Effect of rifampicin-based antitubercular therapy on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis

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Declaration

I, Karen Cohen, hereby declare that this research report is based on my independent work. Neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

This work was not published prior to registration for this degree

Signature: ……

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Literature review

Introduction

Sub-Saharan Africa is overwhelmed by dual epidemics of human immunodeficiency virus (HIV) and tuberculosis (TB) infection. Non-nucleoside reverse-transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART) is recommended for first-line treatment in adult HIV treatment programmes in resource-limited settings [1]. Many South African HIV-infected patients initiate ART while on TB treatment, 38 percent in one local study [2]. In addition, although ART reduces the incidence of TB, incidence in patients on ART is higher than in the HIV uninfected population [3], therefore incident TB on ART requiring concomitant treatment is very common. Efavirenz is regarded as the NNRTI of choice for TB co-infected patients [1] as outcomes are superior compared to those achieved with nevirapine-based ART [4] and concomitant TB treatment does not significantly reduce efavirenz concentrations [5]. However nevirapine is cheaper than efavirenz and is used extensively used in lower income countries with limited access to efavirenz [1]. Data characterising the extent to which concomitant rifampicin-based TB treatment decreases nevirapine plasma concentration therefore remain important.

Nevirapine is metabolised by cytochrome P450 (CYP) isoenzymes, predominantly CYP3A4 and CYP2B6 [6]. Rifampicin potently induces many genes controlling drug metabolism and transport, and up-regulates expression of several CYP isoenzymes, including CYP3A4 [7]. Rifampicin may therefore reduce concentrations of many concomitantly administered medicines, including nevirapine, which is a substrate of CYP3A4 [6]. Isoniazid, an inhibitor of CYP3A isoenzymes, may increase concentrations of concomitantly administered medicines [8, 9]. When isoniazid is administered together with rifampicin in TB treatment regimens, this may modify the effect of rifampicin on the metabolism of concomitantly administered nevirapine.

Reduction in nevirapine concentrations is of clinical importance as it may result in treatment failure, requiring an ART regimen switch. This is particularly problematic in resource-limited settings where ART regimen options are limited and second-line ART is considerably more expensive than first-line ART. A cohort study conducted in Cape Town in 2008 (n=2035) showed inferior treatment responses in patients on rifampicin-based TB treatment who were initiated on nevirapine-based ART, when compared with patients initiated on efavirenz-based ART [4]. At 6 months 16.3% (95% CI 10.6, 23.5%) of patients initiated on nevirapine failed virologically, compared to 8.3% (95% CI 6.7, 10.0%) of patients initiated on efavirenz [4]. It is likely that the difference in rates of virological failure result, at least in part, from sub-therapeutic nevirapine concentrations due to concomitant rifampicin-based TB treatment. Reduction in concentrations may be particularly important at the time of initiation of ART. It is standard practice to administer a lower “lead-in” dose of 200 mg nevirapine daily for 2 weeks, before nevirapine auto-induction is established. Studies in Malawi and Thailand found that when nevirapine was dosed at 200 mg daily for lead-in together with rifampicin-based TB treatment, 59% and 79% of participants respectively had sub-therapeutic nevirapine concentrations during lead-in [10, 11]. This is probably because rifampicin had already fully induced nevirapine metabolism. Nevirapine was initiated with lead-in dosing in the Cape Town cohort described above, and the investigators hypothesised that this was the cause of inferior outcomes [4]. Omission of lead-in dosing is now recommended when nevirapine is administered together with rifampicin-based TB treatment [1]. However, the CARINEMO randomised controlled trial (RCT) of efavirenz-

based versus nevirapine-based ART with TB treatment in HIV-TB co-infected patients in Mozambique, which omitted lead-in dosing for nevirapine, also showed poorer outcomes in the nevirapine arm [12]. The CARINEMO study set out to determine if nevirapine was non-inferior to efavirenz, with a non-inferiority margin of 10%. In the intention to treat analysis nevirapine was inferior to efavirenz: 60% of patients on nevirapine and 68.4% of patients on efavirenz had a viral load of below 50 copies/mL at 6 month (difference 8.4%, 1-sided 95% CI 15.0%) [12].

This is a review of studies exploring the pharmacokinetic interaction between nevirapine and rifampicin-based TB treatment. In addition, studies exploring nevirapine metabolism are reviewed. The data collection for the research project presented in this thesis took place in 2005 and 2006, and the work was published in 2008 (Cohen K, van Cutsem G, Boulle A, McIlleron H, Goemaere E, Smith PJ, Maartens G. 2008. Effect of rifampicin-based antitubercular therapy on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis. *J Antimicrob Chemother* 61, 389–393)[13]. In this review I will present the data that were available at the time of publication of our study, and the data that have been published since, in order to place my work in context with subsequent advances in the field. In addition, I will discuss the gaps in current knowledge, and questions requiring further research.

In this review, I have focused on data characterising the pharmacokinetic interaction. I have therefore not included data on safety and efficacy outcomes with co-administration of nevirapine-based ART and rifampicin or rifampicin-based TB treatment presented in reports on the included studies.

The most recent antiretroviral therapeutic drug monitoring (TDM) guidelines, published in 2006, recommend a target nevirapine trough concentration (C_{min}) of greater than 3 mg/L, as evidence to date suggests that there is an increased risk of virological failure below this cut-off [14-17]. Previous TDM guidelines suggested targets of 3.1 mg/L and 3.4 mg/L, and these values are used as the cut-off to define therapeutic concentrations in some of the earlier studies discussed below [15]. In my review of interaction studies, I have focused on the effect of rifampicin or rifampicin-based TB treatment on C_{min}, as this is the parameter that has been correlated with efficacy of nevirapine. However, area under the curve (AUC) characterises total drug exposure, and assessing changes in AUC will more accurately characterise the magnitude of a drug interaction. Where AUC has been calculated and reported, these results are also presented.

Objectives of literature review

The objectives of this literature review are:

1. To review studies exploring the effect of rifampicin or rifampicin-based TB treatment on nevirapine concentrations.
2. To review studies exploring metabolism of nevirapine.

Methods

Definitions

Rifampicin-based TB treatment: Rifampicin administered in combination with isoniazid, with or without concomitant pyrazinamide and ethambutol.

Nevirapine-based ART: Nevirapine administered in combination with two nucleoside reverse transcriptase inhibitors (NRTIs).

Inclusion criteria

For studies exploring nevirapine metabolism, inclusion criteria are as follows:

- *In vivo* or *in vitro* study in humans or human tissue
- Measurement of nevirapine metabolite concentrations

For studies exploring the interaction between nevirapine and rifampicin or rifampicin-based TB treatment, inclusion criteria are as follows:

- Participants either healthy volunteers or HIV-infected patients
- Adult participants (>=18 years old)
- Administered nevirapine with either rifampicin or rifampicin-based TB treatment.
- Nevirapine concentrations measured both on and off rifampicin or rifampicin-based TB treatment.
    - This includes within patient comparisons (with at least one sampling occasion in the presence of rifampicin or rifampicin-based TB treatment, and one sampling occasion without rifampicin or rifampicin-based TB treatment) , and between patient comparisons (where nevirapine concentrations are compared between a group of participants on rifampicin or rifampicin-based TB treatment, and a group without rifampicin or rifampicin-based TB treatment.)

Studies with both intensive and sparse pharmacokinetic sampling are included. Non-compartmental analyses, as well as analyses performed using population methods are included.

Search strategy

I searched the electronic journal database Pubmed. Nevirapine was registered with United States Food and Drug Administration (FDA) in 1996, so the search included all articles published from 1990 onwards in order to capture any articles published before nevirapine registration. Pubmed search terms were as follows:

("nevirapine"[MeSH Terms] OR "nevirapine"[All Fields]) AND (("rifampin"[MeSH Terms] OR "rifampin"[All Fields] OR "rifampicin"[All Fields]) OR ("rifampin"[MeSH Terms] OR "rifampin"[All Fields]) OR ("isoniazid"[MeSH Terms] OR "isoniazid"[All Fields]) OR ("tuberculosis"[MeSH Terms] OR "tuberculosis"[All Fields]) OR ("antitubercular agents"[MeSH Terms] OR ("antitubercular"[All Fields] AND "agents"[All Fields]) OR "antitubercular agents"[All Fields] OR "antitubercular"[All Fields] OR "antitubercular agents"[Pharmacological Action]) OR pharmacokinetic[All Fields] OR ("drug interactions"[MeSH Terms] OR ("drug"[All Fields] AND "interactions"[All Fields]) OR "drug

interactions"[All Fields] OR ("drug"[All Fields] AND "interaction"[All Fields]) OR "drug interaction"[All Fields]) OR metabolite[All Fields] OR hydroxynevirapine[All Fields] OR 12-hydroxynevirapine[All Fields]) AND ("1990/01/01"[PDAT] : "2012/12/31"[PDAT])

In addition, I searched conference proceedings from the Conferences on Retroviruses and Opportunistic infections, the International Workshop on clinical Pharmacology of HIV Therapy, the International AIDS conference and the International Aids Society Conference from 2009 to 2012, to identify any studies which may not have been published yet. I also reviewed all the studies referenced by the Liverpool Pharmacology Department Drug interaction information charts for the interaction between nevirapine and rifampicin, and nevirapine and isoniazid, to look for any additional studies missed by the above search [18]. I included the most recently updated version of the FDA approved package insert for Viramune® as a reference for premarketing studies of nevirapine metabolism[19]. I also reviewed references of articles that met inclusion criteria to look for any additional studies meeting inclusion criteria.

Data collection

I screened all retrieved abstracts identified by the search strategy, using the inclusion criteria outlined above. I then extracted the data from the studies meeting inclusion criteria, using a data extraction form.

Assessment of study quality

The Federal Drug Administration (FDA) in the United States and the European Medicines Agency (EMA) have guidelines for drug interaction studies for the pharmaceutical industry, for product registration and labelling purposes [20, 21]. However, I could find no formal, validated tool for assessing the quality of drug interaction studies. For purposes of assessing the quality of drug interactions studies included in this review, I developed a list of 9 criteria for assessment of study quality. These are in part derived from the FDA and EMA guidance documents and commentary on the FDA guidance [20-23] .

These criteria aim to address the following questions:

- Is the study adequately powered to detect an interaction? A power calculation, and assumptions made for that calculation, should be documented in the research report.

- Does the design allow the study to characterise the magnitude of the drug interaction that is likely to occur when the drugs hypothesised to be interacting are used concomitantly in patient management?
    - The ideal study design to allow this may differ depending on the specific interaction being studied, and how the drugs under study are used clinically. In this particular case, rifampicin in usually administered to HIV infected patients together with isoniazid, as part of a TB treatment regimen, rather than as monotherapy. Nevirapine, rifampicin, and isoniazid are generally administered to patients for long periods of time, rather than as single doses or short courses of therapy. The extent of induction or inhibition observed may be concentration dependent [8, 24]. Induction of CYP isoenzymes occurs over days. At least 7- 14 days of daily rifampicin

administration are required to approach maximal induction [7, 25]. In addition both nevirapine and rifampicin undergo auto-induction, and rifampicin may take up to 40 days to reach the fully auto-induced state [19, 25, 26]. Induction by rifampicin takes 7-14 days to wane [7]. In contrast to induction, inhibition may occur more quickly, and wane more quickly [27]. The extent of an inhibitory drug interaction observed is therefore likely to be influenced by the timing of administration of the drugs being studied. Reporting of dosing times is therefore important.

- Therefore, to investigate this study question, ideally both rifampicin and isoniazid should be administered, doses used in clinical practice should be administered, steady state and full induction must have been reached, and auto-induction must have occurred, and induction should have waned completely before repeating pharmacokinetic sampling without co-administered TB treatment. Dosing times in relation to pharmacokinetic sampling of all potentially interacting drugs should be reported

- Does the study design minimise important biases, and deal with important confounders?
  - Weight is likely to influence nevirapine concentrations. Changes in weight (in studies with repeated pharmacokinetic sampling separated by a month or more) and differences in weight (in studies with between group comparisons of pharmacokinetic parameters) should therefore be reported and taken into account in the analysis. (This potentially confounding effect may be dealt with by cross-over designs with randomised sequence, but this is not possible in studies in patients due to treatment requirements.)
  - Both HIV and TB disease states may alter drug absorption and pharmacokinetics, and therefore, while healthy volunteer data may be of some use, studies in patients with the diseases being treated are important to characterise the extent of any drug interaction and its likely impact on treatment efficacy.
  - To minimise confounding and potential bias due to between-participant differences, a fixed sequence one way crossover or randomised crossover design (allowing for within-participant comparisons of pharmacokinetic parameters) is a stronger study design than parallel group designs (with between participant comparisons).

- Is the pharmacokinetic data summarised in a way that captures both the midpoint and the variability in the data? Have investigators defined a threshold for concluding that the interaction is clinically significant, rather than merely a statistically significant?
  - The FDA and EMA recommend that changes in the key pharmacokinetic parameters should be presented using geometric means and geometric mean ratios and 90% confidence intervals [20, 21].
  - The FDA recommends that a pre-specified equivalence or “no effect” interval should be stated, outside of which the interaction is regarded as clinically significant [20]. Defining such a boundary for this particular interaction question is difficult. The clinical relevance of this interaction is determined by its impact on treatment efficacy and/or safety, rather than merely the magnitude of the proportional change in pharmacokinetic parameters. The magnitude of change that is significant may differ between populations, as nevirapine pharmacokinetic parameters differ

between populations [28]. There is a defined target nevirapine C_{min} that is supported by efficacy data [15], which might be used to calculate a population-specific margin for proportional change in C_{min}, but no defined target AUC . I have therefore not included this point as a criterion for appraising quality of studies included in this review.

The criteria that I have used to appraise quality of studies included in this review are as follows. They are criteria specific to this interaction question.

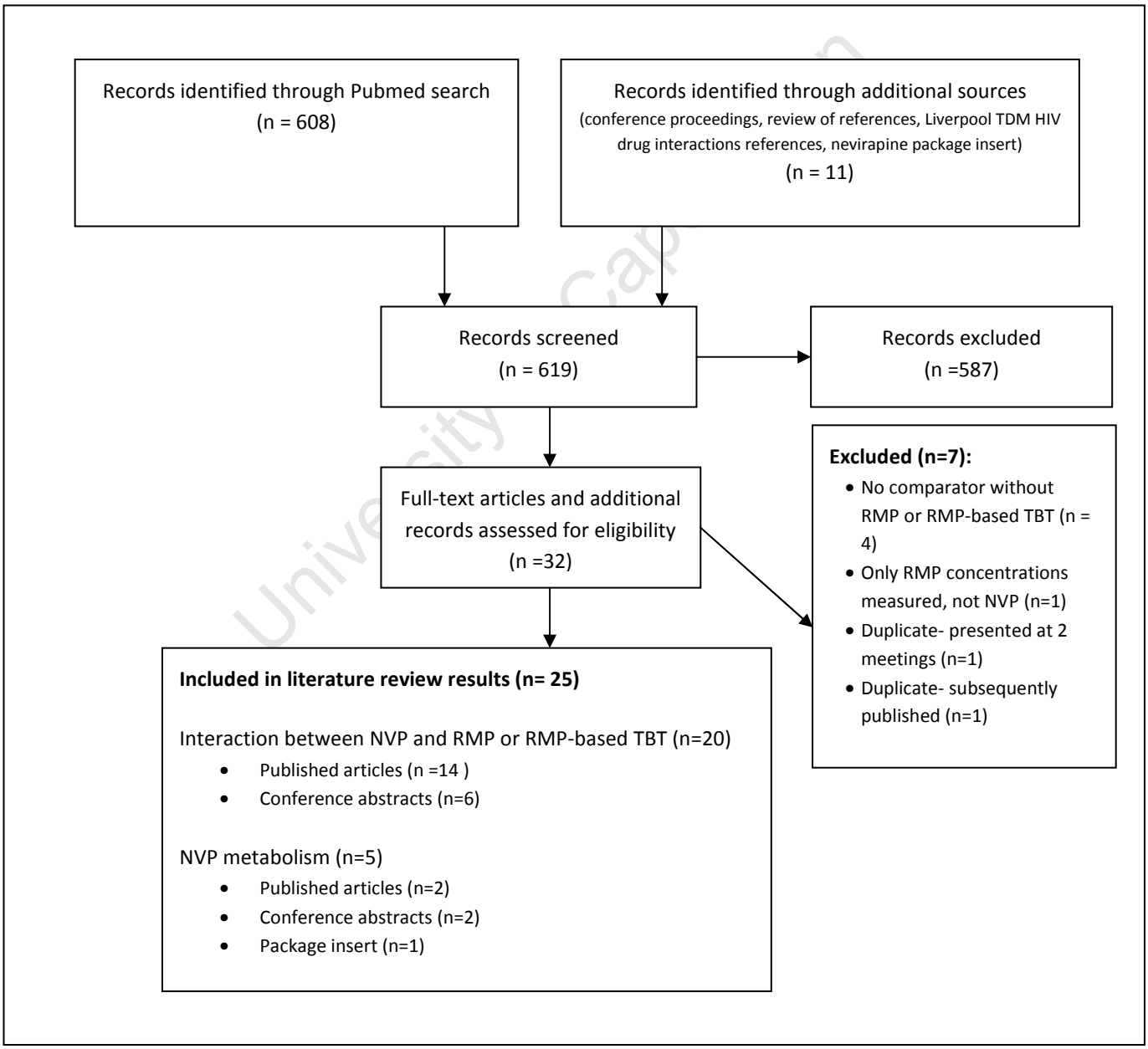
1. Was the study conducted in HIV and TB co-infected participants?
2. Is a sample size calculation presented?
3. Is change in weight (for within-participant comparisons with repeated pharmacokinetic sampling more than a month apart), or difference in weight (for between group comparisons) reported?
4. Is rifampicin-based TB treatment, including both rifampicin and isoniazid, administered?
5. Were the doses used in the study the same as those used in clinical practice?
6. Was pharmacokinetic sampling performed after at least 14 days of nevirapine at full dose, or 14 days of nevirapine in combination with rifampicin-based TB treatment at full dose? For studies with repeated pharmacokinetic sampling, was the second sampling occasion at least 14 days after adding or removing rifampicin?
7. Was the timing of administration of all potentially interacting drugs being studied reported- i.e. nevirapine, rifampicin and isoniazid?
8. Does the study design include a within-participant comparison of pharmacokinetic parameters?
9. Are changes in pharmacokinetic parameters (AUC, C_{max} and C_{min}) presented as geometric mean ratios with 90% CI?

I reviewed all interaction studies using these questions, and recorded the answer as “yes”, “no, “unclear” or “not applicable”.

Results

I performed the Pubmed Search on 14 December 2012 and retrieved 608 abstracts (figure 1). I identified 11 further records through the additional sources outlined above. I screened 619 records, and excluded 587 which did not meet inclusion criteria on review of the abstract. I reviewed 32 records for eligibility. 7 were excluded; reasons outlined in figure 1. There are 25 records included in the results below; 5 exploring metabolism of nevirapine, and 20 exploring the interaction between nevirapine and rifampicin or rifampicin-based TB treatment.

Figure 1 Flow diagram of study selection



Nevirapine metabolism

I identified four studies [6, 29-31], two of which were only available in abstract form [30, 31]. In addition, I reviewed the information from premarketing studies presented in the FDA approved package insert [19].

An in vitro study performed in human hepatic microsomes showed that nevirapine is metabolised by cytochrome P450 isoenzymes to form 2-, 3-, 8- and 12- hydroxynevirapine, predominantly via CYP3A4 and CYP2B6 [6]. The metabolite 2- hydroxynevirapine is formed exclusively by CYP3A4 and 3- hydroxynevirapine exclusively by CYP2B6. The metabolites 8- and 12-hydroxynevirapine are formed predominantly by CYP3A4, but other CYP450 isoenzymes (CYP2B6 and CYP2D6 for 8-hydroxynevirapine, and CYP2D6 and CYP2C9 for 12-hydroxynevirapine) also play a role [6].

Nevirapine was shown in vitro and in phase 1 studies to induce CYP2B6 and 3A [19]. It therefore auto-induces its own metabolism, resulting in a 1.5 to 2 fold increase in apparent oral clearance, and a decrease in half life from 45 hours in single dose studies to 25 to 30 hours following multiple dosing [19, 29].

A healthy volunteer study was conducted in 8 adult men, dosed with nevirapine 200 mg daily for 2 weeks, increased to 200 mg 12 hourly for a further 2 weeks, then administered a radioactively-labelled nevirapine dose [29]. Nevirapine was found to be excreted in the urine predominantly as glucuronidated conjugates of 2-, 3-, 8- and 12-hydroxynevirapine.

A pharmacokinetic study (presented at IAS in 2009 and as yet unpublished) of nevirapine and its metabolites in 10 African American healthy volunteers sampled for 13 days after a single 200 mg nevirapine dose confirmed that 12-hydroxynevirapine was the main metabolite, with 2- and 3-hydroxynevirapine as well as 4- carboxynevirapine also detected, but no 8- hydroxynevirapine [30]. There was considerable variability in nevirapine clearance, area under the curve (AUC) and half life ($T_{1/2}$) of nevirapine metabolites [30]. The authors speculated that this variability may be due to genetic polymorphisms of the CYP isoenzymes responsible for nevirapine metabolism [30].

A further analysis by this group, presented at CROI in 2012, compared nevirapine metabolite profiles from the above single dose study with metabolite concentrations at steady state in 10 HIV-infected Cambodians on nevirapine-based ART, (duration of treatment unspecified in the abstract). There was a marked decrease in the metabolic index (ratio of metabolite to nevirapine area under the curve (AUC)) of 2-hydroxynevirapine when comparing single dose to steady state, from a median of 0.25 (IQR 0.12, 0.66) to 0.04 (IQR 0.03, 0.05). As 2-hydroxynevirapine is produced exclusively by CYP3A4, this finding suggests that CYP3A4 is being inhibited by nevirapine at steady state in this study population. The metabolic index for 3-hydroxynevirapine doubled, in keeping with induction of CYP2B6. These investigators did not find a significant change in the metabolic index for 12-hydroxynevirapine, which is produced by CYP3A4, CYP2D6 (considered to be uninducible) and CYP2C9, as discussed above. This suggests that nevirapine auto-induction primarily affects CYP2B6 [31].

Effect of rifampicin or rifampicin-based TB treatment on nevirapine pharmacokinetics

I identified 20 studies, of which 6 were only available in abstract form from conference proceedings [13, 32-50]. Characteristics, methods and major findings of the 20 studies are summarised in Table 1-4. Three studies explored the interaction between nevirapine and rifampicin; table 1 [32-34]. In one study, the majority of participants on rifampicin-based TB treatment [41], and in the remaining 16 studies the comparison was nevirapine with and without rifampicin-based TB treatment [13, 35-40, 42, 43, 45-50].

The study findings as well as weaknesses and strengths are discussed in more detail below. The studies are presented in order of publication/conference presentation under each sub-heading.

Studies exploring the pharmacokinetic interaction between nevirapine with rifampicin (Table 1)

An unpublished study presented at the International AIDS conference in 1998 (with the primary aim of characterising the effect of nevirapine on rifampicin concentrations) compared nevirapine concentrations in 22 HIV infected individuals on nevirapine to historical controls (number unspecified) [32]. Participants were started on rifampicin 600 mg daily, and then received nevirapine for 28 days (200mg daily lead in for 14 days, then 200 mg 12 hourly) before pharmacokinetic sampling for nevirapine concentrations. Nevirapine C_{min} was 68% lower in the presence of rifampicin. Rifampicin peak concentration (C_{max}) and area under the curve (AUC) were not affected by nevirapine. This is a weak study design to explore the effect of rifampicin on nevirapine pharmacokinetics. Results are likely to be confounded by other covariates, and the study has never been published or subjected to peer review.

Thirteen HIV-infected Indian participants on nevirapine-based ART for more than a month were intensively sampled before commencing rifampicin 450-600 mg daily, and again one week after [33]. In the presence of rifampicin nevirapine median C_{min} decreased by 53%, with a 46% decrease in median AUC. Nevirapine C_{min} was sub-therapeutic (<3mg/L) in 8 patients, and daily dose of nevirapine was increased by 50% to 300 mg 12 hourly in 7 of these participants, resulting in therapeutic concentrations in all 7.

Twelve Indian healthy male volunteers were administered a single dose of nevirapine, and intensive PK sampling up to 336 hours was performed [34]. They were then administered 7 days of rifampicin, and intensive sampling was repeated after a second nevirapine dose. There was a 79% decrease in AUC_{0-t} and a 60% decrease in concentration at 24 hours (C_{24}) in the presence of rifampicin. This is a single dose study, and may not reflect the extent of the interaction once nevirapine auto-induction has taken place.

Table 1: Studies exploring pharmacokinetic interaction between nevirapine (NVP) and rifampicin (RMP)

First author (publication date) site	Number of participants	Study population	Weight kg Mean ±SD or median (IQR)	RMP dose	Study design	Change/difference in NVP PK parameters
Robinson (abstract 1998, unpublished)	22 on NVP –ART Number of controls not given	HIV-infected Historical controls (no details given)	not specified	600 mg daily	RMP for 14 days RMP + NVP-ART for 28 days Intensive PK sampling day 14 (RMP) and 42 (RMP + NVP). Sampling schedule not stated.	C_{min} 68 % ↓ in the presence of RMP. No significant change in RMP C_{max} and AUC with NVP
Ramachandran (2006) India	13	HIV-infected on NVP-based ART	mean (range): 58 (38, 91)	450-600 mg daily	Sampled before commencing RMP and again after 7 days of RMP Intensive sampling 0.5, 1, 1.5, 2, 4, 6, 12 hrs after dosing. Within-participant comparison. Dose of NVP ↑ 50% if Cmin < 3 mg/L, and 1 and 12 hr conc day 0 and day 7 of RMP	Mean C_{max}: 42% ↓* Median C_{min}: 53% ↓* Median AUC: 46% ↓* C_{min} mean ±SD mg/L on standard dose NVP: 2.6 ±1.4 with RMP, 5.5 ± 2.4 without RMP Cmin>3 mg/L in 7/7 with NVP dose ↑
Pujari (abstract 2006, unpublished) India	12	Healthy volunteers	median 51, no range given	450-600 mg daily	Single dose of nevirapine Intensive sampling 0.5, 1, 1.5, 2, 4, 6, 12, 24, 48, 72, 336 hrs after dosing. 7 days of RMP. Intensive sampling then repeated.	Mean C_{max}: 20% ↓ (p=0.13) Mean C_{24}: 60% ↓* Mean AUC: 79% ↓*

*p value<0.05

Abbreviations: conc concentration, DOT directly observed therapy, GMR geometric mean ratio, IQR interquartile range, NVP-ART nevirapine –based antiretroviral therapy, RMP – rifampicin, RMP-TBT rifampicin-based TB treatment , SD standard deviation, TB tuberculosis, TBT TB treatment ,C_{min} trough concentration

Studies with intensive sampling exploring the pharmacokinetic interaction between nevirapine and rifampicin-based TB treatment (Table 2)

Ribera et al conducted a small pharmacokinetic study in HIV and TB co-infected patients in Spain, published in 2001 [35]. Five patients taking ART including nevirapine 200mg 12 hourly (duration of ART not stated) had intensive sampling performed before initiating rifampicin-based TB treatment, and again 12 or more days after TB treatment initiation. Results of this study suggested that concomitant rifampicin-based TB treatment increased nevirapine elimination and decreased nevirapine exposure. There was a 42% decrease in the median AUC in the presence of rifampicin-based TB treatment. The study was underpowered, and although the median C_{min} decreased by 20%, the change was not statistically significant. Of note, all 5 participants had a C_{min} of less than 3mg/L (range 1.1-2.8 mg/L) in the presence of rifampicin-based TB treatment, and 4/5 had concentrations below 3 mg/L before TB treatment. These low concentrations in a small sample may be due to chance, or reflect poor adherence. This study also included a comparison of rifampicin pharmacokinetic parameters between this group and 5 participants on rifampicin-based TB treatment only, and found no significant differences.

We performed intensive pharmacokinetic sampling in 16 South African HIV and TB co-infected participants concomitantly treated with nevirapine-based ART and rifampicin-based TB treatment [13]. Participants were sampled during the continuation phase of TB treatment, and after TB treatment completion. We found that rifampicin-based TB treatment significantly decreased nevirapine plasma concentrations in South African participants. The extent of reduction in C_{min} varied between participants, with a mean decrease in C_{min} of 26% (95% CI 6, 46). Nevirapine concentrations were sub-therapeutic (<3mg/L) in 6/16 participants during TB treatment, and none after TB treatment completion. The ratio of nevirapine AUC to 12-hydroxynevirapine AUC was significantly lower in the presence of TB treatment, consistent with induction of nevirapine metabolism. Half of participants had a rifampicin C_{max} lower than the recommended reference range [51], in keeping with other data in South African HIV and TB co-infected patients [52].

After our study was published, 4 further intensive pharmacokinetic studies in African patients were presented.

Matteelli et al conducted a study in 16 HIV and TB co-infected patients in Burkino Faso [36]. Participants were on rifampicin-based TB treatment, and pharmacokinetic sampling was performed 4 and 12 weeks after NVP-based ART initiation and again 4 weeks after TB treatment completion. Nevirapine concentrations at 4 weeks of ART were similar to those seen in our study, C_{min} median (range) 3.8 (0.9, 9.8) mg/L and $AUC_{(0–12)}$ median (range) 43.7 (19.6, 134.2)mg/h/L. The C_{min} geometric mean ratio comparing week 4 to post TB treatment was 0.67 (90% CI 0.64, 0.90). However, the difference between pharmacokinetic parameters was less when comparing week 12 to post TB treatment values (see table 2 for details).These investigators suggest that rifampicin induction may wane over time, causing nevirapine concentrations to increase from 4 and 12 weeks in their study participants. However this hypothesis is at odds with our study findings- we sampled patients later in TB treatment than in this study, during the continuation phase of TB treatment and found a bigger difference in nevirapine C_{min} with and without TB treatment than their week 12 results suggest. The investigators also suggest that nevirapine concentrations increased with improved absorption and recovery. However, this does not explain why the differences they found comparing week 12 to post

TB treatment are less than those observed in our study, as our participants would already have recovered with any attendant improvement in absorption. Participants in Matteelli et al's study had advanced disease and 4 of the 16 died. (Two participants died during TB treatment, one before 12 weeks. Two died after TB treatment completion, one of them before the post-TB sampling occasion.) The number of participants included in each comparison is not stated in the paper. The statistical methods used were for paired samples, therefore participants with data available at only one time point could not have been included the comparisons, and should not have been included in the estimates at each time point. It is possible that the difference between 4 and 12 week concentrations is in part due to participant attrition (at least 1 participant had died before the 12 week time point, and a further 1 or 2 before the post TB time point).

A pharmacokinetic substudy of the CARINEMO RCT intensively sampled 20 Mozambican participants (taking rifampicin based TB treatment and initiated on nevirapine 200 mg with no lead-in dosing) at 2 and 4 weeks after ART initiation, and 16/20 participants a month after TB treatment completion [37]. This study found that 19/20 had nevirapine C_{min} > 3 mg/L at 2 weeks, and all were therapeutic at 4 weeks. Mean ± SD C_{min} concentration was 4.9 ± 1.8 mg/L with TB treatment and 5.7 ± 1.8 mg/L without TB treatment; a 14% decrease. Of interest, C_{min} concentrations were higher than those found in our study. This difference may be due to chance or the fact that our participants had higher body weight with mean weight of 66 ± 17 kg in our study versus a median of 53 kg (IQR 48, 56) in the Mozambican study.

In Malawi, nevirapine concentrations were measured in 20 patients stable on nevirapine-based ART before and 14 days after initiation on rifampicin-based TB treatment [38]. There was a 22% decrease in geometric mean AUC in the presence of TB treatment, and 6/20 (30%) had sub-therapeutic nevirapine C_{min} on rifampicin-based TB treatment.

Villani et al conducted a further study in Burkino Faso (the title in the abstract book specifying South African participants is an error) in 20 HIV-1 and TB co-infected participants on rifampicin-based TB treatment initiated on nevirapine-containing ART [39]. A similar study design to Mattelli et al was used, with intensive sampling at week 4 and 16 after initiation of nevirapine-based ART and again 4 weeks after TB treatment completion. In contrast to this group's previous study [36] C_{min} and AUC were similar at week 16 and week 4 (see table 2 for details), refuting this group's hypothesis regarding waning of rifampicin induction. In this study 15% of participants at 4 weeks and 35% at 16 weeks had C_{min}<3 mg/L, with none sub-therapeutic after TB treatment completion. Rifampicin concentrations were measured at week 4 and 16, and were similar at both time points. Median rifampicin C_{max} was 4.1 mg/L (IQR 1.3, 5.4) at 4 weeks, and 2.3 mg/L (IQR 1.5, 4.3) at week 16. These rifampicin concentrations are lower than those found in our study, and decrease between time points. This may be due to poor adherence, rifampicin auto-induction (which may not have been maximal at the first sampling point) or failure to adjust rifampicin doses as participants recovered and gained weight.

Table 2: Studies with intensive sampling exploring the pharmacokinetic interaction between nevirapine (NVP) and rifampicin-based TB treatment (RMP TBT)

First author (pub date) site	Number of participants	Study population	Weight kg mean ±SD / median (IQR)	RMP dose	Study design	Sampling schedule	Change/difference in NVP PK parameters
Ribera* (2001) Spain	10 (5 on NVP)	HIV + TB co-infected. 5 on NVP-ART; initiated on RMP- TBT. 5 initated on RMP-TBT only	not specified	600 mg daily	Sampled before commencing TB treatment and after 12 or more days of TB treatment Within-participant comparison NVP Between group comparison of RMP	0.5, 1, 1.5, 2, 4, 6, 12 hrs after NVP dose	Median C_{max}: 19% ↓** Median C_{min}: 20% ↓ Median AUC: 42% ↓** C_{min} median (range) mg/L: 1.8 (1.1, 2.8) with TBT, 2.3 (1.4, 3.4) without TBT
Cohen (2008) South Africa	16	HIV + TB co-infected; on RMP-TBT and NVP-ART	RMP-TBT: 66 ±17 After TBT: 69 ±16	450-600 mg daily, 5 days a week	Sampled during continuation phase of RMP-TBT and 10 days or more after TBT completion. Within-participant comparison	0.5, 1, 1.5, 2, 4, 6, 12 hrs after NVP dose	GMR (90% CI) on/after RMP-TBT C_{max}: 0.61 (0.49, 0.79) C_{min}: 0.68 (0.53, 0.86) AUC_{0-12}: 0.64 (0.52, 0.80) Mean C_{min}↓: 26% (95% CI 6, 46) C_{min} median (IQR) mg/L on/after TBT: 3.2 (2.8, 4.5)/ 4.4 (3.6, 6.9)**
Matteelli (2009) Burkino Faso	16	HIV + TB-co-infected. On RMP-TBT; initiated on NVP-ART	median 52 (range 33, 67)	450-600 mg daily	Intensive PK sampling 4 and 10 weeks after NVP initiation and 4 weeks after end of TBT Within-participant comparison	0, 1, 2, 4, 6, 12 hrs after NVP dose	C_{min} median (range) mg/L: a) NVP+ TBT 4 weeks 3.3 (0.6, 10.5) b) NVP + TBT 12 weeks 3.9 (1.0, 11.0)) c) NVP 4 weeks post TBT 4.2 (1.5, 8.3) a vs b p=0.86; a vs c p=0.01; b vs c p=0.17 median C_{min} 20% ↓; median AUC 26% ↓ (a vs c) median C_{min} 7% ↓; median AUC 8 % ↓ (b vs c) C_{min} GMR (a vs c) 0.69 (90% CI 0.58, 0.83) AUC GMR (a vs c) 0.73 (90% CI 0.60, 0.88)
Bonnet (abstract 2009, unpublished) Mozambique	20	HIV + TB-co-infected. On RMP- TBT; initiated on NVP-ART	53 (48, 56)	not stated	C_{min} 2 weeks after starting TBT Intensive sampling 4 weeks after starting TBT and 4 weeks after TBT completion Within-participant comparison	0.5, 1, 1.5, 2, 4, 6, 12 hrs after NVP dose	GMR (90% CI) without/ with RMP-TBT C_{max}: 1.13(0.99, 1.28) C_{min}: 1.14(0.99, 1.31) AUC_{0-12}: 1.20(1.02, 1.42) C_{min} mean ±SD mg/L on/ after RMP TBT 4.9 ±1.8 mg/L /5.7 ±1.8 AUC_{12}mean ±SD h.mg/L on / after TBT 70.05 ±20.96/ 80.03 ±24.05
Chaponda (abstract 2010, unpublished) Malawi	20 on NVP-ART initiated on RMP-based TBT	HIV + TB-co-infected. On NVP-ART; initiated on RMP-TBT.	Not stated	not stated	Intensive sampling before initiating TBT and after 14 days. C_{min} only day 3 and 7 Within-participant comparison	0, 1, 2, 4, 8 hrs after NVP dose C_{min} only day 3 and 7	22% ↓in geometric mean AUC
Villani (2012) Burkino Faso	20	HIV and TB-co-infected	Not stated	not stated	Intensive PK sampling 4 and 16 weeks after NVP initiation and 4 weeks after end of TBT Within-participant comparison	0, 1, 2, 4, 6, 12 hrs after NVP dose	C_{min} median (IQR) 4 wks/16 wks/post TBT mg/L 4.6 (3.2, 5.9) /3.5 (2.4, 5.1) /6.5 (4.1, 9.5) AUC median (IQR) 4 wks/16 wks/post TBT mg.h/L 65.8 (52.5, 94.9)/ 53.6 (39.5, 75.3) 91.2 (67.9,112.0)

* Changes in median PK parameters have been recalculated from values quoted in the paper; values for percentage change in the median in the paper are incorrect

**p value<0.05

Abbreviations: CI confidence interval, C_{max} peak concentration, C_{min} trough concentration, IQR interquartile range, GM geometric mean, GMR geometric mean ratio, NVP-ART nevirapine – based antiretroviral therapy, RMP – rifampicin, RMP-TBT rifampicin-based TB treatment , SD standard deviation, TB tuberculosis, TBT TB treatment

Studies with sparse sampling exploring the pharmacokinetic interaction between nevirapine and rifampicin-based TB treatment (Table 3)

A small study conducted in London in 1999 compared nevirapine C_{min} concentration in 7 co-infected patients on rifampicin-based TB treatment, dosed twice weekly, with 13 controls not on TB treatment [40]. This study found that TB treatment had little effect on nevirapine concentrations, with a non-significant 8% decrease in C_{min}. Mean ±SD C_{min} in participants on TB treatment was 4.3 ±1.5 mg/L.

Auter et al analysed nevirapine TDM requests in Thai patients on nevirapine-based ART and compared random concentrations in 74 patients taking concomitant rifampicin with 74 controls not taking rifampicin [41]. Method of selecting the controls is not described. Isoniazid was recorded as a concomitant medicine in 64/74 on rifampicin. Nevirapine C_{min} concentrations were 34% lower in the presence of rifampicin, with a mean ±SD concentration of 5.3 ±2.7 mg/L in the presence of rifampicin compared to 8.0 ±3.3 mg/L without rifampicin (95/128 participants had C_{min} concentrations). Trough concentrations in both groups are high, with only 9 C_{min} concentrations below a TDM target of 3.1mg/L, 7 of these in the rifampicin group.

Manosuthi et al compared nevirapine concentrations in two groups of Thai patients, 70 with and 70 without rifampicin-based TB treatment [43]. The mean nevirapine C_{min} concentration was 18% lower in the presence of TB treatment. After 8 weeks of ART 30% of participants on TB treatment and 7% of participants not on TB treatment had nevirapine concentrations below 3.4 mg/L. In a second publication, nevirapine concentrations in the group on rifampicin-based TB treatment were repeated after completion of TB treatment, and nevirapine C_{min} was 15% lower in the presence of TB treatment by this within-participant comparison [42].

Stöhr et al retrospectively analysed data extracted from the Liverpool TDM registry and UK collaborative HIV cohort on 179 patients on nevirapine-based ART [44]. On multivariate analysis they found that black race (39% higher), concomitant rifampicin-based TB treatment (40% lower), concomitant tenofovir (22% higher), and concomitant protease inhibitor (28% higher) were independent predictors of nevirapine concentrations

Uttayamakul et al measured nevirapine C_{min} concentrations in 59 Thai HIV and TB co-infected patients on rifampicin-based TB treatment at 6 and 12 weeks after ART initiation and 1 month after TB treatment completion [45]. Participants were genotyped for CYP2B6 and CYP3A4 polymorphisms. The CYP2B6 516G>T polymorphism had a T allele frequency of 30%, but only 2 (4%) were TT homozygotes (see table 3 for details of genotype frequency). The genotype distribution in this patient group is not in Hardy Weinberg equilibrium (calculated from genotype frequency, Chi squared= 3.96, p=0.047). This is not addressed by the authors, and may indicate a problem with the genotyping technique. These investigators found similar nevirapine concentrations on and off rifampicin-based TB treatment to those previously observed in Thai patients, with a 16% decrease due to rifampicin based TB treatment. The analysis by CYP2B6 516G>T was underpowered, and it is difficult to work out from the text which comparisons found significant differences, but concentrations of nevirapine were highest at all time points in the TT group (see table 3 for details). Data in this paper suggest that CYP2B6 516G>T genotype is an important determinant of nevirapine concentrations, and impaired CYP2B6 function in those with the T allele results in decreased nevirapine elimination even in the presence of rifampicin-based TB treatment. It is interesting to note that nevirapine concentrations in the TT homozygote group were

higher on rifampicin-based TB treatment at the first time point on rifampicin-based TB treatment than they were after TB treatment completion, although this study is underpowered to reach definitive conclusions, with only 2 TT homozygotes in the nevirapine group.

Sinha compared nevirapine C_{min} concentrations in 63 participants on nevirapine-based ART and rifampicin based TB treatment, with concentrations in 51 participants on nevirapine-based ART alone[46]. Concentrations were lower with TB treatment (although not significantly so; probably because the study was underpowered).

A cross sectional study of random nevirapine concentrations in 40 Indonesian patients on nevirapine-based ART, 16 of them on rifampicin based TB treatment, found that mean random concentration was 27% lower in the presence of rifampicin. Dose to sampling time is not stated. 1/24 participants had C_{min} < 3 mg/L on TB treatment[47].

Population pharmacokinetic analyses exploring the interaction between nevirapine and rifampicin-based TB treatment (Table 4)

A non linear mixed effects model, which included the data in our analysis, as well as additional intensive and sparse pharmacokinetic data from participants in Cape Town, was constructed by Elsherbiny et al. In this model, rifampicin-based TB treatment increased nevirapine clearance by 37% [48]. Elsherbiny et al simulated nevirapine plasma concentration profiles at nevirapine doses of 300 mg, 400 mg and 500 mg. These simulations suggested that a dose of 300 mg 12 hourly would result in C_{min} concentrations above 3mg/L in most patients from this population.

A second non linear mixed effects model constructed by Svensson et al, included our data, together with data from 2 additional data sets[50]. This analysis suggested that the predominant effect of rifampicin-based TB treatment was in decreased nevirapine bioavailability rather than increased clearance. This is a surprising finding, as nevirapine has low extraction due to metabolism at gut level, with bioavailability of 90% after single dose[19] and is a weak substrate of P-glycoprotein (which is induced by rifampicin)[50].The authors suggest nevirapine auto-induction may decrease bioavailability, so that rifampicin's effect on gut metabolism may be more marked than is expected from single dose studies. This requires further research. Seventeen percent of individuals in the model had reduced nevirapine clearance. Study participants were not genotyped, but it is possible that those with reduced clearance are TT homozygotes for the CYP2B6 516G>T polymorphism, with impaired CYP2B6 function. The prevalence of lower clearance is in keeping with the prevalence of this polymorphism previously found in the South African population[5].

An unpublished population pharmacokinetic analysis was conducted using intensively sampled data from 24 participants in Burkino Faso on rifampicin-based TB treatment initiated on nevirapine-based ART[49]. Participants were sampled 1 and 2 months after nevirapine initiation, and 1 and 6 months after TB treatment completion. These investigators found a 21% decrease in nevirapine clearance after stopping TB treatment. The change in clearance ranged between 0 and 44%, with 30% of participants having no change in clearance. The authors suggest that this variability may be due to CYP2B6 genetic polymorphisms.

Table 3: Studies with sparse sampling exploring the pharmacokinetic interaction between nevirapine (NVP) and rifampicin-based TB treatment (RMP TBT)

First author (publication date) site	Number of participants	Study population	Weight kg Mean ±SD or median (IQR)	RMP dose	Study design	Change/difference in NVP PK parameters
Dean (1999) London	7 on NVP-ART and RMP-based TBT 13 on NVP + nelfinavir + 2 NRTIs	HIV + TB co-infected Comparator group are HIV-infected	not specified	600 mg twice weekly	Between group comparison of C_{min}	Mean $C_{min.}$ ↓ 8% (p=0.54)
Autar (2005) Thailand	74 on RMP + NVP-ART 74 on NVP- ART	HIV-infected Indication for RMP not described.	RMP: 54 (49,61) Controls: 58 (51, 63)	64/74 on RMP and isoniazid. (450-600 mg daily)	Analysis of results of NVP TDM requests. Single samples at random time-points within dosing interval. 50/ 74 C_{min} on RMP, 45/74 C_{min} in control group	Mean C_{min} ↓ 34%* C_{min} mean ±SD mg/L: 5.3 ±2.7 with RMP, 8.0 ±3.3 without RMP
Manosuthi (2006) Thailand	70 on RMP- TBT + NVP–ART 70 on NVP– ART	HIV + TB co-infected Comparator group are HIV infected	Mean 54. No SD given.	RMP-based TBT (450-600 mg daily)	C_{min} 12 hrs after DOT at 8 and 12 weeks after initiation of ART Between group comparison	Mean C_{min} ↓18% * C_{min} mean ±SD mg/L: 5.4 ±3.5 with RMP, 6.6 ±3.1 without RMP
Manosuthi (2007) Thailand	70 on RMP- TBT + NVP–ART	HIV + TB co-infected Comparator group HIV infected	TB: 55 ±9 Controls: 54 ±10	RMP-based TBT (450-600 mg daily)	Within-participant comparison of C_{min} 12 hrs after DOT at 8 and 12 weeks and C_{min} after completion of TBT (interval from end of TBT to sampling not stated)	Mean C_{min} ↓15% * C_{min} mean ±SD mg/L: 5.4 ±3.5 with TBT, 6.4 ±3.4 without TBT
Stohr (2008) London	10 on NVP-ART and RMP-based TBT 169 on NVP-ART	HIV + TB co-infected Comparator group HIV infected	71 (65, 78)	Not stated	Retrospective analysis of results of NVP TDM requests	NVP conc predictors: black race (39% ↑), concomitant rifampicin (40% ↓), concomitant tenofovir (22% ↑), concomitant protease inhibitor (28% ↑)
Uttayamakul (2010) Thailand	59 on RMP-TBT initiated on NVP-ART GG: 26 (44%) GT: 31 (53%) TT 2(3%)	HIV + TB-co-infected	54 ±9	RMP-based TBT (450-600 mg daily)	Between participant comparison, by CYP2B6 516G>T genotype. Within participant comparison on and off TBT C_{min} measured at week 6 and 12 of ART and 1month after TBT completion	C_{min} mean ±SD wk6 / wk 12/after TBT mg/L GG 5.4 ±0.5/ 5.3 ±0.5/ 6.4 ±0.6 GT 5.7 ±0.5/ 5.6 ±0.5/7.03 ±0.6 TT 14 ±9.5/ 7.9 ±2.8/ 9.4 ±0.2 Whole group 5.8 ±4/5.7 ±2.6/ 6.8 ±3.4* NVP 16% ↓ with RMP-based TBT
Sinha (2011) India	63 on RMP- TBT + NVP–ART 51 on NVP–ART	HIV + TB-co-infected	TB group 48 ±8 Comparator group 51 ±10	Not stated	C_{min} measured in the morning at 2 weeks, 4 weeks, 42 days and 6 months	C_{min} mean ±SD mg/L TBT vs control group: 2 wks 2.2 ±1.5 vs. 3.3 ±5.0 (p = 0.10) 4wks 2.8 ±1.6 vs. 3.7 ±3.6 (p = 0.08) 42 days 3.1 ±3.3 vs. 4.0 ±2.6 (p = 0.10)
Nafraidi (2012) Indonesia	16 on RMP- TBT + NVP-ART 24 NVP- ART	HIV + TB-co-infected Comparator group HIV-infected	TBT 52 ±9 Comparator group 59 ±9	RMP-based TBT Dose of RMP not stated	Cross sectional study comparing random NVP concentrations between the groups. Dose to sampling times not given.	NVP mean ±SD mg/L NVP 7.5 ±2.2 ; NVP + TBT 5.5 ±2.7* Mean random NVP conc 27% ↓ with TBT

*p value<0.05

Abbreviations: DOT directly observed therapy, IQR interquartile range, NVP-ART nevirapine –based antiretroviral therapy, RMP – rifampicin, RMP-TBT rifampicin-based TB treatment , SD standard deviation, TB tuberculosis, TBT TB treatment ,C_{min} trough concentration

Table 4: Population pharmacokinetic analyses exploring the interaction between nevirapine (NVP) and rifampicin-based TB treatment

First author (publication date) site	**Number of participants**	**Study population**	**Weight kg Mean (SD) or median (IQR)**	**RMP/RMP-based TBT (RMP dose)**	**Study design**	**Change/difference in NVP PK parameters**
Elsherbiny (2008) South Africa	27 on NVP-ART and RMP-based TBT 26 on NVP-ART	HIV + TB co-infected Comparator group are HIV-infected	Median 68 (range 43, 105)	RMP-based TBT (450-600 mg daily)	Nonlinear mixed effects modelling, including intensively and sparsely sampled participants Those with TB sampled on and after TBT	NVP clearance ↑37% with RMP-based TBT
Regazzi (abstract 2010, unpublished) Burkino Faso	24 on RMP- TBT initiated on NVP-ART	HIV + TB-co-infected	Not stated	RMP-based TBT Dose not stated	Sampled at 1 month and 2 months after initation of NVP, and 1 and 6 months after TBT completion Sampled 0, 1, 2, 4, 6, 12 hrs after NVP dosing Population pharmacokinetic analysis performed using P-PHARM software	21% ↓in nevirapine clearance without RMP-based TBT
Svennson (2012) South African	27 on NVP-ART and RMP-based TBT 88 on NVP-ART	HIV + TB co-infected Comparator group are HIV-infected	TBT median 67 (range 43-102)	RMP-based TBT (450-600 mg daily)	Nonlinear mixed effects modelling, including intensively and sparsely sampled participants Those with TB sampled on and after TBT Data from 3 studies included in the model	NVP bioavailability ↓ 61% by RMP=based TBT 17% of study population have reduced nevirapine clearance

Abbreviations: IQR interquartile range, NVP-ART nevirapine –based ART, RMP – rifampicin, RMP-TBT rifampicin-based TB treatment, SD standard deviation, TB tuberculosis, TBT TB treatment

Table 5: Assessment of study quality

First author (date if > 1 study)	HIV and TB co-infected?	Sample size calculation?	Weight change/ difference between groups reported?	Rifampicin and isoniazid administered?	Doses used in clinical practice?	14 days of administration before each PK sampling?	Timing of drug administration reported for all relevant drugs?	Within participant comparison	Geometric mean and 90% CI?
Robinson	U	N	N	N	Y	Y	N	N	N
Ramachandran	Y	N	NA	N	Y	N	Y	Y	N
Pujari	N	N	N	N	N	N	N	Y	N
Ribera	Y	N	NA	Y	Y (I)	N	N	Y	N
Cohen	Y	Y	Y	Y	Y (I)	N	Y	Y	Y
Matteelli	Y	N	N	Y	Y (I)	Y	N	Y	Y
Bonnet	Y	N	N	Y	U	Y	N	Y	Y
Chaponda	Y	N	N	Y	U	Y	N	Y	N
Villani	Y	N	N	Y	U	Y	N	Y	N
Dean	Y	N	N	Y	N	U	N	N	N
Autar	Y	N	Y	Y	Y (I)	U	N	N	N
Manosuthi (2006)	Y	Y	Y	Y	Y (I)	Y	N	N	N
Manosuthi (2007)	Y	N	N	Y	Y (I)	U	N	Y	N
Stohr	Y	N	N	Y	U	U	N	N	N
Uttayamakul	Y	N	N	Y	Y (I)	Y	N	Y	N
Sinha	Y	N	Y	Y	U	Y	N	N	N
Nafrialdi	Y	Y	Y	Y	U	U	N	N	N
Elsherbini	Y	NA	N	Y	U	N	U	Y	NA
Regazzi	Y	NA	NA	Y	U	Y	U	Y	NA
Svennson	Y	NA	NA	Y	U	N	N	Y	NA

Abbreviations: I dose of isoniazid not reported, NA not applicable, N no, Y yes, U unclear

Assessment of quality of included studies

My assessment of the quality of included studies, based on the 10 criteria outlined in the methods above, is presented in table 5. The studies appear in table 5 in the same order as in the text and tables 1-4 above.

Most studies were conducted in HIV-infected participants. In the majority of studies rifampicin-based TB treatment rather than rifampicin alone was studied. Most studies conducted pharmacokinetic sampling after 14 or more days of nevirapine, or nevirapine plus rifampicin/rifampicin-based TB treatment administration, with the second sampling occasion at least 14 days after adding or removing rifampicin rifampicin-based TB treatment. In the remaining 6 studies, the time interval ranged from 7 to 12 days.

Only 3/17 studies for which this criterion was relevant presented a sample size calculation. Only 4 studies reported on weight changes between sampling occasions, or weight differences between groups. Only 2 studies clearly reported the timing of administration of nevirapine as well as rifampicin or rifampicin-based TB treatment. Nine studies reported doses of nevirapine and rifampicin that are used in clinical practise, but none of the 17 studies where both rifampicin and isoniazid were administered as components of TB treatment reported the isoniazid dose. Only 3/17 for which this criterion was relevant presented geometric mean ratios with a 90% confidence interval in the results.

Summary of findings to date

Nevirapine concentrations are reduced by concomitant rifampicin, but nevirapine does not affect rifampicin concentrations. The reduction in nevirapine concentrations is greater with rifampicin alone than when rifampicin is administered in combination with isoniazid as part of a TB treatment regimen. Nevirapine C_{min} concentrations differ between populations, with Thai and Indonesian patients having higher concentrations than African, Indian and Spanish patients, both with and without rifampicin/ rifampicin-based TB treatment. Modelling suggests that a 50% dose increase in nevirapine dose when administered with rifampicin-based TB treatment may be appropriate in African patients. Polymorphisms of CYP isoenzymes, specifically the CYP2B6 516G>T polymorphism, influence nevirapine concentrations, and may modify the interaction between nevirapine and rifampicin-based TB treatment.

Gaps in current knowledge and questions for future research

Differing effects of rifampicin alone, and rifampicin administered with isoniazid

The first studies exploring co-administration of nevirapine with rifampicin did not distinguish between administration of rifampicin alone and co-administration of rifampicin with other drugs used in TB treatment, particularly isoniazid. Comparison of study results suggests that this approach was overly simplistic. Results of studies where nevirapine is administered with rifampicin differ from those with rifampicin-based TB treatment. Studies with rifampicin alone found a 53-68% decrease in C_{min} [32-34], and a 46-79% decrease in AUC [33, 34]. In contrast, studies with rifampicin-based TB treatment found more modest decreases in nevirapine concentrations, with C_{min} decrease ranging from 7 to 34% [13, 35-43, 45-50] and decrease in AUC ranging from 22 to 42%[13, 35, 36, 38, 39]. This difference in the magnitude of the interaction observed is likely to be due to the presence of isoniazid in TB treatment regimens. Isoniazid has been shown *in vitro* to be a potent mechanism-based inhibitor of CYP3A

isoenzymes at concentrations achieved by therapeutic doses [8, 9]. Therefore changes observed in nevirapine pharmacokinetics are likely to result from the combined effect of rifampicin, which causes gene up-regulation and increased CYP3A expression, and isoniazid, which inhibits functioning of CYP3A. The extent of the inhibitory effect of isoniazid is dose dependent, and may therefore vary with acetylator status, as slow acetylators have slower isoniazid metabolism and higher isoniazid concentrations [8] In addition, other covariates, such as weight, changes in weight in studies with within-patient repeated measures, and pharmacogenetic factors are also likely to have bearing on the magnitude of changes observed, yet these factors were not always taken into account in analyses to date. The possibility that rifampicin auto-induction, (which takes 40 days to reach full auto-induction) [25] also contributes to interactions has also not been taken into account in studies to date.

A South African pharmacokinetic substudy of an isoniazid preventative therapy RCT, including 21 HIV-infected participants on nevirapine-based ART, compared nevirapine concentrations between the 8 participants randomised to isoniazid and the 13 randomised to placebo [53]. In this study median nevirapine AUC was 24% higher in the presence of isoniazid (AUC_{0-12} median (IQR) mg·h/l 62.5 (52.9, 87.8) without and 77.8 (56.6, 85.5) with isoniazid). Median C_{min} was 33% higher in the presence of isoniazid (C_{min} median (IQR) mg/L 4.5 (3.9, 6.2) without and 5.9 (4.0, 6.5) with isoniazid). The study was underpowered, and these differences were not significant (p=0.66 and p=0.61 respectively). An adequately powered study is needed to confirm or refute the hypothesis that inhibition of CYP3A4 by isoniazid decreases nevirapine metabolism. Ideally this should be demonstrated with a within-participant, rather than between-participant, comparison of nevirapine pharmacokinetic parameters in the presence of isoniazid. In addition, further studies are required to characterise the effects of rifampicin alone compared to rifampicin with isoniazid. This would ideally require a steady state, within participant comparison of nevirapine concentrations. Nevirapine concentrations should be compared at 4 points: when administered with rifampicin alone, with isoniazid alone, with rifampicin and isoniazid, and without either drug. However, this design poses ethical challenges, as the study would need to be conducted in HIV-infected participants, because of safety concerns with repeated dosing of nevirapine in healthy volunteers[19]. Rifampicin administered with nevirapine at standard doses without isoniazid is likely to cause sub-therapeutic nevirapine concentrations, which may result in resistance and compromise future ART options.

Such studies would need to include characterisation of acetylator status of participants, and take into account other important covariates such as weight, and genetic polymorphisms affecting nevirapine metabolism by CYP450 isoenzymes.

Optimal dosing of nevirapine with rifampicin-based TB treatment

The optimal dose of nevirapine when administered with rifampicin-based TB treatment is the most important question to inform clinical practice, as nevirapine at standard doses is associated with poorer virological outcomes in African patients on rifampicin-based TB treatment [4, 12]. Nevirapine is likely to remain part of treatment regimens because of cost constraints and contraindications to efavirenz in some patients. The question of optimal dosing of nevirapine with TB treatment in resource-limited settings, and particularly in African patients therefore needs to be resolved.

Eighteen Ugandan participants on rifampicin-based TB treatment (not included in the literature review above as participants were only sampled on rifampicin-based TB treatment, with no comparator group off TB treatment) were randomised to 200 mg daily or 200 mg 12 hourly for the first 2 weeks of ART[54]. This study confirmed that nevirapine concentrations are sub-therapeutic with once daily lead-in dosing, with a geometric mean C_{min} 1.5 (90% CI 1.1, 2.1) mg/L in the lead-in group. This is in keeping with other studies[10, 11], and lead-in nevirapine dosing is no longer recommended with TB treatment[1]. However, the question of dosing beyond the lead-in period has not been resolved. The Ugandan RCT continued all study participants on nevirapine 200 mg 12 hourly, and found that C_{min} concentrations were less than 3 mg/L in 64% of participants in this study at day 21, after 7 days of full dose nevirapine[54]. African studies included in this review found that 30 to 38% of participants had sub-therapeutic nevirapine C_{min} [13, 36, 38, 39]. A 50% nevirapine dose increase to 300 mg 12 hourly may therefore be appropriate in African patients, as modelling of South African data suggested [48].

A Thai study explored increasing nevirapine doses, and randomised 32 Thai patients on rifampicin-based TB treatment to receive standard dose (nevirapine 200 mg once daily for 14 days, then 200 mg twice daily); or high dose nevirapine (200 mg twice daily followed by 300 mg twice daily) [10]. However, this study was stopped by the Drug Safety Monitoring Board (DSMB) because 4 patients in the high dose group and 1 in the standard dose group developed hypersensitivity reactions. Little clinical detail regarding the hypersensitivity reactions is given, and it is unclear why the DSMB stopped the study, as the difference in proportion with hypersensitivity in the 2 groups is not significant (Fishers exact p value (calculated from the data) = 0.33). Thai patients have smaller body weights and higher nevirapine concentrations than African patients so any risk of adverse reactions with higher doses may not apply to African patients. To date, no African studies have been conducted exploring safety and efficacy of nevirapine administered at a dose of 300 mg daily in patients treated with rifampicin-based TB treatment. Such a study is needed to inform clinical practice. It is possible that different dosing guidelines might be needed for different populations, because of differences in important covariates such as weight and pharmacogenetic factors.

Contribution of pharmacogenetic factors to variability in changes in nevirapine pharmacokinetics in the presence of rifampicin-based TB treatment

There is variability between individuals in the magnitude of the change in nevirapine concentrations when administered with rifampicin-based TB treatment. Pharmacogenetic factors such as CYP isoenzyme polymorphisms are likely to play a role in the variability observed. The CYP2B6 516G>T single nucleotide polymorphism, which is common in African patients, results in impaired 2B6 function, and other isoenzyme pathways are likely to predominate in nevirapine elimination in individuals who are TT homozygotes. Nevirapine concentrations were paradoxically higher with rifampicin-based TB treatment than without in Thai CYP2B6 G>T single nucleotide polymorphism TT homozygotes [45]. Polymorphisms which result in impaired CYP isoenzyme function may alter the predominant CYP isoenzyme utilised for drug elimination. Polymorphisms may therefore determine whether the effect of rifampicin or the effect of isoniazid on nevirapine concentrations predominates.

In addition, acetylator status may contribute to variability. Slow acetylator status is common, and 18% of South Africans were found to be slow acetylators [52]. The inhibitory effect of isoniazid on nevirapine metabolism via CYP3A may be greater in slow acetylators than fast acetylators. Further studies are needed to explore the combined effect of single nucleotide polymorphisms, and acetylator status. Such

studies would need to be adequately powered to reach definitive conclusions, and would ideally need to combine pharmacogenetic sampling with sequencing for single nucleotide polymorphisms, assessment of acetylator status, and measurement of nevirapine and nevirapine metabolite concentrations.

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Effect of rifampicin-based antitubercular therapy on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis

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***Background and objectives*: Nevirapine-containing antiretroviral therapy (ART) and rifampicin-based antitubercular therapy are commonly co-administered in Africa, where nevirapine is often the only available non-nucleoside reverse transcriptase inhibitor. Rifampicin induces the metabolism of nevirapine, but the extent of the reduction in nevirapine concentrations has varied widely in previous studies. We describe the steady-state pharmacokinetics of nevirapine during and after antitubercular therapy in South African patients.**

***Methods*: Sixteen patients receiving ART including standard doses of nevirapine were admitted twice for intensive pharmacokinetic sampling: during and after rifampicin-based antitubercular therapy.**

***Results*: Geometric mean ratios for nevirapine pharmacokinetic parameters during versus after antitubercular therapy were 0.61 [90% confidence interval (CI) 0.49–0.79] for C_{max}, 0.64 (90% CI 0.52–0.80) for area under the curve up to 12 h (AUC_{0-12}) and 0.68 (90% CI 0.53–0.86) for C_{min}. Nevirapine C_{min} was subtherapeutic (<3 mg/L) in six patients during antitubercular therapy (one of whom developed virological failure) and in none afterwards. There was no correlation between rifampicin concentrations and the degree of nevirapine induction assessed by the proportional change in nevirapine concentrations between the two admissions. The ratio of nevirapine AUC_{0-12} to the AUC_{0-12} of its 12-hydroxy metabolite was significantly lower in the presence of antitubercular therapy, consistent with induced metabolism.**

***Conclusions*: Nevirapine concentrations were significantly decreased by concomitant rifampicin-based antitubercular therapy and a high proportion of patients had subtherapeutic plasma concentrations. Further study in African patients is required to determine the implications for treatment outcomes.**

Keywords: pharmacokinetics, interaction, 12-hydroxynevirapine

Introduction

Tuberculosis (TB) is the leading cause of morbidity and mortality in HIV-infected patients in Africa.[1] As access to antiretroviral therapy (ART) expands, a substantial number of patients will require treatment for TB while receiving ART. Nevirapine-based combination ART is used extensively in resource-limited settings, where few alternative regimens are available. Nevirapine is metabolized by cytochrome P450 isoenzymes, predominantly CYP3A4 and CYP2B6, to the hydroxymetabolites 2-, 3-, 8- and 12-hydroxynevirapine.[2] Rifampicin is a key component of antitubercular regimens. Rifampicin induces the metabolism of many drugs, including nevirapine. Previous pharmacokinetic studies have shown a variable reduction in nevirapine trough concentrations with concomitant rifampicin, ranging from 10% to 68%.[3–6]

The largest studies of the interaction between nevirapine and rifampicin have been in Thai patients.[6,7] Despite some reassuring data suggesting that more than 86% of Thai patients on concomitant rifampicin-based antitubercular therapy attain therapeutic

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nevirapine concentrations,[8] their low body weight and slower nevirapine clearance[9] cast doubt on the generalizability of the results.

We report the steady-state pharmacokinetics of nevirapine during and after rifampicin-based antitubercular therapy in a group of South African patients with HIV-associated TB.

Materials and methods

Study design and setting

HIV-infected adults (≥18 years) on a combination ART regimen consisting of two nucleoside reverse transcriptase inhibitors and nevirapine who were in the continuation phase of rifampicin-based antitubercular therapy were recruited at a donor-funded (Médecins Sans Frontières) ART clinic in Khayelitsha, Cape Town, South Africa. Participants were admitted for intensive pharmacokinetic blood sampling while taking rifampicin-based antitubercular therapy, and again 10 days or more after completion of antitubercular therapy. Nevirapine was administered throughout at standard doses of 200 mg 12 hourly. Rifampicin was dosed at 600 mg 5 days a week in those weighing ≥55 kg and 450 mg for those <55 kg.

An estimated sample size of 16 participants had 80% power at a 5% level of significance to detect a 25% reduction in nevirapine C_{min} when administered concomitantly with rifampicin, calculated based on previously published pharmacokinetic parameters.[5] Patients were excluded if they had poor venous access, a Karnofsky score <70, known severe renal, hepatic or intestinal disease (malabsorption or severe diarrhoea), pregnancy, or were taking any other medication known to have a pharmacokinetic interactions with nevirapine. Adherence to ART and antitubercular therapy was assessed by means of self-report, using a structured questionnaire which recorded any doses of ART or antitubercular treatment omitted in the 4 days before admission. Haemoglobin, alanine transaminase (ALT) and albumin were measured on both admissions. CD4+ lymphocyte counts and quantitative HIV-1 RNA (viral loads) were obtained from the participants' 6 monthly routine monitoring results.

The protocol was approved by the research Ethics Committee of the University of Cape Town. All participants gave signed informed consent.

Pharmacokinetic assessment

An observed dose of nevirapine was given and the exact time of administration recorded. Antitubercular therapy was administered together with nevirapine at the first sampling occasion. Venous blood was collected at −0.5, 0.5, 1, 1.5, 2, 4, 6, 10 and 12 h on both admissions. The exact times of sampling were recorded. Blood was immediately centrifuged and the plasma was stored at −80°C until analysis.

Assay methodologies for quantifying nevirapine and 12-hydroxynevirapine were derived from a previously published method.[10] Plasma concentrations of nevirapine were determined by Liquid Chromatography Mass Spectrometry methods using a Waters Alliance 2690 High Pressure Liquid Chromatograph (HPLC) coupled to a Waters/Micromass ZMD single quadrapole mass spectrometer. The mobile phase consisted of 50% acetonitrile in 4 mM ammonium acetate and 0.1% trifluoroacetic acid. A 20 by 2.1 mm Hypersil Gold C18 column (Thermo) was used at a flow rate of 0.3 mL/min. Neostigmine served as internal standard. Detection in positive ionization mode of nevirapine was at 276.2 (*m/z*) and neostigmine at 223.2 (*m/z*). Acetonitrile (50 μL) containing 1 mg/L internal standard was added to 20 μL of each sample or control to precipitate protein. Samples were vortexed for 30 s, centrifuged for 5 min at 750 **g** and 2 μL of the supernatant was injected onto the column. The standard curve was linear in the range 0.2–20 mg/L. The lower limit of quantification (LLQ) was set at 0.2 mg/L.

Plasma concentrations of 12-hydroxynevirapine were quantified by tandem mass spectrometry using an Applied Biosystems API 3200 linear ion trap. HPLC was performed on an Agilent 12000 series instrument using a Gemini C18 3 μm particle size, 50 by 2.1 mm column (Phenomonex). The mobile phase comprised 15% acetonitrile and 85% ammonium formate. The flow rate was 0.3 mL/min and injection volume 5 μL. The following SRM transitions of [M-H]+ precursor ions to product ions were selected: 12-hydroxynevirapine *m/z* 283.2–265.2; physostigmine (internal standard) *m/z* 276.3–162.3. The internal standard was made up to a concentration of 0.5 mg/L in acetonitrile. One hundred microlitres of plasma was transferred to a 1.5 mL polypropylene tube and 300 μL of internal standard solution was added. After mixing for 10 s, the tube was centrifuged for 5 min at 750 **g**. Supernatant (10 μL) was transferred to a new 1.5 mL tube and 1000 μL of the mobile phase was added; 5 μL was injected onto the column. The standard curve was linear in the range 0.025–5 mg/L. LLQ was set at 0.025 mg/L.

Plasma concentrations of rifampicin were determined using an Applied Biosystems API 2000 tandem mass spectrometer, using a previously published method.[11] The mobile phase consisted of 50% methanol, 20% acetonitrile and 30% formic acid (0.1%). A 20 by 2.1 mm Hypersil Gold C18 column (Thermo) was used at a flow rate of 0.3 mL/min. Rifapentine served as internal standard. Detection of rifampicin in positive ionization mode was at 823.5–791.5 (*m/z*) and rifapentine at 877.27–845.30 (*m/z*). Acetonitrile (150 μL) containing 1 mg/L internal standard was added to 50 μL of each sample or control to precipitate protein. Samples were vortexed for 30 s, and centrifuged for 5 min at 750 **g**. Supernatant (2 μL) was injected onto the column. The standard curve was linear in the range 0.1–30 mg/L. LLQ was set at 0.1 mg/L.

Quality control samples covering the range of the standard curve were included with each assay run. Inter- and intra-day percentage coefficients of variation were <10% for all controls. The laboratory is a member of the Association for Quality Assessment in Therapeutic Drug Monitoring and Toxicology international inter-laboratory quality control programme.

Observed peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}) and minimum plasma concentration (C_{min}) in the dosing interval were determined by inspection of individual concentration–time curves. C_{min} was defined as the lowest concentration after T_{max}. Non-compartmental analysis was performed (using WinNonlin version 4, Pharsight Corporation, Mountain View, CA, USA) to calculate area under the curve to 12 h (AUC_{0-12}) using the linear trapezoidal rule with linear interpolation and half-life ($t_{1/2}$). The target minimum plasma concentration for nevirapine was ≥3 mg/L, based on the trough concentration recommended in current antiretroviral therapeutic monitoring guidelines.[12]

Statistical analysis

Statistical analysis was performed using STATA version 9.2 (Stata corp. College Station, TX, USA). Descriptive statistics of patient characteristics and pharmacokinetic data were summarized using means and standard deviations if normally distributed, and medians and interquartile ranges if non-normally distributed. Comparison of participant characteristics, laboratory results and pharmacokinetic parameters in the presence and absence of antitubercular therapy was performed using a paired *t*-test or Wilcoxon signed rank test. Geometric means and geometric mean ratios with 90% confidence intervals (CIs) were calculated for C_{max}, AUC_{0-12} and C_{min}. Spearman's rank correlation coefficients were calculated to explore correlation between nevirapine and rifampicin pharmacokinetic parameters.

Results

There were 16 participants (13 women) with a median age of 35 years (IQR 27–39). Median CD4 count closest to the first pharmacokinetic sampling was 115 cells/mm^3 (IQR 63–252). Twelve participants had World Health Organization stage 4 HIV disease. All participants were taking an ART regimen comprising nevirapine, lamivudine and either stavudine or zidovudine throughout. At the time of first pharmacokinetic sampling, all participants were taking rifampicin and isoniazid, and five participants were taking ethambutol. Median duration of ART at the time of first pharmacokinetic sampling was 165 days (IQR 114–221); 3/16 participants were established on ART before initiating antitubercular therapy and 13 participants had commenced ART during antitubercular therapy. Median time from completion of antitubercular therapy to the second admission for intensive sampling was 56 days (IQR 32.5–98.5). ALT was moderately elevated in 3/16 participants at the first admission and 4/16 participants at the second admission (less than four times the upper limit of normal in all instances). One participant reported having missed a single dose of nevirapine 2 days before the first admission. All other participants reported 100% adherence in the 4 days prior to both admissions.

Participant characteristics and pharmacokinetic parameters of nevirapine during and after antitubercular therapy are given in Table 1. Median weight gain between admissions was 2.5 kg (IQR 0.1–6.9). When participants were receiving antitubercular therapy, C_{max}, C_{min} and AUC_{0-12} were significantly lower. The mean reduction in nevirapine C_{min} in the presence of rifampicin-based antitubercular therapy was 26.3% (95% CI 6.4–46.3). Geometric mean ratios for nevirapine pharmacokinetic parameters during versus after antitubercular therapy were 0.61 (90% CI 0.49–0.79) for C_{max}, 0.64 (90% CI 0.52–0.80) for AUC_{0-12} and 0.68 (90% CI 0.53–0.86) for C_{min}.

Nevirapine concentration–time curves during and after antitubercular therapy are shown in Figure 1. Nevirapine C_{min} was subtherapeutic (<3 mg/L) in 6/16 participants taking rifampicin and in none after antitubercular therapy (Figure 2).

Three participants, with a nevirapine C_{min} while on antitubercular therapy of 1.3, 5.9 and 6.4 mg/L, respectively, had a detectable viral load (>400 copies/mL) within 6 months of the first admission. In all of the other participants, including five with a C_{min}<3 mg/L, viral loads measured during the 6 months after the first admission were suppressed (<400 copies/mL).

Median (IQR) AUC_{0-12} of 12-hydroxynevirapine was similar during and after antitubercular therapy at 3.2 mg·h/L (2.5–3.9) and 3.0 mg·h/L (2.6–4.1), respectively ($P = 0.5$). However, there was a significant change in the ratio of nevirapine AUC_{0-12} to the 12-hydroxy metabolite AUC_{0-12}, with a median ratio of 14.7 (IQR 12.1–18.0) in the presence of rifampicin-based antitubercular therapy and 20.4 (IQR 18.3–25.3) after rifampicin-based antitubercular therapy ($P = 0.0004$).

Median rifampicin C_{max} was 7.8 mg/L (IQR 5.9–9.6) and median rifampicin AUC_{0-12} was 34.7 mg·h/L (IQR 26.3–56.6). There was no evidence of an association between rifampicin AUC_{0-12} and the proportional change in nevirapine concentrations between the two admissions.

Table 1. Participant characteristics and nevirapine pharmacokinetic parameters for 16 participants intensively sampled during and after rifampicin-based antitubercular therapy

		Nevirapine during rifampicin-based antitubercular therapy	Nevirapine after rifampicin-based antitubercular therapy	*P* value[a]
Participant characteristics	mean weight kg (SD)	65.8 (16.6)	69.3 (16.0)	**0.014***
	median albumin g/L (IQR)	40.5 (35–44)	41 (39–45)	0.736**
	mean haemoglobin g/dL (SD)	12.1 (0.6)	12.5 (0.5)	0.261*
Pharmacokinetic parameter, median (IQR)	C_{max} mg/L	4.6 (3.9–6.1)	7.5 (5.7–10.2)	**0.003****
	C_{min} mg/L	3.2 (2.8–4.5)	4.4 (3.6–6.9)	**0.006****
	AUC_{0-12} mg·h/L	42.0 (37.4–60.0)	70.1 (52.9–95.2)	**0.005****
	$t_{1/2}$ h	26.2 (18.6–36.6)	21.7 (17.3–29.9)	0.234**
	T_{max} h	1.4 (1.1–1.8)	1.5 (1.0–1.8)	0.641**

*Paired *t*-test.
**Wilcoxon signed rank test.
[a]*P* values <0.05 are shown in bold.

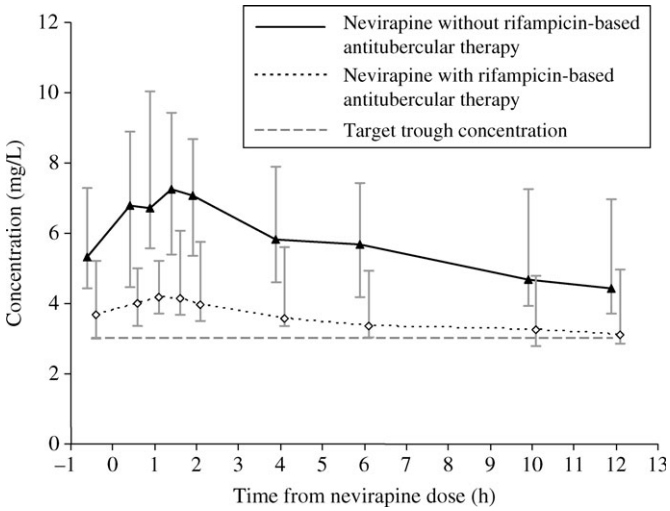

Figure 1. Nevirapine median concentration–time profile during and after rifampicin-based antitubercular therapy. Bars represent interquartile ranges.

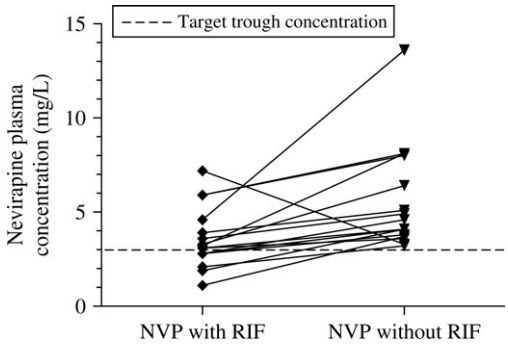

Figure 2. Nevirapine C_{min} in 16 participants during and after rifampicin-based antitubercular therapy.

Discussion

We found significant reductions in nevirapine C_{min}, C_{max} and AUC_{0-12} in participants while they were taking rifampicin-based antitubercular therapy. Nevirapine C_{min} concentration was below the recommended lower limit of 3 mg/L in 6/16 (38%) participants taking antitubercular therapy. Although five of these six participants had a good short-term virological outcome, this is nevertheless a worrying finding, as the trough concentration is the key pharmacokinetic parameter for efficacy.[12] We found a significant decrease in the ratio between the AUC_{0-12} of nevirapine and its inactive 12-hydroxymetabolite (produced primarily by CYP3A4[2]) in the presence of rifampicin-based antitubercular therapy. This indicates that the change in nevirapine pharmacokinetic parameters is due to enhanced metabolism of nevirapine by CYP3A4, with increased flux through the metabolic pathway in the presence of rifampicin. Half of the participants had rifampicin peak concentrations lower than the recommended reference range of 8–24 mg/L. This is in keeping with the findings of two recent African studies, which showed low rifampicin concentrations in a high proportion of TB patients,[13,14] which was associated with HIV infection in one study.[13] A previous pharmacokinetic study found that nevirapine did not affect rifampicin concentrations.[5]

The reduction in nevirapine trough concentrations with concomitant rifampicin has varied widely in previous studies. This variability is due in part to differing study designs. The largest reductions of nevirapine trough concentrations of 53%[6] and 68%[3] were found in HIV-infected patients without TB, who were receiving rifampicin without other concomitant antitubercular therapy. It is likely that the inducing effect of rifampicin is ameliorated by isoniazid, which is an inhibitor of CYP3A—the major cytochrome P450 isoenzyme involved in nevirapine metabolism.[15] In addition, CYP2B6 polymorphisms are known to influence nevirapine pharmacokinetics[16,17] and may influence the magnitude of the inducing effect of rifampicin; thus another source of variability in study results may be differences in the frequencies of CYP2B6 polymorphisms in different populations.

Two adequately powered Thai studies conducted in patients with TB reported reductions of nevirapine trough concentrations of 15.6%[18] and 37.2%,[8] which are similar to the 26.3% that we found. However, the mean trough nevirapine concentrations with concomitant rifampicin-based antitubercular therapy in the Thai studies were 5.5 and 5.4 mg/L,[8,18] considerably higher than the 3.2 mg/L that we found. The likely explanation for the higher nevirapine trough concentrations is their lower body weight (~10 kg lower than our patients' mean weight of 65.8 kg), given that nevirapine clearance is similar in Thai and South African patients.[9]

One approach to compensate for the reduction in nevirapine concentrations when co-administered with rifampicin is to increase the nevirapine dose. In an Indian study, a 50% dose increase, selectively given to a small group of seven patients who had subtherapeutic trough nevirapine concentrations when the standard dose was co-administered with rifampicin, resulted in trough concentrations in the therapeutic range.[6] However, given the variability in nevirapine concentrations, this dosing strategy may result in very high nevirapine concentrations in some individuals with resultant toxicity and requires further study.

The key question is whether the observed reduction in nevirapine concentrations with concomitant rifampicin results in an increased risk of virological failure. A retrospective Spanish study of 32 patients reported that 74% of patients treated with concomitant nevirapine- and rifampicin-based antitubercular therapy attained undetectable viral loads, but there was no control group.[19] A Thai cohort study of 70 patients on nevirapine-based combination ART and concomitant rifampicin-based antitubercular therapy found that virological suppression was similar to a control group,[7] with virological suppression rates remaining similar up to 60 weeks.[18] However, as mentioned above, Thai patients have lower body weight and higher trough nevirapine concentrations.

A limitation of our study is that the majority of the patients are women; there may be sex differences in the pharmacokinetics of both nevirapine and rifampicin. Although pharmacokinetic sampling was performed after observed drug dosing, dosing in the days prior to admission was not directly observed, and adherence cannot be guaranteed. Our study is also not powered to explore the association between nevirapine trough concentration and virological outcomes.

In conclusion, concomitant rifampicin-based antitubercular therapy significantly reduces nevirapine concentrations, and subtherapeutic trough nevirapine concentrations occur in a significant proportion of patients. Our data together with that from

other small studies suggest that virological responses are reasonable, but there is a need for larger cohort studies, particularly in sub-Saharan Africa.

Acknowledgements

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Transparency declarations

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Research protocol

This research protocol included 4 objectives. Objective 1 forms the subject of this research dissertation, namely, “to compare nevirapine pharmacokinetics with and without rifampicin-based antitubercular therapy, by means of non-compartmental analysis.”

Objectives 2, 3 and 4, and the additional data collected to meet those objectives, formed the basis of analyses performed by other investigators. Those analyses are therefore not presented in this research report.

Pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV and tuberculosis-infected patients.

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Introduction

Many HIV-infected patients in sub-Saharan Africa are co-infected with tuberculosis. Tuberculosis is a leading cause of morbidity and mortality in HIV-infected South Africans[1]. Antiretroviral access in sub-Saharan Africa is expanding. Highly active antiretroviral therapy (HAART) reduces the incidence of tuberculosis in HIV infected patients by more than 80% in Cape Town, South Africa[1], but a substantial number of patients will still present with active tuberculosis while on antiretroviral therapy(ART).

Treatment of tuberculosis with a rifampicin-based regimen, consisting of rifampicin, isoniazid, pyrazinamide and ethambutol (with the addition of streptomycin in patients who have previously had an episode of tuberculosis), is standard practice in South Africa[2]. However, when a patient on HAART is started on treatment for tuberculosis, the possibility of clinically significant drug interactions, potentially altering the pharmacokinetics of both antiretroviral and antimycobacterial drugs, must be taken into account[3] [4]. Interactions between non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors and rifamycins, as well as additive side effects and toxicities must be addressed when managing an HIV infected patient with tuberculosis.

Rifampicin is a potent inducer of cytochrome P450, isoenzyme 3A4. This results in enhanced drug metabolism and may lead to sub-therapeutic concentrations of non-nucleoside reverse transcriptase inhibitors and many protease inhibitors.[5] Nevirapine is metabolized by cytochrome P450, by isoenzymes 3A4 and also acts as an inducer of cytochrome P450 3A4[5, 6].

An antiretroviral regimen consisting of 2 nucleoside reverse transcriptase inhibitors (NRTIs), and either efavirenz or nevirapine, is the recommended first line ART regimen by the World Health Organisation for resource-limited settings and has been implemented in South Africa. Nevirapine-based ART is the 1st line regimen of choice in South Africa in women of reproductive potential who cannot guarantee contraception, because of the teratogenic potential of efavirenz. Nevirapine is a component of inexpensive generic twice-daily fixed dose ARV combinations that are used extensively in the developing world. Fixed dose combination therapy is likely to be widely implemented as it improves adherence. In sub-Saharan Africa, the high disease burden

of both HIV and tuberculosis means that many patients will require concurrent treatment with rifampicin and nevirapine.

There is limited data on the interaction between nevirapine and rifampicin. An underpowered pharmacokinetic study in 5 Spanish patients[7] treated with both nevirapine and rifampicin showed a 31% decrease in area under the curve (AUC), a 31% decrease in C_{max} and a 21% decrease in C_{min}, which was not statistically significant. Another study in 22 patients showed a 68% reduction in the clearance of nevirapine in the presence of rifampicin in comparison to historical controls.[15]

An observational study of 36 patients in Spanish hospitals treated with nevirapine and rifampicin showed good virological response in co-treated patients. However, 8 patients (22%) in this cohort developed significant toxicity, leading to discontinuation of one or both drugs in 5 patients. 4 patients developed hepatitis, which was thought to be due to rifampicin, and 4 patients presented with adverse effects thought to be due to nevirapine (1 rash, 1 rash with hepatitis, and 2 gastrointestinal disturbances).[8]

The recently updated Center for Disease Control and Prevention (CDC) guidelines [9] for treatment of tuberculosis in HIV-infected patients state that a regimen comprising of nevirapine and 2NRTIs may be used with rifampicin, although they comment that this is supported by limited data. The Western Cape antiretroviral treatment protocol gives the option of switching nevirapine to efavirenz when tuberculosis is diagnosed and anti-tuberculous treatment commenced, or continuing nevirapine with monthly liver function monitoring.[10]

Data from a population pharmacokinetic study of nevirapine[11] suggests that there may genetic variability in nevirapine clearance between ethnic groups. There are few data about the pharmacokinetics of nevirapine in South African patients, and the pharmacokinetics of nevirapine and rifampicin, when administered concomitantly, has not been studied in our population. There is no published data about the pharmacokinetics of isoniazid when administered with nevirapine. There is high inter-individual and inter-occasional variability in rifampicin kinetics, and the extent of exposure to rifampicin may be a determinant of the extent of rifampicin-inducible CYP3A4 expression[12]

More information about the pharmacokinetic interaction between nevirapine and rifampicin, and the impact of this interaction on treatment efficacy and adverse reactions in South African patients is urgently needed. The impact on pharmacokinetics of covariates such as gender is (known to be important determinant of pharmacokinetics for several drugs), body mass index, concomitant medication (including over the counter and herbal remedies) concomitant recreational substance use or abuse and concomitant illness needs to be explored.

In addition to non-compartmental analysis, a population approach using nonlinear mixed-effect modeling will be used in this study. This approach allows the use of sparse sampling to predict individual pharmacokinetic measures from a structural model describing the drug's pharmacokinetics in the population. Furthermore, it is a powerful tool to account for the pharmacokinetic effect of measured covariate factors (fixed effects) and random (unexplained) effects which together comprise intra- and inter-subject pharmacokinetic variations[13,14].

Pharmacokinetic assessments are limited by the need to transport blood samples in a good condition to the laboratory. This must be done rapidly or by freezing the samples. This is impractical and expensive. During this study a method will be validated for determining nevirapine concentrations on dried blood spots that can be stored and transported inexpensively.

Study aim

To describe nevirapine pharmacokinetic parameters in South African HIV-infected patients taking nevirapine based antiretroviral therapy, in the continuation phase of rifampicin- based short course chemotherapy.

Study objectives

In South African HIV infected patients taking nevirapine-based antiretroviral therapy:

1. To compare nevirapine pharmacokinetics with and without rifampicin, by means of non-compartmental analysis
2. To build pharmacokinetic models describing nevirapine (with and without concomitant rifampicin and isoniazid), rifampicin and isoniazid in HIV-infected patients on nevirapine-based antiretroviral therapy, using a population approach.
3. To validate a whole blood method for measurement of nevirapine concentrations, using 0.2mL blood samples dried onto filter paper
4. To assess the influence of patient factors, such as gender, weight, alcohol consumption, smoking, body mass index (BMI), ALT, albumin, total bilirubin, haemoglobin and disease stage on the pharmacokinetic parameters of nevirapine, isoniazid and rifampicin.

Methodology

Design

This study has a cross-sectional, and a repeated cross-sectional component, including both intensive and sparse pharmacokinetic sampling.

Intensive sampling

- Between 16 and 27 subjects (Group A- intensive), who are on a ART regimen consisting of 2 NRTIs and nevirapine 200mg 12 hourly, and in the continuation phase of tuberculosis therapy, will be recruited sequentially. They will be admitted for pharmacokinetic blood sampling.
- Not less than 14 days after completion of tuberculosis treatment, the same 16 to 27 subjects will be admitted again for PK sampling.
- During the 1st admission, pharmacokinetic profiles of nevirapine, rifampicin and isoniazid will be determined on each subject
- During the 2nd admission, the pharmacokinetic profile of nevirapine without rifampicin and isoniazid will be determined.
- 10 participants who have been taking nevirapine for at least 3 weeks, and are not on rifampicin (Group B-intensive) will be recruited for intensive sampling to characterize the nevirapine pharmacokinetic profile

Patients will be admitted for 24 hours for all admissions. Nevirapine is dosed 12 hourly. Serial blood sampling to 24 hours will allow for sampling of nevirapine concentrations across 2 dosing intervals and assessment of interoccasional variability. During the 1st admission for Group A, rifampicin and isoniazid concentrations will be measured in samples taken during the1st 12 hours.

Sparse sampling

- Additional participants (Group A-sparse) will be recruited to make up a total sample size of 27 in Group A including intensively and sparsely sampled participants). Participants who are on an antiretroviral regimen consisting of 2 NRTIs and nevirapine 200mg 12 hourly, and in the continuation phase of tuberculosis therapy, will be recruited at their routine clinic visit. Exact time of last doses of nevirapine and TB treatment will be recorded, and 2 blood samples will be taken not less than 1 hour apart, for determination of nevirapine, rifampicin and isoniazid concentrations.
- In the same patients, nevirapine concentrations will be determined in two blood samples, not less than 1 hour apart, collected at a routine clinic visit at least 14 days after completion of TB treatment.
- 17 patients (Group B-sparse) who have been treated for at least 3 weeks on nevirapine, but are not being treated for tuberculosis will be recruited at their routine clinic visit. Exact time of last dose of nevirapine will be recorded, and 2 blood samples will be taken not less than 1 hour apart,

Data from both intensively and sparse sampled patients will be used to create a structural model for nevirapine, rifampicin and isoniazid.(using nonlinear mixed effects methods)

Sample size justification

The sample sizes required are based on the C_{min} values published by Ribera et al.[7] Sample size for non-compartmental analysis:

Parametric assumptions are used and estimates are powered to detect a 25% reduction in nevirapine C_{min} when given with rifampicin (power 0.8; alpha 0.05; one sided t-tests.) Should the correlation between the C_{min} with rifampicin and the C_{min} without rifampicin be 0.2, and the correlations between the 2 profile measurements generated with rifampicin and those generated without rifampicin are each 0.5, we require 16 participants to complete intensive PK sampling on both occasions. This sample size was calculated

using a paired t test, using the mean difference and the standard deviation of the mean difference [sqrt((sd1*sd1)+(sd2*sd2)-(2*0.25*sd1*sd2))]

Sample size for population modeling:

Parametric assumptions are used and estimates are powered to detect a 25% reduction in nevirapine C_{min} when given with rifampicin (power 0.9; alpha 0.05; one sided t-tests).

Repeated cross-sectional component (Group A – intensive+sparse):

Should the correlation between the C_{min} with rifampicin and the C_{min} without rifampicin be 0.2, and the correlations between the 2 profile measurements generated with rifampicin and those generated without rifampicin are each 0.5, we require 22 participants to complete PK sampling on both occasions. This sample size was calculated using a paired t test, using the mean difference and the standard deviation of the mean difference [sqrt((sd1*sd1)+(sd2*sd2)-(2*0.25*sd1*sd2))]

We have therefore chosen a sample size of 27 participants treated with nevirapine and in the continuation phase of tuberculosis treatment (Group A), to allow for a 25% loss to follow-up in this group.

Cross-sectional component (Group B):

A t-test for independent samples was used and a correlation of 0.5 was assumed between the 2 measurements in Group A and the 2 measurements in group B respectively, to determine the number of patients in group B needed for comparison of the nevirapine C_{min} to that of Group A with rifampicin (27 patients). To detect a 25% reduction nevirapine C_{min} of the latter group, Group B should comprise 27 patients (alpha 0.05; power 0.9).

Site

Participants will be recruited at the antiretroviral treatment site at the Site B, Khayelitsha community health centre in Cape Town, South Africa. The intensive sampling component of the study will require 2 overnight admissions to the Division of Pharmacology PK ward.

Subjects

Group A

Inclusion criteria

- HIV-infected
- On an antiretroviral regimen consisting of nevirapine 200mg 12 hourly and 2 NRTIs
- In the continuation phase of standard tuberculosis treatment
- >18 years
- Able to give informed consent

Exclusion criteria

- Poor venous access
- Karnofsky score<70
- Known severe renal, hepatic or GIT disease
- Malabsorption or severe diarrhoea
- Other medication that may (on the basis a known interaction, or a strong theoretical basis) affect nevirapine, rifampicin or INH concentrations.

Group B

Inclusion criteria

- HIV-infected
- On an antiretroviral regimen consisting of nevirapine 200mg 12 hourly and 2 NRTIs
- >18 years
- Able to give informed consent

Exclusion criteria

- Poor venous access
- Karnofsky score<70
- Known severe renal, hepatic or GIT disease
- Malabsorption or severe diarrhoea
- Other medication that may (on the basis a known interaction, or a strong theoretical basis) affect nevirapine, rifampicin or INH concentrations.

Subject Restrictions for intensive sampling group (Group A-intensive):

Medicines: No use of any new medication, including over-the-counter, and herbal remedies, without discussion with the principal investigator, from 14 days before the 1st admission to completion of sampling.

Diet: Standardised meals to be given during admissions for PK sampling

Movement: Subjects will be admitted at 8am and will be required to stay in the ward until completion of blood sampling the following morning

Adherence: Adherence over the week prior to admission will be assessed by means of a questionnaire and pill-count from pill box. Pillbox will be filled at the MSF clinic 1 week before admission

Ethical considerations

This study will be conducted according to the guidelines of the Helsinki declaration of 2000 and according to the ICH principles of Good Clinical Practise. Approval of the protocol and informed consent documents will be sought from the research ethics committee of the University of Cape Town before commencing with the study.

All protocol amendment shall be submitted to the UCT ethics committee for approval. The ethics committee will be notified of all protocol deviations, and will be notified of any serious adverse event within 72 hours. All patient data will be treated in the strictest confidence. A record of all adverse events will be retained. Patients in the intensively sampled group will be remunerated for inconvenience. The remuneration amount will be R150 per admission. No remuneration will be given to the sparsely sampled group.

Data Collection:

The following information will be obtained from the patient's previous records: last CD4 count and viral load, and date.

The following information will be collected at baseline: age, weight, height, gender, race, WHO clinical stage, duration of antiretroviral therapy, alcohol / recreational drugs/ cigarettes consumption history, concomitant medication, other diseases, time of last food consumption, and details of food consumed.

The following will be performed at each sampling occasion:

- ALT, albumin and total bilirubin

- Laboratory haemoglobin
- Completion of questionnaire about side effects
- Completion of a questionnaire about adherence
- Pill-count

Subjects will be assigned sequential numbers on enrolment.

Analytical methods

Sample collection

Venous blood will be collected in 4 ml LiH Pst Gel plastic vacuum tubes, stored on ice until sample collection at that time point is complete for all patients, then immediately centrifuged for ten minutes at 3 000 rpm. Plasma will be transferred to labelled 1.2 ml microcentrifuge tubes, which will be stored at -80°C until analysis (The label on each tube will include the subject number, the visit (1 or 2) and the sampling time

Total volume of blood drawn throughout the entire study period in the intensively sampled group (minimum 6 weeks duration): 200 mL. No more than 70 mL of blood will be drawn at any one admission.

A method is being developed whereby the nevirapine, rifampicin and isoniazid concentrations will be determined using a 200 µl whole blood sample dried onto filter paper and stored at room temperature in a sealed plastic bag with desiccant. The method will be convenient for the scant pharmacokinetic sampling under field conditions. The method will be validated using a small portion from each blood sample collected.

Pharmacokinetic profiling

Patients enrolled in "Group A- intensive" will be admitted in the morning to the Division of Pharmacology's PK ward at 8am. At 9am, enrolled patients will receive their morning dose of nevirapine, together with the morning dose of NRTIs. Standard meals will be given during the day.

Drugs will be administered with 200ml of water, and the exact time of administration recorded. The investigator or an assistant will directly observe drug ingestion.

Blood samples will be taken at approximately the following time points and the exact time of sampling will be recorded:

1st admission: 0, 0.5, 1, 1.5, 2, 4, 6, 10, 12, 12.25, 12.75, 14, 22 and 24 hours. Nevirapine will be determined in all samples; rifampicin an isoniazid will be determined from 0-12 hours

Pharmacokinetic sampling will be repeated a 2nd time to measure nevirapine concentrations, 14 days or more after rifampicin and isoniazid have been discontinued. The sampling schedule will be as follows

2nd admission: 0, 0.5, 1, 1.5, 2, 4, 6, 10, 12, 12.25, 12.75, 14, 22 and 24 hours.

"Group A-scant" and "Group B" participants will be sampled twice (at least 1 hour apart) at each pharmacokinetic sampling, which will be performed at a routine clinic visit as described previously.

For each sample a 0,2 ml whole blood sample will be collected onto filter paper and dried before storage (at room temperature) in a sealed plastic bag with desiccant These samples will be used to validate the "dried whole blood" methodology for assessment of nevirapine concentrations.

Sample analysis

Assays for determination of nevirapine, rifampicin and isoniazid plasma concentrations (from plasma samples (intensively sampled participants) and dried blood spots (all samples) will be performed using validated Liquid Chromatography Mass Spectrometry (LCMS) methods in the Division of Pharmacology Laboratory.

Validation of dried whole blood methodology for measurement of nevirapine concentrations

Results obtained from dried whole blood will be compared with results obtained from plasma,, in order to validated the dried whole blood methodology

Statisical analysis

Statistical methods

Group A-intensive: Noncompartmental analysis will be performed to calculate the following parameters for each drug taken on each occasion, using WinNonlin Enterprise version 4 :

- Plasma peak concentration (Cmax)
- Time to plasma peak concentration (Tmax)
- Apparent half-life ($t_{1/2}$)

- Area under the plasma concentration-time curve from 0 until 12 hours for nevirapine, and from 0-24 hours for rifampicin and isoniazid (AUCt 0-24) and from 0 - ∞ (AUCi)
- Plasma trough concentration (Cmin)

The whole blood drug concentrations from all patients will be used in a population analysis. The structural models for each drug will be derived (in the first instance using concentrations from the intensively sampled participants), allowing the prediction of the PK measures for the participants undergoing scant sampling. In addition, the effect of the covariate factors and the coadministration of the TB drugs on the models will be assessed.

The primary objective will be to compare the PK measures of N (AUC, Cmax, Cmin) between those patients receiving concomitant R and H, and those receiving N without TB treatment. Appropriate nonparametric or parametric tests will used to determine the differences between the groups.

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25 February 2005

REC REF: 362/2004

Dr K Cohen
Pharmacology

Dear Dr Cohen

PHARMACOKINETICS OF NEVIRAPINE IN COMBINATION WITH RIFAMPICIN-BASED SHORT COURSE CHEMOTHERAPY IN HIV AND TUBERCULOSIS-INFECTED PATIENTS

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has ***formally approved*** *the above-mentioned study on the 23 February 2005.*

Your comments to the queries are noted with thanks.

Please quote the Reference number in all correspondence.

Your$ sincerely

PROF T. ZABOW
CHAIRPERSON

Version 2 march 2005

Patient information sheet: Pharmacokinetics of nevirapine in HIV infected patients in South Africa, taking nevirapine with and without rifampicin.

Investigators

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Dr Eric Goemaere *Médecins Sans Frontières*

You are being invited to participate in a study looking at nevirapine blood levels in HIV infected people, some of whom are being treated for tuberculosis (TB).

Why are we doing this study?

Tuberculosis is a common and important problem among people with HIV infection. People who get tuberculosis are treated with a combination of medicines, including one called rifampicin. Rifampicin can have an effect on other medicines that a person is taking at the same time, such as some medicines that fight HIV (antiretroviral medicines), by making the body break down the other medicines more quickly. It is possible that rifampicin could affect the antiretroviral drug nevirapine in this way. This is important because if antiretroviral medicines are broken down more quickly than normal, they may not work as effectively as they should. There is at present little information about blood levels of nevirapine in HIV infected people in South Africa, and little information about what happens to nevirapine levels when HIV infected people are treated with rifampicin. We are carrying out this study to find out more about these questions, and we invite you to take part in it.

This study will not benefit you right now, but the results of the study will help doctors to choose the best combination of antiretroviral medicines to give people with HIV infection who get tuberculosis, and so will help people like you in the future.

If you have any questions about this study, please ask us. You do not have to take part in this study: if you do not take part, it will not affect the medical care you receive. You can decide to stop taking part in the study at any time, without giving a reason, and without affecting your future medical care.

If you take part in this study, what will happen?

Sparse sampling group:

If you agree to take part in this study, we will ask you some questions about your health, and all the medicines you are taking today and those you have taken in the last two weeks. We will look at your hospital and clinic records to check details of illnesses you have had previously, recent medicines, and the results of routine blood tests. We will measure how tall you are, and record your weight. Blood will be taken twice, at different times not less than 1 hour apart, on the day that you visit the clinic. We will take 16mL (about 4 teaspoons) of blood for the study and test it to see how much of the antiretroviral drug nevirapine, and the antituberculosis drugs rifampicin and isoniazid, are in it, as well as testing your liver function and haemoglobin.

(this paragraph is only for people with TB currently taking rifampicin)
We would like to take a second 4 samples of blood (totaling 16mL or about 4 teaspoons) from you when your tuberculosis treatment has been finished for at least 2 weeks. This will also be tested to see how much nevirapine is in it, as well as your liver function and haemoglobin.

Intensively sampled group

If you agree to take part in this study and enter the intensively sampled group, we will ask you some questions about your health, all the medicines you are taking today and those you have taken in the last two weeks. We will look at your hospital and clinic records to check details of illnesses you have had previously, recent medicines, and the results of routine blood tests. We will measure how tall you are, and record your weight. Blood will be taken to check your liver function and haemoglobin. You will be admitted to a hospital ward 2 times, for a period of 24 hours at each occasion. The 2nd sampling admission will occur 4 weeks or more after you have completed all your treatment for tuberculosis.

During the admissions a venous cannula (like a drip) will be inserted into a vein, and blood will be taken from you at set time points

Blood samples will be taken at the following time points:

1st admission (after your swallow your morning dose of nevirapine and TB treatment): 0, 0.5, and 1, 1.5, 2, 4, 6, 10, 12, 12.25, 12.75, 14, 22 and 24 hours.

2nd admission (after your swallow your morning dose of nevirapine): 0, 0.5, 1, 1.5, 2, 4, 6, 10, 12, 12.25, 12.75, 14, 22 and 24 hours

A total volume of no more than 70mL (5 tablespoons) of blood will be taken at each admission. This blood will be used to measure the concentrations of the drugs nevirapine, rifampicin and isoniazid. In addition, at each visit, tests will be done to check your liver function and haemoglobin, and you will be asked about any side effects of your medicine. You will also be asked about your compliance with your medication.

Confidentiality of information collected during this study

All information collected during the course of this study will be kept securely and confidentially: Dr Cohen and Dr Van Cutsem are responsible for this. Reports about the study and results that may be published in scientific journals will not include any information which identifies you personally.

The committee giving ethical approval for this study is the University of Cape Town Ethics committee. If you have any problem with this study please contact the Ethics committee directly, telephone number_____

(This document will be available in the most frequent local languages and will be read by, read to or translated to the participant.)

Version 2 march 2005

Patient consent form: Nevirapine mid-dosing interval plasma levels in HIV infected patients in South Africa, taking nevirapine with and without rifampicin.

I have fully understood the above information about this study, which I have read, or which has been read or translated to me. I understand what will be required of me if I take part in the study.

My questions concerning this study have been answered by ……..... (name of study staff member)

I agree to take part in the study: **YES / NO** (answer to be circled)

I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.

Participant's signature: …… Date: ……..
Participant's name: ……..

If the information sheet and consent form were translated or explained to the participant, please enter the name of the translator here and their signature:

Translator's signature: …….. Date: ……..
Translator's name: ……

If the participant gave verbal consent, please enter the name of the person who witnessed the consent here and their signature:

Witness' signature: …….. Date: ……..
Witness' name: ……..

Name and signature of investigator or designated co-investigator taking consent
Investigator Signature: …….. Date: ……..

Investigator Name: ……..