} and C_{12h} of nevirapine were 70 (56,103) h.mg/L, 7.5 (6.2,10) mg/L, and 4.3 (2.9,6.9) mg/L, respectively (Table 1), ~20% lower than in children weighing ≥ 6kg, although differences were not statistically significant (ranksum p>0.08). Moreover, mean values in infants 3-<6kg were higher than reported in adults[8]. Stavudine and lamivudine PK parameters were broadly comparable with those reported in children weighing ≥ 6kg and adults[15, 16]. Lamivudine C_{12h} was somewhat higher and stavudine C_{max} somewhat lower than heavier children.

Inter-infant variability in nevirapine PK parameters was moderate-to-large. Coefficients of variation were 31%, 40% and 55% for C_{max}, $AUC_{0\text{-}12h}$, and C_{12h}, respectively, similar to those reported previously in children ≥6kg (38% C_{max}, 41% $AUC_{0\text{-}12h}$, 50% C_{12h}). Nevirapine C_{12h} were subtherapeutic (defined as <3.0 mg/L)[2] in 27% (4/15) 3-<6kg children versus only 5% (3/63) children ≥ 6kg (exact p=0.02). The 3-<6kg children with C_{12h}<3.0 mg/L were aged 3.6, 3.6, 4.8 and 8.4 months. Visual inspection (Figure 1 (a)) suggested risks might be higher in those aged <5 versus ≥5 months (3/6 versus 1/9, respectively), but numbers were too small to reach statistical significance (exact p=0.24). Children aged <5 months received adequate nevirapine doses (range 324-406 mg/m^2/day).

VL at ART initiation was high (median (IQR) 347610 (70404,1689820) copies/mL) (Figure 1 (b)). The median (IQR) VL at week 4 (PK-day) was 1820 (804,2920) in eight

infants with (supra)therapeutic C_{12h} versus 1793 (158, 5027) copies/mL in four infants with subtherapeutic C_{12h} (ranksum p=0.87, Figure 1 (c)). There was no evidence of any association between VL and nevirapine PK parameters at PK day (Spearman p>0.3). All VLs after week 4 were <5000 copies/mL (WHO failure threshold) except one of 16021 copies/mL at week 36 in a child with week 12 and 24 VLs <80 and 1670 copies/mL, respectively. This child had C_{12h}<3.0 mg/L at week 4.

None of the 15 children discontinued Triomune Baby due to adverse events (AEs), and no serious adverse events or clinical AEs were reported. Thirty-eight asymptomatic laboratory events occurred in the first 8 weeks on ART in 14/15 children; all resolved without ART modification. The most common were single elevated AST or ALT (n=3 grade 3; n=2 grade 2; n=10 grade 1), with other AEs being hyperbilirubinaemia (n=1 grade 4; n=4 grade 2; n=4 grade 1), anaemia (n=2 grade 3; n= 1 grade 2; n=7 grade 1), neutropenia (n=1 grade 3; n=2 grade 1) and thrombocytopenia (n=1 grade 3). There was no evidence of association between raised AST/ALT and supratherapeutic (>8.0 mg/L) nevirapine C_{12h} plasma levels (exact p=0.20), nor between the number of AEs per child (median 3 (range 0-5)) and nevirapine PK parameters (Spearman p>0.8).

DISCUSSION

In this study, 16 Zambian, HIV-infected 3-<6kg infants receiving paediatric FDC Triomune Baby tablets twice-daily had plasma nevirapine concentrations that were slightly lower, but generally comparable with ≥ 6kg children, and were higher than historical adult data. However, large interinfant variability (55%) led to a relatively high proportion of children, particularly those <5 months, with low nevirapine trough concentrations. Stavudine and lamivudine PK parameters of were broadly comparable to heavier children/adults.

Our results agree with previous studies[4-7] showing younger children have higher risks of subtherapeutic nevirapine levels compared with older children. For example, using divided adult Triomune[30] tablets (200 mg nevirapine, 30 mg stavudine, 150 mg lamivudine), subtherapeutic nevirapine levels were more common in children <3 years[7], and in the three lowest weight-bands (40% versus 8% subtherapeutic in <12kg versus >12kg respectively). However, nevirapine doses in adult FDCs are known to be too low for children[3]. More recently, in children receiving paediatric FDCs with a higher nevirapine NRTI ratio, age remained the major predictor for low nevirapine levels[6]; a substantial proportion (35%) had subtherapeutic nevirapine trough levels, more pronounced in children ≤3years[6]. This is consistent with our study using a comparable paediatric FDC where 27% of children weighing 3-<6kg (most <10 months) had a subtherapeutic nevirapine C_{12h} concentration versus only 5% children ≥6kg[4].

Low nevirapine plasma levels may occur from noncompliance, malabsorption, drug interactions, low dose, increased metabolism or pharmacogenetics. Clinical signs of malabsorption and concomitant medications that could interfere with ART were substudy

exclusion criteria; furthermore we found no evidence that levels were related to weight- or height-for-age (Spearman p>0.7). We used WHO paediatric dose recommendations to prevent nevirapine underdosing[17]; nevirapine dose ranged from 320-438 mg/m^2/day meaning overt underdosing cannot explain our findings. Drug metabolism varies with age, so the low plasma levels in our youngest children could be explained by increased metabolism at younger ages. Children have an increased ability relative to adults to eliminate drugs metabolized by some cytochrome P450 (CYP) isoenzymes, including CYP*3A4* and CYP*2B6* which metabolise nevirapine. However, the relationship is not linear[6,18,19], and after birth newborns initially have a decreased ability to eliminate drugs. It is unknown at precisely which age the switch from reduced to increased metabolic capacity occurs but our data suggest this is before month 6 for nevirapine. Lastly, relatively little is known about the influence of CYP*2B6* and *3A4* gene polymorphisms on nevirapine PK parameters in African children[20-22]. Whilst pharmacogenetics can explain the high inter-subject variability, polymorphisms should be similar in children weighing 3-<6kg and ≥6kg, although chance imbalances due to small numbers could partly explain our results.

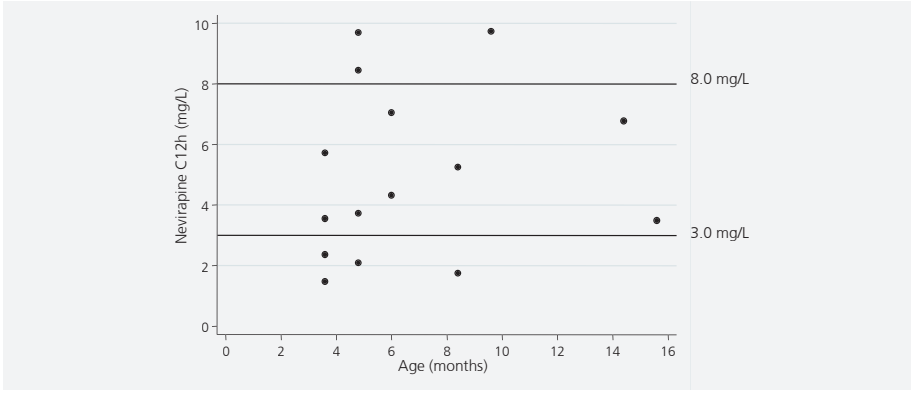
VL responses in these young children were generally good with 42% having VL <1000 copies/mL at week 4, with no associations between VL and nevirapine plasma PK parameters.. Low nevirapine plasma concentrations (<3.0 mg) are usually associated with an increased risk of subsequent virological failure and nevirapine resistance[2, 23, 24]. One reason why we could not confirm this association may be that children remain in the 3-<6kg weight band for only a short time (median (IQR) 113 (59-156) days in our study) before doses are increased. One child with subtherapeutic week 4 nevirapine C_{12h} subsequently failed virologically, although as always non-adherence cannot be excluded. Numbers are too small and follow-up too short to generalise further. Cutaneous hypersensitivity and hepatic toxicity are the most important nevirapine-associated side-effects[8, 25-27]. However, adult studies have not consistently identified a relationship between toxicity and nevirapine PK parameters, and such a relationship has never been observed in children. Here no infant developed a rash (despite 8/15 initiating nevirapine at full dose within the CHAPAS-1 randomisation)[9] and we found no association between AEs and nevirapine PK parameters, consistent with our findings in heavier children[4]. These results suggest that virological failure due to underexposure and potentially lower-than-optimal dosing is of greater concern than toxicity due to overdosing in these young infants.

Apart from small numbers, one major study limitation was lack of sufficient plasma for VL assays. Power to detect associations between nevirapine levels and treatment responses was therefore low, and nevirapine resistance could not be assayed to confirm treatment failure over poor adherence. A study strength was demonstration that PK data can be obtained on very young infants using a limited sampling model[11]. This used fewer blood sampling timepoints to predict a precise AUC_{0-12h} for nevirapine, lamivudine and stavudine in HIV-infected children.

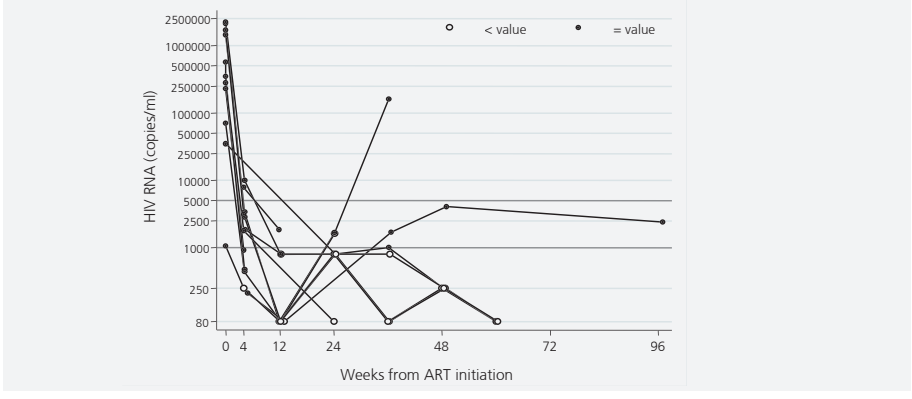
In conclusion, based on this study exposure to nevirapine in 15 African, HIV-infected infants with body weight 3-<6kg taking Triomune Baby following WHO guidelines was on average adequate but, with large inter-infant variability a rhigher proportion had sub-therapeutic nevirapine plasma concentrations (than heavier children. However, given fast growth rates after starting ART such that most infants spend only a short time in the lowest weight band, and encouraging VL responses four weeks after ART initiation, WHO weight band tables for nevirapine-containing FDCs appear reasonable for 3-6kg infants. Larger population PK studies in young children taking FDCs in the CHAPAS-3 trial are ongoing, and will be linked to longer-term VL outcomes.

FIGURE 1 ▸ Nevirapine C_{12h} concentrations, age and viral load (by week after antiretroviral therapy initiation)

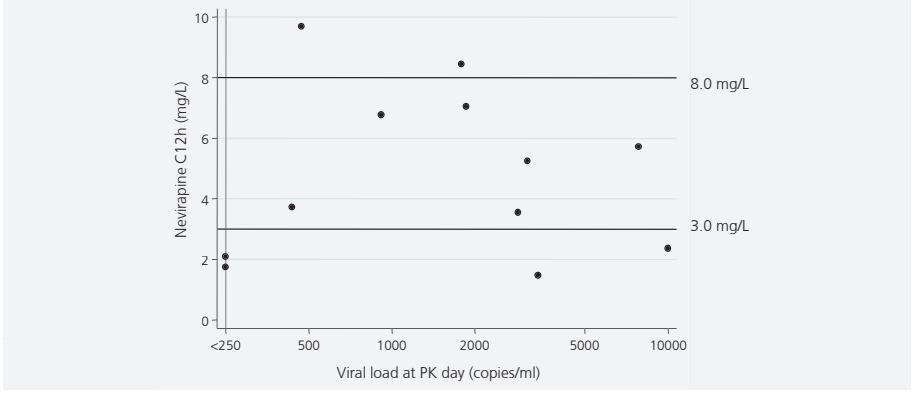
(A) NEVIRAPINE C_{12h} (MG/L) BY AGE



(B) VL (COPIES/ML) PER INFANT BY WEEK AFTER ART INITIATION



(C) NEVIRAPINE (MG/L) PER VIRAL LOAD AT PK DAY



Note for Figure 1 (b): VL was available for 11 (73%), 12 (80%), 9 (60%), 10 (67%), 8 (53%), 7 (47%), 5 (33%) and 1 (7%) infants at baseline, week 4, 12, 24, 36, 48, 60 and 96, respectively.

Table 1 Pharmacokinetic parameters of nevirapine, lamivudine and stavudine in children weighing 3 kg to less than 6 kg, compared with heavier children and adults.

	Weight-band 3-<6 kg (n=15)	Weight-band ≥6 kg (n=63*)	P (ranksum)	GMR (3-<6 vs ≥6 kg) (90% CI)	Literature data adults
Nevirapine					
C_{12h} (mg/L)	4.3 (2.9-6.9) [2.8]	5.4 (4.2-7.0) [2.9]	p=0.22	0.78 (0.62,0.98)	3.7[8]
C_{max} (mg/L)	7.5 (6.2-10) [2.6]	9.5 (7.9-11) [3.8]	p=0.08	0.84 (0.70,0.99)	5.7[8]
AUC_{0-12h} (h.mg/L)	70 (56-104) [31]	87 (71-106) [39]	p=0.15	0.83 (0.69,1.00)	54.5[8]
Lamivudine					
C_{12h} (mg/L)	0.14 (0.084-0.17) [0.051]	0.081 (0.062-0.11) [0.048]	p=0.005	1.59 (1.21,2.07)	0.09[15]
C_{max} (mg/L)	1.6 (0.71-2.1) [0.85]	1.2 (0.90-1.6) [0.68]	p=0.59	0.97 (0.75,1.28)	1.2[15]
AUC_{0-12h} (h.mg/L)	6.5 (3.8-7.8) [3.4]	5.3 (3.8-6.9) [2.2]	p=0.41	1.10 (0.88,1.36)	4.7[15]
Stavudine					
C_{12h} (mg/L)	<0.015 (<0.015-<0.015) [-]	<0.015 (<0.015-<0.015) [-]	-	-	0.009[16]
C_{max} (mg/L)	0.28 (0.22-0.34) [0.15]	0.42 (0.34-0.52) [0.15]	p<0.001	0.61 (0.51,0.73)	0.54 [16]
AUC_{0-12h} (h.mg/L)	0.88 (0.69-1.2) [0.34]	0.94 (0.80-1.3) [0.40]	p=0.31	0.86 (0.72,1.04)	1.28[16]

** lamivudine and stavudine analyses failed for one child*

Note: Values are median (IQR) [SD]. Ranksum test compares <6 vs ≥6kg weight-bands.

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CHAPTER 06

Is nevirapine dose escalation appropriate in young, African, HIV-infected children?

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ABSTRACT

Objectives

Young children metabolize nevirapine faster than older children/adults. We evaluated nevirapine pharmacokinetics with/without dose-escalation in Zambian, HIV-infected infants/children and its relationship with safety/efficacy.

Design

A retrospective pharmacokinetic substudy of the CHAPAS-1 trial.

Methods

HIV-infected, Zambian children were randomized to initiate ART with full-dose twice-daily nevirapine versus 2-week nevirapine dose-escalation. Samples taken 3-4 hours postmorning-dose two weeks after nevirapine initiation were assayed for nevirapine levels. Viral load was measured on available samples at weeks 4 and 48; adverse events were prospectively reported.

Results

Of 162 (77%) children with week-2 samples, 79 (49%) were randomized to nevirapine dose-escalation. At ART initiation, median (IQR) age, weight and CD4% were 5.2 (1.5-8.7) years, 13.0 (8.1-19.0) kg and 13 (8-18)% respectively; 81 (50%) were male. With full-dose, few children <2 years (3/23, 13%) or >2 years (4/60, 7%) had subtherapeutic nevirapine levels (defined as <3.0 mg/L), but with dose-escalation, 7/22 (32%) <2 years versus 7/57 (12%) >2 yrs had subtherapeutic nevirapine levels (p=0.05). There was no difference between week-2 nevirapine levels in those with VL >250 c/ml versus <250 c/mL at week 4 (p=0.97) or week 48 (p=0.40). 11/162 children had grade 1/2 rash; all were >2 years (p=0.04), and 10 were randomized to full-dose.

Conclusions

Subtherapeutic nevirapine levels 3-4h post-dose were more frequent in young children on dose-escalation. Younger children were at lower risk for rash. To simplify ART initiation and reduce the risk of sub-optimal dosing, full-dose nevirapine at ART initiation should be considered for African HIV-infected children <2 years of age.

INTRODUCTION

In resource-limited settings, nevirapine is generally the most frequently used non-nucleosidereverse transcriptase inhibitor (NNRTI) and is frequently the only option available for first-line combination antiretroviral treatment (cART) of HIV-infected infants/children (if not perinatally exposed to nevirapine)[1]. The drug has many advantages as it is relatively inexpensive, has been used extensively with good virological efficacy and an acceptable safety profile, and is available in pediatric fixed-dose combination (FDC) tablets.

At nevirapine initiation, a dose-escalation strategy (half nevirapine dose for the first 14 days) is recommended[1] to minimize the risk of early adverse reactions[2]. Nevirapine induces its own metabolism and without dose-escalation high plasma concentrations occur during the first weeks of therapy. Elevated nevirapine levels have been associated with higher risks of rash and hepatotoxicity[3, 4].

However, young children metabolize nevirapine more rapidly than older children or adults[5-7] and hence the impact of auto-induction on nevirapine pharmacokinetics may be less relevant. Indeed, early nevirapine-induced adverse events have been reported to be less frequent among children, particularly the youngest, compared to adults[5]. On the contrary, dose-escalation in young children may result in lower and suboptimal nevirapine levels during the dose-escalation period which might increase the potential for slower viral load suppression or virological failure[8]. Here we evaluate the pharmacokinetics of nevirapine with and without dose-escalation strategy in Zambian HIV-infected infants and children, and investigate its relationship with safety and efficacy.

METHODS

CHAPAS-1 (ISRCTN31084535) was an open, randomized trial, performed at the University Teaching Hospital, Lusaka, Zambia, to investigate appropriate pediatric dosing of pediatric FDC tablets of nevirapine/lamivudine/stavudine: Triomune Baby (50 mg nevirapine, 6 mg stavudine and 30 mg lamivudine) and Junior (double Baby dose)[7]. Eligible HIV-infected, Zambian children, aged 3 months to 14 years, weighing <30 kg, and fulfilling WHO criteria for ART initiation, were enrolled June 2006-June 2008 and followed to October 2008 (trial closure). Exclusion criteria were previous exposure to ART (including to prevent mother-to-child transmission), severe laboratory abnormalities, active opportunistic infections/other illnesses and use of concomitant medication which may interfere with ART. Children were randomized 1:1 to initiate cART following WHO 2006 weight-band dosing[1] with either nevirapine full-dose using Triomune Baby/Junior taken as one tablet twice daily from ART initiation or nevirapine dose-escalation using 50% of the normal daily dose for Triomune Baby/Junior in the morning together with FDC Lamivir-S Baby (30 mg lamivudine and 6 mg stavudine) or Junior (double Baby dose) in the evening during the first two weeks of treatment. After two weeks, dose-escalation children stopped Lamivir-S Baby/Junior and continued with full dose Triomune Baby/Junior twice

daily. All carers (and children where appropriate) gave written informed consent. The study was approved by Ethics Committees in Lusaka, Zambia and London, UK.

In this retrospective pharmacokinetic substudy, a single plasma sample taken 3-4 hours post morning-dose of Triomune Baby/Junior two weeks after initiating ART was assayed. Nevirapine plasma levels were measured using ultra-performance liquid chromatography with a lower limit of quantification of 0.05 mg/L[9]. Subtherapeutic plasma concentrations were defined as <3.0 mg/L[3]. HIV-RNA viral load was measured on available stored samples at weeks 4 and 48, as previously described[7]. Nevirapine plasma concentrations were compared between full-dose and dose-escalation groups, under and over 2-year-olds, those with/without viral load <250 copies/mL, and those with/without adverse events using rank-sum tests. Exact tests were used similarly to compare proportions with subtherapeutic concentrations.

RESULTS

211 HIV-infected children were randomized to full-dose versus dose-escalation. 162 (77%) children had week-2 samples available for determining nevirapine plasma concentrations; 79 (49%) of them randomized to the dose-escalation group. At ART initiation, median (interquartile range; IQR) age, weight and CD4% in the 162 included children were 5.2 (1.5-8.7) years, 13.0 (8.1-19.0) kg and 13 (8-18)% respectively (very similar to the whole trial[7]). 81 (50%) children were male. They were moderately-to-severely stunted and wasted (median (IQR) height-for-age z-score -3.23 (-4.09,-2.26); weight-for-age z-score -3.09 (-4.15,-2.17)). The median (IQR) prescribed daily nevirapine doses at enrolment were 362 (344-400) mg/m^2 and 175 (152-193) mg/m^2 in the full-dose and dose-escalation groups, respectively.

Two weeks after ART initiation at enrolment, median (IQR) nevirapine plasma concentrations were 9.2 (6.4-12) versus 4.9 (3.6-6.4) mg/L in the in the full-dose versus dose-escalation groups respectively (p<0.001; ranksum). Smaller differences between full-dose and dose-escalation groups were observed in those below 2 years of age (5.3 (4.2-9.0) in the full-dose versus 4.8 (2.9-6.4) mg/L in the dose-escalation groups). In children over 2 years, the median (IQR) nevirapine plasma concentrations were 10.0 (7.9-12.2) versus 5.0 (3.9-6.6) mg/L in the full-dose versus dose-escalation groups, respectively (interaction p<0.001; Table 1, Figure 1 (a)). In the full-dose group, 3 of 23 (13%) children <2 years and 4 of 60 (7%) children >2 years had subtherapeutic nevirapine plasma concentrations (p=0.39; Exact). However, with dose-escalation, 7 of 22 (32%) children <2 years of age versus 7 of 57 (12%) children >2 years of age had subtherapeutic nevirapine plasma concentrations (p=0.05; Exact).

There was no difference between week 2 nevirapine plasma concentrations in those children with viral load >250 copies/mL versus <250 copies/mL at week 4 (p=0.97; ranksum) or week 48 (p=0.40; ranksum). Four weeks after ART initiation 12 of 28 (43%) children in the full-dose group versus 11 of 34 (32%) children in the dose-escalation group had a viral load <250 copies/mL (p=0.44; exact), compared to 44 of 61 (72%) children in the full-dose group versus 45 of 60 (75%) children in the dose-escalation group at 48 weeks (p=0.84; exact).

Eleven of 162 (7%) children developed a rash, which were all graded as 1 or 2. All (100%) children with rash were older than 2 years of age (11/117 (9%); p=0.04, exact, versus 0/45 under 2 years), and 10/11 were in the full-dose group (p=0.009; exact versus dose-escalation). Only one child was between 2-3 years of age (2.4 years) on full-dose. In children over 2 years, median (IQR) nevirapine plasma concentration was 15.1 (10.4-19.6) mg/L in those with rash (n=11) versus 6.8 (4.5-9.7) mg/L in those without rash (n=106) (p<0.001; ranksum; Figure 1 (b)).

TABLE 1 Nevirapine plasma concentrations of children in the study

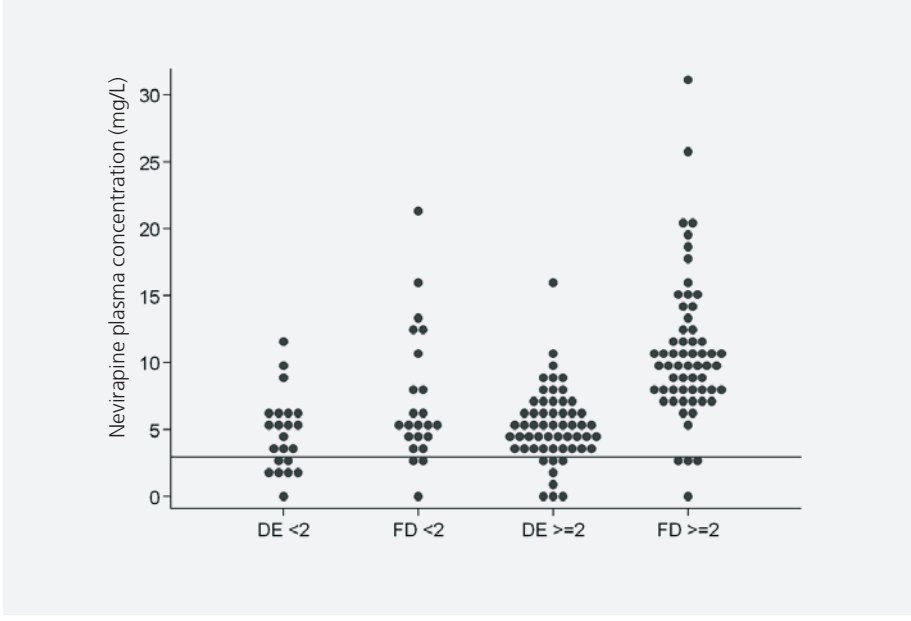
	Children <2 years (n=45)	Children >2 years (n=117)	P-value
Nevirapine plasma concentration, mg/L			
Full-dose group (n=83)	5.3 (4.2-9.0)	10.0 (7.9-12.2)	0.001
Dose-escalation group (n=79)	4.8 (2.9-6.4)	5.0 (3.9-6.6)	0.41
P-value comparing full-dose versus dose-escalation	0.14	<0.001	*
Subtherapeutic nevirapine concentrations, n (%)			
Full-dose group (n=83)	3 of 23 (13%)	4 of 60 (7%)	0.39
Dose-escalation group (n=79)	7 of 22 (32%)	7 of 57 (12%)	0.05
P-value comparing full-dose versus dose-escalation	0.16	0.35	**

** Interaction (heterogeneity) p<0.001*

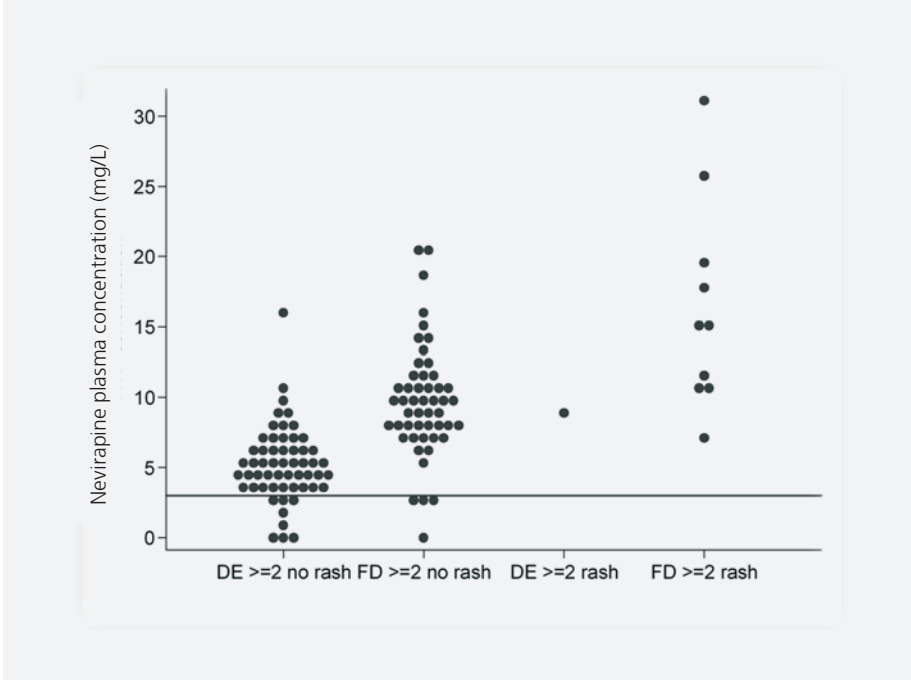
*** Interaction (heterogeneity) p=0.65. Nevirapine concentrations compared using ranksum tests and median regression: subtherapeutic concentrations compared using exact tests and logistic regression.*

FIGURE 1 ▸ Nevirapine plasma concentrations of children in the study

(A) NEVIRAPINE PLASMA CONCENTRATIONS, DOSING GROUPS AND AGE



(B) NEVIRAPINE PLASMA CONCENTRATIONS AND RASH



Note: DE=dose-escalation; FD= full-dose

DISCUSSION

This randomized trial is the first, and currently only, to evaluate plasma nevirapine concentrations under full-dose or dose-escalation strategies during the initial two weeks of treatment in children. Despite the relatively small sample size, with nevirapine dose-escalation at cART initiation more subtherapeutic nevirapine plasma concentrations 3-4 hours post-dose were observed in children <2 years of age compared to older children. This finding is compatible with earlier studies describing more rapid nevirapine systemic clearance in younger than in older children[5, 6].

Of note, the P1060 trial[8] found less favorable outcomes with nevirapine compared with ritonavir-boosted lopinavir among young children (median 1.7 years of age), regardless of whether they were previously exposed to nevirapine or not; virological failure (including deaths) by 24 weeks was significantly (15.6 percentage points) higher with nevirapine versus ritonavir-boosted lopinavir. Although no pharmacokinetic data were available, dose-escalation in infants was hypothesized to be a potential contributing factor for inferior response to nevirapine therapy[8]. We did not find evidence for an association between week-2 nevirapine plasma concentrations with short (week 4) or longer term (week 48) viral load suppression (which might be due, at least in part, to small numbers of samples assayed), but the high observed proportion of subtherapeutic nevirapine plasma concentrations two weeks after cART initiation in young children supports the hypothesis from the P1060 trial that the nevirapine dose-escalation strategy might be suboptimal in children <2 years of age. Unfortunately we did not assay VL in 24 week samples, so we are unable to directly compare our results with P1060. Interestingly however, we observed 11% higher viral load suppression <250 copies/ml in the full-dose group at week 4; whilst the small number of samples that could be assayed (n=62) precluded us from excluding chance as an explanation, this does suggest that it is plausible that full-dose initiation could have some virological benefits.

Another argument supporting the use of full-dose nevirapine from ART initiation in young children is that none of those in the present study developed rash, which has previously been described to be less frequent in younger children[5, 7]. All rashes occurred in those over 2 years (only one child between 2-3 years old), and all but one in those starting full-dose nevirapine; further our results demonstrate the first pharmacokinetic-pharmacodynamic association between high nevirapine plasma concentrations and rash, since all those with rash had high week 2 levels (most >10 mg/L) in the potentially toxic range[3].

A third argument for full-dose nevirapine from ART initiation in young children is convenience. The use of three separate liquid formulations of these drugs is impractical, confusing for caregivers, and expensive; in studies in children aged 2-3 years carers strongly preferred tablet to syrup medications[10]. A half-dose of Triomune Baby/Junior from ART initiation should not be used, since the resulting substantial initial lamivudine underdosing at a time when very young children have very high viral loads could lead to early resistance development given its low genetic barrier. Another way to escalate the

nevirapine dose is to use one morning dose of Triomune Baby/Junior plus one evening dose of Lamivir-S Baby/Junior during the first two weeks of cART (as in CHAPAS-1). These low-cost pediatric FDCs enable safe and simple dose-escalation in contrast to cutting parts of adults NRTI FDCs. However, initiating nevirapine using full-dose is a less complicated approach; based on our findings this could certainly be considered in young African children <2 years of age.

One limitation of our study was that it was unknown if trial drugs were taken the days prior study visit; however the proportions with very low concentrations suggesting non-adherence were similar across randomised and age groups (Figure 1 (a)). The exact time between nevirapine ingestion and blood sampling was also not recorded, but its relatively long elimination half-life (25-30 hours) coupled with the fact that its subtherapeutic concentration (defined as <3.0 mg/L) applies for the entire 12-hour dosing interval[3] makes nevirapine tolerant to minor variability in time from ingestion to sampling, here estimated as 3-4 hours. The other main limitation was the relatively small sample size, a consequence of failure to locate specimens taken at weeks 2 and 4 three years after study closure.

In conclusion, using a dose-escalation strategy, subtherapeutic nevirapine levels 3-4 hours post-dose were more frequent in younger than older children. Younger children had a low risk for rash with a full-dose initiation strategy. To simplify ART initiation in young children and reduce the risk of suboptimal dosing, full-dose nevirapine at ART initiation should be considered for African HIV-infected children <2 years of age. Children >2 years of age could continue to receive dose-escalation to avoid the development of rash, or should have easy access to clinics to enable timely review if rashes recur with temporary discontinuation and nevirapine re-initiation at half-dose[7].

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CHAPTER 07

Paediatric under-dosing of efavirenz: a pharmacokinetic study in Uganda

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ABSTRACT

Objectives

To evaluate international pediatric efavirenz dosing recommendations using full pharmacokinetic information.

Design

Open-label, multi-centre, pharmacokinetic study

Methods

41 HIV-infected Ugandan children (3-12 years) on efavirenz+ lamivudine+ abacavir were enrolled in a study of twice- to once-daily lamivudine+abacavir 36 weeks after ART initiation in the ARROW trial. Once-daily efavirenz doses were 200, 250, 300*, 350* mg for children weighing 10-<15, 15-<20, 20-<25, 25-<30kg respectively,using 200/50 mg capsules or *halved 600 mg tablets. Intensive plasma PK sampling (t=0,1,2,4,6,8,12h post observed ingestion) was performed at steady state (PK1) and repeated 4 weeks later (PK2, including a further 24h sample).

Results

41 and 39 children had evaluable efavirenz profiles at PK1 and PK2, respectively. Seventeen (41%) were boys. Five, 16, 17, 3 were in the 10-<15, 15-<20, 20-<25, 25-<30kg weight-bands. The geometric mean (%CV) AUC_{0-24} was 50.8 (90.8%) and 55.5 (82.7%) h.mg/L at PK1 and PK2 respectively. Six children at PK1 and seven at PK2 had subtherapeutic C_{8h} and/or C_{12h} (<1.0 mg/L), 7/41 (17%) at either visit. At PK2, 15/39 (38%) children had C_{24h}<1.0 mg/L (median (IQR) [range] 1.1 (0.7-2.9) [0.3-18.4] mg/L). Ten children at PK1 and 11 at PK2 had C_{8h} and/or C_{12h} >4.0 mg/L; 12/41 (29%) at either visit.

Conclusions

African children aged 3-12 years, on efavirenz dosed according to 2006 WHO/ manufacturer's recommendations, had lower and highly variable efavirenz PK parameters compared to adult data from manufacturer's leaflet. There were no differences across weight-bands, suggesting no major effect of using half-tablets. Higher pediatric efavirenz doses, as per WHO 2010 recommendations, should be used and investigated further, but may risk increasing the proportion of children with potentially toxic levels.

INTRODUCTION

The revised WHO guidelines of 2010 for the treatment of HIV-1 infected children older than three years of age recommend two nucleoside reverse transcriptase inhibitors (NRTI) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI) for first-line antiretroviral therapy (ART)[1]. Efavirenz, an NNRTI, has potent antiviral activity and a long elimination half-life (40-55 hours), which makes it suitable for once daily dosing[2]. It is also suitable for co-administration with anti-tuberculosis medications. For these reasons, the drug is therefore one of the most preferred first-line antiretroviral agents and is currently used widely in HIV-infected children. The licensed and recommended pediatric dosages for efavirenz are based upon weight-bands (Table 1), with allometric doses targeting at least 300 mg/m^2 in each. However doses have not been established for children younger than three years and/or weighing below 10 kg, due to problems in achieving appropriate drug levels despite giving high doses[1,3].

Until now, there is limited knowledge about steady state pharmacokinetics of efavirenz in children, particularly in African populations. Studies have reported high proportions of children with subtherapeutic plasma concentrations of efavirenz[4-7] receiving doses in accordance with current licensed doses[2] and previous 2006 WHO guidelines, which were mainly based on data assessed in adult patients[8,9].

However, most pediatric studies to date have analyzed sparse pharmacokinetic (PK) samples rather than full pharmacokinetic curves, including the only two studies that have been reported in African children[4,6]. Ren *et al* found a high prevalence (40%) of sub-therapeutic plasma concentrations of efavirenz in a small cohort of 15 children, based on 3 samples taken 12-24 hours after observed dose. Hirt *et al* conducted a population pharmacokinetic study based on 3 samples taken before, 1 and 3 hours after efavirenz administration in 48 children in Burkina Faso (9 of whom had full pharmacokinetic samples taken) and found an association between estimated $AUC_{0\text{-}24}$ >51 h.mg/L and virological efficacy. They concluded that younger children should receive higher efavirenz doses than currently recommended. In both studies[4,6] doses were derived from the licensed dose (Table 1)[2]. Together with earlier studies, these findings suggest that a large proportion of children taking efavirenz following the pediatric licensed or WHO 2006 dosing guidelines might need a higher dose.

Since there are few studies validating international weight-band dosing recommendations for efavirenz to date, in particular in African HIV-infected children, we conducted a pharmacokinetic substudy to determine whether the 2006 WHO weight-band dosing, similar to the manufacturer's recommendations, resulted in optimal exposure in 41 Ugandan children over 3 years old in the AntiRetroviral Research fOr Watoto (ARROW) trial.

METHODS

Population and study design

ARROW is an open-label randomised trial comparing routine laboratory (toxicity, CD4) versus clinically driven monitoring strategies, and also comparing three different NRTI-based ART strategies in 1,206 symptomatic HIV-infected infants and children in Uganda and Zimbabwe (www.arrowtrial.org). Forty-one children aged 3-12 years from two Ugandan ARROW centers (the Joint Clinical Research Centre (JCRC), Kampala and the Paediatric Infectious Disease Centre (PIDC), Mulago Hospital) who had been taking lamivudine + abacavir twice daily with once-daily efavirenz for at least 36 weeks in ARROW and were not expected to change weight-bands in the next 4 weeks participated in a two-period, crossover, open-label PK study comparing twice versus once daily lamivudine+abacavir[10]. Efavirenz was dosed once daily according to WHO 2006 pediatric recommendations as 50 mg or 200 mg capsules or halved 600 mg tablets (Table 1), except that children weighing 14-<15 kg received 200 mg (the licensed dose) rather than 250 mg efavirenz to harmonize with NRTI weight-bands. The 200 mg, 250 mg, 300 mg and 350 mg efavirenz dose consisted of one 200 mg capsule, one 200 mg plus one 50 mg capsule, one halved 600 mg tablet and one halved 600 mg tablet plus one 50 mg capsule, respectively. Efavirenz 600 mg tablets were unscored, but were cut in the pharmacy before dispensing at four weekly visits. Children on any concomitant medication with known interactions to any drug in the ARV regimen or with anemia or illnesses that could influence the pharmacokinetics of efavirenz, such as diarrhea, vomiting, renal or liver disease were not eligible. Children who missed any dose of any antiretroviral drug in the 3 days prior to the pharmacokinetic evaluation (confirmed by pill count and questionnaire) were excluded. All carers gave fully informed written consent for both the main trial and the PK study, and children provided additional assent as appropriate according to age and knowledge of HIV-status. The pharmacokinetic study was approved by the Ethics Committee from each participating centre as well as by the Uganda National Council of Science and Technology.

Efavirenz blood sampling and analyses

Four weeks before the pharmacokinetic evaluation was undertaken (32 weeks after starting ART), children were changed to efavirenz administration in the morning if they were taking efavirenz in the evening. At week 36 after starting ART (once-daily efavirenz plus twice daily NRTIs) a 12-hour pharmacokinetic sampling session was done. Samples were taken immediately before directly observed medication intake (t=0) and at 1, 2, 4, 6, 8 and 12 hours later. Breakfast (non-standardized, but mostly milk/milky tea with samosas/bread/chapati) was provided two hours after the morning dose. One and a half ml of blood was collected per time point. Plasma was separated and stored at -80°C until transportation on dry-ice for analysis of efavirenz (and NRTI) plasma concentrations. After the pharmacokinetic evaluation at week 36 the children were switched to a completely once daily regimen (morning administration). At week 40 the intensive plasma pharmacokinetic sampling was repeated, including an extra pharmacokinetic sample at 24 hours after observed intake. Serum biochemistry and hematology were performed at week 36.

Plasma concentrations of efavirenz were assayed by a validated high-performance liquid chromatography method at the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, using a validated high-performance liquid chromatography assay with UV detection[11]. The lower limit of quantification was 0.05 mg/L. Three samples had concentrations below the detection limit. They were treated as zero for the determination of PK parameters in WinNonlin, and as 0.05 mg/l for estimation of geometric mean concentration.

Statistical methods

Since WHO growth charts only go up to ten years of age, weight-for-age and height-for-age z-scores were calculated using the 1990 British Growth Charts[12] in STATA statistical software, version 11.1 (STATA Corp., College Station, Texas, USA). Pharmacokinetic parameters (C_{24h}, C_{max}, the area under the concentration-time curve 0-24 hours post-dose (AUC_{0-24h}) and CL/F) of efavirenz were calculated by noncompartmental analysis of the plasma concentration data using WinNonlin software version 5.2 (Pharsight Corporation, Mountain View, CA). AUC was calculated using a trapezoidal rule. C_{24h} at week 36 was estimated by extrapolation of the 12-hour PK curve using the exponential equation for first-order pharmacokinetics: $\ln(C_{24h})=\ln(C_{12h})-(\Delta t_{0-12h}*k)$ with $k=\ln 2/t_{1/2}$. Elimination half-life was calculated on ≥3 time points after C_{max}. Four children had $R^2<0.85$ for the log-linear regression to calculate the elimination rate constant or elimination half-life. C_{24h} was therefore not extrapolated. Efavirenz pharmacokinetic parameters were compared across weight-bands and predictors of $\log_{10} AUC_{0-24}$ and CL/F/kg including sex, age, weight- and height-for-age and dose, were assessed using mixed models, fitting random effects for each child. As CYP*2B6* 516G>T genotype is known to strongly increase plasma EFV exposure[13-15], but was not measured in this study. We used finite normal mixture modeling to estimate the geometric mean EFV exposure in and the size (percentage of the population) of three population subgroups to correspond to GG, GT and TT genotypes.

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RESULTS

Forty-one children (24 girls, 17 boys) were included in this ARROW pharmacokinetic substudy. Four children increased weight-bands between weeks 36 and 40 (2 from 10-<15 to 15-<20 kg; 2 from 20-<25 to 25-<30 kg) and are included in all analyses as the primary goal was to evaluate the weight-band based dosing. Two children had implausible time concentration curves (possible labeling errors) at week 40 which were excluded from analysis. The median (interquartile range, IQR) age and bodyweight were 7.6 (5.6-9.1) years and 20.0 (16.6-23.0) kg, respectively at 36 weeks after starting ART. Eighteen and 23 children were aged 3-6 years and 7-12 years, respectively. The majority were moderately stunted (median (IQR) height-for-age -1.85 (-2.78 to -1.11)) and wasted (median (IQR) weight-for-age -1.56 (-2.15 to -0.82)). Five (12%), 16 (39%), 17 (41%) and 3 (7%) were in weight-bands 10-<15/15-<20/20-<25/25-<30 kg, receiving 200, 250, 300 and 350 mg efavirenz, respectively at the first PK day (Table 1, 300 and 350 mg doses including halved

TABLE 1 ▸ Efavirenz pediatric dosing guidelines

Body weight (kg)	Approx. surface area (m^2) *	Sustiva leaflet 2004/2008					ARROW protocol					WHO guidelines 2006					WHO guidelines 2010				
		EFV daily dose					EFV daily dose †					EFV daily dose					EFV daily dose				
		mg	mg/kg		mg/m^2		mg	mg/kg		mg/m^2		mg	mg/kg		mg/m^2		mg	mg/kg		mg/m^2	
			min	max	min	max		min	max	min	max		min	max	min	max		min	max	min	max
10 to <12	0.49 to 0.56	200	16.7	20.0	360	410	200	16.7	20.0	360	410	200	16.7	20.0	360	410	200	16.7	20.0	360	410
12 to <14	0.56 to 0.62	200	14.3	16.7	320	360	200	14.3	16.7	320	360	200	14.3	16.7	320	360	200	14.3	16.7	320	360
14 to <15	0.62 to 0.65	200	13.3	14.3	310	320	200	13.3	14.3	310	320	**250**	16.7	17.9	380	400	**300**	20.0	21.4	460	480
15 to <17	0.65 to 0.71	250	14.7	16.7	350	380	250	14.7	16.7	350	380	250	14.7	16.7	350	380	**300**	17.6	20.0	420	460
17 to <20	0.71 to 0.79	250	12.5	14.7	320	350	250	12.5	14.7	320	350	250	12.5	14.7	320	350	**300**	15.0	17.6	380	420
20 to <25	0.79 to 0.92	300	12.0	15.0	330	380	300	12.0	15.0	330	380	300	12.0	15.0	330	380	300	12.0	15.0	330	380
25 to <30	0.92 to 1.1	350	11.7	14.0	320	380	350	11.7	14.0	320	380	350	11.7	14.0	320	380	**400**	13.3	16.0	360	430
30 to <32.5	1.1	350	10.8	11.7	320	320	**400**	12.3	13.3	360	360	400	12.3	13.3	360	360	400	12.3	13.3	360	360
32.5 to <35	1.1 to 1.2	400	11.4	12.3	330	360	400	11.4	12.3	330	360	400	11.4	12.3	330	360	400	11.4	12.3	330	360
35 to <40	1.2 to 1.3	400	10.0	11.4	310	330	400	10.0	11.4	310	330	400	10.0	11.4	310	330	**600**	15.0	17.1	460	500
≥40	≥1.3	600		15.0		460	600		15.0		460	600		15.0		460	600		15.0		460

** based on standard from pediatric oncology[24]; † using halved 600 mg tablets in those weighing 20-30 kg*

Note: bold values show increases compared to the dosing recommendation in the previous column

adult 600 mg tablets). Median (IQR) [range] doses per bodyweight were 13.6 mg/kg (12.8-14.6) [11.6-16.7] and 13.9 mg/kg (12.8-14.7) [11.9-16.7] at first and second PK days respectively; and 345 mg/m^2 (323-360) [291-400] and 352 mg/m^2 (329-367) [296-395] respectively per unit surface area. Doses in mg/kg received were highest in the 15-<20 kg weight-band (median 14.7) and lowest in the 20-<25 kg weight-band (median 13.0) (Kruskal-Wallis p=0.001). Doses in mg/m^2 were lowest in the 10-<15 kg weight-band (median 315) and were higher in other weight-bands (medians between 342 and 363; Kruskal-Wallis p=0.0002).

The geometric mean efavirenz plasma concentration versus time curves obtained at the pharmacokinetic evaluation days 36 and 40 weeks after starting ART were very similar (Figure 1). Seven of the 41 (17%) children in total had subtherapeutic (<1.0 mg/L) plasma concentrations at 8 hours (C_{8h}) and/or at 12 hours (C_{12h}) after observed intake at one or both PK days (Figure 2). Six of the 41 (15%) children had subtherapeutic C_{8h} and/or C_{12h} plasma concentrations at week 36 and 7/39 (18%) children at week 40. Twenty-two (59%) of the 37 children in whom C_{24h} could be extrapolated at week 36 had values <1.0 mg/L, and 15/39 (38%) observed C_{24h} plasma levels at week 40 were <1.0 mg/L. Supra-therapeutic and potentially toxic C_{8h} and/or C_{12h} plasma levels (>4.0 mg/L) were found in 12 (29%) children: 10 (24%) children at week 36 and 11 (28%) children at week 40.

FIGURE 1 Mean efavirenz levels at week 36 (PK1) and week 40 (PK2)

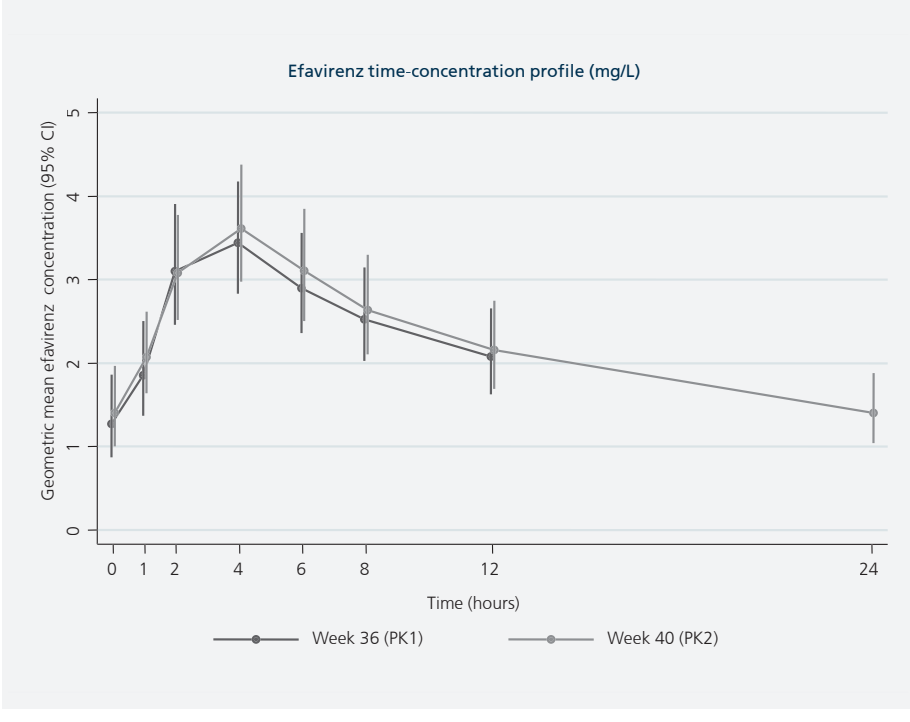
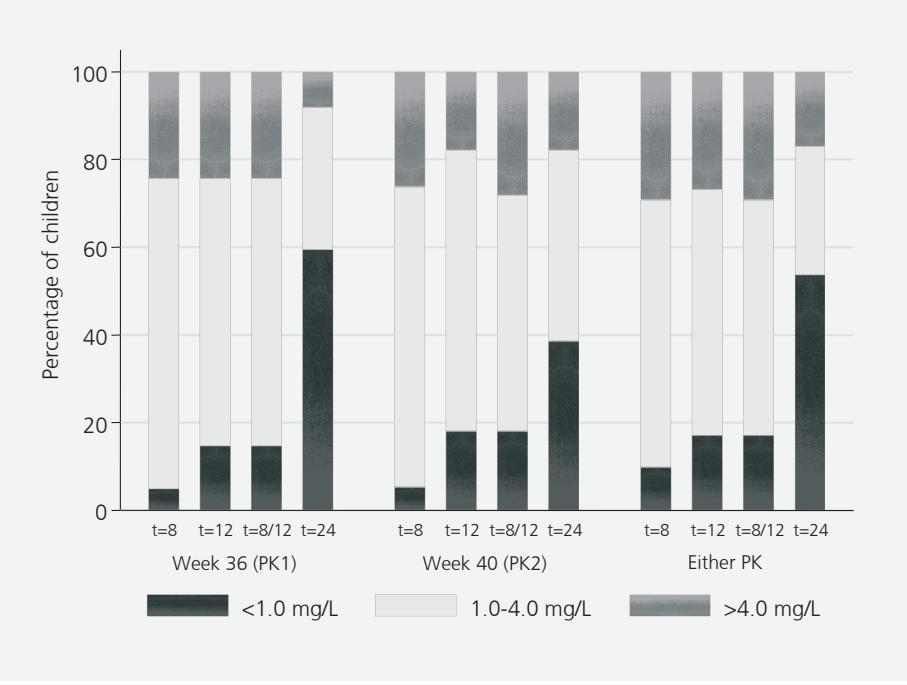


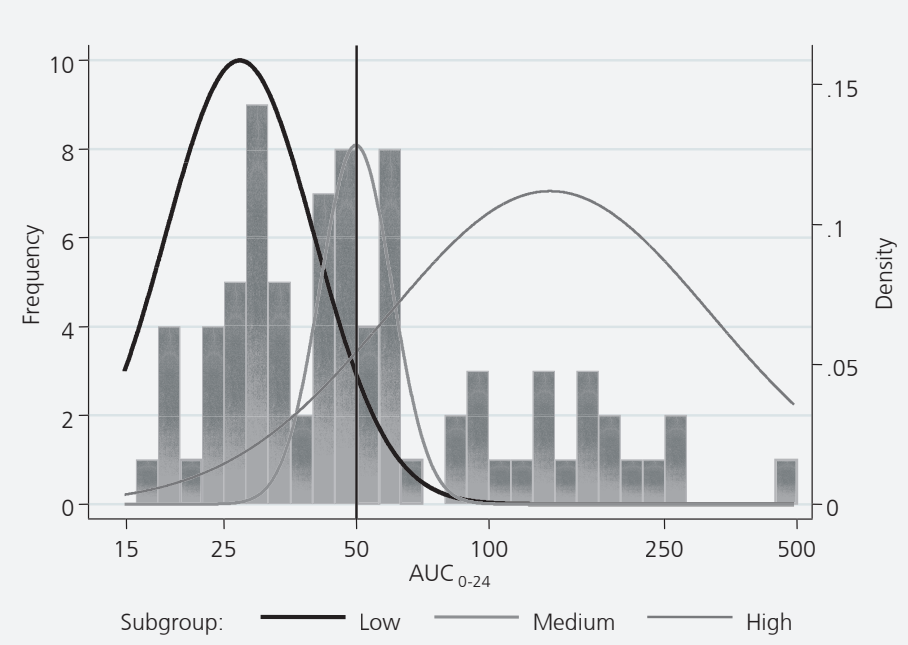
FIGURE 2 ▸ Efavirenz concentrations 8, 12 and 24 hours after observed intake



Note: n=41 and 39 at week 36 and 40 respectively. C_{24h} extrapolated at week 36 in 37 children, and observed at week 40.

The geometric mean pharmacokinetic parameters of efavirenz are presented in Table 2. In the 35 children who stayed on the same efavirenz dose and had both PK evaluations, there was no evidence of variation in C_{max}, $AUC_{0\text{-}24h}$, and clearance between the two pharmacokinetic evaluation days (geometric mean ratio (90% CI) 0.99 (0.88-1.10) p=0.83, 0.92 (0.82-1.02) p=0.18, 1.09 (0.98-1.21) p=0.18 respectively). Efavirenz concentrations were mainly lower than those previously reported in adult patients[2]. In particular 26/41 (63%) and 20/39 (51%) children had $AUC_{0\text{-}24}$ <50 h.mg/L at week 36 and 40 respectively, 27 (66%) at either PK visit. A large intersubject but moderate intrasubject variability was found in efavirenz pharmacokinetic parameters: being 57% and 28% for C_{max}, 81% and 28% for $AUC_{0\text{-}24}$, 84% and 27% for clearance, and 113% and 39% for C_{min} (estimated not observed in 37 patients at week 36). There was no evidence of significant difference in efavirenz across the four weight-bands for C_{max} (p=0.58), $AUC_{0\text{-}24}$ (p=0.12), clearance (p=0.22) or C_{min} (p=0.52). However, with only 41 children power was relatively low. Compared to the 15-<20 kg weight-band (which had the most children), $AUC_{0\text{-}24}$ was 29% lower (95% CI 55% lower to 10% higher) in those weighing 10-<15 kg, and 25% lower (95% CI 53% lower to 20% higher) in those weighing 20-<25 kg. $AUC_{0\text{-}24}$ was more similar in the small number of children weighing 25-<30 kg (11% higher, 95% CI 38% lower to 99% higher).

FIGURE 3 Subpopulations of AUC_{0-24} efavirenz within the ARROW PK substudy



Although pharmacogenetic testing was not performed in this study, we used finite normal mixture modeling to identify the three most likely sub-populations for AUC_{0-24}. 40% of the children had a geometric mean AUC_{0-24} of 27.2 h.mg/L, 32% 49.9 h.mg/L, and 28% 137.7 h.mg/L (Figure 3). However, there was no evidence of association between sex, age, weight, receipt of halved tablets, weight- or height-for age with AUC_{0-24} ($p>0.2$). There was a marginal trend towards higher AUC_{0-24} with greater doses, (+8.8 per mg/kg higher (95% CI-1.2,+18.9); +20.3 per 50 mg/m^2 higher (-3.2,+43.8); both p=0.09). No child substituted efavirenz for toxicity, and there were no grade 3/4 clinical events considered definitely/ probably related to efavirenz in these children, with the exception of 3 rashes during the first week on ART (children had week 36 AUC_{0-24} 42, 56 and 258 h.mg/L).

DISCUSSION

A group of 41 HIV-infected Ugandan children aged between 3 and 12 years, receiving efavirenz once daily and using the 2006 WHO weight-band dosing recommendations had lower and highly variable pharmacokinetic parameters of efavirenz compared to historical data from adults. The efavirenz C_{max}, C_{min} and AUC_{0-24} in our patients were 15%, 36% and 10% lower, respectively than those observed in adult patients receiving once daily efavirenz 600 mg (Table 2)[2]. If we compare our week 36/40 data to previously reported efavirenz data in African children, C_{max}, C_{min} and AUC_{0-24} were similar or even somewhat lower than those

TABLE 2 ▸ Pharmacokinetic parameters of efavirenz

Efavirenz	Week 36 (PK1) geometric mean [95% CI] (range)	Week 40 (PK2) geometric mean [95% CI] (range)	Lit. data adults[2] arithmetic mean [SD]
C_{max} (mg/L)	4.10 [3.37-4.98] (1.45-14.87)	4.10 [3.41-4.93] (1.18-21.97)	4.072 [1.16]
AUC_{0-24} (h.mg/L)	50.79 [39.77-64.88] (16.04-258.84)	55.49 [43.91-70.11] (18.18-479.91)	58.08 [23.04]
CL/F (l/h)	5.29 [4.12-6.80] (0.77-15.99)	4.94 [3.90-6.25] (0.52-17.92)	NA
C_{24h} (mg/L)	0.84 [0.60-1.18]* (0.17-9.28)	1.40 [1.03-1.90] (0.27-18.39)	1.77 [1.01]

** Extrapolated (n=37) Note: ranges provided with geometric means to describe the variation across children.*

of previous studies: 4.10/4.10 mg/L, 0.84/1.40 mg/L and 50.79/55.41 h.mg//L, respectively in our study compared to 3.71 mg/L[4], 1.18 to 1.64 mg/L[4,6] and 65.2 h.mg/L[4], respectively. Based on the study of Marzolini relating efavirenz t o efficacy and toxicity in adults, a minimum target of 1.0 mg/L 8-20 hours post-dose and a maximum target of 4.0 mg/L is advocated[16]. Of note, this study did not measure the true trough concentration at 24 hours, but was based on samples taken 8-20 hours post-dose. Fifteen percent (week 36) and 18% (week 40) of the children in the current study had subtherapeutic plasma concentrations (<1.0 mg/L) 8-12 hours post-dose, which is consistent with other pharmacokinetic studies of efavirenz in African children. The study by Ren *et. al*, where the 15 children received lower efavirenz doses[2] than in our study[1,17], found 40% of the patients had estimated C_{min} extrapolated from samples taken 12-24 hours post-dose <1.0 mg/L[6], compared to 59% extrapolated and 38% observed C_{24} in our study. Ren *et. al* also found a higher chance of detectable viral load in children with subtherapeutic plasma concentrations. Another study evaluating efavirenz exposure in African children demonstrated that 19% had estimated C_{min} <1.0 mg/L[4], and again, subtherapeutic exposure was linked to suboptimal antiviral efficacy.

In addition to adult and pediatric data showing an association between a C_{min} target of 1.0 mg/L and virological efficacy, two recently published studies have found an exposure-efficacy link for efavirenz AUC in children[4,18]. Both found an efavirenz AUC_{0-24} above ~50 h.mg/L significantly improved virological efficacy. In our study the geometric mean AUC_{0-24} of efavirenz is only just above this 50 h.mg/L threshold (Table 2). Twenty-six of 41 (63%) children at week 36 and 20 of the 39 (51%) evaluable pharmacokinetic profiles at week 40 had an efavirenz exposure lower than 50 h.mg/L.

Taken together, our data, added to the previously reported information, strongly suggests that children should receive efavirenz doses higher than the WHO 2006 recommendations

to increase the percentage of children with C_{min} and/or AUC in the target ranges, leading to maximal antiviral efficacy. The fact that these WHO 2006 recommendations[17] are very similar to the manufacturers leaflet[2] daily dose (50 mg higher only for those children weighing 14-<15 kg and 30-32.5 kg) highlights the importance of our results for children in resource-rich as well as resource-limited settings. On the other hand, due to the large interpatient variability (81% in our patients), we found a considerable proportion of children (29%) with an efavirenz C_{max} in the supratherapeutic and potentially toxic range (>4.0 mg/L). Because of the relatively large proportions of children with efavirenz exposure outside the therapeutic range, it is not difficult to understand that only a small majority of children (22 of the 41 (54%)) was dosed adequately (1.0-4.0 mg/L) 8-12 hours post-intake, which is consistent with data from the previous two studies in African children: 47% and 66%[4,6]. The latest 2010 WHO dosing guidelines have higher efavirenz doses than evaluated in our study (and than licenced) for children weighing 14-<20, 25-<30 and 35-<40 kg: of note, these higher doses were chosen not only to reflect concerns about underdosing raised by the two previous African studies[4,6], but also to remove the 50 mg capsules from dosing tables, as these were becoming no longer available. Our data suggest that, whilst these doses should lead to greater exposure and thus greater virological efficacy, the necessary trade-off is that more than one third of children will be exposed to potentially toxic efavirenz levels. Whilst overt grade 3/4 toxicity was not reported and CNS side-effects is transient in most adults, CNS side-effects associated with efavirenz in children (colorful dreams, impaired concentration) can be problematic, particularly at school, without ever reaching high toxicity grades or without recognition that their source may be an adverse event and their importance should not be ignored. However, WHO 2010 guidelines retain the same 300 mg dose for children weighing 20-<25 kg as we evaluated in ARROW: 21/31 (68%) AUC_{0-24} in this weight-band were below the 50 h.mg/L target with this 300 mg dose, suggesting this should be increased. All children in ARROW weighed 14-30 kg, so our data cannot inform whether the WHO 2010 doses for the 10-14 kg (200 mg) or 30-<35 kg (400 mg) weight-bands are sufficient. However, efavirenz CNS side-effects can be more problematic in adolescents where problems with concentration can have major impacts on schooling, so avoidance of very high levels may be more important in those >30 kg.

Lower plasma concentrations found for efavirenz in these African children showed one important distinction compared to adults, namely that the apparent clearance for efavirenz in our patients is faster than that observed in adult patients[18]. These data support the hypothesis that efavirenz metabolism is affected by age and that children are pharmacokinetically different from adults. Another factor that could influence efavirenz metabolism is genetic polymorphisms in CYP*2B6*, the enzyme responsible for metabolism of efavirenz. African patients have been found to have higher frequencies of 516G>T polymorphism for the CYP*2B6* enzyme[19-22], which is associated with much higher and potentially toxic efavirenz concentrations[7]. Additionally, Leger *et al* showed that distinct *CYP2B6* polymorphisms decreased plasma efavirenz exposure in patients of African descent[23]. Although we do not have pharmacogenetic data available for our patient population, the

trimodal distribution of efavirenz AUC as shown in Figure 3 suggests a division of patients being either normal metabolizers (homozygote wild-type CYP*2B6*), intermediate metabolizers (heterozygote CYP*2B6* mutants) and slow metabolizers (homozygote CYP*2B6* mutants). Our small patient set is roughly divided in three equal-sized subpopulations with a gene frequency of 0.44 for the T allele.

One limitation of our study is that it included only Ugandan (East African) children: however, results are broadly compatible with earlier studies in South Africa[4,6] and Burkina Faso (West Africa)[4,6], and we were able to include a larger number of children than Ren *et al*. As far as we know, our study is the first using full pharmacokinetic information to evaluate the 2006 WHO weight-band dosing table which harmonized the licensed dosing recommendations for efavirenz with weight-bands for other antiretrovirals. This dosing table used split adult 600 mg tablets to provide 300 mg as all or part of another dose in those 20-<30 kg. Whilst exposure appeared slightly lower in children receiving these split tablets in the 20-<25 kg weight-band in our study, given the low exposures in all weight-bands it is difficult to attribute all or even much of this to a possible effect of splitting tablets. Using split adult tablets enables a far larger number of children to receiving life-saving ART, and is also recommended in WHO 2010 dosing guidelines: our findings highlight the importance of testing all pediatric dosing recommendations regardless of dosing modality. In terms of methodology, both previous studies used population pharmacokinetic models to determine of pharmacokinetic parameters, with the disadvantage that full efavirenz exposure can only be estimated by complex models. Here we used a full pharmacokinetic curve for every individual child after observed intake, which is more reliable for the estimation of pharmacokinetic parameters. Whilst grade 3/4 and ART-modifying toxicity was collected as part of the ARROW trial, lower grade adverse events which may be important for children, such as poor concentration, were not collected. Thus, while we observed no severe or serious toxicity associated with high efavirenz levels, we cannot rule out other toxic effects.

The final limitation of the study is the lack of virological data to evaluate the association of pharmacokinetic parameters of efavirenz with virologic response. However, previous studies have demonstrated a strong concentration-effect relationship, not only in adults (subtherapeutic $C_{8\text{-}24h}$<1 mg/L)[16], but also in children ($AUC_{0\text{-}24}$ <~50 h.mg/L)[4,18]. The lack of virological data in this study is thus less important than for drugs where such associations have not been previously described. Hence, we are confident that our efavirenz exposure data are truly suboptimal, and that those treating children with efavirenz in resource-limited and resource-rich settings should move urgently to the adjusted WHO 2010 dosing recommendations. However, even with these new recommendations, dosing in some weight-bands (particularly from 20-<25 kg but also possibly 10-<14 kg and 30-<35 kg) remains relatively low. A new PK substudy within the CHAPAS-3 trial has just started to evaluate increased efavirenz dosing in African children. Extensive monitoring of efavirenz-associated toxicity, particularly active solicitation for lower grade side-effects, and retrospective evaluation of virological efficacy will be performed for this important, but difficult to handle, antiretroviral agent.

Ugandan children aged between 3-12 years, on efavirenz once daily and using the 2006 WHO weight-band dosing recommendations, had lower and highly intersubject variable pharmacokinetic parameters of efavirenz compared to data from adults. There were no statistically significant differences across weight-bands, suggesting no major effect of some using half-tablets. Our findings suggest that children receiving efavirenz dosed according to 2006 WHO guidelines (or licensed recommendations on which these were based) could be at a higher risk of virological failure, and all pediatricians in resource-limited and resource-rich countries should move to WHO 2010 guideline dosing as a matter of urgency. Nevertheless, doses in some weight-bands may need to be increased still further, and this should be investigated promptly. Yet, the higher proportion of children expected to have high and potentially toxic levels remains a concern.

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CHAPTER 08

Middosing interval efavirenz plasma concentrations in HIV-1 infected children in Rwanda: treatment efficacy, tolerability, adherence and the influence of CYP2B6 polymorphisms

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ABSTRACT

This study evaluated middosing interval efavirenz plasma concentrations and the influence of CYP*2B6* polymorphisms in relation to efficacy, tolerability and adherence in 97 Rwandan HIV infected children (3-16 years). Plasma drug concentrations and CYP*2B6* polymorphisms were determined. Ten children were excluded for non-adherence. Large intersubject variability in efavirenz plasma concentrations was found. Of the 87 remaining, efavirenz concentrations were therapeutic, supratherapeutic and subtherapeutic in 67%, 20% and 14%, respectively. No associations were found between efavirenz concentrations and CNS disturbances or virological failure. Minor allele frequencies were 0.32 (516G>T), 0.33 (785A>G) and 0.09 (983T>C). Polymorphisms in CYP*2B6* were strongly associated with high efavirenz levels.

INTRODUCTION

Current Rwandan national guidelines recommend two nucleoside reverse transcriptase inhibitors (NRTIs) plus a non-NRTI for first-line antiretroviral therapy (ART) of HIV-infected children >3 years of age[1,2]. Efavirenz is given as an alternative to nevirapine, when the child develops nevirapine associated side-effects or receives anti-tuberculosis treatment[1,2]. The drug is clinically and virologically effective, safe and well tolerated in children[3,4]. Its prolonged half-life enables once daily dosing which facilitates adherence to ART[3].

An efavirenz plasma concentration of 1.0-4.0 mg/L 8-20 hours after ingestion is recommended as appropriate for achieving viral suppression and for limiting side-effects[6]. Studies evaluating efavirenz pharmacokinetics in African children are sparse but indicated a high prevalence of virologic failure after exposure to efavirenz plasma concentrations <1.0 mg/L[4] and a disadvantage of efavirenz is the large inter- and intrapatient pharmacokinetic variability, both in adults and children[7-13]. High variability in efavirenz plasma levels is associated with various factors, like ethnicity, body weight, dosing, non-adherence and concomitant medication[14,15]. Notably, since efavirenz is mainly metabolized by cytochrome-P450 CYP3A4, both elevated and decreased efavirenz plasma concentrations have been associated with CYP*2B6*-polymorphisms[12, 16-19]. Studies have shown a high prevalence of some genotypic variants in CYP*2B6* enzyme in African adults that would lead to higher efavirenz plasma levels, and potentially more toxicity[20,21].

This study aimed to evaluate the middosing interval efavirenz plasma concentrations and the influence of CYP*2B6*-polymorphisms in relation to treatment efficacy, tolerability, and adherence in Rwandan HIV-infected children.

METHODS

Study population

Ninety-seven children (3-16 years) on efavirenz based regimens from the Department of Paediatrics, Kigali University Teaching Hospital and the Treatment and Research AIDS Centre; Kigali, Rwanda participated in the study. Efavirenz was prescribed once-daily as a (halved) 600 mg tablet, and/or capsules containing 200 mg and/or 50 mg in weight-band doses according to Rwandan 2009 National Guidelines and WHO 2006 paediatric recommendations[1;5]. No children were on anti-tuberculosis treatment or other co-medication interfering with the efavirenz based regimen. All legal guardians gave informed written consent. Children ≥12 years provided additional consent. The study was approved by the Rwandan National Ethical Committee.

Study procedures

Demographic data were collected using patient's medical record and a case report form with standardised questionnaires. All caregivers and children if ≥12 years were interviewed and all children underwent physical examination.

Adherence was assessed using questionnaires, self-reports and monthly pharmacy refill forms. For children with undetectable efavirenz plasma concentrations, adherence was also assessed by determination of lamivudine plasma levels. For evaluation of safety and acceptability, a complaint checklist was developed indicating whether the child had experienced adverse events (AEs) in the past month. AEs were stated as unlikely, possibly, probably or definitely related to the drugs.

Blood sampling and laboratory investigations

The study visit was planned 8-20 hours after the last efavirenz intake to collect one blood sample for determination of mid-dosing interval efavirenz plasma concentrations, CYP*2B6*-genotype testing, haematology, biochemistry, CD4 count and HIV1-RNA concentration (viral load, VL). The bioanalyses of efavirenz and (if applicable) lamivudine were done at the Department of Pharmacy, Radboud University Medical Centre, Nijmegen, The Netherlands using two validated HPLC assays with ultraviolet detection[23, 24]. Genotype testing for CYP*2B6* was performed at the Pharmacogenetics Core Laboratory, Department of Clinical Chemistry, Erasmus Medical Centre, Rotterdam, The Netherlands, using validated PCR-RFLP assays with primers 5′-CTGTTGCAGTGGACATTTG-3′ and 5′-ATCTCACTCCTGCACTCAC-3′ for 1459C>T, with digestion of the 460 bp product with BglII (New England Biolabs), yielding 460 bp for wild type, and 258 and 202 bp for variant alleles. For 785A>G primers 5′-GACAGAAGGATGAGGGAGGAA-3′ and 5′-CTCCCTCTGTCTTTCATTCTGT-3′ were used: the 640 bp product was digested with BstN1 (New England Biolabs) for 2 h at 60 °C. The fragments for wild-type alleles were 312, 136, 109 and 83 bp, and for variant alleles 295, 136, 109, 83 and 17 bp. For 516G>T, primers 5′-GGTCTGCCCATCTATAAAC-3′ and 5′-CTGATTCTTCACATGTCTGCG-3′ were used: the product of 526 bp was digested with Bsr1 (New England Biolabs) yielding fragments for wild-type of 268, 241 and 17 bp, and for variant alleles 509 and 17 bp. All three PCR-RFLP assays were validated by direct sequencing. Assays for haematology, biochemistry, CD4 counts and VL (lower limit of detection 40 copies/mL) were done at the National Reference Laboratory at Kigali, Rwanda. Virologic failure was defined as VL ≥ 400 copies/mL.

Statistical methods

Logistic regression models were used for associations between efavirenz plasma concentrations and VL suppression (<40 copies/mL), CD4 count (≥350 cells/mm^3). Separate univariate logistic regression analyses were done to determine correlations between efavirenz plasma concentrations and reported side-effects. Analyses were conducted with STATA version 10.

RESULTS

General characteristics

The median (IQR) age of the 97 children (55% female) was 12.0 (10.0-13.7) years. Efavirenz was combined with lamivudine and either zidovudine (70%), stavudine (28%), or tenofovir (1%). The average efavirenz dose was 11.4 (10.0-12.9) mg/kg and median efavirenz duration was 3.7 (1.5-4.9) years. 5/97 children (5%) were on efavirenz for ≥2 but <6 months. Median (IQR) CD4 cell count was 643 (444-925) cells/mm^3. All children were in good clinical condition although the majority was moderately wasted and stunted with median (IQR) WAZ -1.3 (-2.4;-0.3) and HAZ -1.2 (-2.2;-0.6). One (1%), 6 (6%), 17 (18%), 17 (18%), 36 (37%) and 20 (21%) children were in weight-bands 10-14.9, 15-19.9, 20-24.9, 25-29.9, 30-39.9 and ≥40 kg, receiving 200, 250, 300, 350, 400 and 600 mg efavirenz, respectively.

Pharmacokinetics

Median (IQR) time of plasma sampling was 15.9 (14.8-16.8) hours after the last efavirenz intake. Twenty-two (22.7%) children had subtherapeutic efavirenz plasma concentrations (<1.0 mg/L). Ten children with undetectable plasma concentrations of both efavirenz and lamivudine were considered non-adherent and excluded from further analysis. Eighty percent of them had reported to be adherent by self-report. From the remaining 87 children, the median (IQR) efavirenz plasma concentrations were 2.05 (1.41-3.17) mg/L. Twelve (13.8%) children had subtherapeutic efavirenz plasma concentrations (<1 mg/L), while 58 (66.7%) had therapeutic (≥1.0-4.0 mg/L) and 17 (19.5%) supratherapeutic efavirenz plasma levels (>4.0 mg/L). Four children had a concentration of >12.0 mg/L. A large intersubject variability in efavirenz middosing interval plasma concentrations was found (coefficient of variation of 107%).

Efficacy, adherence and safety

Sixty-one out of 87 (70%) remaining children were fully virologically suppressed (< 40 copies/mL), 26 (30%) were not. Seventeen (20%) had a VL ≥400 copies/mL. No association was found between virological response and efavirenz plasma concentration groups (OR:1.7, 95CI% 0.7-4.3) (Table 1). However, the proportion of children with virologic failure was higher in the sub- and therapeutic group (>20%) compared to the group with supratherapeutic levels (6%). The majority of children (87%) self-reported to be well adherent (intake >95% of prescribed doses in previous month) including children with treatment failure. Adherence was not found to be associated with middosing interval efavirenz plasma concentrations: OR (95% CI): 1.1 (0.3-4.1).

Of 41 AEs reported, all were grade 1 or 2. Fourteen percent reported ≥1 CNS side-effect, including insomnia, nightmares, concentration disturbances, and dizziness (all grade 1). We did not find a significant relationship between efavirenz plasma concentrations and CNS side-effects (OR: 0.8, 95CI%:0.3-4.1). Skin problems reported by another 14% were atypical and not compatible with allergic reactions to antiretroviral drugs. Seventeen children (20%) had elevated AST and ALT concentrations. No serious AEs were reported.

TABLE 1 ▸ Associations between efavirenz plasma concentrations and efficacy and safety.

	efavirenz and lamivudine undetectable (n=10)	efavirenz plasma level <1.0 mg/L (n=12)	efavirenz plasma level 1.0-4.0 mg/L (n=58)	efavirenz plasma level >4.0 mg/L (n=17)	Odds ratio (95% CI)*
HIV-RNA (c/mL)					
median (IQR)	97200 (13270 - 703500)	66050 (17354 - 548350)	1240 (178-4590)	88 (51-14000)	0.9 (0.9-1.0)
Proportion < 40 c/mL, *n (%)*	0 (0)	8 (67)	39 (67)	14 (82)	
Proportion >40 c/mL, *n (%)*	10 (100)	4 (33)	19 (33)	3 (18)	1.7 (0.7-4.3)
Proportion ≥400 c/mL, *n (%)*	8 (80)	3 (25)	13 (22)	1 (6)	0.5 (0.2-1.4)
CD4 count					
cells/mm3 *(median (IQR))*	684 (445 - 916)	672 (441-815)	684 (445-922)	580 (507-864)	0.9 (0.9-1.0)
Adherence					
Adherent by self report	8 (80)	2 (17)	7 (12)	3 (18)	0.8
Side-effects					
CNS disturbances, *n (%)*	2 (20)	2 (17)	7 (12)	3 (18)	0.8 (0.3-4.1)

** Multivariate analysis, including possible confounders adherence (even if no association still added to model), age, weight-for-age z-score, height-for-age z-score and sex; factors not included were NRTI backbone, duration of efavirenz use, time between drug ingestion and blood sampling, co-medication and biochemistry as no correlation was observed with these and the middosing interval efavirenz plasma concentration.*

Efavirenz plasma concentrations and pharmacogenetics

Genotyping results are shown in Table 2. The CYP*2B6* 516GT-genotype was present in 46% (37/80), 516GG in 45% (36/80) and 516TT-genotype in 9% (7/80) children. CYP*2B6* 785AA, 785AG and 785GG genotypes were present in 45% (37/83), 46% (38/83) and 9% (8/83) children, respectively. The CYP*2B6* 785A>G and the CYP*2B6* 516G>T gene polymorphisms were mostly found together in our population (CYP*2B6**6, *19, *20 or *26 (www.cypalleles.ki.se)). The minor allele frequency for 516G>T, 785A>G and 983T>C was 0.32, 0.33 and 0.09, respectively. All polymorphisms were in Hardy-Weinberg equilibrium (p>0.05).

Median efavirenz concentrations were strongly associated with CYP*2B6* 516G>T, 785A>G and 983T>C polymorphisms (Table 2). Six out of seven (86%) children with the CYP*2B6* 516TT homozygous genotype had supratherapeutic (>4.0 mg/L) mid-dosing interval efavirenz plasma concentrations whereas no individual in this group had an efavirenz plasma concentration <1.0 mg/L. Four out of 36 (11%) 516GG children had subtherapeutic efavirenz plasma concentrations and none of the children had a plasma level >4.0 mg/L. Similar data were observed for the CYP*2B6* 785A>G and 983T>C polymorphisms.

TABLE 2 Gene polymorphisms in the G516T, T983C and A785G genotypes of the CYP2B6 enzyme

Variables	Number of children n (%)	efavirenz middosing interval plasma concentration, median (IQR)	p-value*
CYP*2B6* 516G>T	**80**		
GG	36 (45)	1.7 (1.3-2.2)	
GT	37 (46)	2.4 (1.8-3.1)	<0.001
TT	7 (9)	8.4 (5.0-13.0)	
CYP*2B6* 983T>C	**87**		
TT	71 (82)	1.8 (1.4-2.2)	
TC	16 (18)	3.1 (1.4-8.3)	<0.001
CC	0	-	
CYP*2B6* 785A>G	**83**		
AA	37 (45)	1.7 (1.4-2.2)	
AG	38 (46)	2.2 (1.7-3.1)	0.01
GG	8 (9)	7.3 (5.2-12.0)	

**p-values were calculated using Wilcoxon rank-sum test*

DISCUSSION

In this study, a high level of intersubject variability in middosing interval efavirenz plasma concentrations was observed. Only two-third of children had efavirenz concentrations within the therapeutic range. High variability (i.e. 107%) resulted in 14% children with subtherapeutic levels and 20% with potentially toxic plasma levels. Virologic failure was found in nearly a fifth of children with detectable efavirenz levels. Although the difference was not significant in this small study, a higher proportion of virologic failure was observed in children with (sub)therapeutic efavirenz levels.

In pediatric studies from South Africa and Burkina-Faso, a large intersubject variability was also found (137% and 143%) and two-thirds of the children with persistent sub-therapeutic efavirenz levels experienced virological failure[7, 10, 15]. In these studies, results were not corrected for adherence. In two other pediatric studies, sub-therapeutic efavirenz exposure was also linked to suboptimal antiviral efficacy[7, 25].

Variability in efavirenz plasma concentrations can occur due to different factors. Low concentrations of antiretrovirals can result from poor adherence, and subsequently lead to virological failure[26, 27]. The majority of children reported to be fully adherent by self-report. The fact that nearly half of children with subtherapeutic efavirenz levels also had undetectable lamivudine concentrations confirms that self- or proxy-reporting of adherence is unreliable and therapeutic drug monitoring provides more accurate information on adherence.

We also explored the influence of *CYP2B6*-polymorphisms on efavirenz plasma concentrations. The 516G>T polymorphism frequency of 0.32 was comparable to other reports[17] and a significant correlation between this polymorphisms and middosing interval }efavirenz plasma concentrations was found. This is consistent with other studies, like the ACTG-trial (one-third African patients), a Zimbabwean study in adults and with the study by ter Heine et al[12, 21, 28] in a Dutch pediatric population including 89% black children, which showed that clearance was 30% lower for 516GT compared to 516GG patients. The Zimbabwean study recommended a dose reduction of 35% for 516TT patients.

We found that CYP*2B6* 785AA patients had significantly lower efavirenz levels compared to 785GG or 785AG patients. The 785A>G allele frequency was 0.33 in our study population, which was not earlier explored in paediatric populations, but are comparable to findings by Mukonzo *et al* in Ugandan adults[20]. The increase in CYP*2B6* activity by the additional 785A>G polymorphism appears not to overcome the decrease in CYP*2B6* expression or activity produced by the 516G>T variant(29;30). Therefore, these findings indicate that the 516G>T effect is more relevant. In addition, the 983T>C polymorphism was also associated with efavirenz plasma concentrations. The prevalence is comparable to African adult populations[16, 21, 31].

CNS side effects, were not significantly associated with middosing interval efavirenz plasma concentrations comparing groups with efavirenz concentrations of <1.0, 1.0-4.0, >4.0 mg/L, which is in agreement with paediatric studies from different continents[15, 17, 25]. Reported skin problems were mild and not typical for efavirenz associated drug reactions, which usually occur in the first weeks after initiation[2].

One of the limitations of this study was the small number of children. It was also not possible to observe the ingestion of efavirenz the evening before study visit to reduce the number of non-adherent participants, nor were we able to collect full pharmacokinetic curves for every individual as this is a more reliable estimation of pharmacokinetic parameters. However, many paediatric studies have this shortcoming. Sparse blood sampling in children is recommended and hence middosing concentration levels are frequently used[9]. Currently only one pharmacokinetic study of efavirenz in African children is published with full pharmacokinetic curves[7, 10, 15].

Conclusions

This study presented a large variability of efavirenz middosing interval concentrations in Rwandan HIV-1 infected children, when they were dosed according to the national guidelines. The World Health Organisation recently moved to higher paediatric weight-band-based doses, these recommendations should continue to be reviewed for risks of high efavirenz concentrations and potential toxicity problems. Both adherence and CYP*2B6*-genetic polymorphisms are major factors influencing variability in mid-dosing interval plasma concentrations. Significantly higher efavirenz levels are correlated to CYP*2B6* 516TT, 785GG and 983CC individuals. Therefore, children with these CYP*2B6* variants may have an increased chance of toxicity.

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CHAPTER 09

Pharmacokinetics of zidovudine dosed twice-daily according to WHO weight-bands in Ugandan HIV-infected children

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ABSTRACT

Data on zidovudine pharmacokinetics in children dosed using WHO weight-bands are limited. 45 HIV-infected, Ugandan children, 3.4 (2.6-6.2) years, had intensive pharmacokinetic sampling. Geometric mean zidovudine $AUC_{0\text{-}12h}$ was 3.0 h.mg/L, which is higher than previously observed in adults, and was independently higher in those receiving higher doses, younger and underweight children. Higher exposure was also marginally associated with lower hemoglobin.

INTRODUCTION

Current WHO 2010 guidelines for the treatment of HIV-infected children recommend two nucleoside reverse transcriptase inhibitors (NRTIs) as part of first-line combination antiretroviral therapy (ART)[1]. For infants/children, the preferred NRTI backbone is twice-daily lamivudine+zidovudine, which is effective, inexpensive, and available as fixed-dose-combination (FDC) scored adult tablets (Combivir)[2] which can be split for pediatric dosing, or generic dual or triple (with nevirapine) pediatric FDCs. However, whereas studies have investigated the pharmacokinetics of the current lamivudine doses[3], surprisingly most zidovudine pharmacokinetic data is based on old 6-8 hourly and/or higher dosing[4, 5]. Data on zidovudine pharmacokinetics and pharmacodynamics at currently recommended lower/less frequent doses in children remain sparse, particularly in African children[6]. Pharmacokinetic-pharmacodynamic studies are particularly important as anemia is a common, plausibly dose-related, toxicity[7, 8]. As ~90% HIV-infected children needing ART are in Africa, we investigated zidovudine exposure in Ugandan, HIV-infected children receiving WHO-recommended twice-daily weight-band based dosing (not studied to date).

METHODS

ARROW was an open randomized trial comparing monitoring and first-line ART strategies in HIV-infected Ugandan/Zimbabwean children eligible for and initiating ART[9]. Allocation to zidovudine-containing regimens or not was part of the first-line ART strategy randomization[9]. Once stable on ART (>24 weeks after initiation) children from two Ugandan ARROW centers (Joint Clinical Research Centre, Kampala; Paediatric Infectious Disease Centre, Mulago, Kampala) were approached for additional consent to participate in two intensive crossover pharmacokinetic substudies. The first compared twice- versus once-daily lamivudine and abacavir in children aged 3-12 years, before and four weeks after move to once-daily dosing, 36 weeks after ART initiation[3]. All children were taking efavirenz; some were also taking zidovudine as a fourth drug at the first (36-week) pharmacokinetic sampling day. The second substudy compared twice-daily zidovudine, lamivudine and abacavir as syrups versus tablets in children aged 1-4 years, at and four weeks after moving from syrups to tablets[6] (median (IQR) 56 (40,70) weeks on ART).

This analysis included all available zidovudine pharmacokinetic data from both substudies. Children on concomitant medication which could interfere with ART, or who had illnesses that could influence ART pharmacokinetics were excluded, as were children who reported missing any ART dose in the previous three days. All caretakers provided fully informed written consent. The pharmacokinetic substudies were approved by the Ethics Committee from each centre.

Zidovudine was dosed twice-daily as syrups or halved or whole 300 mg solid formulation, scored FDC tablets (provided by GlaxoSmithKline; Table 1). Doses followed WHO 2006 recommendations, except that children weighing 12-15kg received 240 mg zidovudine syrup daily instead of 220 mg, children weighing 20-21kg on lamivudine/zidovudine

FDCs received 300 mg zidovudine rather than 450 mg and children 21-<25kg received 450 mg zidovudine to harmonize with the lamivudine weight-band. Blood samples of 1.5 mL were taken at t=0, 1, 2, 4, 6, 8 and 12 hours after observed ART intake. Breakfast (mostly milk/milky tea with samosas/bread/chapati) was provided two hours post morning dose. Plasma concentrations were assayed by a validated high-performance liquid chromatography-tandem mass spectrometry method[10], with lower limit of quantification 0.0025 mg/L, by Worldwide Bioanalysis, GlaxoSmithKline, Research Triangle Park, North Carolina. Zidovudine pharmacokinetic parameters (C_{12h}, C_{max}, $AUC_{0\text{-}12h}$) were calculated using WinNonlin version 5.2 (Pharsight Corporation, Mountain View, CA). In exploratory analyses, associations between zidovudine $AUC_{0\text{-}12h}$ and sex, age, dose (mg/m^2), weight- and height-for-age, and formulation were investigated using multivariable mixed models including a child-level random-effect (STATA version 11.1, STATA Corp, College Station, USA).

RESULTS

Zidovudine pharmacokinetic data were included from 45 children (17 (38%) male). Twenty-eight (62%) children aged 1-4 years had two pharmacokinetic profiles (one each on syrup and tablets) and 17 (38%) aged 3-12 years had one profile on tablets. One child had C_{0h}>3·C_{12h} and was excluded, leaving 72 pharmacokinetic profiles available for analyses. Median (interquartile range, IQR) age and weight at the first pharmacokinetic day were 3.4 (2.6-6.2) years and 12.6 (12.3-18.0) kg, respectively. Median (IQR) weight-for-age and height-for-age z-scores were -1.09 (-1.62,-0.56) and -1.85 (-2.68,-1.20), indicating moderate wasting and stunting. Of the 72 evaluable profiles, median (IQR) zidovudine morning and total daily doses were 242 (218-278) and 466 (432-546) mg/m^2 respectively (10 (9.4-12.2) and 20 (18.5-23.9) mg/kg respectively). Eight (11%), 20 (27%), and 36 (50%) profiles were from children on 100 mg syrup, 120 mg syrup, and 150 mg lamivudine/zidovudine FDC tablets twice-daily, respectively; and 8 (11%) were on 150 mg morning and 300 mg evening tablets. The geometric mean (95% confidence interval (CI)) $AUC_{0\text{-}12h}$, C_{max} and C_{12h} was 3.0 (2.7-3.3) h.mg/L, 1.8 (1.6-1.9) mg/L and 0.009 (0.007-0.010) mg/L, respectively (Figure 1 (a)) with CV% of 40%, 42% and 70%, respectively. Zidovudine $AUC_{0\text{-}12h}$ was 27-150% higher than previously reported in adults[2, 11]. GM C_{max} were 1.84 mg/L and 1.58 mg/L in children <4 years and >4 years (p=0.12, ranksum), respectively.

In multivariable models, higher zidovudine exposure ($AUC_{0\text{-}12h}$) was independently associated with higher dose, younger age and lower weight-for-age. $AUC_{0\text{-}12h}$ was 0.43 h.mg/L higher for every 50 mg/m^2 higher zidovudine dose [95% CI 0.15,0.71] (p=0.003). Associations between age and zidovudine exposure varied across the age range (test for non-linearity p=0.001). Independently of the dose effect, zidovudine exposure was 1.06 h.mg/L lower for every year older up to 4 years of age [0.48,1.63] (p<0.001), but there was no association >4 years (p=0.72) (Figure 1 (b). Thus, for the same dose in mg/m^2, youngest children had higher plasma zidovudine exposure. Exposure was 0.72 h.mg/L lower for every unit higher weight-for-age [0.30,1.13] (p=0.001). Adjusted for these factors, there was no independent effect of sex (p=0.56), height-for-age (p=0.57) or for-

mulation (syrups/tablets) (p=0.75). There was a trend towards higher C_{max} in children <4 years (GM=1.9 mg/L (95% CI 1.7-2.1)) versus >4 years (GM=1.5 mg/L (1.3-1.9) p=0.096). Thirty-seven children had viral load (VL) measured within 4 weeks of PK day. Thirty-two (86%) had VL <80c/mL; only one (3%) had VL >400c/mL (17174c/mL). This child had an $AUC_{0\text{-}12h}$ of 3.7 h.mg/L.

Thirty-four children had hemoglobin assayed within one day of the pharmacokinetic sampling. There was marginal evidence for 0.31 g/dL lower hemoglobin per 1 h.mg/L higher zidovudine $AUC_{0\text{-}12h}$ [-0.65,+0.03] (p=0.07) (Figure 1 (c)).

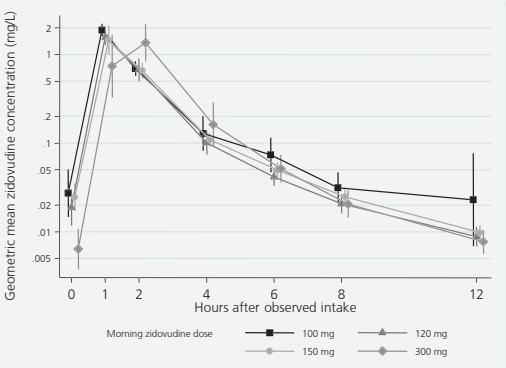
TABLE 1 Zidovudine pediatric dosing

Weight band (kg)	Approx. Surface Area (m^2)	WHO guidelines 2006		WHO guidelines 2010		ARROW protocol			Choke-phai-bulkit et al[12]
		Zidovudine		Zidovudine		Zidovudine		Combi-vir	GPOVIR
		syrup	tablet	syrup	tablet	syrup	tablet	tablet[2]	tablet
		daily dose (mg)							
10-<12	0.49 to 0.56	200	-	**240**	-	200	-	-	180
12-<14	0.56 to 0.62	220	-	**240**	-	**240**	-	-	**240**
14-<15	0.62 to 0.65		300	-	**300**	240	**300**	**300**	240
15-<17	0.65 to 0.71	-	300	-	**300**	-	**300**	**300**	240
17-<20	0.71 to 0.79	-	300	-	**300**	-	**300**	**300**	**300**
20-<21	0.79 to 0.82	-	300	-	**450**	-	**450**	300	360
21-<25	0.82 to 0.92	-	300	-	**450**	-	**450**	**450**	360
25-<30	0.92 to 1.1	-	450	-	**600**	-	450	450	420
≥30	≥1.1	-	600	-	**600**	-	**600**	**600**	-

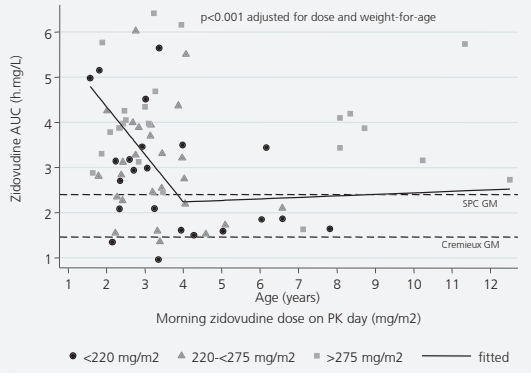
Note: shaded cells show agreement between ARROW protocol doses and WHO 2006 guidelines, and bold cells between ARROW protocol doses and WHO 2010 guidelines. Underlining shows where zidovudine doses were increased between 2006 and 2010 WHO guidelines. The ARROW protocol was submitted for ethical approval before WHO 2006 guidelines were published, and also harmonised doses with weight-bands for lamivudine and other drugs.

FIGURE 1 ▸ Mean zidovudine concentrations, exposure, age & hemoglobin at pharmacokinetic sampling

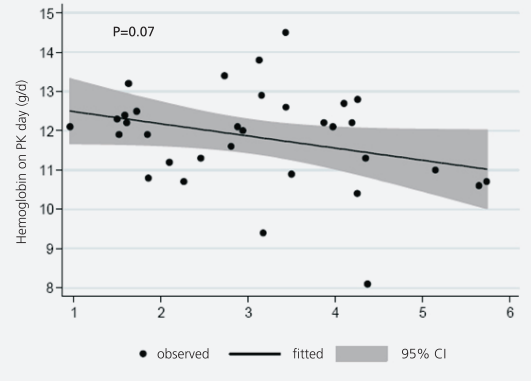
(A) MEAN ZIDOVUDINE PLASMA CONCENTRATIONS



(B) RELATIONSHIP BETWEEN ZIDOVUDINE EXPOSURE ($AUC_{0\text{-}12h}$) AND AGE (YEARS) AT PHARMACOKINETIC SAMPLING



(C) RELATIONSHIP BETWEEN HEMOGLOBIN AND ZIDOVUDINE EXPOSURE ($AUC0_{\text{-}12h}$) AT PHARMACOKINETIC SAMPLING



Note: in panel (b) fitted effect of age is shown for a child with median weight-for-age (-1.09) and median dose (242 mg/m²). Points demonstrate the relationship between age and dose which is adjusted for within the multivariable model.

DISCUSSION

Here, we have found higher zidovudine exposure in Ugandan children aged 1-12 years dosed twice-daily according to WHO 2006 weight-bands compared to exposure previously reported in adults receiving the standard dose of 300 mg zidovudine twice-daily[2,11]. Exposure was also higher (GMs were 2.36h.mg/L and 1.58h.mg/L) than in the only two previous pediatric zidovudine pharmacokinetic studies[5, 12], which used lower doses than our study (Table 1 and 360 vs 360 -480 mg/m^2/day, respectively). Subsequent 2010 WHO guidelines[1] have further increased the recommended zidovudine dose for all children except those weighing 15-20 or >30kg (Table 1), without new evidence, but reflecting concerns that children had often been under-dosed with antiretrovirals. Thus exposure in children currently receiving zidovudine in Africa is likely to be even higher than observed here.

As well as the expected association between higher zidovudine exposure and higher zidovudine mg/m^2 dose in this study, we also found strong independent evidence that higher exposure was associated with lower age in children <4 years and with lower weight-for-age. Whilst one study suggested that zidovudine clearance increased most rapidly during the first weeks of life, reaching adult levels at 2 years of age[8], a small study in six Dutch children[5] showed elimination rate increased with age between 2 and 14 years, implying further maturation of metabolism during childhood. This agrees with our study, where we found that exposure from the same mg/m^2 dose decreased in children from 1 through to 4 years. An alternative explanation might be greater absorption in younger children; although we did not find any evidence that this might be due to liquid vs solid formulations, there was a trend to higher C_{max} in younger children. We did not find any association between exposure and age among 19 children >4 years, but moderate-to-large variability may have obscured this.

Available information about the relationship between zidovudine exposure and toxicity is limited[7, 8]. Although only 34 children had paired hemoglobin and pharmacokinetic measurements, we found marginal evidence for an association between higher zidovudine exposure and lower hemoglobin values. This is similar to a previous population pharmacokinetic study[8], which found mild anemia occurred in 23% of those with average zidovudine concentration >350 ng/mL (versus 8% without). However these children were receiving zidovudine as part of only mono or dual therapy; as chronic HIV-related anemia is common in HIV disease, the contribution of replicating HIV to these findings is unclear. Further, all but two hemoglobin values in our study were in the normal range (one grade 1, one grade 2 toxicity). Whether higher exposure increases the risk for more severe toxicity over the longer-term is unknown.

A limitation of our study is that we included only Ugandan (East African) children, thus limiting generalisability to other populations where host genetics may result in different pharmacokinetics. A population pharmacokinetic study including data from 100 children from six pediatric trials, including this study, is ongoing and is expected to provide further

insight in the pharmacokinetics and potential co-factors which may impact the pediatric pharmacokinetics of zidovudine. Another limitation is that the true C_{max} (T_{max} is 0.5 hours[2]) could have been missed and consequently $AUC_{0\text{-}12h}$ might be underestimated. Lastly, because of challenges in sampling relatively young children over 24h, we were unable to directly estimate the impact of unequal am/pm dosing in those weighing 20-30kg.

In summary, zidovudine is a common component of first-line pediatric ART, especially in resource-limited countries and only limited data are available on the widely applied twice-daily dosing regimen. Children dosed following WHO 2010 guidelines, younger children, and those with low weight-for-age are likely to have even higher zidovudine exposure than that observed here and substantially higher than previously reported in adults. Our findings suggest that this higher exposure could be associated with greater suppression of hemoglobin levels within the normal range and probably with no change in efficacy, since viral load suppression was already very good using WHO 2006 dosing. The impact on severe anemia warrants further investigation, particularly with regards to current WHO 2010 dosing.

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09

CHAPTER 10

The pharmacokinetics and acceptability of lopinavir/ritonavir minitab sprinkles/tablets/syrups in African HIV-infected children

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Submitted

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ABSTRACT

Objective

To evaluate the pharmacokinetics/acceptability of lopinavir/ritonavir (LPV/r) in a novel minitab sprinkle formulation (40/ 10 mg, Cipla Pharmaceuticals) versus innovator syrup or Cipla tablets (100/ 25 mg) in HIV-infected Ugandan infants/children.

Design

Open-label, comparative bioavailability (randomized cross-over) studies in three age groups.

Methods

Twice-daily minitabs were compared versus syrup in infants 3-<12 months (cohort-2) and children 1-4 years (cohort-3) and versus tablets in children 4-<13 years (cohort-1). 12-hour intensive pharmacokinetic sampling after observed intake of LPV/r plus two NRTIs following WHO 2010 dosing with food was performed 4 weeks post-enrolment. Children then switched formulation and sampling was repeated at week-8. Acceptability data were also collected.

Findings

77 infants/children were included in cohort-2 (n=19)/ 3 (n=26)/ 1 (n=32). Among 132 evaluable PK profiles, 13/ 21/ 25 pairs in cohort-2/ 3/ 1 were available for within-child comparison. For minitabs versus syrup, geometric mean (GM) (95% CI) $AUC_{0\text{-}12h}$ was 88.6 (66.7-117.6) versus 77.6 (49.5-121.5) h.mg/L in cohort-2 (GM ratio (GMR) (90% CI) = 1.14 (0.71-1.85)) and 138.7 (118.2-162.6) versus 109.1 (93.7-127.1) h.mg/L in cohort-3 (GMR (90% CI)=1.27 (1.10-1.46)). For minitabs versus tablets, GM (95% CI) $AUC_{0\text{-}12h}$ was 83.1 (66.7-103.5) versus 115.6 (103.0-129.7) h.mg/L; GM ratio (GMR) (90% CI) 0.72 (0.60-0.86). Subtherapeutic levels (<1.0 mg/L) occurred in 0 (0%)/ 2 (15%) minitabs/syrup in infants (p=0.48), no children 1-4 years and 4 (16%)/ 1 (4%) minitabs/tablets (p=0.35). 13/17 (76%) and 19/26 (73%) carers of infants and children 1-4 years respectively chose to continue minitabs after week 8, mainly for reasons of convenience; only 7/29 (24%) older children (five <6 years) remained on minitabs.

Conclusions

LPV/r exposure from minitabs was comparable with syrup, but lower than from tablets, with no significant differences in subtherapeutic concentrations. Minitabs were more acceptable than syrups for younger children, but older children preferred tablets.

INTRODUCTION

The new 2013 WHO guidelines recommend a ritonavir-boosted protease inhibitor (bPI) plus two nucleoside reverse transcriptase inhibitors (NRTIs) for first-line antiretroviral therapy (ART) in HIV-infected children <3 years, particularly if perinatally exposed to NNRTI, and for second-line ART in HIV-infected children receiving 2 NRTIs plus a non-nucleoside reverse transcriptase inhibitor (NNRTI) first-line[1]. In 2011, only 28% of children urgently needing to initiate treatment were receiving first-line ART, compared to 58% of adults[2], and only 3% of children on ART were on ritonavir-boosted PI second-line therapy. As children face a life-time requirement for ART, the number of children in need of second-line treatment is expected to rise substantially in the future.

Simplifying NNRTI plus 2 NRTI pediatric first-line ART regimens by making fixed dose combination (FDC) solid formulations, dosed according to simplified WHO weight band tables, has significantly aided the programmatic scale up of first-line pediatric treatment, albeit that coverage remains low compared with adults (28% vs 58% in 2011[2]). An additional factor constraining wider use of second-line ART, and bPI-based first-line ART in children <3 years exposed to perinatally to NNRTI, is the lack of affordable and appropriate pediatric bPI-based formulations for resource-limited settings. The only currently available combination bPI is ritonavir-boosted lopinavir (LPV/r) in liquid formulation for young children and as pediatric tablets for older children. The syrup is unpleasant tasting, has a high alcohol concentration (42%), and contains propylene glycol, which is especially undesirable in children <2 years of age[3]. Moreover, LPV/r syrup requires refrigeration, which makes it challenging for storage, transport and use in resource-limited settings. The tablet formulation is relatively large and must not be crushed; a recent study found a 40% decrease in bioavailability with crushed compared to whole tablets[4]. As well as being the only combined bPI formulation, LPV/r is the only bPI licensed for young children. Further, ritonavir as a booster for other PIs is only available for children as an even more unpleasant tasting liquid or a large tablet (minimum 100 mg dose). Therefore, alternative formulations of LPV/r with better taste and which do not require cold-chain transport and storage are urgently needed, particularly in resource-limited settings.

LPV/r has recently been developed by a generic pharmaceutical company (Cipla Pharmaceuticals) as a heat-stable tablet containing half the dose (100/25 mg LPV/r; Lopimune) of the currently FDA approved and available adult tablets, and the same dose as pediatric tablets from the innovator company, and also as a novel minitab sprinkle formulation which is stored in capsules (40/ 10 mg LPV/r; Lopimune). The capsules can be opened and given even to the smallest children, as the sprinkles within them can be mixed with food or milk. In the CHAPAS-2 (Children with HIV in Africa - Pharmacokinetics and Adherence of Simple Antiretroviral Regimens) study, we evaluated the pharmacokinetics and acceptability of the minitab formulation versus the innovator syrup (80/20 mg LPV/r per mL; Kaletra, Abbott) and versus the generic pediatric tablets in HIV-infected Ugandan infants and children.

METHODS

CHAPAS-2 was an open, randomized, phase I, two-period cross-over comparative bio-availability trial in HIV-infected infants/children from 3 months to <13 years of age taking or about to start first-line (infants exposed to perinatal nevirapine for pMTCT) or second-line ART with LPV/r plus 2 NRTIs (older children) from two pediatric clinics in Kampala, Uganda (Joint Clinical Research Centre, Baylor-Uganda; ISRCTN01946535). Infants/children were not eligible if they were expected to change weight bands resulting in a dose increase after enrolment and before the second pharmacokinetic sampling day at week 8, or if they were on concomitant medication known to interact with their ART, or if they had liver enzymes grade 2 or higher, anemia (hemoglobin <8.5 g/dL) or other illnesses (e.g. severe diarrhea, vomiting, renal or liver disease) that could influence LPV/r pharmacokinetics. Enrolled chil-dren who missed any dose of any antiretroviral drug in the three days before the pharma-cokinetic sampling session (assessed by adherence questionnaire) were excluded from pharmacokinetic analyses, as were children with pharmacokinetic evidence of poor compliance (C_{0h} below the lower limit of quantification (LLOQ) and C_{12h} >3.0 mg/L). All carers, and children where appropriate, gave written informed consent and assent respectively. The study was approved by the Ethics Committee from each participating site, the Uganda National Council of Science and Technology and by University College London, UK.

At enrolment, all infants 3-<12 months were included in a non-randomized two-period crossover design (cohort-2) and all children 1-4 years of age in a 1:1 randomized two-arm, two-period crossover design (cohort-3). All children 4-<13 years of age, weighing <25 kg were randomized 1:1 in a two-arm, two-period crossover study comparing the novel LPV/r minitab sprinkle formulation with the new generic pediatric tablets (cohort-1) (Supplementary Figure 1). The two first designs compared the novel LPV/r minitabs with innovator syrup. Children were only included in cohort-1 if they were able to swallow pediatric tablets. Children in cohort-1 and cohort-3 were randomized using computer-generated randomization lists produced by the trial statistician at the MRC Clinical Trials Unit (CTU) in London, UK. Randomization was done by phoning the CTU. LPV/r was dosed twice-daily according to WHO 2010 pediatric weight-band based dosing recommendations (Supplementary Table 1) as syrup, minitab-contained capsules (which have to be opened) and whole pediatric tablets.

Four weeks after enrolment, when steady state was achieved on allocated treatment (syrup cohort-2; minitabs/syrup cohort-3 and minitabs/tablets cohort-1), an intensive 12-hour pharmacokinetic session was performed. Samples were taken immediately before directly observed intake of the morning LPV/r dose (t=0 hours) and 1, 2, 4, 6, 8, and 12 hours later. Children, if not breastfed, fasted >3 hours before the pharmacokinetic session and breakfast (mainly porridge) was given with the morning dose. Following the week 4 sampling session, all infants and children switched LPV/r formulation. Cohort-2 switched from syrup to minitabs (arm 3), and cohort-3 switched from minitabs to syrup (arm 4) or vice versa (arm 5) and cohort-1 switched from tablets to minitabs (arm 1) or vice

versa (arm 2) (Supplementary Figure 1). At week 8, four weeks after switching LPV/r formulation, a second intensive pharmacokinetic sampling session was performed. Data on acceptability of each LPV/r formulation were collected from standardized questionnaires at baseline (if already on LPV/r at enrolment), and weeks 4, 8 and 12 after enrolment. At week 8, children and carers chose which formulation they wished to continue.

Concentrations of LPV and ritonavir in plasma samples were determined using ultra performance liquid chromatography with UV detection[5]. The analytical assay LPV and ritonavir ranges were 0.109-31.2 mg/L and 0.044-29.4 mg/L, respectively. Intraday and interday precision ranged from 0.6 to 4.2% (coefficient of variation (CV)), and 0.3 to 1.8%, respectively. The assay accuracy range was 98.2-105.6%.

For cohort-1 and cohort-3, the sample size of 24 children provided at least 80% power for the width of the 90% confidence interval (90% CI) for the geometric mean ratio (GMR) between formulations to lie within 0.80 to 1.25 (bioequivalence[6]) if no difference was observed (GMR=1), based on an estimated standard deviation of change in log10 area under the concentration-time curve 0-12 hours post dose ($AUC_{0\text{-}12h}$) between sprinkle and tablet/syrup of approximately 0.26. For primary analysis, steady state LPV pharmacokinetic parameters ($AUC_{0\text{-}12h}$, maximum concentration (C_{max}) and concentration at 12 hours post dose (C_{12h})) were determined using Phoenix version 6.3 (Pharsight Corporation, CA, USA) and compared between formulations within-child using GMRs and their corresponding 90% CIs. The proportion of children with subtherapeutic LPV concentrations (defined as <1.0 mg/L[7]) were compared between formulations using the exact-test. LPV $AUC_{0\text{-}12h}$ were compared across weight bands using analysis of variance on log-transformed data (equivalent to the geometric mean (GM)).

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RESULTS

In total 79 children were recruited in CHAPAS-2 from August 2011 until September 2012. One was ineligible (not receiving LPV/r, on efavirenz-based first-line) and one withdrew consent shortly after enrolment (because the carer was not able to attend the sample session days), leaving 77 children for analyses (19 infants in cohort-2, 26 children in cohort-3 and 32 children in cohort-1). Median (interquartile range, IQR) age and CD4% were 0.5 (0.4-0.7), 2.0 (1.8-2.8), and 6.2 (5.8-7.8) years and 24% (19-30), 32% (28-37) and 32% (27-37) in cohort-2, -3 and -1, respectively (Table 1). Infants/children were moderately wasted and stunted; median weight-for-age z-scores were -1.11, -1.19 and -0.82 and median height-for-age z-scores were -1.99, -2.05 and -1.24 in cohort-2, -3, and -1, respectively.

Primary pharmacokinetic analyses were based on 13 infants in cohort-2 (6 excluded), 21 children in cohort-3 (5 excluded) and 25 children in cohort-1 (7 excluded), each with two pharmacokinetic profiles. Children excluded from primary pharmacokinetic analyses had been wrongly dosed (3 cohort-1), unable to swallow pediatric tablets (1 cohort-1), non-

compliant at one of the two sampling sessions (C_{0h}<LLOQ and C_{12h}>3.0 mg/L; 1 cohort-1, 4 cohort-2, 4 cohort-3), changed weight-bands between week 4 and 8 (2 cohort-1, 1 cohort-3), developed tuberculosis (1 cohort-2), or had transferred to another clinic so only one PK curve was undertaken (1 cohort-2).

Pharmacokinetic analyses

In cohort-2 (infants 3-<12 months), LPV concentrations and pharmacokinetic parameters were slightly higher with minitabs compared to innovator syrup (Figure 1 (b), Table 2). The GMR(minitabs:syrup) for $AUC_{0\text{-}12h}$, C_{max} and C_{12h} all lay within the range of 0.80-1.25, but the lower and the upper 90% CI limits fell outside this range (Table 2). This was a consequence of high but similar inter-individual variability with both minitabs (CV% 46%, 38% and 68%, $AUC_{0\text{-}12h}$, C_{max} and C_{12h} respectively) and innovator syrup (51%, 40% and 73%, respectively). Two (15%) and zero (0%) of 13 (15%) children had a subtherapeutic concentration on syrup and minitabs respectively (Figure 2; p=0.48; Exact).

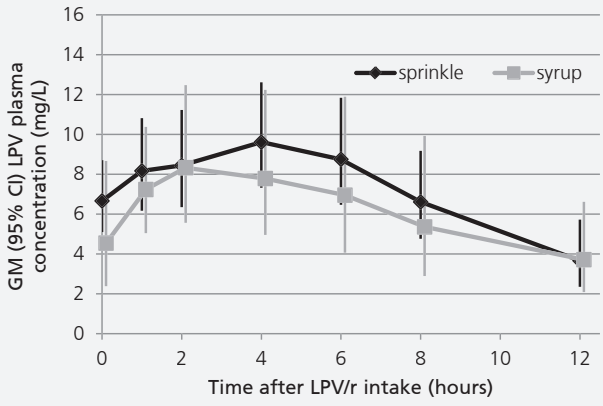
Similar to our data in infants (cohort-2), in cohort-3 (children 1-<4 years), LPV concentrations and pharmacokinetic parameters were slightly higher with minitabs compared to innovator syrup (Figure 1 (c), Table 2). The GMR(minitab:syrup) for $AUC_{0\text{-}12h}$, C_{max} lay within the range of 0.80-1.25, with C_{12h} GMR just above this, with the upper 90% CI limits all above 1.25 (Table 2). There was no impact of randomization order on any estimated GMR (p>0.4). The $AUC_{0\text{-}12h}$, C_{max} and C_{12h} CV% were 33%, 27% and 50%, respectively in minitabs, compared to 33%, 27% and 55% with syrup. None (0%) of the children had subtherapeutic concentrations 12 hours after intake of either formulation (Figure 2).

In cohort-1 (children 4-<13 years), LPV concentrations of the new generic pediatric tablets were higher compared to the novel minitabs (Figure 1 (a)). Compared to historical data, LPV pharmacokinetic parameters were higher with tablets, but similar with minitabs (Table 2). The GMR(minitabs:tablets) (90% CI) for $AUC_{0\text{-}12h}$, C_{max}, and C_{12h} were 0.72 (0.60-0.86), 0.74 (0.64-0.85) and 0.59 (0.43-0.81), respectively, all lying outside the bioequivalence range of 0.80-1.25. There was no impact of randomization order on any estimated GMR (p>0.15). The inter-individual variability (CV%) of the pharmacokinetic parameters was moderately high for minitabs (49%, 42% and 76%, for $AUC_{0\text{-}12h}$, C_{max}, and C_{12h} respectively), compared with tablets (28%, 19% and 66%, respectively). One (4%) and four (16%) of the 25 included children had subtherapeutic LPV trough concentrations after receiving tablets and minitabs, respectively (Figure 2; p=0.35; Exact).

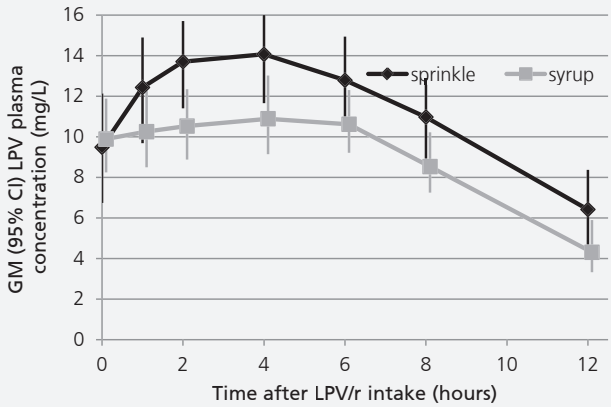
Ritonavir pharmacokinetic data and comparisons were consistent with the LPV data in all three cohorts. There were no differences across weight-bands in LPV or RTV pharmacokinetic parameters in the different cohorts, or in cohorts 2 and 3 combined (p>0.23; ANOVA).

FIGURE 1 Geometric mean LPV plasma concentrations in infants/children after intake of LPV/r.

(A): Cohort-2 (sprinkle vs syrup in infants 3-<12 months)



(B): Cohort-3 (sprinkle vs syrup in children 1-<4 years)



(C): Cohort-1 (sprinkle vs tablet in children 4-<13 years)

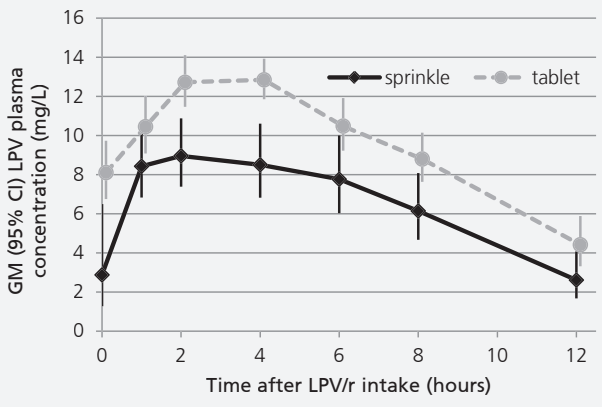
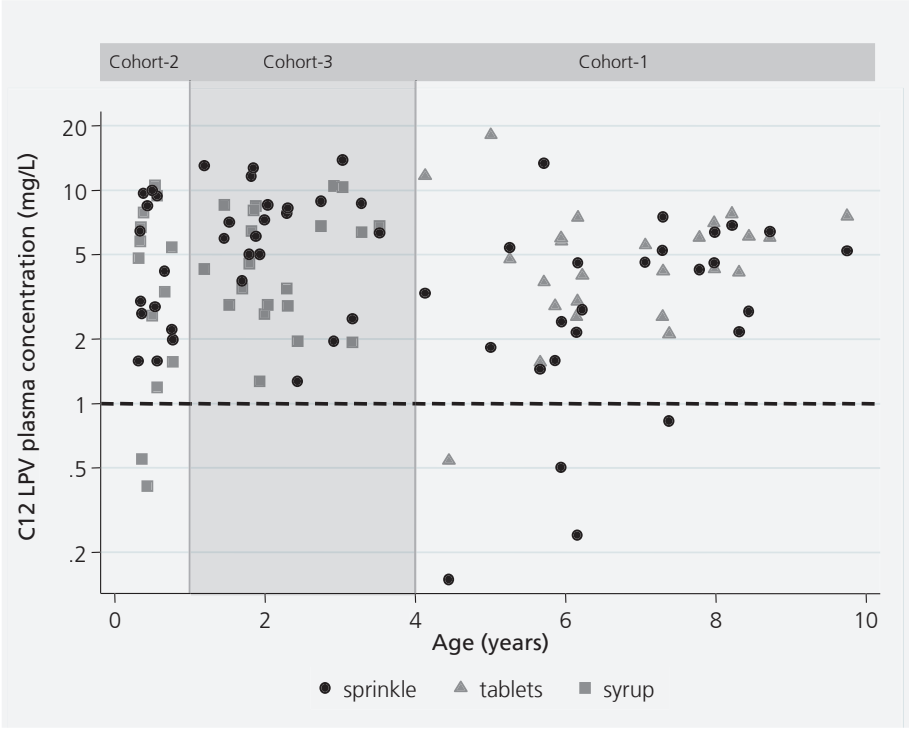


FIGURE 2 ▸ LPV plasma concentrations 12 hours after intake of LPV/r versus age in cohorts 2, 3 and 1.



Acceptability

Administration of the minitabs required that they should be taken with food. In cohort-2, 83% of infants were breastfed; in older children the most common food caregivers gave minitabs with was porridge (34% cohort-1, 62% cohort-3).

Among younger children in cohorts 2 and 3, more problems were reported with taking the syrups than the sprinkles (Figure 3). In contrast, the older children, all but one of whom were already established on tablets for >6 months, reported more problems with taking minitabs. The problem reported most often with all formulations was taste; taste was worse with minitabs than tablets (50% versus 0%), but similar with minitabs and syrup (53% minitabs versus 67% syrups, cohort-2, and 38% minitabs versus 38% syrup, cohort-3) (Figure 3). At week 8, 71% cohort-1 children who had switched from tablets to minitabs reported problems with taste, whereas this was 47% and 36% in those that switched from syrups to the minitabs, cohort-2 and cohort-3 respectively. Difficulty swallowing was reported as a problem in 13% minitabs versus 0% tablets (cohort-1), 20% minitabs versus 60% syrups (cohort-2) and 27% minitabs versus 19% syrup (cohort-3). Storage, transport and conspicuousness were less problematic for minitabs compared

with syrups. However, several caregivers were concerned about the number of capsules needing to be opened to give minitabs to the older children.
On ease of switching from the original formulation to minitabs, 76%, 94% and 79% of the carers in cohort-1, cohort-2 and cohort-3 respectively reported that it was easy or very easy.

At enrolment 37%, 12% and 41% of caregivers in cohort-2, cohort-3 and cohort-1 thought they would prefer minitab sprinkles. This compares with 72%, 64% and 19% reporting preference for minitabs at week 12. At week 8, 14/18 (78%) caregivers in cohort-2 and 19/26 (73%) in cohort-3 chose to continue minitabs rather than syrups; however, in cohort-1 only 7/32 (22%) caregivers/children chose minitabs, five of the seven children being under 6 years.

Caregivers provided multiple comments about the formulations at each visit. It was clear that minitabs were preferred to syrup in cohorts 2 and 3 largely because they were considered to be easier to administer, store (including not requiring refrigeration) and transport. For those preferring syrup, key issues were: first that minitabs were also bitter; second that carers felt more confident that they could administer the whole dose with syrup than with minitabs even if the child struggled; third, the requirement to give sprinkles with food was of concern to some carers, because some felt they needed to sweeten food with sugar or give with honey (which is expensive) to mask the taste. One carer in cohort-2 reported that the child subsequently refused the food which had been previously administered with minitabs.

Only one grade 4 adverse event (AE) was reported (malaria with severe anemia, Hb <6.5 g/dL) in a child on innovator syrup. This AE was also reported as a serious AE (SAE) and was considered unrelated/unlikely related to the study medication. Apart from this SAE, no other grade 3 or 4 AEs were reported among children taking any of the formulations.

TABLE 1 ▸ Baseline characteristics of children in CHAPAS-2

	Cohort-1 sprinkles vs tablets in children 4-<13 years (n=32)	Cohort-2 sprinkles vs innovator syrup in infants 3-<12 months (n=19)	Cohort-3 sprinkles vs innovator syrup in children 1-<4 years (n=26)
Age, years	6.2 (5.8-7.8)	0.5 (0.4-0.7)	1.9 (1.8-2.7)
Male, n (%)	14 (44%)	11 (58%)	14 (54%)
Height, cm	112 (107-117)	63 (60-65)	80 (77-86)
Weight, kg	19.3 (18.0-20.9)	6.3 (5.9-6.7)	10.7 (9.4-12.6)
Weightbands, n (%)			
5-<7 kg	0	15 (79%)	0
7-<10 kg	0	4 (21%)	8 (31%)
10-<12 kg	0	0	11 (42%)
12-<14 kg	1 (3%)	0	3 (12%)
14-<20 kg	16 (50%)	0	4 (15%)
20-<25 kg	13 (41%)	0	0
>25 kg	2 (6%)	0	0
BMI, kg/m^2	15.2 (14.3-15.8)	16.1 (15.1-17.5)	16.5 (15.5-17.6)
CD4%	32 (27-37)	24 (19-30)	32 (28-37)
Height-for-age z-score	-1.24 (-0.30 to -2.05)	-1.99 (-1.02 to -2.66)	-2.05 (-1.37 to -3.43)
Weight-for-age z-score	-0.82 (-0.18 to-1.70)	-1.11 (-0.76 to -2.42)	-1.19 (-0.18 to -2.11)
First-line Second-line	0 33 (100%)	20 (100%) 0	26 (100%) 0
Already on LPV/r for >1m at enrolment If yes, years on LPV/r	31 (97%) 2.3 (1.2-3.3)	10 (53%) 0.3 (0.2-0.4)	25 (96%) 1.1 (0.9-1.5)
Daily LPV dose, mg/kg	22.3 (21.1-24.7)	38.1 (36.1-40.7)	28.3 (25.9-30.0)
Daily LPV dose, mg/m^2	553 (530-591)	723 (696-769)	627 (565-663)

Values are n (%) for categorical variables and median (interquartile range, IQR) for continuous variables.

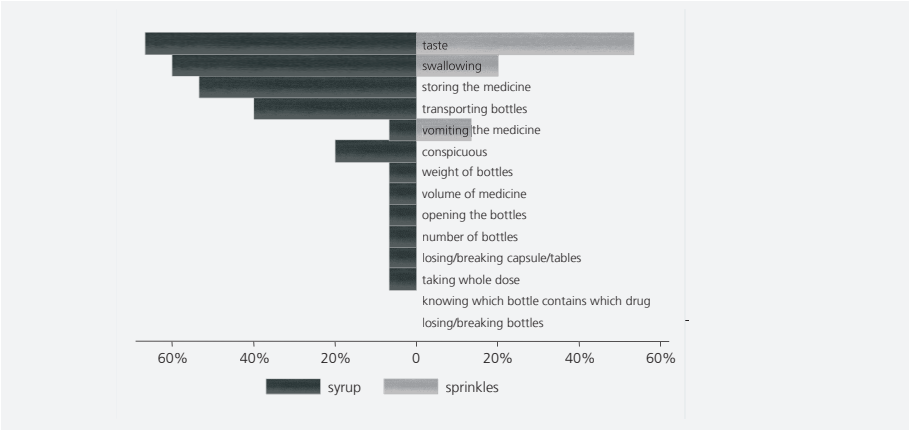
TABLE 2 Pharmacokinetic parameters of LPV in cohorts-1-3

Cohort-2	Sprinkles	Syrup	Sprinkles: Syrup	Historical data in children [3*]	IMPAACT 1083 study [9†]
PK parameter	**GM (95% CI) [range]**	**GM (95% CI) [range]**	**GMR (90% CI)**		
$AUC_{0\text{-}12h}$ (h.mg/L)	88.6 (66.7-117.6) [41.1-174.7]	77.6 (49.5-121.5) [12.2-175.5]	1.14 (0.71-1.85)	72.6 (31.1)	119.8 (29.3-261.0)
C_{max} (mg/L)	10.7 (8.6-13.4) [6.3-20.6]	10.6 (7.8-14.3) [3.7-17.7]	1.01 (0.72-1.42)	8.2 (2.9)	13.4 (3.8-29.2)
C_{12h} (mg/L)	3.9 (2.6-6.0) [1.6-10.0]	3.1 (1.7-6.0) [0.4-10.5]	1.25 (0.61-2.54)	3.4 (2.1)	5.5 (0.03-13.8)
Cohort-3	**Sprinkles**	**Syrup**	**Sprinkles: Syrup**	**Historical data in children [3*]**	**IMPAACT 1083 study [9†]**
PK parameter	**GM (95% CI) [range]**	**GM (95% CI) [range]**	**GMR (90% CI)**		
$AUC_{0\text{-}12h}$ (h.mg/L)	138.7 (118.2-162.6) [72.5-228.5]	109.1 (93.7-127.1) [57.3-191.3]	1.27 (1.10-1.46)	72.6 (31.1)	119.8 (29.3-261.0)
C_{max} (mg/L)	15.3 (13.4-17.4) [9.6-24.8]	12.6 (11.1-14.3) [7.3-18.9]	1.21 (1.09-1.36)	8.2 (2.9)	13.4 (3.8-29.2)
C_{12h} (mg/L)	6.4 (4.8-8.6) [1.3-13.8]	4.3 (3.3-5.7) [1.3-10.5]	1.49 (1.12-1.97)	3.4 (2.1)	5.5 (0.03-13.8)
Cohort-1	**Sprinkles**	**tablets**	**Sprinkles: tablets**	**Historical data in children [3*]**	**IMPAACT 1083 study [9†]**
PK parameter	**GM (95% CI) [range]**	**GM (95% CI) [range]**	**GMR (90% CI)**		
$AUC_{0\text{-}12h}$ (h.mg/L)	83.1 (66.7-103.6) [22.6-222.7]	115.6 (103.0-129.7) [68.4-204.6]	0.72 (0.60-0.86)	72.6 (31.1)	119.8 (29.3-261.0)
C_{max} (mg/L)	10.3 (8.6-12.2) [4.0-25.1]	13.9 (12.8-15.1) [8.7-18.8]	0.74 (0.64-0.85)	8.2 (2.9)	13.4 (3.8-29.2)
C_{12h} (mg/L)	2.6 (1.7-4.1) [0.2-13.4]	4.4 (3.3-5.9) [0.5-18.1]	0.59 (0.43-0.81)	3.4 (2.1)	5.5 (0.03-13.8)

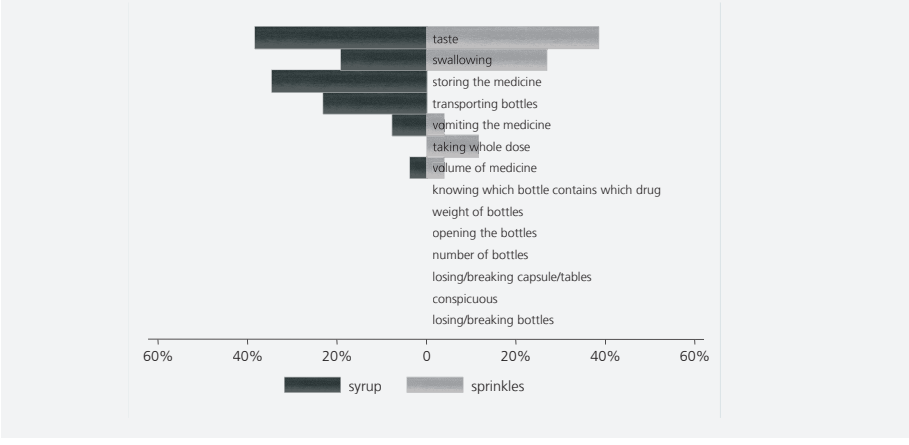
**mean (SD) steady state LPV PK parameters of 53 children 6 months to 12 years of age receiving 230/57.5 mg/m² twice daily LPV/r innovator syrup. † GM (range) LPV PK parameters of 48 children 4.2 (0.4-12.9) years of age receiving 316 (258, 451) mg/m² twice daily LPV/r of the innovators heat-stable pediatric LPV/r tablets or liquid formulation.*

FIGURE 3 ▸ Percentage reporting each problem for the different formulations.

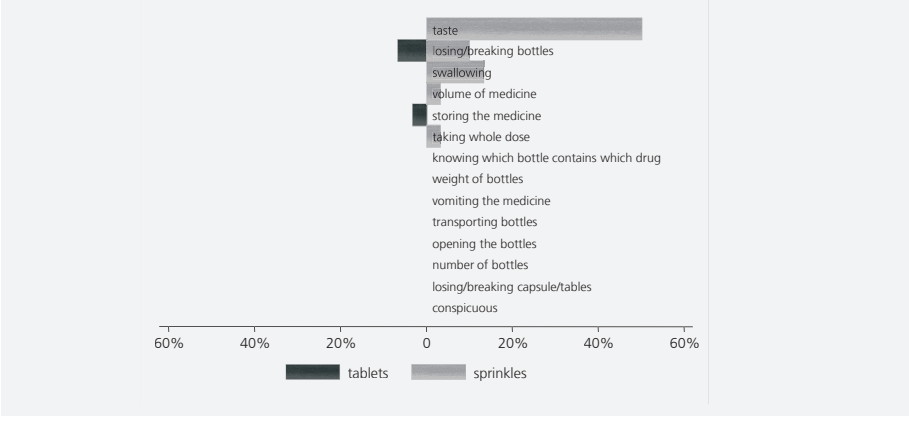
(A): Cohort-2 (sprinkle vs syrup in infants 3-<12 months)



(B): Cohort-3 (sprinkle vs syrup in children 1-<4 years)



(C): Cohort-1 (sprinkle vs tablet in children 4-<13 years)



DISCUSSION

This phase I bio-equivalence study has shown slightly higher, but still broadly equivalent, LPV/r exposure from the newly developed minitab sprinkle formulation together with food in infants/children from 3 months to 4 years of age compared with LPV/r exposure from the currently used innovator syrup and historical data[3]. Exposure from minitab sprinkles in older children (4-<13 years of age) was also generally consistent with our younger cohort. However, exposure to LPV/r from the generic tablets was significantly higher than from minitabs. Variability in LPV/r pharmacokinetic parameters was moderate to high in all formulations, but no LPV/r exposure differences were found between weight-bands for twice daily dosing recommendations (Supplementary Table 1) between the three formulations. Carers found minitabs more acceptable than syrups for their infants/children, particularly for transport and storage reasons. However, for older children already able to swallow tablets, these were more acceptable than minitabs, where taste remained a concern.

LPV/r exposure in children in this study was slightly higher than adults and historical data from children on the same formulation[3, 8], probably because the children in our study received higher doses (median range 553-723 mg/m^2/day) following the WHO 2010 weight-band based dosing recommendations (Supplementary Table 1) rather than being dosed at 230/57.5 mg/m^2 twice daily following the manufacturers leaflet[3]. Exposure from the two novel formulations was higher than from the syrup, despite equivalent mg dosing, thus bioequivalence could not be confirmed. Despite the higher LPV/r exposure found in these children, LPV/r treatment was well tolerated. Only one (serious) adverse event was reported, which was not related to study medication. Our findings are compatible with the interim results of the IMPAACT 1083 study[9], which also described drug exposure and safety profiles in infants and children dosed according to the WHO weight-band dosing guidelines. $AUC_{0\text{-}12h}$, C_{max}, and C_{12h} were estimated as 119.8 h.mg/L, 13.4 mg/L and 5.5 mg/L, respectively, in children 0-13 years of age on the innovator heat-stable pediatric LPV/r tablets or liquid formulation. Similarly to our findings, in the 1083 study there were no grade 3 or higher adverse events definitely related to study treatment.

Apart from relatively small numbers (59 children with two full pharmacokinetic profiles), one limitation is that our study included only Ugandan children. However, genetic polymorphisms in children on LPV/r have been shown to have minimal clinically relevant influence on LPV exposure and clinical outcomes[10], so this is unlikely to have materially affected results. Another study limitation was the lack of concurrent virological data, although the fact that exposure from the new formulations was higher than previously reported suggests that this is likely to have remained adequate. Variability in the pharmacokinetic parameters within and between our study arms was high, but the proportion of subtherapeutic trough concentrations (<1.0 mg/L) was absent or low in most groups. In only 2 groups (children 4-<13 years on minitabs and infants 3-<12 months on syrups) were trough concentrations sub-therapeutic in 15–16%. This

proportion is similar to the 13% observed in the IMPAACT 1083 study; this study also reported favorable 24 week HIV-1 RNA responses and increases in CD4 percentage[9].

Overall, the pharmacokinetics of the novel minitab sprinkle and generic pediatric tablet formulation of LPV/r with the newly developed dosing schedule, based on WHO dosing recommendations for children in resource-limited settings, are acceptable. The acceptability data for the minitab sprinkle formulation investigated in our study also suggest that carers found it to have important advantages over the syrup formulations. Most young children (1-<4 years) in cohort-3 and around half the infants (3-<12 months) enrolled in cohort-2 had already been taking LPV/r syrup for >1 month (median 1.1 years in these young children from both cohorts). Despite being stable on syrup for some time, most chose to continue minitabs at the end of the study, mainly because of improved ease of transportation and/or storage than syrup. However, for older children (all able to swallow tablets) minitabs were less acceptable and taste in particular was a concern. A few carers in the younger cohorts (1 and 2) also preferred syrup to minitabs, citing poor taste of the minitabs, requirement to take with food (particularly if food refusal occurred by association or if additional expensive food items such as honey were deemed necessary). Finally, as a consequence of mixing the minitab sprinkle with a small amount of food, some carers were unsure that the whole dose was taken when administering minitabs.

The unmet need for child-friendly formulations to facilitate the scale-up of second-line ART access for older children, and bPI-based first-line access for infants in resource-limited settings is clear. We have shown that the novel, heat-stable, solid drug minitab sprinkle formulation of LPV/r evaluated in the CHAPAS-2 trial had an acceptable pharmacokinetic profile when dosed according to weight-band dosing recommendations and was generally more acceptable and preferred to syrup in the short term.

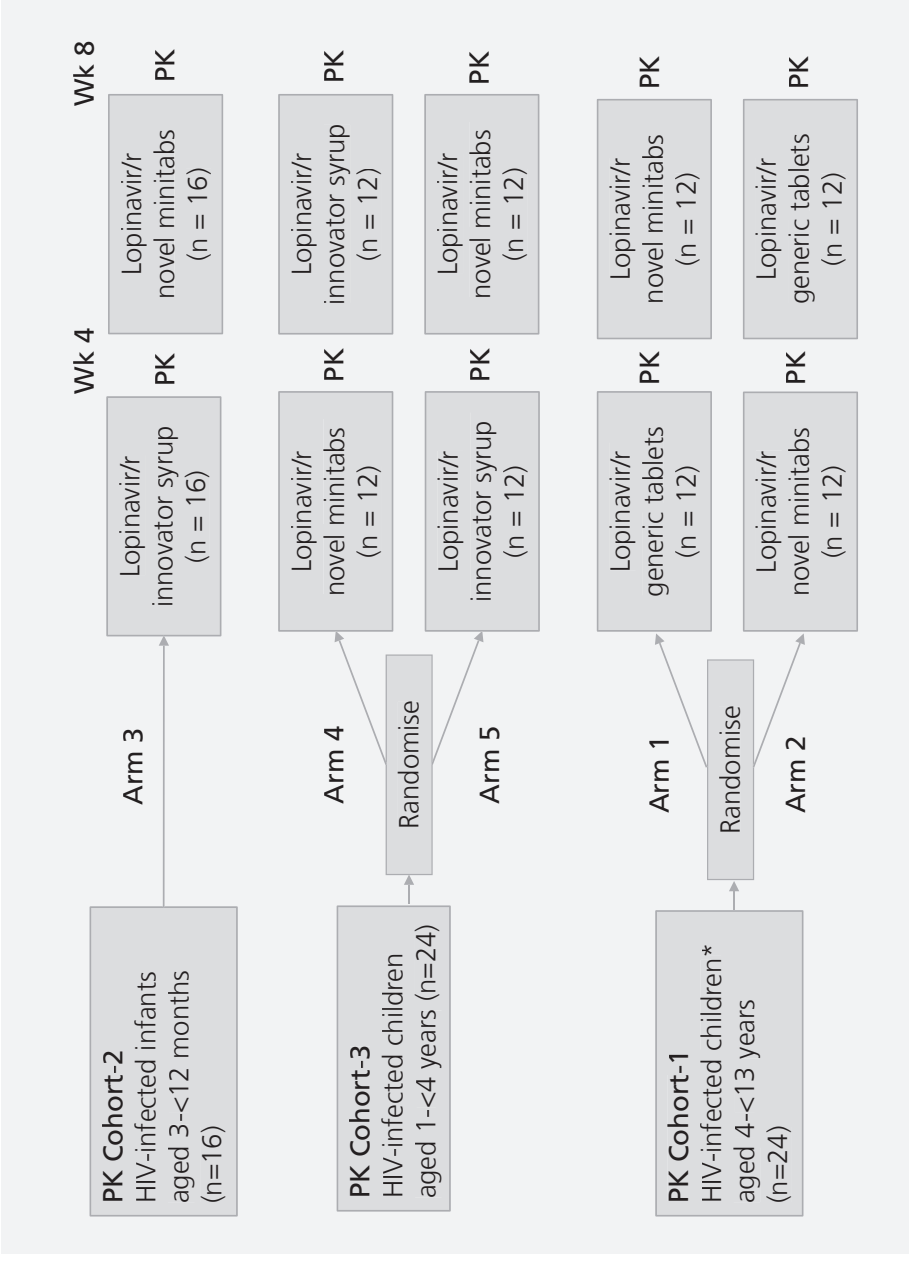
For countries choosing to follow new WHO 2013 recommendations and move to LPV/r as first-line ART for children <3 years, the minitab sprinkle formulation (being submitted for regulatory approval) offers an alternative to syrup which has well known limitations in terms of acceptability, administration and logistics of transportation and storage. Further follow-up of the CHAPAS-2 cohorts is planned to describe longer term acceptability and efficacy through to one year. In addition, further pharmacokinetic and|acceptability studies for an improved finer 'granule' sprinkle formulation with better taste masking are planned.

SUPPLEMENTARY TABLE 1 LPV paediatric dosing guidelines

body weight (kg)	body surface area (m^2)*	WHO guidelines 2010												CHAPAS-2 Protocol											
		Liquid (80/20 mg/mL)					Paediatric tablet (100/25 mg)							Innovator syrup (80/20 mg/mL)						Novel sprinkle (40/10 mg)					
		LPV daily dose*					LPV daily dose*							LPV daily dose						LPV daily dose					
		mg	mg/kg		mg/m^2		mg	mg/kg		mg/m^2				mg	mg/kg		mg/m^2			mg	mg/kg		mg/m^2			
			min	max	min	max		min	max	min	max				min	max	min	max			min	max	min	max		
5-<7	0.3 0.38	240	34	48	632	800	-	-	-	-	-	240	320	240	34	48	632	800	240	34	48	632	800	-	-	-	-
7-<10	0.38 0.49	240	24	34	490	632	-	-	-	-	-	240	320	240	24	34	490	632	240	24	34	490	632	-	-	-	-
10-<12	0.49 0.56	320	27	32	571	653	300	25	30	536	612	320	320	267	32	571	653	320	320	27	32	571	653	300	25	30	536	612
12-<14	0.56 0.62	320	23	27	516	571	300	21	25	484	536	320	320	23	27	516	571	320	320	22	27	516	571	300	21	25	484	536
14-<20	0.62 0.79	400	20	29	506	645	400	20	29	506	645	400	400	20	29	506	645	400	400	20	29	506	645	400	20	29	506	645
20-<25	0.79 0.92	480	19	24	522	608	400	16	20	435	506	480	480	19	24	522	608	480	**500**	19	24	522	608	**500**	20	25	543	633
25-<30	0.92 1.1	560	19	22	509	609	600	20	24	545	652	560	560	19	22	509	609	560	560	19	22	509	609	600	20	24	545	652
30-<35	1.1 1.2	640	18	21	533	582	600	17	20	500	545	**560**	560	16	19	467	509	**560**	560	16	19	467	509	600	17	20	500	545

**Based on standard from paediatric oncology[11]. † Target daily LPV dosage of WHO 2010 is 230-350 mg/m^2 twice-daily (=460-700 mg/m^2 LPV daily dose) Note: bold indicates where CHAPAS-2 protocol dosing differed from WHO 2010.*

SUPPLEMENTARY FIGURE 1 ▸ CHAPAS-2 trial design.



** who were able to swallow LPV/r paediatric tablets.*

Note: all infants/children were either initiating LPV/r based ART or were already on LPV/r-based ART and interested/willing to try the new generic sprinkle formulation. Children on LPV/r were already taking tablets in cohort-1 and most already taking syrup in cohort-2 and cohort-3.

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10

CHAPTER 11

General Discussion

Since the turn of the millennium, remarkable successes have been achieved in the fight against HIV/AIDS worldwide. In Sub-Saharan Africa, the region most severely affected by the life-threatening disease, a massive scale-up of access to combination antiretroviral treatment (cART) have been realized during the past ten years and this has saved millions of lives[1, 2]. Despite the increasing number of HIV-infected individuals receiving ART, the drugs are still not universally accessible. To date, the global coverage, in particular for the most vulnerable populations in need for cART, is modest[3]. It is, however, rapidly expanding in order to reach the goal of UNAIDS' commitment that by 2015 15 million people, including pregnant women and children, should be using the life-saving medicines[3]. To allow cART scale-up in resource-limited settings, the World Health Organization (WHO) recommends a population-based strategy, which is mainly focused on standardized cART protocols, such as simplified drug regimens and appropriate fixed-dose combination (FDC) drug formulations for HIV infected individuals.

In line with WHO's public health approach for resource-limited settings, this thesis has two objectives: The first section investigated simple, accessible and affordable strategies for the prevention of mother-to-child transmission (pMTCT) of HIV using enzyme inducers for the elimination of nevirapine resistance after single-dose exposure as part of the antiretroviral prophylaxis for pMTCT. The second section aimed to optimize antiretroviral regimens for children to facilitate the scale-up of HIV treatment in resource-limited settings by evaluating new appropriate antiretroviral paediatric drug formulations and validating newly developed WHO weight-band based dosing recommendations for the use in resource-constrained settings. The current chapter (general discussion) addresses the challenges in translating our research findings into practice in resource-limited settings focusing on antiretroviral prophylaxis for pMTCT of HIV, HIV-treatment for children in resource-limited settings, research capacity development and our international collaboration for clinical research.

Antiretroviral prophylaxis for pMTCT of HIV

The World Health Organization (WHO) issued the first recommendations on pMTCT in resource-limited settings in the year 2000. It recommended a single-dose nevirapine at labor onset, which has been shown to reduce MTCT by ~50%[4, 5]. These recommendations were revised in 2004 and later in 2006[6, 7], since nevirapine resistance development became a major concern. Single-dose nevirapine alone was no longer endorsed as a preferred option, and was recommended only as part of a longer prophylactic regimen. The field is dynamic, as in 2010 and soon thereafter in 2012 the WHO presented its new guidelines on pMTCT[8], recommending two options: Option A and Option B/B+. Option A recommends antenatal prophylaxis with zidovudine followed by intrapartum and postpartum prophylaxis with single-dose nevirapine and zidovudine plus lamivudine. Option B recommends cART until after finishing breastfeeding and Option B+ recommends lifelong cART, regardless of CD4 cell count[8]. The programmatic update of WHO indicates that Options B and specifically B+ is likely to be preferable to Option A. The shift

in pMTCT policy is driven by the lack of operational feasibility of the complex regimen in Option A, the clear benefits to pMTCT models of Option B that emphasizes the expansion and access of HIV treatment and the contribution of option B's cost-effectiveness[9].

However, because of the fragility of health systems, new guidelines, such as Option B/B+, are difficult to implement. By the end of 2011, 57% of HIV-infected women worldwide were estimated to have received an antiretroviral regimen during pregnancy; only 30% of those who were eligible for treatment had initiated cART[3]. The current Tanzanian National guidelines on pMTCT recommending Option A were developed in July 2012[10], but implementation of these recommendations nationwide, especially in rural regions, is still behind. Lack of infrastructure and constraints on human and financial resources contribute to poor uptake of new recommendations in resource-limited settings, as in Tanzania[11, 12]. Single-dose nevirapine alone has turned into a fading concept, but in practice, this simple intervention is still widely used by thousands of women in resource-limited settings, particularly in more rural areas where access to cART is not feasible or unavailable[3].

For as long as pMTCT programs are still including single-dose nevirapine, the risk for nevirapine resistance development remains a major concern. In our systematic review (**Chapter 2**) it was demonstrated that single-dose nevirapine alone results in a prevalence of 31% nevirapine resistance. Addition of antepartum zidovudine reduced the proportion of nevirapine resistance by 30% to 21%. Combining antepartum zidovudine with a postpartum course of two other antiretroviral drugs for 4-7 days reduces the risk of nevirapine resistance mutations to 0.011%. Longer duration of the postpartum course (20-30 days) appeared to be most effective, as it nearly eliminated nevirapine resistance. This sounds promising, but may require even more self-discipline of the mothers' drug compliance in these yet complex regimens.

We therefore set up a trial investigating adding simple strategies of enzyme inducers (a single-dose carbamazepine or seven days of phenytoin at labor onset) to single-dose nevirapine for pMTCT in HIV-infected pregnant women. A pharmacological, rather than antiretroviral, approach of adding a CYP*3A4* enzyme inducer has been shown to decrease nevirapine elimination half-life in healthy women[13], with greatest reductions from carbamazepine and phenytoin. In our first trial (VITA-1; **Chapter 3**), a simple intervention of adding a single-dose carbamazepine to single-dose nevirapine at labour onset significantly reduced nevirapine plasma concentrations one week after delivery in HIV-infected Tanzanian women, with a trend towards fewer resistance mutations[14]. Intensifying the current Option A with a 7-days postpartum regime of phenytoin once daily from labour onset (VITA-2; **Chapter 4**) demonstrated a larger enzyme induction effect with a significant reduction in the elimination half-life of nevirapine in HIV-infected, pregnant women compared to a single-dose carbamazepine. Seven days phenytoin was furthermore safe and effective with no new nevirapine resistance mutations observed.

Where it is not possible to provide cART, phenytoin and carbamazepine are cheap and available in almost every clinic. These interventions may also be useful when women stop nevirapine at the end of breastfeeding within Option B. Implementation of this intervention 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. It would likely retain the benefits of single-dose nevirapine in reducing transmission, with plausibly lower rates of emergence of major resistance mutations. Therefore, adding an enzyme inducer to single-dose nevirapine shows new possibilities for pMTCT programs to reduce the selection of nevirapine resistance mutations in settings where other options are limited and 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.

It is important to note that conducting these VITA trials has been challenging. Due to lack of research capacity and experience within the team at the start of the VITA-1 trial, many women were lost to follow-up after delivery and data management procedures were insufficiently developed for optimal operation. It was therefore possible that the guidelines of Good Clinical Practice (GCP) provided by the International Conference on Harmonization (ICH) were not fully followed. Support from other African (University Teaching Hospital, Lusaka, Zambia) and European (Medical Research Council, Clinical Trials Unit, London, UK) clinical research groups became extremely valuable in a successful completion of the trial. Hence, the execution of the subsequent multi-centre VITA-2 trial was tremendously improved, with considerably greater GCP-compliance.

Partially due to financial support derived from funding of the VITA studies a new research building has been constructed at the Kilimanjaro Christian Medical Centre that currently enables enhanced collaboration between different clinical research disciplines (PhD candidates, research nurses, data management team, meeting rooms, computer rooms with wireless internet). The VITA studies have shown that conducting clinical research in low-experienced resource-limited settings is not only about asking relevant scientific questions, here as simplified and better strategies in the reduction of nevirapine resistance after-single-dose exposure for pMTCT in regions where this intervention is still being used, but also about research capacity development at the clinical research site in favor of future clinical trials (see section *research capacity development*).

HIV-treatment for children in resource-limited settings

To date only 28% of the children who need cART are reported to be receiving the medicines[3, 15]. The scale-up of pediatric treatment programs has always lagged behind that of adults. Treatment options for children remain insufficient and have restricted roll-out in resource-limited settings, where most (90%) children with HIV infection live[3]. Therefore, one of the specific challenges in the scale-up of treating HIV-infected children is the unmet need for more appropriate pediatric drug formulations that are inexpensive and easily usable.

When pediatric drug formulations are unavailable, adult tablets are used in an attempt to create child-size doses. Carers of pediatric patients may break or crush the (mostly unscored) tablets meant for an adult patient. This may lead to administration of inappropriate doses, as they are unequally divided. Furthermore, the dosage after breaking might not allow administration of a dosage small enough for an infant or young child[16].

Even when appropriate formulations of antiretroviral agents are available for children, pharmacokinetic data are often insufficient to appropriately guide drug dosing, especially in the youngest children, who metabolize these drugs differently[17, 18]. Due to growth and development of these children pharmacokinetic parameters change. It causes large interpatient and intrapatient variability in the pharmacokinetics of antiretroviral drugs complicating optimal dosing. Adequate drug exposure is essential in HIV treatment of children, who generally start treatment earlier and will need to take it for far longer than adults; it is therefore a major goal in the development of appropriate pediatric fixed-dose combination tablets (FDCs).

In the CHAPAS-1 (Children with HIV in Africa-Pharmacokinetics and Adherence of Simple antiretroviral regimens) trial, appropriate dosing of FDC tablets, approved based on early pharmacokinetic data generated within the trial, was assessed. It is known that children, particularly the youngest, metabolize nevirapine more rapidly than adults[19-21]. The tablets therefore have higher nevirapine to lamivudine/stavudine dose ratio compared to adults FDCs. Our findings in **Chapter 5** have confirmed that younger children (weighing 3-6 kg) have higher risks of subtherapeutic nevirapine levels compared to older children. Also, evaluating plasma nevirapine concentrations under full-dose or dose-escalation strategies during the initial two weeks of treatment in children, more subtherapeutic nevirapine plasma concentrations 3-4 hours post-dose were observed in children <2 years of age compared to older children with nevirapine dose-escalation at cART initiation (**Chapter 6**). Drug metabolism varies with age, and so the low nevirapine plasma levels in the youngest children in our studies could be explained by increased metabolism at younger age.

Studies, such as the abovementioned trials, are key to safety and rational dosing recommendations of new FDCs established for children. Regulatory approval needs to be gained through the US Food and Drug Administration or European Medicines Agency to ensure that those formulations become more widely available to children living with this life-threatening disease. The WHO pediatric antiretroviral working group (PAWG) advised a higher nevirapine to lamivudine/stavudine dose ratio compared to adults FDCs, based on the findings of CHAPAS-1 and it is hoped that in the future children <2 years will be recommended by the WHO's PAWG to start full-dose nevirapine at ART initiation in order to avoid subtherapeutic nevirapine levels.
Only few studies have validated international weight-band dosing recommendations for efavirenz. In **Chapters 7** and **8**, the pharmacokinetics of efavirenz were evaluated in African HIV-infected children, dosed according to weight-band based 2006 WHO or

manufacturer's recommendations. Low and highly variable efavirenz PK parameters and middosing interval concentrations were reported with subtherapeutic efavirenz trough levels (<1.0 mg/L) in 14-59% of the Rwandan and Ugandan children. It was obvious that efavirenz underdosing was of a major concern using the previous WHO 2006 guidelines. These and earlier studies[22, 23] have therefore been warning WHO and national authorities to urgently move to higher paediatric weight-band based dosing for efavirenz, although to date, the current higher WHO 2010 dosing guidelines have not been validated. This is, however, especially necessary as doses in some weight-bands may need to be increased still further. A new pharmacokinetic substudy within the CHAPAS-3 trial has been done in African children dosed with a new generic pediatric efavirenz tablet following the current dosing recommendations. First study results have shown similar values as our previous studies, but collection and analysis of pharmacokinetics, toxicity and efficacy data is still ongoing. Final outcomes of this study should advise WHO's PAWG for better rational efavirenz dosing recommendations.

A number of FDC tablets, such as Triomune Baby and Junior, with dose ratios correct for children have and are being developed in order to simplify first-line treatment and increase the number of children on cART[24]. Generic pharmaceutical manufacturing companies are leading many of these initiatives and trying to fill the pressing gap in resources for the unmet need of cART in the pediatric population in Africa. With more people receiving cART in resource-limited settings, treatment failure and the need to switch to second-line regimens is likely to increase, but second-line drugs are clearly under prescribed in developing countries. In 2011, only 3% adults and children receiving cART in resource-limited settings were on a ritonavir-boosted protease inhibitor (PI) as second-line cART, even though the proportion with virological failure on first-line cART is substantially higher[25, 26].

The only currently available second-line drug in resource-limited settings is ritonavir-boosted lopinavir in liquid formulation for infants and in paediatric tablets for older children. Whereas these formulations have many limitations, ritonavir-boosted lopinavir remains the preferred PI for use in infants and children and, therefore, alternative formulations with better taste and which do not require cold-chain transport and storage are urgently needed in resource-limited settings. Recently, two new paediatric lopinavir/ritonavir formulations have been developed by a generic pharmaceutical company (Cipla Pharmaceuticals), a heat-stable tablet containing half the dose of the currently available adult tablets and a novel sprinkle formulation in capsules, which can be opened and given even to the smallest children, as it allows the drug to be easily mixed in with food. In the CHAPAS-2 study (**Chapter 10**), where we evaluated the pharmacokinetics and acceptability of these novel/generic formulations in HIV-infected Ugandan infants and children, it was demonstrated that these formulations had acceptable pharmacokinetic profiles and were preferred by the children and their carers compared to the currently available innovator syrup. Also, the current WHO weight-band dosing recommendations appeared to be appropriate for use in children.

It is hoped that the novel sprinkle formulation and the new generic paediatric tablet will soon become accessible at low cost to international roll-out programmes. To date, affordable second-line formulations are rare. Second-line treatment is still 4-5 times more expensive than first-line regimens[26]. Another hurdle for patients to receive second-line cART is the inability to measure viral load. Uncertainty in the diagnosis of treatment failure together with unavailability of these drugs is a barrier for prescribers to switch to second-line[27]. Infrastructure, personnel, and supply chain constraints all affect the ability to monitor, and tests for measuring efficacy are generally unavailable, particularly at primary care facilities[28]. However, the DART trial has demonstrated that on the basis of clinical or immuno-clinical criteria, such as simple point-of-care CD4 tests, it can be decided to switch to second-line in adults[29, 30]. In children, monitoring of weight gain can be used as an important additional clinical aid for identification of first-line failure, as demonstrated in the ARROW trial (www.arrowtrial.org)[28] Subsequently, the need for third-line therapy for children is expected to grow in future years[27]. Ritonavir-boosted darunavir and raltegravir are options for third-line regimens by WHO guidelines[29]. However, this treatment is even more hindered by the lack of pediatric formulations and high costs, with dosing especially problematic for young children[31]. Importantly, the aim of future (CHAPAS) trials should focus on inexpensive, new generation drugs for second-line and third-line treatment with appropriate formulations for children.

A final challenge in the scale-up of HIV-treatment for children is the need for large investments from international government initiatives and private foundations. In the last decade a massive increase in substantive funding streams for global health has occurred[32]. At the current pace at which treatment and clinical care is scaled up, reaching the targets of 2015 is feasible[3]. However, the continued global economic crisis has put the humanitarian aid for developing countries under pressure. A strategic evaluation of how to make each dollar/euro spent on HIV treatment programs more efficient and sustainable is crucial[32]. Donors and governments should not compete with each other, but should more effectively collaborate. The international community should learn from this situation that it should not only respond with short term solutions that often reflect the donor's priorities, but that it is important to fund durable health systems, including the availability of appropriate drug formulations, that can withstand crises.

Joining forces to develop research capacity

It has been widely recognized that resource-limited settings need more appropriate drug formulations, but also stronger health systems in the fight against HIV/AIDS. This must involve clinical research. However, total research outputs from Africa are low; the number of research publications in peer-reviewed journal from the African continent is smaller than the number of research publications produced individually by several countries[33, 34]. Research capacity development is crucial for the success of performing clinical trials and to strengthen health systems in resource-limited settings. In this last section, practice-based lessons are provided from three clinical trials contributed to this thesis: VITA, CHAPAS and ARROW.

The first lesson that can be learned from these clinical trials is that management and communication structures are fundamental for a trial. In ARROW and CHAPAS, coordination, planning and completion of the trial plus proper administrative procedures were led by a well-trained trial management team from the Medical Research Council Clinical Trials Unit (MRC CTU), London, UK. In these studies, the trial manager regularly visited the research sites to discuss the progress and difficulties. Also regular and structured telephone conferences were important to ensure the progress at every research site. Trial managers are essential as trials need to follow guidelines and procedures (ICH-GCP and the study protocol itself), the trial paper work is enormous and there is always need to motivate others in a team. Since MRC CTU has over 20 years expertise in conducting field studies and clinical trials in the developing world, they were asked to share their experience with the VITA study team. The VITA-1 trial (**Chapter 3**) was primarily set up at Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania in collaboration with Radboud University Nijmegen Medical Centre (RUNMC), Nijmegen, The Netherlands. However, both institutes had little experience in conducting clinical trials. The subsequent VITA-2 trial (**Chapter 4**) was established as a multi-country and a more ICH-GCP compliant study at KCMC and at the University Teaching Hospital (UTH), Lusaka, Zambia with great contributions from the MRC CTU.

Secondly, infrastructures are important for the high scientific workload in ICH-GCP compliant clinical trials. For ARROW and CHAPAS, particular research clinics were raised with clinical care units for the children enrolled in the study, but also for data management and trial meetings. During the VITA studies, the Kilimanjaro Clinical Research Institute building was constructed, containing study, meeting and archiving rooms, a data management department and clinical care units. Well equipped laboratories with clinical research capacity were already in place or further expanded. Given the lack of equipment for the bio-analysis of antiretroviral agents, it was decided to store plasma samples and ship them to the laboratories of RUNMC that ensured standardization and quality assurance of the laboratory procedures. Recently, a north-south collaboration was started between RUNMC and Makarere University Hospital, Kampala, Uganda. It is hoped that exchanging experience of laboratory staff from African and European countries enables the development of standardized and qualitative bio-analysis programs for laboratories in resource-limited settings in the enhancement of research capacity for future trials.

Thirdly, it is important to bring research sites to ICH-GCP standards and sustain quality of sites by (refresher) trainings and maintaining staff. Staff from all clinical sites received additional training in ICH-GCP and in the specific procedures which they were involved in. When ARROW and CHAPAS started, the infrastructure and well-trained staff were already in place, since the same study clinics and study team were involved in previous trials[35]. These two trials were fully ICH-GCP compliant. In contrast, the VITA project was yet completely lacking in research capacity. Many medical professionals in the VITA studies did not have any prior research experience. As was done in CHAPAS and in ARROW,

monitoring visits to the participating VITA sites in Zambia and Tanzania were conducted and were partly dedicated to teaching and training of basic research skills to site clini-cians, nurses and data managers. Also, the so-called sandwich model was introduced; two PhD students from Tanzania and the Netherlands were working together at the clinical trials to exchange knowledge, such as in data analysis, which remains in many studies predominantly under the control of the Northern partner. Annual workshops were used to provide skills on ICH-GCP and HIV-disease management (HIV-PENTA trainings) and to strengthen the North-South collaboration.

Finally, the abovementioned clinical trials, in particular CHAPAS and ARROW, have shown that key to success in conducting clinical trials is attributable to the commitment of project staff and goodwill of all northern and southern partners and their professional leadership and collaboration. CHAPAS and ARROW have been accomplished very well; the research team of the VITA project has learned very much, particularly in clinical pharmacology studies of HIV, for future research at this site. The next step for these developed research collaborations in Sub-Saharan Africa is to partner with less developed sites in the regions to facilitate training and to further expand research capacity.

Conclusions and future perspectives

Clinical pharmacology studies are highly relevant for improving and expanding the roll-out of HIV-treatment and clinical care in pregnant women and children in resource-limited settings. Strategies, such as option A (with the addition of an enzyme inducer?), should be used in pMTCT when WHO's Option B/B+ is not available or unfeasible. However, it is hoped that the roll-out of Option B/B+ for pMTCT will be expanded soon to be globally accessible in meeting one of the ambitious targets of UNAIDS/WHO to reduce the overall mother-to-child HIV transmission rate to <5% at the population level by the end of 2015.

More appropriate pediatric drug formulations that are inexpensive and easily usable are urgently needed to widen global access of HIV-treatment for children. WHO PAWG's standardized dosing guidelines should be evaluated in the global goal to optimize treatmentin resource-limited settings. Future trials should focus on inexpensive, new generation drugs with correct dosing guidelines and appropriate formulations for second-line and third-line treatment in children.

Research capacity development is essential in performing clinical trials and strengthening health systems in resource-limited settings. This thesis has shown that north-south collaboration plays an important role in exchanging knowledge about clinical pharmacology studies. The next step for these developed research collaborations in Sub-Saharan Africa is to partner with less developed sites in the regions to facilitate training and to further expand research capacity'

More than twenty years ago, professor Chifumbe Chintu (one of the investigators of CHAPAS) from UTH, Lusaka, Zambia developed one of the first African-led, North-South collaborations in research for pediatric infectious diseases[36]. He and his team had a motto launched that also comprises the conclusion of this thesis: *'Holding hands together and moving forward in the fight against HIV/AIDS in Africa.'*

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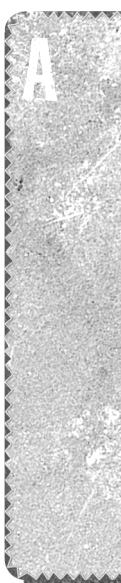
APPENDIX

Summary
Samenvatting
List of Publications
Dankwoord / Acknowledgements
Curriculum Vitae



SUMMARY

To date, more than 30 million people have died from HIV/AIDS and currently 34 million people are estimated to be living with HIV. The southern part of Africa is the region most severely affected, and it accounts for approximately 68% of the global total persons living with HIV. Since the prevalence is high in women of reproductive age, mother-to-child transmission of HIV (MTCT) is also common in the region. In 2011, 330.000 children acquired HIV infection (90% due to MTCT) and more than 90% of those were living in Sub-Saharan Africa.

Since the turn of the millennium, a massive scale-up of access to ART in resource-limited countries, especially in Africa, has been realized with political commitments and extensive international financial support. After introduction of combination antiretroviral therapy (ART) in resource-limited settings the overall growth of the HIV epidemic appears to have stabilized, as the number of people newly infected globally each year is continuing to fall and fewer people are dying from AIDS-related causes worldwide. Despite these major gains, ART is still unfortunately not universally accessible. The global coverage for the most vulnerable population in need for ART is still low to moderately low; only 28% of children 0-14 years of age in need of ART and 50% of pregnant women and infant pairs in need of antiretroviral prophylaxis for the prevention of MTCT (pMTCT) are currently receiving the life-saving medicines. Significant progress has been made, but the HIV problem has not yet been solved.

This thesis presents studies that were undertaken to optimize HIV treatment in HIV-infected pregnant women and children in Africa by studying the clinical pharmacology of antiretroviral drugs in the most vulnerable populations in resource-limited settings.

The first part of this thesis deals with clinical pharmacology studies in HIV-infected pregnant women using single-dose nevirapine as part of antiretroviral prophylaxis for pMTCT. We investigated simple, accessible and affordable strategies for the prevention and the reduction of MTCT of HIV in Sub-Saharan Africa using enzyme inducers for the elimination of nevirapine resistance after single-dose nevirapine as part of pMTCT.
The second part of this thesis focuses on the clinical pharmacology of ART in HIV-infected African children to optimize paediatric antiretroviral regimens for the scale-up of HIV treatment. We evaluated new appropriate antiretroviral drug formulations and we validated newly developed WHO weight-band based dosing recommendations for the use in children living in resource-limited settings.

Part I: Clinical pharmacology studies in HIV-infected pregnant women
Single-dose nevirapine is still being used globally in pMTCT, with a risk of 1-76% of women developing resistance against nevirapine from just this single-dose. **Chapter 2** of this thesis includes a systematic review together with a meta-analysis of different drug interventions,

which have been investigated to date, for reducing the development of nevirapine resistance. Pooling data from 18 eligible studies, single-dose nevirapine alone resulted in a prevalence of 31% (95% CI 7.6-54) nevirapine resistance in the mother. Addition of antepartum zidovudine reduced the proportion of women with detectable nevirapine resistance by 30% to 21% (95% CI 8.6-33). Combining antepartum zidovudine with a postpartum course of two other antiretroviral drugs for 4-7 days nearly eliminated the risk of nevirapine resistance mutations, to a prevalence of 0.011% (95% CI -0.11-0.13). The summary effect of longer 20-30 days of postpartum drug regimens combined with antepartum zidovudine and single-dose nevirapine was associated with similar incidence of nevirapine resistance, namely 0.003% (95% CI -0.054-0.060). The WHO guideline option A (antepartum zidovudine, single-dose nevirapine and one week of lamivudine/zidovudine postpartum) should be followed wherever feasible to achieve the minimum risk of nevirapine resistance in regions where single-dose nevirapine is still being used.

Most interventions to reduce the risk of nevirapine resistance development are antiretroviral-based, but a totally different approach is a pharmacological strategy of adding a CYP*3A4* enzyme inducer to the single-dose of nevirapine. In **Chapter 3** a clinical trial (VITA-1) was presented investigating the effect of a single-dose carbamazepine on nevirapine pharmacokinetics and the development of nevirapine resistance in 158 HIV-infected pregnant women in Tanzania. This trial provides the first data on adding a single-dose of an enzyme inducer (carbamazepine) to single-dose nevirapine for pMTCT in HIV-infected, pregnant women. Single dose carbamazepine significantly reduced nevirapine plasma concentrations one week after delivery by 36%, with a trend towards fewer resistance mutations. We also found a trend towards lower rates of nevirapine resistance in women with undetectable nevirapine concentrations across the whole population. Our study demonstrated the validity of this approach, and showed that that enzyme inducers, such as carbamazepine, have new possibilities for pMTCT programs to reduce the development of nevirapine resistance in settings where other options are limited.

In **Chapter 4**, we demonstrated the results on nevirapine pharmacokinetics of a pilot study investigating the effect of a seven-day course of phenytoin, as enzyme inducer (VITA-2 trial) in 50 Zambian and Tanzanian HIV-infected pregnant women. Adding a seven-day course of phenytoin, as enzyme inducer, from labour onset produced a large and significant reduction in the elimination half-life of nevirapine in HIV-infected, pregnant women using single-dose nevirapine as part of pMTCT prophylaxis. Seven days phenytoin was safe and effective with no new nevirapine resistance mutations observed. Since prolonged subtherapeutic nevirapine exposure is known to lead to nevirapine resistance emergence and since phenytoin is safely and widely used in women, to minimise pMTCT, single-dose nevirapine can be used with a seven-day course of phenytoin as an alternative if other antiretroviral drugs are unavailable.

Part II: Clinical pharmacology studies in HIV-infected children

Adequate drug exposure is essential in HIV treatment of children, who generally start treatment earlier and will need to take it for far longer than adults. However, obtaining optimal drug exposure in children is challenging, as they cannot simply be regarded as small adults. Pharmacokinetics of antiretroviral drugs are highly variable in the paediatric population as children mature and grow rapidly.

In an open randomized trial, called CHAPAS-1 (Children with HIV in Africa-Pharmacokinetics and Adherence of Simple antiretroviral regimens), appropriate dosing of fixed-dose combination tablets, approved based on early pharmacokinetic data generated within the trial, was assessed. The tablets have higher nevirapine to lamivudine/stavudine dose ratio compared to adults FDCs. It is known that children, particularly the youngest, metabolize nevirapine more rapidly than adults. In **Chapter 5** (substudy of CHAPAS-1) we reported the exposure to nevirapine in 16 Zambian, HIV-infected infants weighing 3-6 kg taking these paediatric fixed-dose combination tablets twice daily. The infants had plasma nevirapine concentrations that were slightly lower, but generally comparable with heavier children, and were higher than historical data. However, large interinfant variability (55%) led to a relatively high proportion of children, particularly those <5 months, with low nevirapine trough concentrations. Nevertheless, given fast growth rates after starting ART such that most infants spend only a short time in the lowest weight band, and the encouraging viral responses we observed at four weeks after ART initiation, WHO weight-band tables for nevirapine-containing fixed-dose combination tablets appear reasonable for HIV-infected infants weighing 3-6kg.

WHO guidelines recommend nevirapine dose escalation (half dose for the first 14 days) when initiating ART to avoid toxicity from high initial nevirapine levels. Since young children metabolize nevirapine more rapidly, there is the potential for inadequate nevirapine plasma levels during dose escalation, which could result in slower viral load suppression or increased risk of later virological failure. In the subsequent chapter (**Chapter 6**; also a substudy of CHAPAS-1) the pharmacokinetics of nevirapine two weeks after nevirapine initiation with and without using a dose escalation strategy were evaluated in 162 Zambian HIV-infected infants/children. The relationship between starting dose and safety and efficacy was also assessed. Despite the relatively small sample size, with nevirapine dose-escalation at ART initiation more subtherapeutic nevirapine plasma concentrations 3-4 hours post-dose were observed in children <2 years of age compared to older children. This finding is compatible with earlier studies describing more rapid nevirapine systemic clearance in younger than in older children. We also found that younger children had a low risk for rash with a full-dose initiation strategy. To simplify ART initiation in young children and reduce the risk of suboptimal dosing, full-dose nevirapine at ART initiation should be considered for African HIV-infected children <2 years of age. Children >2 years of age could continue to receive dose-escalation to avoid the development of rash, or should have easy access to clinics to enable timely review if rashes recur with temporary discontinuation and nevirapine re-initiation at half-dose.

Paediatric dosing has not only been standardized with fixed-dose combination formulations, but also by using simplified weight-band based dosing recommendations. However, these have typically been determined using extrapolation from licensed mg/kg or mg/m^2 doses, and require validation for optimal HIV treatment. **Chapter 7** described actual efavirenz plasma exposure in a group of 41 HIV-infected Ugandan children aged between 3 and 12 years treated following the 2006 efavirenz weight-band based dosing recommendations of WHO using full pharmacokinetic information. This was a substudy of the ARROW trial, an open-label randomized trial comparing routine laboratory (toxicity, CD4) versus clinically driven monitoring strategies, and also comparing three different first-line ART strategies in HIV-infected infants and children in Uganda and Zimbabwe (www.arrowtrial.org). The children in this study had lower and highly intersubject variable pharmacokinetic parameters of efavirenz compared to historical data from adults. There were no statistically significant differences across weight-bands, suggesting no major effect of some using half tablets. Our findings in **Chapter 7** suggest that children receiving efavirenz dosed according to 2006 WHO guidelines (or the licensed recommendations on which these were based) could be at a higher risk of virological failure, and that all pediatricians in resource-limited and resource-rich countries should move to the higher WHO 2010 guideline dosing as a matter of urgency. Nevertheless, doses in some weight-bands may need to be increased still further, and this also should be investigated promptly.

There are numerous factors which may lead to interindividual variability in pharmacokinetics, such as gender, age, body weight, non-compliance, and genetic factors. **Chapter 8**evaluated mid-dosing interval efavirenz plasma concentrations and the influence of CYP*2B6* polymorphisms in relation to efficacy, tolerability, and adherence in 87 Rwandan HIV-infected children. We found a high level of intersubject variability in mid-dosing interval efavirenz plasma concentrations in Rwandan HIV-1 infected children when they were dosed according to the national guidelines. Only two-third of these children had efavirenz concentrations within the therapeutic range. High variability (i.e. 107%) resulted in 14% children with subtherapeutic levels and 20% with potentially toxic plasma levels. Although the difference was not significant in this small study, a higher proportion of virologic failure was observed in children with (sub)therapeutic efavirenz levels. Finally, we found that both adherence and CYP2B6-genetic polymorphisms were major factors influencing variability in mid-dosing interval plasma concentrations. Significantly higher efavirenz levels were correlated to children having specific CYP*2B6* variants (516TT, 785GG and 983CC).

In **Chapter 9** (a further pharmacokinetic substudy of ARROW) we evaluated a different weight-band based dosing recommendation from WHO, for twice daily zidovudine, which has only been limitedly studied, to investigate whether this provides optimal exposure in Ugandan, HIV-infected children. Zidovudine is a common component of first-line paediatric ART, especially in resource-limited countries. Here, we found higher zidovudine exposure in Ugandan children aged 1-12 years dosed twice-daily according to WHO 2006 weight-bands compared to that previously reported in adults receiving the standard dose of 300

mg zidovudine twice-daily. Exposure was also higher than in the only two previous paediatric zidovudine pharmacokinetic studies evaluating twice daily zidovudine, which used lower doses than our study. Subsequent 2010 WHO guidelines have further increased the recommended zidovudine dose for children, without new evidence, but reflecting concernsthat children had often been under-dosed with ART. Children dosed following WHO 2010 guidelines, younger children, and those with low weight-for-age are therefore likely to have even higher zidovudine exposure than that observed in **Chapter 9** and substantially higher than previously reported in adults. Our findings suggest that this higher exposure could be associated with greater suppression of haemoglobin levels within the normal range and probably with no change in efficacy, since viral load suppression was already very good using WHO 2006 dosing. However, the impact on severe anaemia warrants further investigation, particularly with regards to current WHO 2010 dosing.

The final chapter before the General Discussion expanded on the scale-up of second-line ART access. ART formulations suitable for children should ideally be pleasant-tasting, with no food restrictions, be heat-stable and require administration no more than twice-daily. The present accessible antiretroviral drug for second-line in resource-limited settings is ritonavir-boosted lopinavir. This drug is currently only available as syrup, which tastes bad, requires a stable cold chain and has a relatively short shelf-life, or as paediatric tablets, which are very hard, still relatively large, unscored and cannot be cut into pieces (as this significantly reduces bioavailability). The lopinavir tablets are difficult to be taken by children younger than 5-6 years old - in contrast to many of the FDCs discussed above which are dispersible, scored, and generally preferred by carers. A randomised pharmacokinetic bio-equivalence phase Ib trial (CHAPAS-2; **Chapter 10**) was designed to assess the pharmacokinetics and acceptability of ritonavir-boosted lopinavir in sprinkle and tablet formulations for treatment of HIV infected children in Africa. The inexpensive, novel, heat-stable, solid drug sprinkle formulation of LPV/r evaluated in the CHAPAS-2 study has been shown to have generally acceptable pharmacokinetic profiles and to be pre-ferred by younger children and their carers compared to the currently available innovator syrup. The current WHO weight band dosing recommendations appear to be appropriate for use in the paediatric population.

In the General Discussion (**Chapter 11**) the challenges in translating our research findings into practice in resource-limited settings were discussed, focusing on antiretroviral prophylaxis for pMTCT of HIV, HIV-treatment for children in resource-limited settings, research capacity development and the advantages of international collaboration for clinical research.

Clinical pharmacology studies are highly relevant for improving and expanding the roll-out of HIV-treatment and clinical care in pregnant women and children in resource-limited settings. Conducting the VITA trials investigating the addition of simple strategies of enzyme inducers (a single-dose carbamazepine or seven days of phenytoin at labor

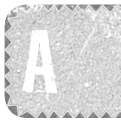
onset) to single-dose nevirapine for pMTCT in HIV-infected pregnant women has been challenging, but support from other African clinical research groups became extremely valuable in successfully completing the trial.

Strategies, such as WHO's option A (possibly with the addition of an enzyme inducer), should be used in pMTCT when WHO's Option B/B+ is not available or unfeasible. However, it is hoped that the roll-out of Option B/B+ for pMTCT will be expanded soon to be globally accessible in order to meet one of the ambitious targets of UNAIDS/WHO to reduce the overall mother-to-child HIV transmission rate to <5% at the population level by the end of 2015.

In terms of optimising HIV treatment for children in resource-limited settings, more appropriate pediatric drug formulations that are inexpensive and easily usable are urgently needed to widen global access to HIV-treatment for children. The WHO's standardized dosing guidelines developed by the pediatric antiretroviral working group should be evaluated in the global goal to optimize treatment in resource-limited settings. Future trials should focus on inexpensive, new generation drugs with correct dosing guidelines and appropriate formulations for second-line and third-line treatment in children.

Research capacity development is essential in performing clinical trials and strengthening health systems in resource-limited settings. This thesis has shown that north-south and south-south collaboration plays an important role in exchanging knowledge about clinical pharmacology studies. The next step for these developed research collaborations in Sub-Saharan Africa is to partner with less developed sites in the regions to facilitate training and to further expand research capacity.

More than twenty years ago, professor Chifumbe Chintu (one of the investigators of CHAPAS) with others from UTH, Lusaka, Zambia developed one of the first African-led, North-South collaborations in research for pediatric infectious diseases. They had a motto launched that also comprises the conclusion of this thesis: *'Holding hands together and moving forward in the fight against HIV/AIDS in Africa.'*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SAMENVATTING

HIV EN AIDS

HIV, het humaan immunodeficiëntie virus, is het virus dat AIDS, het Acquired Immune Deficiency Syndrome, veroorzaakt. Dit virus valt de afweercellen in het lichaam aan, vermenigvuldigt zich daar en vernietigt daarbij de afweercellen. Op die manier is het virus in staat het mechanisme uit te schakelen waarmee het lichaam zich beschermt tegen allerlei ziekteverwekkers, zoals bacteriën, schimmels en virussen. Die ziekteverwekkers worden normaal gesproken zonder problemen door het immuunsysteem vernietigd, maar kunnen nu een ziekmakende infectie veroorzaken. Het zijn in de meeste gevallen ook deze infecties waaraan een AIDS-patiënt overlijdt.

HIV bevindt zich in bloed, sperma, voorvocht, vaginaal vocht en moedermelk. Als HIV in deze lichaamsvochten in contact komt met iemand anders' bloedbaan of slijmvliezen, is er kans op een HIV-infectie, zoals bij onveilig seksueel contact, bij het inspuiten van drugs met gebruikte naalden of spuiten, een baby van een HIV-geïnfecteerde moeder, een bloedtransfusie of bij het prikken aan een naald met HIV-besmet bloed.

Sinds de ontdekking van HIV in het begin van de jaren tachtig, dat tegelijkertijd het begin was van deze grote epidemie, zijn er naar schatting 30 miljoen mensen gestorven aan HIV/AIDS. Op dit moment leven er maar liefst 34 miljoen mensen met HIV wereldwijd en daar komt elke 12 seconden een nieuwe patiënt bij. Sinds de komst van de HIV-remmers en betere wereldwijde beschikbaarheid van deze middelen is het welzijn en de levensverwachting van mensen met HIV/AIDS sterk verbeterd. Maar ondanks de vooruitgang die is geboekt met de bestrijding van deze ziekte in het laatste decennium, blijft de HIV-epidemie één van de grootste problemen voor de wereldwijde volksgezondheid.

HIV IN AFRIKA

Het gebied in Afrika ten zuiden van de Sahara is de zwaarst getroffen regio in de wereld, waar 68% van alle mensen met HIV leven. In tegenstelling tot de meeste andere landen, waar homoseksuele mannen of drugsgebruikers het meeste risico lopen op besmetting, zijn het in het gebied in Afrika ten zuiden van de Sahara voornamelijk vrouwen die slachtoffer zijn geworden van de epidemie. Omdat ook veel vrouwen van vruchtbare leeftijd met HIV zijn besmet, worden er veel baby's met HIV geboren. Alleen al in 2011 hebben 330.000 kinderen wereldwijd een HIV-besmetting opgelopen (in 9 op de 10 gevallen overgebracht door hun HIV-geïnfecteerde moeder) en 90% van die kinderen leeft in het gebied in Afrika ten zuiden van de Sahara. Een behandeling wordt meestal gestart met drie HIV-remmers en wordt ook wel antiretrovirale combinatietherapie genoemd. Helaas is deze antiretrovirale combinatie therapie nog steeds niet wereldwijd beschikbaar. Vanaf 2002 zijn er in Afrika grootschalige HIV-behandelprogramma's opgestart om de antiretro-

virale combinatietherapie zo snel mogelijk beschikbaar te krijgen voor iedereen die dat nodig heeft. Op advies van de Wereldgezondheidsorganisatie (WHO) worden in ontwikkelingslanden een beperkt aantal en goedkope HIV-remmers voorgeschreven en zo min mogelijk laboratorium tests gebruikt. Met deze benadering is inmiddels grote vooruitgang geboekt. Eind 2011 kreeg een recordaantal van 8 miljoen volwassenen en kinderen voor het eerst de levensreddende HIV-remmers, maar nog altijd is de helft van de mensen met HIV niet voorzien van behandeling, terwijl ze dat wel heel hard nodig hebben. Voor kinderen is de beschikbaarheid van HIV-remmers nog veel lager. Slechts 28% (dus minder dan een derde) van de kinderen wordt op dit moment behandeld.

HIV-behandeling voor zwangere vrouwen in Afrika

De kans van overdracht van HIV van een moeder op haar baby kan sterk worden verkleind door HIV-remmers te gebruiken tijdens de zwangerschap, de bevalling en de borstvoeding. In de westerse wereld wordt een HIV-geïnfecteerde vrouw tijdens haar zwangerschap zo snel mogelijk met antiretrovirale combinatietherapie van drie HIV-remmers behandeld om het virus zo goed mogelijk te onderdrukken. Op die manier wordt de kans van overdracht van HIV op de baby verminderd tot vrijwel 0. In Afrika zijn de middelen die we in de westerse wereld gebruiken niet overal beschikbaar. Vanaf 2000 adviseerde de WHO een simpele, goedkope, maar zeer effectieve methode: één dosis van het middel nevirapine, dat net voor de bevalling moet worden gegeven aan de moeder en na de bevalling aan het kind. Dit zorgt voor bijna een halvering aan HIV-besmettingen in de baby's. Een nadeel echter is de ontwikkeling van resistentie, waardoor het middel bij een volgende bevalling of bij chronisch gebruik niet meer werkt. Eén dosis van het middel nevirapine alleen wordt daarom niet langer aanbevolen, maar nog wel als onderdeel van de antiretrovirale combinatietherapie. Deze simpele methoe van één dosis nevirapine zal dus langzaam verdwijnen, maar wordt in de praktijk doorgaans nog steeds gebruikt door duizenden zwangere vrouwen in ontwikkelingslanden. Dit gebeurt met name in de gebieden buiten de steden, waar antiretrovirale combinatietherapie helaas nog niet voorhanden is.

HIV-behandeling voor kinderen in Afrika

In de westerse wereld is de medische zorg voor kinderen met HIV/AIDS goed geregeld. De overdracht van HIV van moeder-op-kind kan bijna geheel voorkomen worden. Daarnaast worden baby's en kinderen goed onderzocht en kunnen ze een antiretrovirale combinatietherapie krijgen als ze dat nodig hebben. Doordat er in de westerse wereld relatief weinig kinderen met HIV leven, is er geen grote financiële stimulans voor grote farmaceutische bedrijven om medicijnen te ontwikkelen die specifiek geschikt zijn voor kinderen. De weinige die er zijn, zijn bovendien een stuk duurder dan de volwassen varianten. Kinderen vormen daarmee het buitenbeentje voor de ontwikkeling van betere en betaalbare HIV-remmers, waardoor de beschikbaarheid van deze middelen voor deze kwetsbare groep patiënten achter blijft (met maar 28% van de kinderen die op dit moment behandeling krijgen). Inmiddels zijn er een aantal generieke farmaceutische bedrijven die het initiatief hebben genomen om dit onvervulde gat voor kinderen te dichten.

THEMA'S IN DIT PROEFSCHRIFT

Het doel van dit proefschrift is het verbeteren van de HIV-behandelingen voor HIV-geïnfecteerde zwangere vrouwen en kinderen in Afrika. Hiervoor hebben we de klinische farmacologie van HIV-remmers in deze kwetsbare groep Afrikaanse mensen bestudeerd. Met de klinische farmacologie wordt naar het effect gekeken van het menselijk lichaam op het geneesmiddel (ook wel farmacokinetiek genoemd) en wordt de relatie tussen de geneesmiddelconcentratie in het bloed en het effect op het menselijk lichaam (ook wel farmacodynamiek genoemd) onderzocht.

Het eerste deel van dit proefschrift beschrijft het onderzoek van simpele, beschikbare en betaalbare methoden voor de preventie van de moeder-op-kind-overdracht van HIV in het gebied in Afrika ten zuiden van de Sahara. Daarbij hebben we onderzoek gedaan bij zwangere vrouwen naar de toevoeging van een extra geneesmiddel, waardoor mogelijk minder resistentie ontstaat tegen het middel nevirapine.

Het tweede deel van dit proefschrift gaat over het verbeteren van HIV-behandelingen voor kinderen in ontwikkelingslanden. Hiervoor hebben we nieuwe, geschikte HIV-geneesmiddelen geëvalueerd en hebben we gekeken of de doseringsrichtlijnen van de WHO goed zijn voor het gebruik van deze HIV-remmers voor kinderen in ontwikkelingslanden.

Deel I: Onderzoek in HIV-geïnfecteerde zwangere vrouwen

Zoals eerder beschreven, wordt één dosis van de HIV-remmer nevirapine net voor de bevalling doorgaans nog steeds gebruikt door duizenden HIV-geïnfecteerde zwangere vrouwen in ontwikkelingslanden, waar antiretrovirale combinatietherapie helaas nog niet voorhanden is. Nevirapine zorgt voor een snelle daling van het virus in het bloed, waardoor de kans wordt verkleind dat het kind besmet raakt met HIV. Een nadeel van nevirapine is het hoge risico op resistentie als het middel eenmalig wordt gegeven zonder andere middelen erbij. In **Hoofdstuk 2** van dit proefschrift is een systematisch review met een meta-analyse (een beknopt overzicht van alle literatuur over dit onderwerp) beschreven, waarin het effect van de toevoeging van andere middelen op de nevirapine-resistentie is onderzocht. Er waren 18 onderzoeken gevonden, die naar dit vraagstuk hadden gekeken. Eén dosis van nevirapine alleen resulteerde in 31% resistente moeders. Deze moeders kunnen mogelijk geen nevirapine meer gebruiken in een volgende zwangerschap, omdat het gewoonweg niet meer werkt. Daarnaast kunnen zij hun resistente virus overbrengen op anderen. Met toevoeging van een andere HIV-remmer, zidovudine, zagen we bij 21% van de moeders resistentie ontstaan. Als er dan ook nog een week lang HIV-remmers na de bevalling worden toegevoegd aan die ene dosis nevirapine plus zidovudine verdwijnt het risico op resistentie bijna geheel. In ons onderzoek zagen we namelijk slechts 0.011% (1 op 10.000) resistente moeders. Als die HIV-remmers nog langer werden gegeven (20-30 dagen) leek het verschil nog iets kleiner, maar eigenlijk niet verschillend met 0.003%. De richtlijnen van de WHO bevelen bij gebrek aan antiretrovirale combinatietherapie aan om zidovudine plus één dosis

nevirapine net voor de bevalling te geven met nog een week lang HIV-remmers na de bevalling. Ons onderzoek bevestigd dat deze aanbeveling goed is met een daling naar slechts 0.011% resistente moeders.

In **Hoofdstuk 3 en 4** zijn de VITA-onderzoeken beschreven. Deze onderzoeken zijn uitgevoerd in HIV-geïnfecteerde zwangere vrouwen in Tanzania en Zambia. Hierbij hebben we een ander middel toegevoegd aan die ene dosis nevirapine, namelijk carbamazepine en fenytoine in een lage dosering. Deze middelen noemen wij enzyminductoren en zorgen voor een versnelde afbraak van nevirapine. In het algemeen wil je niet dat geneesmiddelen met elkaar een wisselwerking hebben, maar hier maken we er juist gebruik van. Als nevirapine sneller uit het bloed wordt geklaard, zal dat ook tot minder resistentie moeten leiden, maar dit was nog nooit eerder onderzocht in deze patiëntengroep. De vrouwen werden in twee groepen verdeeld. Groep 1 kreeg een dosis nevirapine en groep 2 kreeg een dosis nevirapine plus een enzyminductor voor de bevalling. Eén dosis van de enzyminductor, carbamazepine, zorgde voor een daling in nevirapine bloedspiegels van wel 36% (VITA-1). Ook zagen we minder resistentie in deze groep. In **Hoofdstuk 4** (VITA-2) hebben we fenytoine erbij gegeven tot 7 dagen na de bevalling. Dit zorgde voor een 85% daling van de nevirapine bloedspiegels en we zagen ook een trend naar minder resistentie. De groepen waren helaas te klein om over resistentie een echte eerlijke uitspraak te kunnen doen. In beide onderzoeken bleken de middelen veilig en bleek nevirapine nog steeds even effectief tijdens de bevalling. Eén dosis nevirapine zou dus met een zevendaagse toevoeging van fenytoine een goed alternatief zijn, als antiretrovirale combinatietherapie niet beschikbaar is.

Deel II: Onderzoek in HIV-geïnfecteerde kinderen in Afrika

Adequate geneesmiddelconcentraties in het bloed zijn heel belangrijk bij de behandeling van HIV bij kinderen. Maar dat is makkelijker gezegd dan gedaan. Kinderen kunnen niet zomaar worden vergeleken met volwassenen. Het effect van het lichaam op de geneesmiddelconcentraties in het bloed van kinderen is sterk variabel aangezien kinderen zich individueel ontwikkelen en groeien, totdat ze volwassen zijn.

In **Hoofdstuk 5** is een klein onderzoek beschreven van het grote CHAPAS-1 kinderonderzoek. In dit onderzoek zijn nieuw ontwikkelde kindertabletten onderzocht. Deze kindertabletten bevatten drie middelen: nevirapine, lamivudine en stavudine. Nevirapine is een HIV-remmer die door kinderen, voornamelijk door de allerjongsten, sneller wordt afgebroken in het lichaam dan door volwassenen. Daardoor vinden we vaak te lage nevirapine concentraties in het bloed, terwijl de bloedspiegels van de andere twee middelen goed zijn. In de nieuwe kindertablet zit in verhouding meer nevirapine ten opzichte van de andere twee middelen en daarom verwachtten we betere nevirapine concentraties in het bloed van de kinderen te zien. We hebben de concentraties onderzocht in het bloed van 16 HIV-geïnfecteerde Zambiaanse baby's/kinderen tussen de 3-6 kg. De nevirapine concentraties bleken, vooral in de allerjongsten onder de 5 maanden, erg wisselend

en iets lager dan in oudere, zwaardere kinderen, waarin dit onderzoek al eerder was uitgevoerd. De kindjes zitten echter maar korte tijd in de gewichtsgroep 3-6 kg, ze groeien immers snel en krijgen daarna een hogere dosering. Daarnaast werd het virus wel voldoende onderdrukt, waardoor we vinden dat deze kindertabletten in deze dosering, geadviseerd door de WHO, acceptabel zijn voor Afrikaanse HIV-geïnfecteerde kinderen tussen de 3-6 kg.

De WHO beveelt bij het eerste gebruik van nevirapine aan om met een halve dosering te starten voor de eerste 14 dagen, waarna de volledige dosering kan worden gegeven. In het begin worden namelijk vaak hoge nevirapine concentraties in het bloed gemeten met nare bijwerkingen, zoals huiduitslag, tot gevolg. Omdat jonge kinderen de HIV-remmer nevirapine sneller afbreken in het lichaam dan volwassenen, zou het bij deze groep juist kunnen zijn dat ze helemaal geen te hoge nevirapine spiegels hebben in het begin met een volledige dosering. Een halve dosering in de eerste 14 dagen is dus misschien helemaal niet nodig, en zou misschien zelfs tot te lage bloedconcentraties kunnen leiden met een slechtere onderdrukking van HIV tot gevolg. In **Hoofdstuk 6**, ook een CHAPAS-1 subonderzoek, is gekeken naar de nevirapine concentraties in het bloed van 162 HIV-geïnfecteerde Zambiaanse kinderen tussen de 0-12 jaar. De kinderen waren verdeeld over twee groepen. De ene groep kreeg eerst de aanbevolen halve dosering nevirapine tijdens de eerste 14 dagen en de andere groep startte direct met de volledige nevirapine dosering. Twee weken na het starten van de nevirapine werden bloedspiegels gemeten. Zoals we hadden verwacht, zagen we lagere spiegels in de kinderen met de aanbevolen halve dosering. Met name bij veel kinderen jonger dan 2 jaar zagen we te lage nevirapine concentraties. Dat zou tot slechtere onderdrukking van HIV kunnen leiden, maar dat konden we helaas met dit onderzoek niet aantonen. Wel zagen we in de groep met heel jonge kinderen (onder de 2 jaar) die waren gestart met een volledige dosering helemaal geen bijwerkingen, maar bij de oudere kinderen wel. Dit onderzoek heeft dus aangetoond dat bij kinderen onder de 2 jaar direct met een volledige dosis nevirapine kan worden gestart, omdat de kans op bijwerkingen veel kleiner is. Oudere kinderen zouden wel met een halve dosering moeten starten, zoals volwassen, vanwege deze bijwerkingen. Als oudere kinderen toch ook met een volledige dosering willen starten, omdat dat gewoonweg makkelijker is, moeten ze wel snel bij een kliniek kunnen komen, voor het geval dat ze toch huiduitslag krijgen.

In **Hoofdstuk 7 en 8** hebben we onderzoek gedaan naar de doseringsrichtlijnen van de WHO en hebben we gekeken of deze goed zijn voor het gebruik van de HIV-remmer, efavirenz, voor kinderen in ontwikkelingslanden. **Hoofdstuk 7** beschrijft de informatie die we uit de efavirenz concentraties uit het bloed hebben gekregen van 41 Oegandese HIV-geïnfecteerde kinderen tussen de 3-12 jaar. Deze kinderen werden nog gedoseerd volgens de richtlijnen van de WHO uit 2006. Dit was een subonderzoek van een groter onderzoek, dat ARROW heette. De kinderen in het onderzoek hadden lagere en veel meer wisselende efavirenz bloedspiegels dan bij volwassenen was gemeten. Onze

bevindingen geven duidelijk aan dat deze efavirenz doseringen uit 2006 kunnen leiden tot een slechtere onderdrukking van HIV. We geven dan ook aan als conclusie dat de kinderartsen zo snel mogelijk naar de nieuwere richtlijnen van de WHO uit 2010 moeten overstappen. De efavirenz concentraties in het bloed van kinderen die gedoseerd worden met de nieuwe richtlijnen uit 2010 worden op dit moment onderzocht in een vervolgonderzoek.

In **Hoofdstuk 8** is gekeken naar de factoren die de wisselende efavirenz bloedspiegels in kinderen verklaren. Het is bekend dat het geslacht, de leeftijd en het gewicht van een kind, maar ook bepaalde genen een rol kunnen spelen in de hoogte van de efavirenz bloedspiegels. Voor dit onderzoek zijn efavirenz concentraties gemeten in 87 Rwandese HIV-geïnfecteerde kinderen. De efavirenz concentraties in het bloed van deze kinderen wisselden enorm, wanneer ze volgens de richtlijnen van 2006 werden gedoseerd. Slechts twee van de drie kinderen had een goede efavirenz concentratie in het bloed, 14% van de kinderen had een te lage bloedspiegel met grotere kans op slechte onderdrukking van HIV en 20% had een te hoge spiegel met grote kans op bijwerkingen. Ook vonden we dat veranderingen in een bepaald gen (ook wel genetisch polymorphisme genoemd) en het trouw innemen van de HIV-remmer twee belangrijke factoren waren voor de variabele bloedspiegels in de kinderen.

In **Hoofdstuk 9** (een ander subonderzoek van ARROW) hebben we de doseringsrichtlijnen uit 2006 van de WHO voor het tweemaal daags gebruik van de HIV-remmer zidovudine bestudeerd. Zidovudine is een middel dat erg veel in kinderen in ontwikkelingslanden wordt gebruikt. In dit onderzoek vonden we hogere zidovudine concentraties in het bloed van de kinderen vergeleken met gemeten waarden in volwassenen. We hebben ook gezien dat deze hogere spiegels voor wat lagere hemoglobine spiegels kunnen zorgen. Dat kan leiden tot bloedarmoede. Inmiddels zijn de richtlijnen van de WHO voor zidovudine in 2010 verder opgehoogd, omdat kinderen vaak met andere HIV-remmers ondergedoseerd worden (zoals de HIV-remmer efavirenz uit **Hoofdstuk 7**). Het risico op hoge zidovudine concentraties met de nieuwe richtlijnen uit 2010 is dus waarschijnlijk nog groter dan met die uit 2006. We denken dat dit geen verbetering van de onderdrukking van het virus oplevert, want dat was al erg goed. De impact die deze hogere doseringsrichtlijnen uit 2010 voor zidovudine hebben op bloedarmoede wordt nog verder onderzocht.

Het laatste onderzoek uit dit proefschrift gaat over de uitbreiding van de beschikbaarheid van tweedelijns antiretrovirale combinatietherapie. Tweedelijns HIV-remmers worden voorgeschreven als de standaard eerste-keus HIV-remmers niet meer gegeven kunnen worden, omdat ze bijwerkingen geven, niet meer werken (door resistentie) of wisselwerkingen geven met andere geneesmiddelen die de patiënt krijgt. Idealiter zouden HIV-remmers voor kinderen aangenaam moeten smaken, met of zonder eten ingenomen moeten kunnen worden en niet vaker dan tweemaal per dag gegeven moeten worden. De enige beschikbare tweedelijns HIV-remmer in ontwikkelingslanden is lopinavir/ritonavir. Dit middel is in tabletten beschikbaar, maar die mogen niet in stukjes gemalen worden, zoals wel in de

kindertabletten voor de onderzoeken in de **Hoofdstukken 5-9** beschreven. Kinderen jonger dan 5-6 jaar zijn niet in staat deze lopinavir/ritonavir tabletten te slikken. Ook is lopinavir/ritonavir in vloeibare vorm beschikbaar, maar het gebruik daarvan levert ook problemen op. De flessen zijn zwaar en lastig te vervoeren, moeten gekoeld worden bewaard (terwijl veel mensen in Afrika geen koelkast hebben) en hebben een korte houdbaarheidsdatum. Bovendien is het niet altijd even makkelijk om een kind zover te krijgen de drank door te slikken, omdat deze erg vies smaakt. In **Hoofdstuk 10** zijn nieuw ontwikkelde kindertabletten en korreltjes van de HIV-remmer lopinavir/ritonavir vergeleken met de vies smakende drank die in Afrika beschikbaar is. De bloedspiegels van 77 Oegandese kinderen werden onderzocht en vergeleken. De lopinavir/ritonavir concentraties in het bloed van de kinderen die de korreltjes kregen bleken over het algemeen goed overeen te komen met de bloedspiegels van de kinderen die de drank of de nieuwe kindertablet kregen. Bovendien hadden de jonge kinderen en hun ouders ook een voorkeur voor de korreltjes boven de drank. Bovendien bleken de huidige doseringsrichtlijnen voor lopinavir/ritonavir van de WHO goed voor het gebruik in deze Afrikaanse kinderen. Het is te hopen dat de lopinavir/ritonavir korreltjes snel op de markt beschikbaar komen.

In de discussie van **Hoofdstuk 11** zijn de belangrijkste bevindingen in bovengenoemde onderzoeken besproken. Onderzoek van de farmacologie van HIV-remmers is erg belangrijk in de uitbreiding van HIV-behandelingen en de medische zorg voor zwangere vrouwen en kinderen in ontwikkelingslanden. Niet alleen om goede resultaten uit deze onderzoeken te halen, maar ook om kennis van het doen van onderzoek over te brengen naar de Afrikaanse teams, zodat ze later zelfstandig onderzoeken kunnen uitvoeren. In de VITA-onderzoeken bleek ook dat de samenwerking tussen verschillende Afrikaanse onderzoeksgroepen erg waardevol was. Hierdoor hebben we de VITA-onderzoeken tot een goed einde gebracht met mooie resultaten. Het is te hopen dat de HIV-behandelprogramma's voor HIV-geïnfecteerde zwangere vrouwen zo goed en zo snel mogelijk wereldwijd worden uitgebreid voor de bescherming van de baby's die nog geboren gaan worden.

Voor het verbeteren van de HIV-behandelingen voor HIV-geïnfecteerde kinderen in ontwikkelingslanden is het belangrijk dat er geschikte medicijnen voor kinderen beschikbaar komen. Daarbij moeten ook de doseringsrichtlijnen van de WHO goed geëvalueerd worden voor het gebruik van deze HIV-remmers voor kinderen. Daarnaast is het belangrijk dat de nieuwe geneesmiddelonderzoeken gedaan worden voor goedkope en tweedelijns (en derdelijns) HIV-remmers voor kinderen.

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CURRICULUM VITAE

Quirine Fillekes werd geboren op 7 mei 1983 te Harderwijk. In 2001 behaalde zij haar gymnasiumdiploma aan het Agnieten College, locatie Carolus Clusius, te Zwolle. Daaropvolgend begon zij met een studie Farmacie aan de Rijksuniversiteit Groningen, waarvoor zij in 2005 het bachelordiploma behaalde. Als onderdeel van de masteropleiding verrichtte zij een wetenschappelijke stage aan de Rijksuniversiteit Groningen op de afdeling *Biomonitoring and Sensoring*. Hier heeft zij een studie met dieren uitgevoerd met als doel de autoregulatie van de H_3-receptor in het histaminerge hersensysteem middels micro-dialyse te onderzoeken. Tevens heeft Quirine uit interesse voor (klinisch) onderzoek een extra wetenschappelijk onderzoeksproject verricht in de laboratoria van overheidsinstituut Badan-POM in Jakarta, Indonesië. In 2008 ontving zij haar masterdiploma Farmacie en startte vervolgens als apotheker-onderzoeker in de Apotheek van het UMC St Radboud te Nijmegen. Haar promotieonderzoek, dat is beschreven in dit proefschrift, heeft zij uitgevoerd onder begeleiding van prof. dr. D. M. Burger in nauwe samenwerking met de *Medical Research Council Clinical Trials Unit* in Londen, Verenigd Koninkrijk en met verschillende onderzoeksgroepen uit Sub-Sahara Afrika. Per augustus 2013 werkt Quirine als Beoordelaar Farmacovigilantie bij het agentschap College ter Beoordeling van Geneesmiddelen te Utrecht.

Quirine Fillekes was born on the 7th of May, 1983 in Harderwijk, The Netherlands. In 2001 she completed her secondary school education at Agnieten College in Zwolle, The Netherlands. She then studied Pharmacy at the University of Groningen and obtained her bachelor's degree in 2005. Her master's degree followed and as part of her training she did a scientific research internship with the department of *Biomonitoring and Sensoring* at the University of Groningen. Here she worked on a project involving animals to investigate the autoregulation of the H_3-receptor in the histaminergic brain system using microdialysis. Having developed an interest in (clinical) research she then carried out a scientific research project in the Badan-POM laboratories, Jakarta, Indonesia. She obtained her master's degree in 2008. After graduation, Quirine started her PhD in the Department of Pharmacy at the Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. Her PhD work, which is described in this thesis, was supervised by Professor D. M. Burger. She collaborated closely with the Infections group at the *Medical Research Council's Clinical Trials Unit,* London, UK and with various research groups across Sub-Saharan Africa. From August 2013 Quirine will be working as a Pharmacovigilance Risk Assessor of the Medication Evaluation Board in Utrecht, The Netherlands.

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