CLINICAL PHARMACOLOGICAL INVESTIGATIONS OF ANTIRETROVIRAL DRUGS

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KLINISCH FARMACOLOGISCH ONDERZOEK VAN ANTIRETROVIRALE GENEESMIDDELEN (MET EEN SAMENVATTING IN HET NEDERLANDS)

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Healing is a matter of time,
but it is sometimes also a matter of opportunity.
Hippocrates

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PREFACE

Human immunodeficiency virus (HIV) infects and destroys cells of the immune system, specifically CD4 positive cells. When the immune system has been severely weakened and can no longer defend the body from certain life-threatening opportunistic infections and malignancies, the acquired immunodeficiency syndrome (AIDS) is diagnosed.[1,2]

Nowadays, individuals infected with HIV can be effectively treated with combination drug therapy, known as highly active antiretroviral therapy (HAART). HAART typically consists of three or four antiretroviral agents usually from different drug classes.[3] The four classes of antiretroviral drugs currently available for the treatment of HIV include nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and fusion inhibitors (FIs).

Although HAART can suppress viral replication and increase CD4 cell count, and has convincingly demonstrated to result in decreased morbidity and mortality,[4] improvement is still required. Problems such as toxicity, resistance, non-adherence and drug-drug interactions are frequently encountered in daily practice.

The likelihood of treatment success in patients on HAART has directly been related to exposure of the virus to active drug. The presence of antiviral drugs in partially suppressive concentrations might result in emergence of viral resistance, on the other hand, high concentrations may lead to adverse events.[5-8] Variability in plasma drug concentrations is caused by a number of reasons, including poor adherence, drug-drug interactions and patient characteristics.[9]

Associations between concentrations of antiretroviral drugs in body compartments such as blood plasma and treatment outcome (efficacy and adverse events) provide a clear rationale to carefully evaluate the pharmacokinetic characteristics of these drugs. This knowledge can then be applied to further optimise individualised treatment of HIV-infected patients with antiretroviral drugs, for instance by the application of therapeutic drug monitoring (TDM).

The major aim of all studies described in this thesis is to contribute to the optimisation of antiretroviral drug treatment in HIV-infected patients by the assessment and interpretation of pharmacokinetic characteristics of antiretroviral drugs.

In the first chapter the current knowledge on the relationships between pharmacokinetics and treatment outcomes is summarised into practical guidelines for TDM. In subsequent chapters the pharmacokinetics of selected PIs and NNRTIs are studied in representative patient populations. Moreover, determinants

of pharmacokinetic variability are assessed.
Several studies have been performed in view of the large international randomised 2NN study.[10] This was the first study to compare the safety and efficacy of nevirapine and efavirenz containing regimens in treatment-naive HIV-1-infected patients. Within this trial, several clinical pharmacological substudies were performed. A requirement to perform these substudies is the availability of validated bioanalytical methods to assess quantitatively plasma concentrations of nevirapine and efavirenz. In this thesis, the development and validation for the simultaneous determination of the NNRTIs efavirenz and nevirapine in human plasma is described. Afterwards, population pharmacokinetics of nevirapine and efavirenz and its determinants were characterised in several studies. In addition, relationships between the pharmacokinetics and adverse events were investigated.
Altogether, the results of these studies may contribute further to the safe and effective treatment of HIV-infected individuals.

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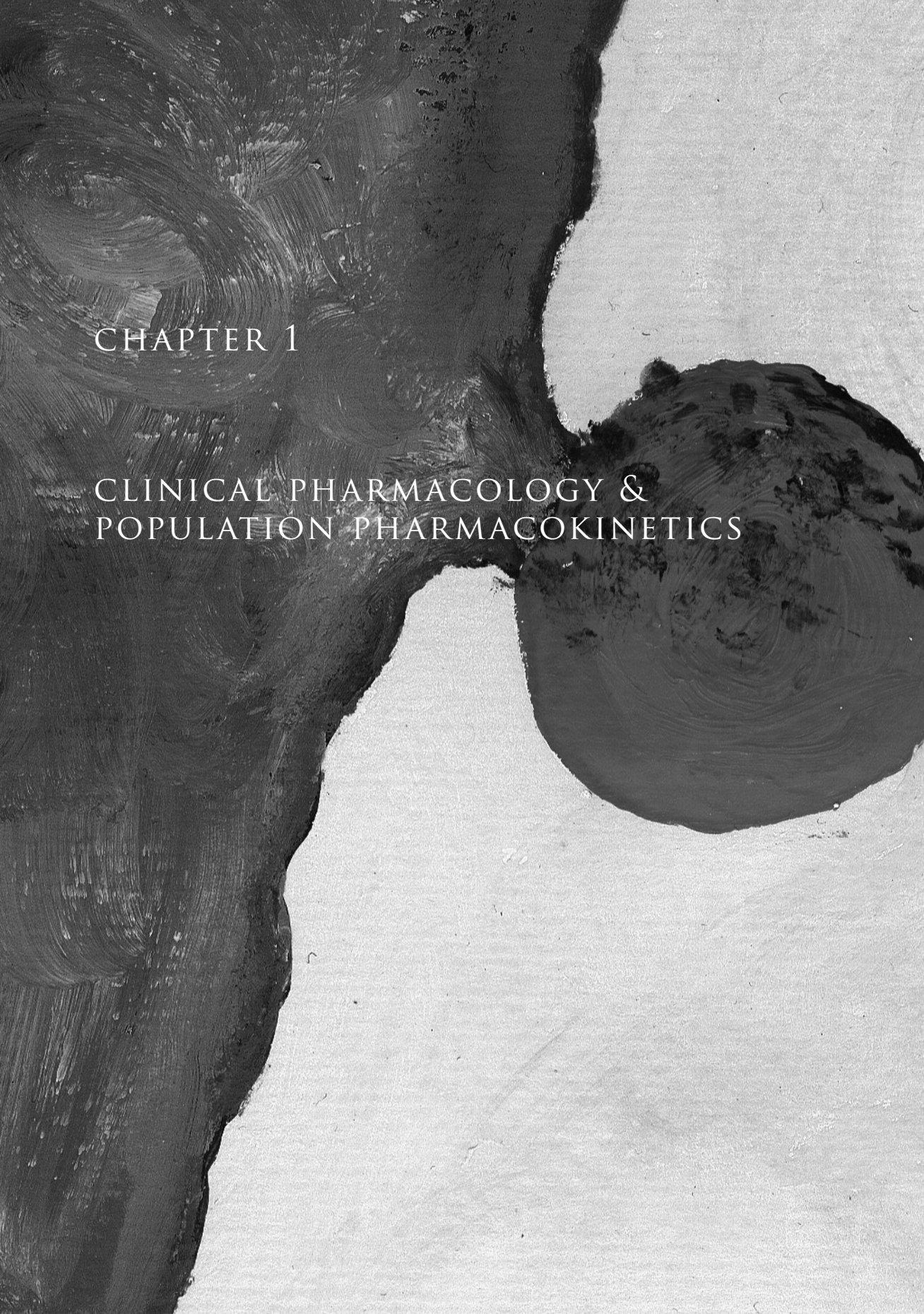
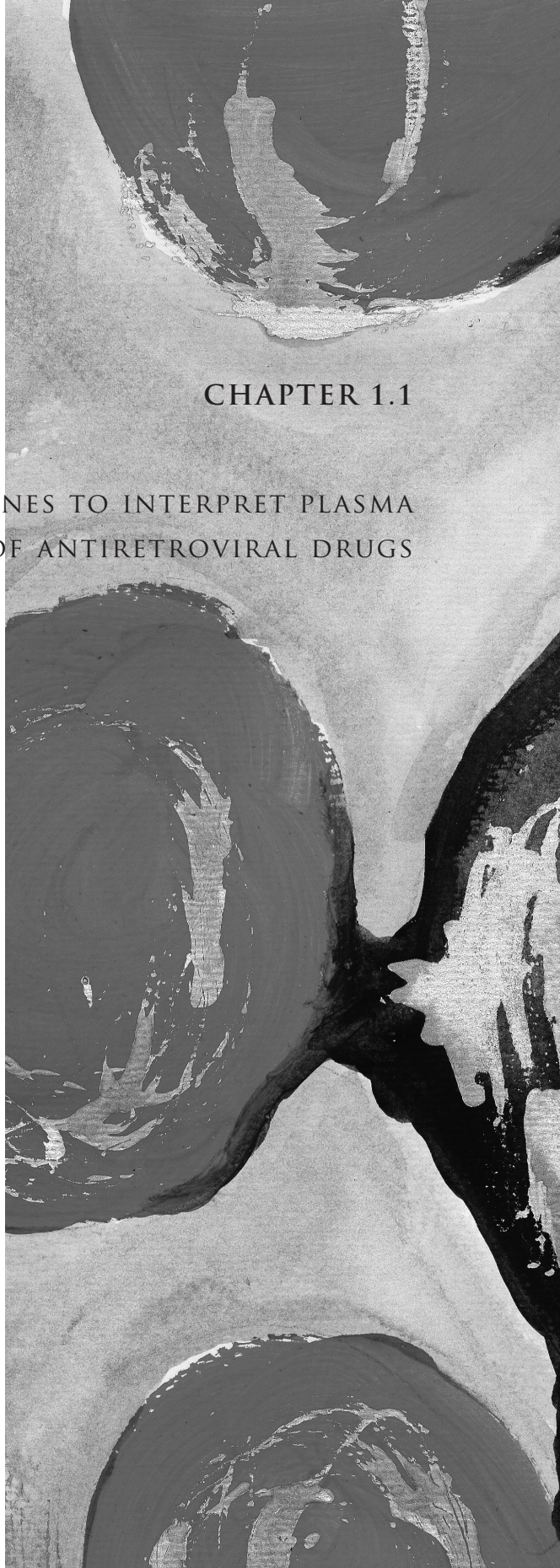


CHAPTER 1

CLINICAL PHARMACOLOGY & POPULATION PHARMACOKINETICS



CHAPTER 1.1

PRACTICAL GUIDELINES TO INTERPRET PLASMA CONCENTRATIONS OF ANTIRETROVIRAL DRUGS

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ABSTRACT

Several relationships have been reported between antiretroviral drug concentrations and the efficacy of treatment, and toxicity. Therefore, therapeutic drug monitoring (TDM) may be a valuable tool in improving the treatment of HIV-1-infected patients in daily practice.

In this regard, several measures of exposure have been studied, e.g. trough- and maximum concentrations, concentration ratios and the inhibitory quotient. However, it has not been unambiguously established which pharmacokinetic parameter should be monitored to maintain optimal viral suppression. Each pharmacokinetic parameter has its pros and cons. Many factors can affect the pharmacokinetics of antiretroviral agents, resulting in variability in plasma concentrations between and within patients. Therefore, plasma concentrations should be considered on several occasions. In addition, the interpretation of the drug concentration of a patient should be performed on an individual basis, taking into account the clinical condition of the patient. Important factors herewith are viral load, immunology, occurrence of adverse events, resistance pattern and co-medication.

In spite of the described constraints, the aim of this review is to provide a practical guide for TDM of antiretroviral agents. This article outlines pharmacokinetic target values for the HIV protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir, and the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine. Detailed advice is provided on how to interpret the results of TDM of these drugs.

INTRODUCTION

In antiretroviral therapy, maintaining maximally suppressive drug concentrations during a complete dosage interval represents a key defence against the emergence of resistance.[1] If plasma concentrations of antiretroviral drugs fall and viral replication increases, the opportunity for mutant virus to be selected occurs. Therefore, the treatment of individuals infected with Human Immunodeficiency Virus (HIV) is increasingly monitored and optimised by the use of therapeutic drug monitoring (TDM) and/or pheno- or genotyping.[2-4] Highly active antiretroviral therapy (HAART) usually consists of two nucleoside reverse transcriptase inhibitors (NRTIs) and one or two protease inhibitors (PIs) or a non-nucleoside reverse transcriptase inhibitor (NNRTI).[5] Relationships between drug concentrations and resistance have been reported for PIs and NNRTIs, whose activity does not

involve intracellular conversion, unlike NRTIs.[6]

Until now it has not been established unambiguously which pharmacokinetic parameter (e.g. minimum plasma concentration (C_{min}), maximum plasma concentration (C_{max}) or area under the plasma concentration-time curve (AUC)) should be monitored to maintain optimal viral suppression.

Relationships have, however, been observed between the efficacy of treatment and minimum or trough plasma concentration,[7-17] concentration ratio [18,19] and the inhibitory quotient.[20] The concentration ratio represents the measured plasma concentration compared with the time-adjusted average concentration, as measured in a reference population of HIV-infected individuals.[21] The inhibitory quotient is defined as the ratio of C_{min} to the effective viral inhibitory concentration necessary to inhibit 50%, 90% or 95% of viral replication ($IC_{50/90/95}$). On the other hand, for several drugs, C_{max} in plasma has been shown to relate to toxicity.[5,22-24] These results indicate that TDM may be a very valuable tool for the optimisation of antiretroviral therapy.

The application of TDM for PIs and NNRTIs is, however, still in its infancy and several problems emerge when using any of the pharmacokinetic parameters as a tool in performing TDM in daily practice. In routine clinical care, it is often difficult to draw a blood sample at a strictly defined time point, e.g. trough sample, particularly when a trough concentration is reached outside working hours, as is the case in many patients receiving a once daily efavirenz containing regimen, for example. An actual C_{max} is difficult to determine because it is not possible to predict when this exact point is reached in a particular patient on a certain day. The difficulty with inhibitory quotients lies in the great variability that is found in both C_{min} and the inhibitory concentration, depending on the way in which these parameters are determined. The potential variability in the C_{min}/IC_{50} ratio between patients can be as large as 100%.[25]

In our hospital, concentration ratios are used to interpret the antiretroviral plasma concentrations of HIV-infected patients attending the outpatient clinic. A disadvantage of the use of concentration ratios is that reference curves are needed, not only for every drug but also for every different drug regimen. The population used to construct the reference curve should reflect the average patient population on which the curve is applied to. Moreover, the concentration ratio concept assumes that ratios are constant during the dosage interval. In the light of the described difficulties, it may be more appropriate to use Bayesian estimates to perform TDM.[26] Research has shown that Bayesian estimation performed better than the concentration ratio concept in predicting nevirapine trough

concentrations (personal observations).

Many factors can affect the plasma concentrations of antiretroviral agents. Variability in pharmacokinetics occurs not only between, but also within patients and accounts for variability in drug levels over time. Therefore, plasma concentrations should always be considered at several occasions when advising the physician. Regardless of which pharmacokinetic tool is used to perform TDM, the interpretation of a patient's drug level should be performed on an individual basis, taking into account the clinical condition of the patient. Factors that need to be considered before treatment advice is given to the physician are viral load, immunology, occurrence of adverse effects, resistance pattern and co-medication.

Because of the higher drug concentrations that are necessary to inhibit drug-resistant HIV-strains, it is crucial to know whether the advice given relates to a patient who is antiretroviral therapy-naive or on a salvage regimen.

Furthermore, issues of compliance and food instructions should be kept in mind, especially when plasma concentrations deviate from average values. Dietary advice is given in conjunction with most antiretroviral drug regimens and plasma concentrations can be influenced to some degree when this advice is neglected. Before changing the dosage or regimen because of deviating plasma concentrations, compliance, food instructions, interacting co-medication and previous plasma concentrations must be considered. Also, one should be watchful for the "white coat syndrome", i.e. a patient may have been adherent to therapy in the days immediately prior to the collection of the blood sample, but was non-compliant between clinic visits. This could lead to a therapeutic drug level in a patient with non-adequate suppression of viral replication. Table 1 highlights the points that are to be considered when interpreting a patient's plasma drug concentrations.

Although prospective studies investigating the additional value of TDM in HIV care are scarce, TDM has proven to be useful in specific situations (e.g. in cases of non-compliance or drug interactions).[21,27] Therefore, current treatment guidelines recommend monitoring drug concentrations on an individual basis until more data are available.[28]

In spite of the described pitfalls, the aim of this review was to provide a practical guide for TDM of antiretroviral agents. Figure 1 presents a description of frequently used dosage regimens and pharmacokinetic targets for PIs and NNRTIs, and detailed advice on what to do when drug levels are not considered adequate. Most recommendations concerning trough concentrations are based on published data of either antiretroviral-naive or pre-treated patients. Although relationships between high plasma concentrations and toxicity have been observed, there are

few C_{max} data available to provide real cut-off values to avoid the occurrence of toxicity. Therefore, most of the maximum target values are given as twice the concentration ratio of C_{max}. Treatment of HIV-infected individuals is a challenging long-term undertaking. The scheme presented in this review will assist clinicians in optimising the management of HIV-positive patients.

Table 1. Points for consideration in TDM of antiretroviral drugs.

• Previous plasma concentration
• Viral load
• Immunology
• Adverse effects
• Resistance pattern
• Interacting co-medication
• Compliance
• Food instructions
• "White coat syndrome"

ANTIRETROVIRAL DRUGS

For an overview of current regimens, readers are referred to the most recent version of the guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents.[5] The following sections discuss pharmacokinetic-pharmacodynamic relationships for NNRTIs and PIs, and present data to elucidate and support the scheme presented in figure 1 (in which practical advice and cut-off points are given for the TDM of these agents).

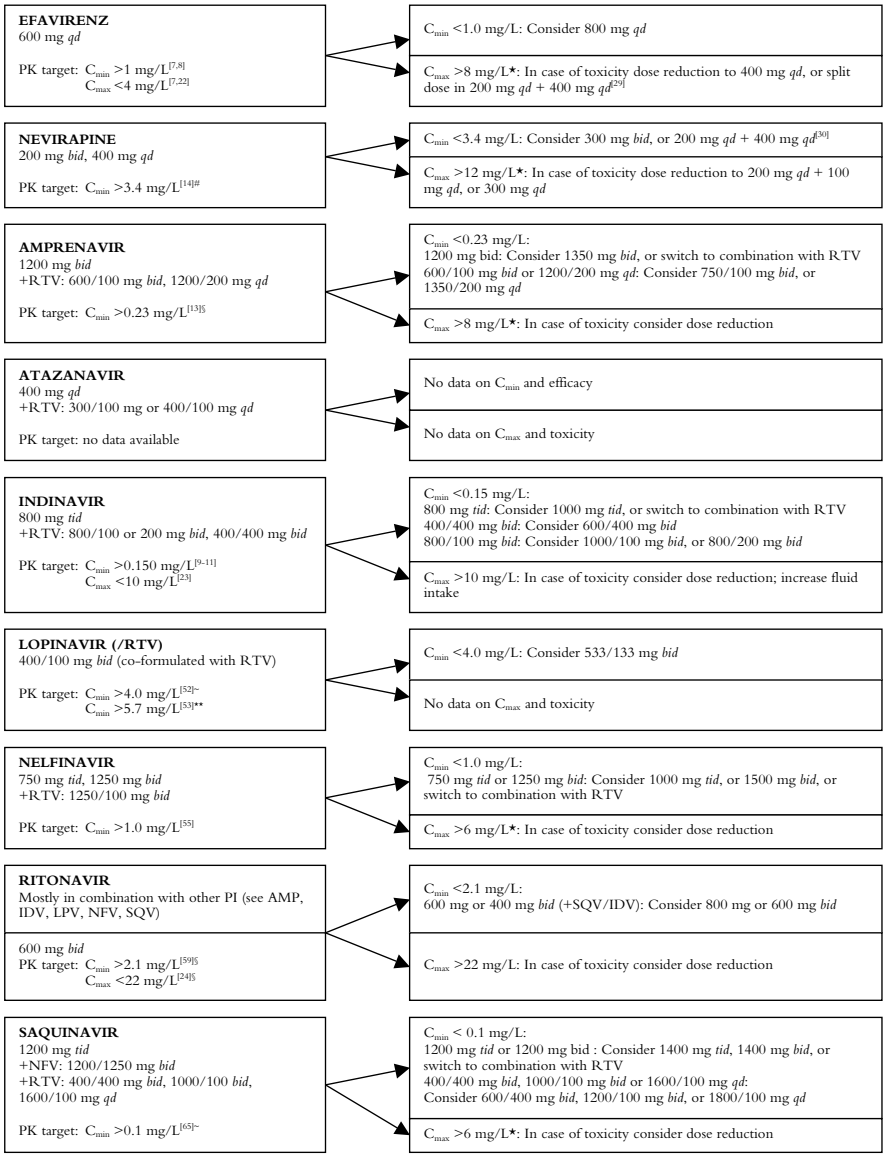
Non-nucleoside reverse transcriptase inhibitors

Efavirenz

Several studies in HIV-1-infected patients have shown that treatment failure and adverse effects are associated with low and high efavirenz plasma concentrations, respectively.[7,8,22]

Virologic failure was observed significantly more in patients with low efavirenz plasma levels (<1 mg/L).[7,8,15] With plasma concentrations over 4 mg/L at mid-interval sampling times, patients were at higher risk for reversible central nervous system adverse effects (i.e. dizziness, abnormal dreams and insomnia).[7,22] Because of the reversibility and temporary nature (particularly in the first few

Figure 1. Practical guide to interpret the results of therapeutic drug monitoring of antiretroviral drugs.



The antiretroviral pre-treatment of the patient population in which the target value was defined, was indeterminate or mixed unless coded as follows: #: antiretroviral-naive patients, §: PI-naive patients, ~: pre-treated patients, **: salvage therapy. ★: C_{max} based on 2 x concentration ratio.

weeks of treatment) of these adverse effects, action (i.e. dosage reduction or dosage separation) will not be necessary in most cases. When persisting adverse effects are accompanied by high plasma concentrations, it is advisable to reduce the dosage to 400 mg once daily or split the dosage into 200 mg once daily plus 400 mg once daily.[29] A cut-off value of 8 mg/L is suggested based on twice the concentration ratio of the C_{max} of efavirenz and extrapolated from the mid-interval data presented in the literature.[7,8,22]

Nevirapine

A study exploring the association of exposure to nevirapine with virological response in antiretroviral-naive HIV-1-infected patients yielded a target trough concentration of 3.4 mg/L.[14] From 12 weeks onwards, the median nevirapine concentration was predictive for success of therapy, in which success was defined as an HIV-1 RNA concentration in plasma of <50 copies/ml. Also, other studies have shown that nevirapine exposure influences virological suppression.[16,17] De Vries-Sluijs et al.[17] have demonstrated that nevirapine drug concentrations <3.0 mg/L were predictive of virological failure. Dose intensification can be applied to patients with a trough concentration <3.4 mg/L. It has been shown that increasing the dosage to 600 mg/day results in a significant increase in nevirapine concentrations without signs of increased toxicity.[30]

Although indications exist that transaminase elevations and rash are related with plasma concentrations,[31,32] the relation of toxicity and nevirapine plasma concentrations has not been confirmed conclusively.[33,34] Until more data are available, dose adjustments in case of high plasma concentrations should be guided by the clinical condition of the patient.[35]

Protease inhibitors

Amprenavir

The target concentration of amprenavir 0.23 mg/L mentioned in figure 1 was proposed in a study by Sadler et al.[13] This value was also adopted by Aarnoutse et al.[2] as target C_{min}, while Acosta et al.[3] gave a range of 0.15 mg/L to 0.4 mg/L. The study of Sadler et al.[13] represents the estimated *in vivo* trough concentration calculated to yield 90% of the maximum antiviral effect over 4 weeks, and was derived from a sigmoid E_{max} model. The study was conducted in PI-naive HIV-positive subjects, therefore care should be taken when transposing this target concentration to pre-treated HIV-infected patients. To inhibit drug-resistant HIV-strains higher drug concentrations might be necessary; however, the established

concentration was approximately 10-fold higher than the median *in vitro* IC_{50} of clinical isolates (0.023 mg/L),[13] even after adjustment for protein binding. Furthermore, this study revealed oral numbness and headache to be adverse effects significantly associated with the C_{max} of amprenavir.

Atazanavir

Atazanavir is a recently approved protease inhibitor with a pharmacokinetic profile that allows once daily administration. Up-to-date sparse data are available on relationships between pharmacokinetics and early virological response and bilirubin elevations,[36,37] therefore no target values of C_{min} and C_{max} can be declared. However, mean plasma concentrations have been established for atazanavir 400 mg once daily (0.118 mg/L (C_{min}) and 5.79 mg/L (C_{max})), and atazanavir 300 mg once daily plus ritonavir 100 mg (0.696 mg/L (C_{min}) and 4.42 mg/L (C_{max})).[38–41] When efavirenz is also part of the antiretroviral regimen, atazanavir should be administered as 400 mg boosted with ritonavir 100 mg.[42]

Indinavir

Both toxicity and efficacy have been related to indinavir plasma concentrations.[9-11,23,43-45] A C_{min} of indinavir of at least 0.15 mg/L proved necessary for adequate viral suppression,[9-11] whereas a C_{max} >7.0 mg/L was significantly associated with increases in CD4 count.[46] In the case of a C_{min} <0.15 mg/L, the indinavir dose should be raised, or a "baby dose" of ritonavir (100 mg or 200 mg) should be added to the regimen. Adding ritonavir to an indinavir containing regimen resulted in an increased trough concentration of indinavir, without a relevant change in C_{max}.[47] When indinavir is already used in a boosted regimen, increasing the ritonavir dose to 200 mg may be a safe alternative when suboptimal (<0.15 mg/L) concentrations of indinavir are found, as demonstrated in the study by Saah et al.[48]

Patients with urological complaints resulting from indinavir use frequently have plasma concentrations exceeding 10 mg/L.[23] When plasma concentrations are >10 mg/L, the dose may be lowered because of the possibly non-reversible character of the toxicity. Based on measured plasma concentrations, the dosage of indinavir/ritonavir may be gradually reduced to 600/100 mg twice daily and, if necessary, eventually to 400/100 mg twice daily. Drinking enough water can also help to prevent urological adverse effects. Administering the medication in combination with food will lower the C_{max} of indinavir.

Lopinavir

With the boosted PI combination of lopinavir/ritonavir, relatively high lopinavir plasma concentrations are achieved, compared with the IC_{50} value. Therefore, in several studies, no real cut-off value could be defined for lopinavir trough levels in antiretroviral therapy-naive patients.[49,50] Hsu et al.[51] suggested that an inhibitory quotient of 15, which could be translated to a C_{min} of 1.0 mg/L, was associated with a high response rate in treatment-naive patients. However, none of the lopinavir concentration parameters were defined as a predictor of virological response in this study. On the contrary, Breilh et al.[52] defined a plasma lopinavir C_{min} efficacy threshold at 4.0 mg/L for HAART-experienced patients. In addition, Boffito et al.[53] defined a target value in heavily pre-treated patients. These investigators have shown that, in salvage therapy, a C_{min} >5.7 mg/L is an independent predictor of response.

Nelfinavir

Several studies have found relationships between the exposure to nelfinavir and its efficacy, based on the concentration ratio. Burger et al.[19] set a concrete pharmacokinetic target at a concentration ratio of 0.9 in antiretroviral therapy-naive patients. Results from the Athena trial, a randomised, prospective TDM study carried out in The Netherlands between 1996 and 2001, showed that patients with a concentration ratio <0.9 had a relative risk of 3.0 (95% CI: 1.2-7.6) for virological failure. Another finding of this same Athena trial showed that TDM of nelfinavir resulted in a significantly lower treatment discontinuation rate in therapy-naive patients after 1 year of follow-up. This was mainly driven by a lower rate of discontinuation because of virological failure.[54]

Pellegrin et al.[55] avoided the use of a concentration ratio, the practical disadvantages of which were mentioned earlier. A nelfinavir C_{min} efficacy-threshold of 1 mg/L was estimated in the whole population, whereas a cut-off point of 0.8 mg/L was set in patients infected with wild-type virus. This value of 0.8 mg/L was also found to significantly improve virological response in HIV-1-infected children.[56] Levels of the active metabolite M8 should also be considered as this compound contributes to overall antiviral activity. Baede-van Dijk et al.[57] reported the median M8/nelfinavir ratio to be 0.29, independent of the time after ingestion. Adding low dose ritonavir to a nelfinavir containing regimen increases the plasma concentrations of nelfinavir and particularly M8.[58] Therefore, assuming that the M8/nelfinavir ratio does not vary with time after ingestion, and that summed concentrations of M8 and nelfinavir are only marginally affected when cytochrome

P450 (CYP) 3A4 inducers are co-medicated, measuring M8 concentrations in addition to nelfinavir concentrations may not be required.[57]

Ritonavir

The therapeutic dosage of ritonavir is 600 mg twice daily, or 400 mg twice daily in combination with saquinavir or indinavir. However, nowadays ritonavir is mainly used in combination with other PIs as a booster of these compounds. A low dose of ritonavir (100 mg or 200 mg), the so-called "baby dose", results in non-therapeutic plasma concentrations of ritonavir and is not considered in this review.

For therapeutic doses of ritonavir, significant correlations between antiviral activity and plasma drug concentrations have been demonstrated in various clinical studies in HIV-1-infected patients.[12,24,59,60] A ritonavir C_{min} of 2.1 mg/L was extracted as a target for adequate viral suppression.[59] Adverse effects have a tendency to occur more frequently with ritonavir C_{max} >26.7 mg/L and C_{min} >12.6 mg/L.[24] In cases where these targets are reached, the dose of ritonavir could be reduced to 500 mg twice daily or 300 mg twice daily (in combination with saquinavir or indinavir).

Saquinavir

For saquinavir, many different dosage regimens are used in common practice, resulting in a wide range of concentrations encountered. Relationships between the exposure to saquinavir and efficacy have been described in various studies.[61-63] A concrete pharmacokinetic target concentration was determined by Hoetelmans et al.[18] In this study, patients with a saquinavir trough concentration >0.05 mg/L had a significantly higher likelihood of having a sustained decline in HIV-1 RNA more than two logs below baseline after 48 weeks of therapy. However, the validity of this target value is uncertain because it is in the same range as the IC_{50} value for saquinavir, which lies between 0.02 mg/L and 0.05 mg/L.[64] Moreover, the mean C_{min} in most dosage regimens is higher than this target value. Therefore, a higher target value of 0.1 mg/L, as established by Valer et al.[65] in heavily pre-treated patients may be used in clinical practice.

CONCLUSION

TDM may be a valuable tool in improving the treatment of HIV-infected patients. Despite some limitations, routine measurement of plasma concentrations not only provides clinicians with practical information about pharmacokinetics and drug

interactions, but may also protect patients from the occurrence of adverse effects and prevent virological failure.

Target values of plasma concentrations of NNRTIs and PIs are presented in this review to make TDM applicable in daily practice. However, these target values merely serve as guidelines and do not represent absolute cut-off points between adequate and suboptimal therapy. It is of paramount importance to consider each patient individually, thereby taking into account the characteristics, history and co-medication of the patient and the characteristics of the virus.

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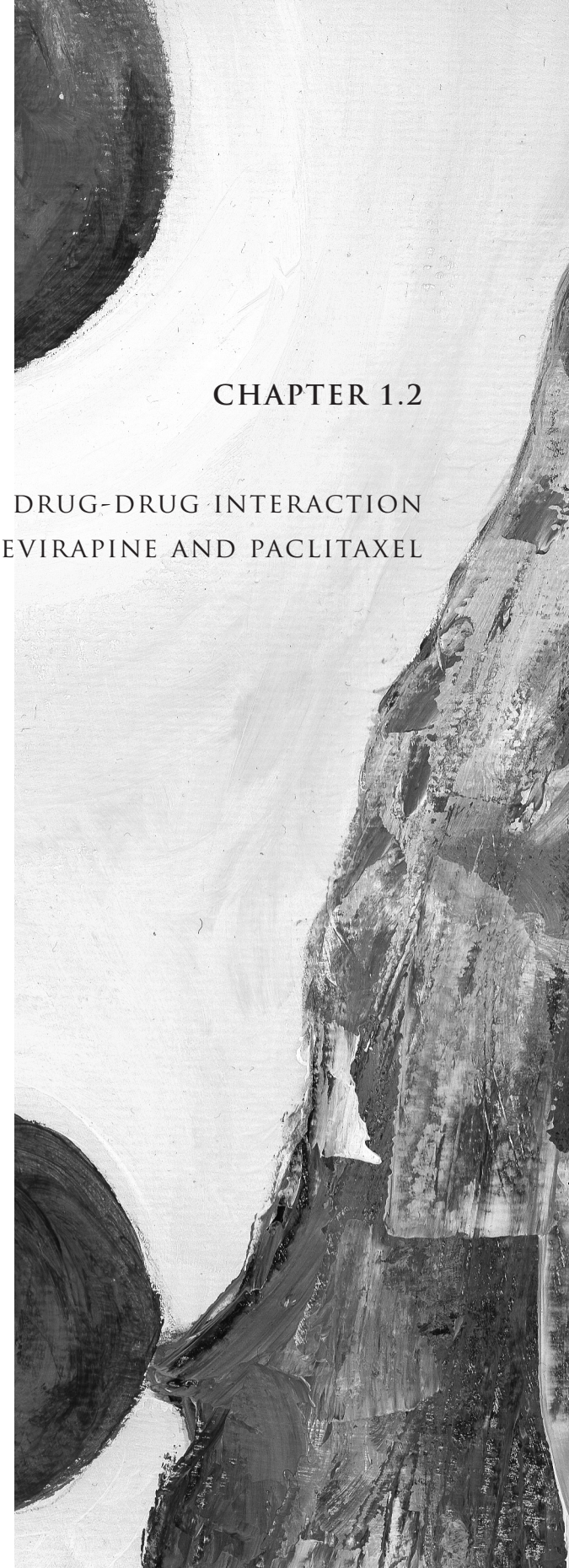

CHAPTER 1.2

NO PHARMACOKINETIC DRUG-DRUG INTERACTION BETWEEN NEVIRAPINE AND PACLITAXEL

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Submitted

ABSTRACT

The aim of the study was to describe the pharmacokinetics of nevirapine and paclitaxel in a patient who used both drugs concomitantly and to investigate any potential pharmacokinetic drug-drug interaction, for which strong theoretical indications exist.

Plasma concentrations of nevirapine (dose: 200 mg twice daily orally) and paclitaxel (dose: 100 mg/m^2 3-h intravenous infusion) were determined in a human immunodeficiency virus 1 (HIV-1)-infected patient with Kaposi´s sarcoma. Since both drugs are metabolised via the same cytochrome P450 isoenzymes, investigation of a drug-drug interaction was considered important.

We found that the plasma concentrations of nevirapine given together with paclitaxel were similar with those given without paclitaxel. The exposures to paclitaxel ($AUC_{0-\infty}$=3787 h⋆ng/mL) and its hydroxy-metabolites when co-administered with nevirapine were comparable to the mean exposure to paclitaxel and its metabolites from 8 historical controls ($AUC_{0-\infty}$=3614 h⋆ng/mL) treated with the same dose.

No pharmacokinetic drug-drug interaction between nevirapine and paclitaxel could be demonstrated in our HIV-1-infected patient.

INTRODUCTION

Kaposi´s sarcoma is the most common human immunodeficiency virus (HIV)-associated malignancy.[1] Optimal antiretroviral therapy is an essential component of Kaposi´s sarcoma management. It has reduced the incidence and prolonged the time to treatment failure in Kaposi´s sarcoma.[2,3] For patients with more extensive disease or failure to respond sufficiently to highly active antiretroviral therapy (HAART), a variety of systemic and sometimes topical therapies is used, including chemotherapy, radiotherapy and/or immunotherapy.[4] Paclitaxel has previously demonstrated impressive antitumour activity in patients with HIV-1-associated Kaposi´s sarcoma.[5] More recently, it has been approved by the EMEA and FDA as second-line monotherapy for advanced Kaposi´s sarcoma.[6]

When an HIV-1-infected patient with Kaposi´s sarcoma has to be treated with antiretroviral therapy and paclitaxel, there is a considerable potential for pharmacokinetic drug interactions.[7-10] Paclitaxel is metabolised by the hepatic cytochrome P450 (CYP) 3A4 and CYP2C8 isoenzymes to 3'-p-hydroxypaclitaxel (3'OHP) and 6α-hydroxypaclitaxel (6OHP), respectively.[11] Nevirapine induces CYP3A4 enzymes [12] and may thus increase paclitaxel metabolism.

We studied the pharmacokinetics and the possible drug-drug interaction of nevirapine and paclitaxel in an HIV-1-infected patient with Kaposi´s sarcoma.

PATIENT AND METHODS

Presentation of case

A 37 year old male patient was diagnosed with Kaposi´s sarcoma on the uvula and the left lower leg in the form of a sensitive and red oedema with superficial injures and wound fluid. Around the swelling, small skin lesions were seen. The patient had a low cellular immunity of 170 CD4 cells/mm^3 and a high plasma viral load of 113,000 copies/mL.

The patient received antiretroviral therapy consisting of stavudine (40 mg twice daily), lamivudine (150 mg twice daily) and nevirapine (200 mg twice daily). The extensive Kaposi´s sarcoma was treated with paclitaxel, given at 100 mg/m^2 (180 mg) as a 3-h intravenous infusion.

Sampling and analyses

Samples for pharmacokinetic analysis were collected by intravenous sampling from the arm contralateral to the one in which the paclitaxel was infused. Samples were collected prior to the start of the paclitaxel infusion, at 0.5, 1 and 2 h during the infusion, at the end of the 3-h infusion, and 0.5, 1.5, 2.5 and 4.5 h after the cessation of the paclitaxel infusion. Paclitaxel and the metabolites 6OHP and 3'OHP were quantitated by a validated and sensitive liquid chromatography-mass spectrometry (LC-MS/MS) method.[13] The area under the plasma concentration versus time curves ($AUC_{0-\infty}$) of paclitaxel and metabolites were determined using the trapezoidal rule from the concentration versus time plots with extrapolation to infinity (WinNonlin Professional, Version 4.1, Pharsight Corporation, Mountain View, CA). Clearance (CL) of paclitaxel was calculated as CL=dose/AUC. Values of the pharmacokinetic parameters maximum concentration (C_{max}), $AUC_{0-\infty}$, half-life ($t_{½}$), volume of distribution (V), CL and time that paclitaxel concentrations are higher than 0.1 μmol/L (T>0.1 μmol) were compared to values from courses (100 mg/m^2 3-h infusion) from 8 patients, as part of a paclitaxel dose-escalating study executed in our Institute.[14]

Nevirapine, stavudine and lamivudine were given 1 h prior to the paclitaxel infusion. Samples for determination of the plasma concentrations of nevirapine were collected prior to intake, at 0.5, 1.5, 2, 2.5, 3.5, 4.5, 5, 6, 7 and 9 h after ingestion. Concentrations were assayed by a validated and sensitive high-performance liquid chromatography (HPLC) assay with ultraviolet (UV) detection.[15] Plasma

concentrations of nevirapine during concomitant paclitaxel use were compared with plasma concentrations of nevirapine from the same patient when no paclitaxel was used (samples were drawn at least 5 days after paclitaxel course).

RESULTS

The pharmacokinetic parameters of paclitaxel (C_{max}, $AUC_{0-\infty}$, $t_{½}$, CL, V and T >0.1 μmol/L), of the HIV-1-infected patient who used concomitantly nevirapine, were comparable with the results of 8 courses of non-HIV-1-infected historical controls (table 1). In figure 1 the pharmacokinetic profiles of paclitaxel and its metabolites are visualised.

The concentration versus time curve of nevirapine during paclitaxel treatment, is shown in figure 2. Also single concentration-time points of nevirapine, when used without paclitaxel, from our patient are presented in the figure. The mean plasma concentrations and standard deviations of nevirapine with and without paclitaxel are 4.79 ±0.56 mg/L and 4.21 ±0.40 mg/L, respectively (p=0.75).

Table 1. Pharmacokinetic parameters of paclitaxel.

	C_{max} (ng/mL)	$AUC_{0-\infty}$ (h⋆ng/mL)	$t_{1/2}$ (h)	CL (L/h)	V (L)	T >0.1 μmol/L (h)
Paclitaxel 100 mg/m^2 (P)	989	3787	1.75	47.5	72.2	10.0
Paclitaxel 100 mg/m^2 (C) (mean ± SD)	967 ±187	3614 ±701	4.44 ±3.85	51.2 ±12.3	130 ±100	6.79 ±0.996
p-value⋆	1.00	0.439	0.245	1.00	0.439	0.121

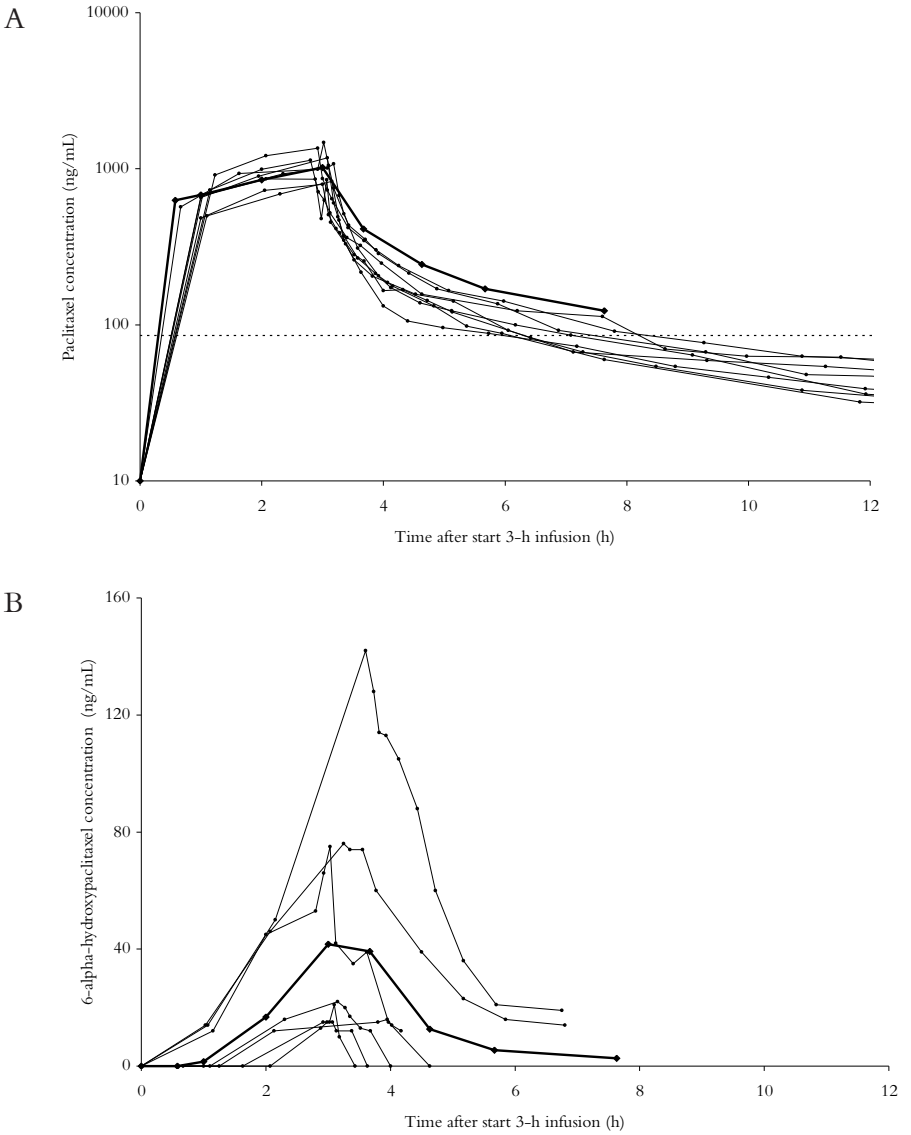
⋆: Mann-Whitney test. C_{max}=maximum concentration, $AUC_{0-\infty}$=area under the concentration-time curve with extrapolation to infinity, $t_{1/2}$=elimination half-life, CL=clearance, V=volume of distribution, T >0.1 μmol/L=time that paclitaxel plasma concentrations are higher than 0.1 μmol/L (85 ng/mL), P=patient (also using nevirapine 200 mg twice daily), C=control group (n=8),[14] SD=standard deviation.

DISCUSSION AND CONCLUSION

We studied the pharmacokinetics and the possible drug-drug interaction of nevirapine and paclitaxel, for which strong theoretical indications exist, in an HIV-1-infected patient with Kaposi´s sarcoma.

Optimal antiretroviral therapy is obtained only when maximally suppressive drug concentrations during a complete dosage interval are maintained. A study exploring the association of exposure to nevirapine with virological response in antiretroviral HIV-1-infected patients yielded a target trough concentration of 3.4 mg/L.[16] In our case it appeared that nevirapine plasma concentrations were not significantly influenced by combined use with paclitaxel and were always higher than the

Figure 1. Plasma concentration-time curves of paclitaxel (A), metabolite 6α-hydroxypaclitaxel (B), metabolite 3'-p-hydroxypaclitaxel (C). Dots connected with a line represent the paclitaxel curves of control patients. Diamonds connected with a line (bold-type) represent paclitaxel concentrations with concomitant use of nevirapine. The dotted line in panel A represents the threshold concentration of 0.1 μmol/L (=85 ng/mL).[14]



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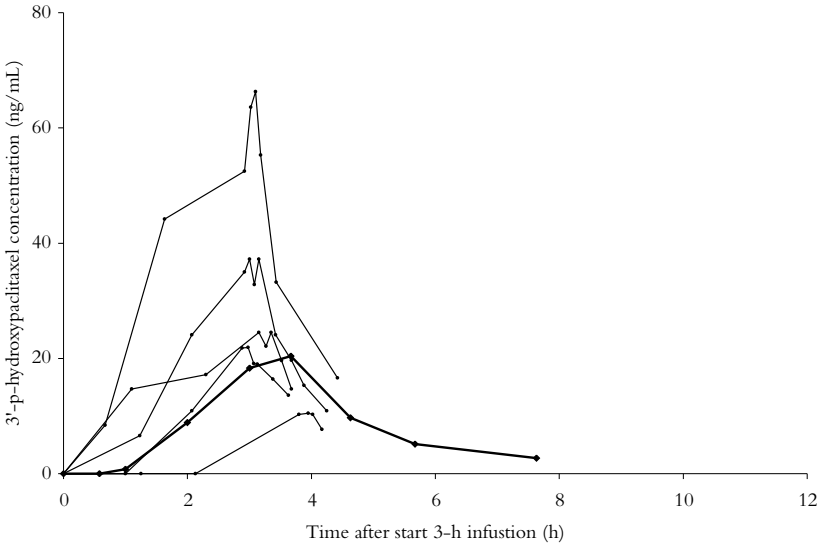
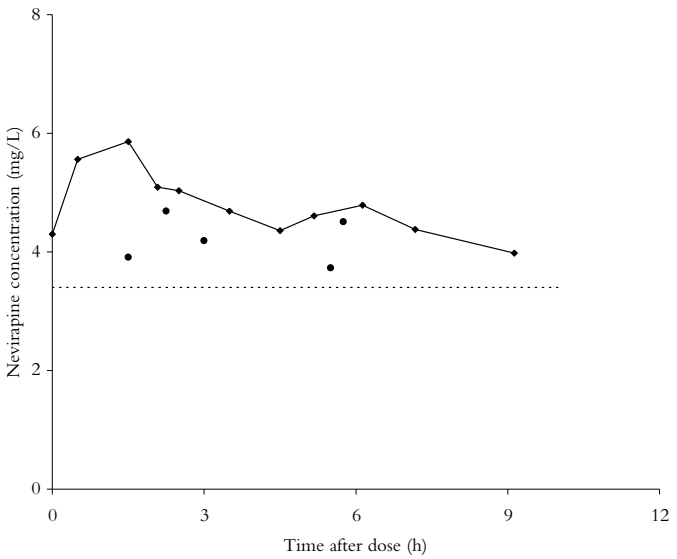


target trough concentration. Thus, paclitaxel and the mixture of polyoxyethylated castor oil and ethanol (50/50, volume/volume), which is used in the intravenous formulation of paclitaxel to improve its aqueous solubility but causes several drug-drug interactions,[17] did not influence the pharmacokinetics of nevirapine. Since adequate nevirapine levels were reached, which resulted in an increase of cellular immunity (340 CD4 cells/mm^3) and undetectable viral load (<50 copies/mL), dose adjustment of nevirapine when used with paclitaxel is not needed. The pharmacokinetic parameters of paclitaxel and its metabolites 6OHP and 3'OHP in our HIV-1-infected patient were comparable with the 8 non-HIV-1-infected historical controls, to whom paclitaxel had been given the same dose of 100 mg/m^2, as a 3-h infusion. To compare the pharmacokinetics of paclitaxel in our patient with those in a control group, it is of paramount importance that this group received the same dose with the same duration of infusion, since paclitaxel is characterised by non-linear pharmacokinetics.[14]

Paclitaxel is metabolised into the metabolites 6OHP and 3'OHP by CYP2C8 and CYP3A4, respectively. Nevirapine induces CYP3A4. This could have led to an increased metabolism of paclitaxel into the 3'OHP metabolite and to a reduction of CYP2C8 metabolism but was not demonstrated.

Thus, despite of the strong theoretical indications for a potentially clinically relevant pharmacokinetic drug-drug interactions between nevirapine and paclitaxel, the pharmacokinetic parameters of paclitaxel and metabolite profiles were comparable for our HIV-1-infected patient and the control group. Also

Figure 2. Plasma concentration-time curve of nevirapine. Diamonds connected with a line represent the nevirapine curve during paclitaxel treatment. Dots represent single nevirapine concentrations without concomitant use of paclitaxel. The doted line represents the target trough concentration of 3.4 mg/L.



the plasma concentrations of nevirapine when dosed concomitantly with paclitaxel were similar to those without paclitaxel. From this case it could not be demonstrated that nevirapine affected the pharmacokinetics of paclitaxel or its metabolic pathway, or vice versa.

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CHAPTER 1.3

POPULATION PHARMACOKINETICS OF EFAVIRENZ IN AN UNSELECTED COHORT OF HIV-1-INFECTED INDIVIDUALS

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Clinical Pharmacokinetics
in press

ABSTRACT

The aim of the study was to characterise the population pharmacokinetics of efavirenz in a representative patient population and to identify patient characteristics influencing the pharmacokinetics of efavirenz, with the ultimate goal to further develop techniques that can be applied to optimise therapeutic drug monitoring of antiretroviral agents.

Ambulatory HIV-1-infected patients using an efavirenz containing regimen were included. During regular visits, blood samples were collected for efavirenz plasma concentrations and clinical chemistry parameters. Concentrations of efavirenz were quantitatively assessed by a validated high-performance liquid chromatographic with ultraviolet detection method.

Using non-linear mixed effect modelling (NONMEM) the pharmacokinetics of efavirenz were described. Disposition of efavirenz was described by a two-compartment model and absorption was modelled using a chain of three transition compartments. Clearance, volume of distribution, intercompartmental clearance, the peripheral volume of distribution and the intercompartmental absorption rate constant were estimated. Further, interindividual, interoccasion and residual variability were estimated. The influence of patient characteristics on the pharmacokinetic parameters of efavirenz was explored.

From 172 patients 40 full pharmacokinetic curves and 315 efavirenz plasma concentrations at a single time point were available, resulting in a database of 1009 efavirenz plasma concentrations. Clearance, volume of distribution, and absorption rate constant were 11.7 L/h (4.3% RSE), 189 L (14.6% RSE) and 3.07 h^{-1} (11.2% RSE), respectively. Residual variability in the model was composed of 0.14 mg/L additive error and 8.85% proportional error. Asian race and baseline total bilirubin (TBR) increased the relative bioavailability of efavirenz with 56% and 57%, respectively. No significant covariates were found for clearance or volume of distribution.

The pharmacokinetic parameters of efavirenz were adequately described with the developed population pharmacokinetic model. Asian race and baseline TBR were found to be significantly correlated with bioavailability of efavirenz. The described model will be an essential tool in further optimisation of efavirenz containing antiretroviral therapy, e.g. by the use of Bayesian estimation of individual pharmacokinetic parameters.

INTRODUCTION

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are one of the four classes of drugs currently approved to treat human immunodeficiency virus (HIV) infection.

Efavirenz is a potent NNRTI, which inhibits non-competitively the human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme and interferes with viral RNA to DNA-directed polymerase activities. HIV-2 reverse transcriptase and human cellular DNA polymerase are not inhibited by efavirenz.[1]

Efavirenz is strongly bound to plasma proteins, primarily albumin (>99%). The penetration into the brain is related to the degree of protein binding, resulting in low levels of efavirenz in the cerebrospinal fluid. Nonetheless, concentrations are still sufficient to inhibit viral replication of non-resistant virus and may thus provide antiviral protection in the spinal cord and brain.[1,2]

Central nervous system symptoms (including headache, dizziness, insomnia and fatigue) and dermatological effects (including maculopapular rash) appeared to be the most common adverse events reported with efavirenz containing antiretroviral regimens.[1]

Efavirenz is metabolised by the cytochrome P450 (CYP) enzyme system, primarily by the CYP3A4 and CYP2B6 isoenzymes. The oxidative inactive metabolites are excreted into bile and urine, with less than 1% appearing as unchanged drug in the urine.[1,3] The long terminal half-life ranging from 52 to 76 hours following single oral doses, and from 40 to 55 hours following multiple oral doses, allows for once daily dosing. The decrease in half-life after multiple dosing is explained by autoinduction of efavirenz metabolism.

Treatment failure and central nervous system side effects are associated with efavirenz plasma levels.[4–6] Limited data on pharmacokinetic parameters of efavirenz and correlations between patient characteristics and pharmacokinetics are available. Patients with hepatic diseases showed a 35% decrease in maximal plasma concentration compared with healthy volunteers following a single 400 mg dose. The apparent half-life increased from 118 to 152 hours, however the area under the concentration-time curve (AUC) did not differ significantly. The standard dose of efavirenz is recommended to patients with chronic liver diseases, but toxicity should be monitored.[7] A population pharmacokinetic analysis from phase II studies of efavirenz found weight to be a significant predictor of clearance.[4] Body weight and race have also been related with efavirenz plasma concentrations.[8,9] Pfister et al. showed that the hepatic clearance of efavirenz appeared to be higher

in white non-Hispanics than in African Americans and Hispanics.[10] In a cohort of HIV-1-infected patients significant racial differences in time to virologic failure that appear to be specific for efavirenz-based therapy, have been observed.[11] However, in a population analysis of efavirenz pharmacokinetics in HIV-positive patients no clinically significant influence of patient characteristics on the efavirenz pharmacokinetic parameters was shown.[12]
Several patient characteristics (e.g. demographics, co-morbidity) may have a large impact on plasma levels. Knowledge of these factors is very useful in the treatment of HIV-1-infected patients. For instance, subpopulations at risk for high or low concentrations may be identified a priori based on these relations.
In order to establish the pharmacokinetics and to identify patient characteristics involved in the variability of pharmacokinetic parameters, a population pharmacokinetic analysis was performed in an unselected cohort of HIV-1-infected individuals. In this study we have focussed on the absorption phase of efavirenz to get more insight into this process by investigating concentration-time points over the total time schedule of a dose (0-24 h). Since toxicity is related with maximal concentrations an adequate description of the absorption process is of paramount importance. The developed model will be used to further optimise therapeutic drug monitoring (TDM) of efavirenz containing antiretroviral therapy, by e.g. the use of Bayesian estimation of individual pharmacokinetic parameters.

METHODS

Patients

Included patients were ambulatory HIV-1-infected patients from the outpatient clinics of the Slotervaart Hospital and the Academic Medical Centre, Amsterdam, The Netherlands. Data were collected during regular outpatient clinic visits at random time points, between September 1997 and January 2002. All patients were using efavirenz as part of their antiretroviral regimen and had at least one efavirenz plasma concentration available for analysis. Most patients received 600 mg oral efavirenz once a day, 5 patients received 800 mg once daily, 7 patients received 200, 300 or 400 mg once daily.

Sampling and bioanalysis

At each visit to the clinic, a blood sample was obtained for the determination of the efavirenz plasma concentration. Within the TDM-program in the participating hospitals, a strict protocol is utilised in which plasma concentrations of antiretroviral drugs are routinely and frequently monitored. As a consequence

patients are conversant with the principle of recording time of ingestion of the last dose. Additionally, sampling times are recorded electronically at the department of clinical chemistry. From this information, time after ingestion is extracted. In addition to the random samples, from 40 patients full pharmacokinetic curves (15-18 time points), assessed over 24 h, were available, which were collected as part of the DONUT study. Full details of this study have been presented elsewhere.[13] All concentrations were considered to be at steady-state, because all individuals were sampled at least two weeks after initiation of their efavirenz containing regimen.
Concentrations of efavirenz were quantitatively assessed by a validated high-performance liquid chromatographic (HPLC) with ultraviolet (UV) detection method.[14] This method was validated over the range 0.01-10 mg/L using 250 μL of plasma. Recovery of efavirenz from human plasma was 106.4%. Within- and between-day precisions were always less than 4.3% for all quality control samples covering the complete calibration curve.

Population pharmacokinetic analyses

The non-linear mixed effect modelling software program NONMEM (version V 1.1) was used to perform the analyses. The first-order conditional estimation (FOCE) procedure was used throughout. For interaction between the interindividual, intraindividual and residual error was accounted by the use of the INTERACTION option on NONMEM. The adequacy of the developed structural models was evaluated using goodness-of-fit plots, precision of parameter estimates and graphical methods. The minimal value of the objective function (OFV) provided by NONMEM was used for the comparisons of the models. Discrimination between hierarchical models was based on the OFV using the log-likelihood ratio test.[15] A p-value of 0.05, representing a decrease in OFV of 3.84, was considered statistically significant (degrees of freedom (df)=1).
Standard errors for all parameters were calculated using the COVARIANCE option of NONMEM. Individual Bayesian pharmacokinetic estimates of the pharmacokinetic parameters were obtained using the POSTHOC option in NONMEM. For each subject, individual pharmacokinetic parameters were calculated taking both individual observations and population effects into account.[15]

Basic pharmacokinetic model

First-order absorption models with and without absorption lag-time were tested. Also different numbers of transition compartments instead of absorption lag-time

were tested to describe the absorption process. To describe the distribution kinetics of efavirenz both one-compartment models and two-compartment models were tested. Population pharmacokinetic parameters clearance, volume of distribution, transition rate constant, intercompartmental clearance and peripheral volume of distribution were estimated. The typical value of transtition rate constant (k_{tr}) can be converted into a mean absorption time (MAT) with the following formula:

$$MAT = (n + 1)/k_{tr}$$

in which n represents the number of transition compartments. The early and subsequently slower decline in the plasma concentration of efavirenz can be describe with two decay rate constants, α and β. Interindividual and interoccasion variability in the pharmacokinetic parameters were estimated with an exponential error model. For instance, variability in clearance was estimated using:

$$CL/F_{ij} = \theta_1 \star \exp(\eta_i + \kappa_j)$$

in which CL/F_{ij} represents the clearance of the i^{th} individual on the j^{th} occasion, θ_1 is the population value of clearance, η_i is the interindividual random effect with mean 0 and variance ω^2 and κ_j is the interoccasion random effect with mean 0 and variance π^2. Residual variability was modelled with a combined additive and proportional error model.

Covariate pharmacokinetic model

To identify possible relationships between the pharmacokinetics of efavirenz and patient characteristics, the following covariates were collected at baseline: gender, race, alanine aminotransferase (ALAT, in U/L), aspartate aminotransferase (ASAT, in U/L), alkaline phosphatase (AP, in U/L), γ-glutamyltransferase (GGT, in U/L), total bilirubin (TBR, in μmol/L), CD4 cells (in /mm^3), CD8 cells (in /mm^3) and viral load (in number of copies/mL). Patients were considered to have a chronic hepatitis B infections when hepatitis surface antigen (HbsAg) could be detected at baseline. When anti-hepatitis C antibodies (anti-HCV) were present at baseline, patients were considered to have a chronic hepatitis C infection. In addition, covariates determined during treatment with efavirenz were collected and included age (in years), body weight (in kg), serum creatinine (CR, in μmol/L). Although the use of co-administered drugs in addition to antiretroviral drugs in our cohort was very limited, potential interfering drugs [16] were included in this analysis. CD4 cell count, CD8 cell count, viral load, age, weight and CR were examined as continuous variables. Gender, race, hepatitis B co-infecion and hepatitis C co-infection were examined as dichotomous variables. The values of ALAT, ASAT, AP, GGT and TBR were transformed to dichotomous variables by using 2 times

the upper limit of normal for ASAT and ALAT and 1.5 times the upper limit of normal for, AP, GGT and TBR as cut-off value. Not all variables were available from all patients. In order to avoid bias, a covariate was included into the model to indicate the missing data. For instance, the influence of a dichotomous covariate X on clearance with missing data of X for some individuals was modelled as:

$$TVCL = \theta_1 \star \theta_2^{X \star (1-MIS)} \star \theta_3^{MIS}$$

in which TVCL is the typical value of clearance in the population, MIS is 1 for records with missing data and 0 for all other records, θ_1 is the typical value of an individual with X=0 (no missing data) and θ_2 is the relative difference in clearance for individuals with X=1 (no missing data) and θ_3 is the relative difference in clearance for individuals with missing data.

A covariant was included in an intermediate model when the inclusion of this covariate was statistically significant or clinically relevant. A covariate was considered statistically significant when the inclusion was associated with a decrease in minimal value of the OFV associated with a p-value of <0.05 (log-likelihood ratio test). Clinical relevance was reached when the typical value of the pharmacokinetic parameter of interest changed at least 10% in the range of the covariate observed.

All significant or relevant covariates were included in an intermediate model. Finally, a stepwise backward elimination procedure was carried out. A parameter was only retained in the model when the influence of this parameter was statistically significant ($p<0.05$) and clinically relevant (10% change in pharmacokinetic parameter).

Statistical refinement

The validity of the inter-individual variability model was checked by evaluating correlations between individual random effects (η) and interoccasion random effects (κ) for all of the pharmacokinetic parameters.[17]

Posterior predictive check

The posterior predictive check technique was applied as an internal validation of the developed population pharmacokinetic model. This validation method was executed to assess whether simulated data sets have the same characteristics as the original data set. From the concentration data of the index data set, trough concentrations were selected. A concentration was considered a trough concentration when the sample was taken within 0.5 h before or after ingestion of 600 mg efavirenz in a once daily regimen. The median of the trough concentrations

was determined. Subsequently, at least 1000 data sets were simulated based on the index data set and the final pharmacokinetic parameters of the basic model without covariates. The median in the index data set was compared with the 90% prediction intervals of the median values, calculated in all 1000 simulated data sets.[18]

To illustrate the posterior predictive check findings 1000 individuals were simulated over the complete dosing interval (0-24 h) to show observed and 90% prediction intervals of simulated data.

RESULTS

Patients

From 172 outpatients 40 full pharmacokinetic curves and 315 plasma concentrations at a single time point were available, resulting in a database of 1009 efavirenz plasma concentrations. Full curves consisted of 15-18 time points. The effect of time after

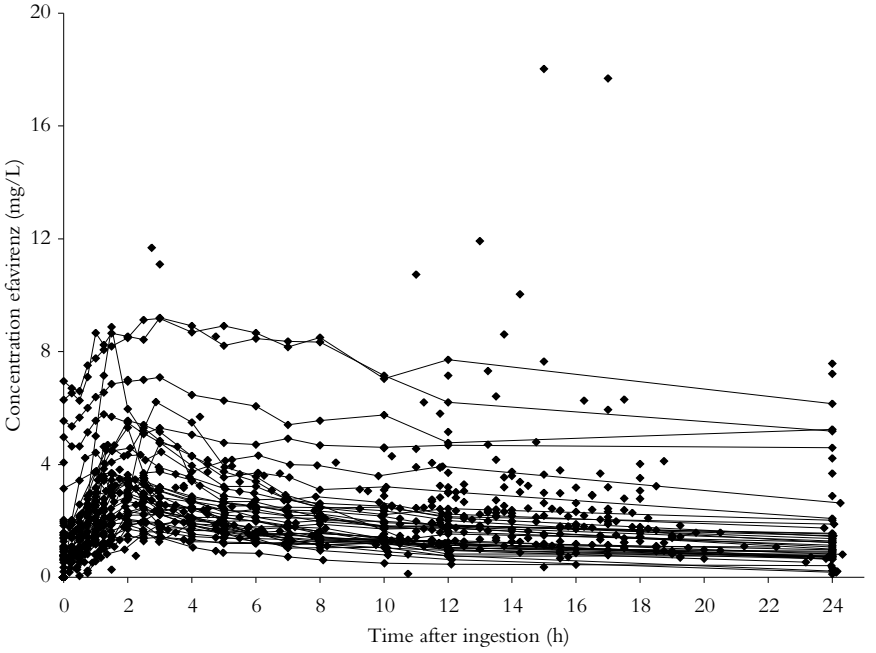
Table 1. Patient characteristics of 172 individuals.

Parameter		median	IQR	no. of patients >1.5 x ULN*	no. of missing
Age (years)		40	35.6-47.4		1
Gender M/F (%)	125/25 (80/20)				22
Weight (kg)		72	66.0-79.0		59
Race					34
Caucasian (%)	103 (74.6)				
Black (%)	20 (14.5)				
Asian (%)	10 (7.2)				
Latino (%)	5 (3.6)				
Clinical chemistry					
Baseline ASAT (U/L)		37	27-55	21	21
Baseline ALAT (U/L)		35	23-61	21	22
Baseline GGT (U/L)		56	31-126	98	25
Baseline AP (U/L)		85	65-108	9	21
Baseline TBR (μmol/L)		9	7-14	11	19
Creatinine (μmol/L)		69	59-79		40
CD4/CD8 cell counts at baseline					
CD4 cell count (/mm^3)		300	125-480		41
CD8 cell count (/mm^3)		970	610-1370		51
Plasma HIV-1 RNA at baseline					
Plasma $\log_{10}$ HIV-1 RNA (copies/mL)		3.06	<1.70-5.10		21
HBV / no HBV (%)	11/115 (8.7/91.3)				46
HCV / no HCV (%)	10/115 (8/92)				47

*: for ASAT and ALAT >2 x ULN. IQR=interquartile range, ULN=upper limit of normal, M=male, F=female, ASAT=aspartate aminotransferase, ALAT=alanine aminotransferase, GGT=gamma-glutamyltransferase, AP=alkaline phosphatase, TBR=total bilirubin, HBV=hepatitis B infection, HCV=hepatitis C infection.

start of efavirenz containing regimen was used as covariate, however no influence could be demonstrated. Two patients had a remarkably high plasma concentration, however no reasons for this deviation could be identified. Patient characteristics of the data set are presented in table 1. The patient population was predominantly male and Caucasian. From 1-34% of the patients (depending on the covariable), characteristics were not available. Most used co-administered drugs in addition to the antiretroviral regimen were prednison (9 patients), co-trimoxazol (8 patients), temazepam (7 patients), rifampicine (6 patients), methadone (6 patients), cetirizine (4 patients) and fluconazol (4 patients). Not taking the data from the full curves into account, a mean of 2 to 3 samples (i.e. 3 to 9 months) per patient (ranging from 1 to 16, i.e. up to 31 months) was used in this study. Figure 1 shows the concentration-time data of efavirenz.

Figure 1. Concentration-time data of efavirenz. Solid diamonds represent plasma concentrations at a single time point, dots connected with lines represent full pharmacokinetic curves.



Pharmacokinetics

The population pharmacokinetics of efavirenz were best described with a two-compartment model with first-order elimination from the central compartment. Because the absorption of efavirenz occurs fast but with a slow onset, a lag-time was included in the model. This parameter resulted in a marked decrease in OFV as compared to the former model (Δ=-146, $p<0.001$), indicating an improved fit of the model to the data. However, an overestimation of the concentrations in the early absorption phase was seen. This systematic deviation could be corrected by using several transition compartments between the depot compartment and the central compartment instead of a lag-time. A chain of three transition compartments between the absorption and the central compartment best described the absorption process. This resulted in a decrease in OFV as compared to the lag-time model (Δ=-95, $p<0.001$). In the elimination phase an underestimation of the concentrations was seen indicating the presence of a peripheral compartment. Subsequently, a second compartment was introduced into the model, resulting in an improvement of the model (ΔOFV=-223). The model was further optimised by including relative bioavailability. In the absence of intravenous data, the population value for bioavailability was fixed to 1, and an interoccasion variability on relative bioavailability was estimated. This resulted in a decrease in OFV (Δ= -46, $p<0.001$). This model was considered the final structural model and was used for the development of the covariate model. The model used is schematically depicted in figure 2.

The results of the basic pharmacokinetic model are summarised in table 2. The estimate of clearance was 11.4 L/h with an interindividual (IIV) and interoccasion variability (IOV) of 44% and 39%, respectively. The estimate of volume of

Figure 2. Schematic representation of the basic pharmacokinetic model.

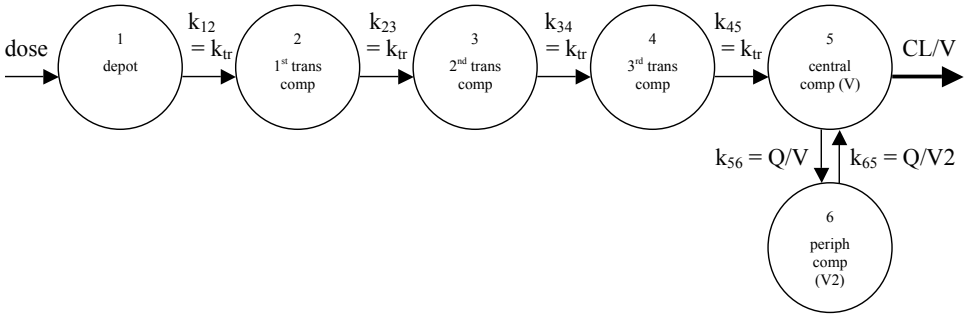


Table 2. Final parameter estimates of basic and final pharmacokinetic model.

	Basic model		Final model		
	Est	RSE (%)	Est	RSE (%)	p-value
CL/F (L/h)	11.4	4.1	11.7	4.3	
V/F (L)	207	13.7	189	14.6	
k_{tr} (h^{-1})	3.07	10.7	3.07	11.2	
Q (L/h)	71.6	21.5	62.9	21.5	
V2 (L)	282	15.5	233	12.8	
$\theta_{ASIAN\ race}$			1.56	25.3	0.0305
$\theta_{race\ missing}$			0.894	11.2	
$\theta_{TBR>1.5\ ULN}$			1.57	13.7	0.0130
$\theta_{TBR\ missing}$			1.01	13.0	
Additive error (mg/L)	0.139	17.3	0.138	17.9	
Proportional error (%)	8.68	14.5	8.85	14.1	
Interindividual variability CL/F (%)	43.6	18.3	39.7	21.6	
Interindividual variability V/F (%)	32.7	53.6	40.1	39.1	
Interindividual variability k_{tr} (%)	38.5	24.2	40.4	25.1	
Interindividual variability Q (%)	62.8	47.5	60.8	38.9	
Interoccasion variability CL/F (%)	38.9	40.9	21.9	23.5	
Interoccasion variability F (%)	37.6	12.9	35.2	17.4	

Est=parameter estimate, RSE=relative standard error, CL/F=clearance, V/F=volume of distribution, k_{tr}=transition rate constant, Q=intercompartimental clearance, V2=peripheral volume of distribution, TBR=total bilirubin, F=bioavailability.

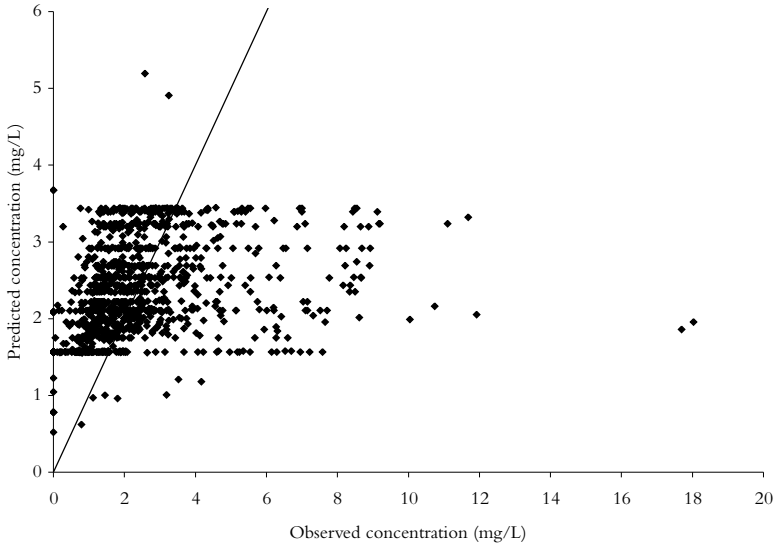
distribution and transition rate constant were 207 L (IIV=33%) and 3.07 h^{-1} (IIV=40%), respectively. The IOV of relative bioavailability was 38%.

The conversion of transition rate constant into mean absorption time leads to a value of 1.3 h, indicating a rapid absorption after oral administration from the depot to the central compartment. The intercompartmental rate constants k_{56} and k_{65} can be calculated by dividing intercompartmental clearance by volume of distribution and peripheral volume of distribution. Subsequently, the decay rate constants α and β can be calculated, resulting in 0.633 h^{-1} and 0.022 h^{-1}, respectively with an associated half-life of 1.10 h^{-1} and 31.35 h^{-1}, respectively. The population was associated with an additive error of 0.14 mg/L and a proportional error of 8.68%.

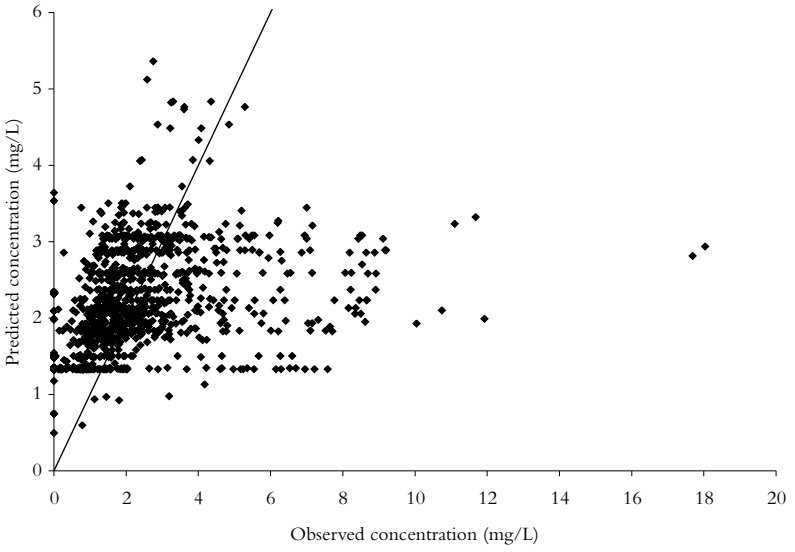
The different covariates were introduced separately into the basic model on clearance, volume of distribution and relative bioavailability, using a univariate procedure. Gender, weight, race, baseline TBR, baseline ASAT, baseline ALAT, baseline AF and hepatitis C co-infection had a statistically significant or clinically relevant relation with clearance and/or relative bioavailability. None of the covariates had a significant or relevant relation with volume of distribution. Concomitantly used drugs did not show a relation with any pharmacokinetic parameter of efavirenz. The relationships between clearance and the covariates gender, weight, race and baseline TBR were included simultaneously in the model. The same was performed to investigate relationships with relative bioavailability. Finally, only baseline TBR and Asian race had a significant and relevant relation

Figure 3. Model predicted concentrations versus observed concentrations of efavirenz using the basic model (A) and the final model (B). The solid line represents the line of identity.

A



B



with relative bioavailability, suggesting that Asians and patients with high baseline TBR could have a higher relative bioavailabiliy value than other patients, with similar clearance and volume of distribution. The final model contained these covariables on bioavailability.

Statistical refinement

Correlations between κ_{CL} and κ_F and between η_V and η_{ktr} were observed. However, models with inclusion of covariance between these parameters did not converge possibly due to overparameterisation.

In table 2 the results of the final pharmacokinetic model are presented. Figure 3 shows the model predicted concentrations from the basic model (panel A) and the final model (panel B) versus the observed concentrations of efavirenz. As can be observed, the final model (B) describes the data better than the basic model (A). In figure 4A, the individual predicted plasma concentrations, based on the final model, are plotted versus the observed plasma concentrations. The model based predictions are symmetrically distributed around the line of identity, indicating that the model adequately describes the pharmacokinetic profile of efavirenz. Figure 4B shows the plot of the weighted residuals versus the predicted concentrations of efavirenz.

The magnitudes of increase in relative bioavailability were 56% and 57% for Asian race and baseline TBR >1.5 x upper limit of normal, respectively. The following equation describes the final model for bioavailabiliy:

$$F = 1 \star 1.56^{\text{Asian}} \star 0.894^{\text{MIS-race}} \star 1.57^{\text{TBR}} \star 1.01^{\text{MIS-TBR}}$$

in which Asian is 1 for Asian individuals and 0 for all others, TBR is 1 for patients with baseline TBR >1.5 x upper limit of normal and 0 for all others, and MIS is 1 for patients with missing value for the indicated variable and 0 for all others.

Posterior predictive check

The observed median of the trough efavirenz concentration was 1.27 mg/L. The 90% prediction interval from the simulated data sets for the basic model without covariates was 1.08-2.56 mg/L. These results of the posterior predictive check showed that the observed median of the trough efavirenz concentration was well included in the 90% prediction interval from the simulated data sets for the basic model, indicating a stable model to describe the efavirenz concentration-time data adequately. To illustrate the findings of the posterior predictive check observed and 90% prediction intervals of simulated data over the complete dosing interval (0-24 h) are shown in figure 5.

Figure 4. Individual predicted concentrations versus observed concentrations (A) and weighted residuals versus the predicted concentrations (B) using the final model for efavirenz. The solid line represents the line of identity.

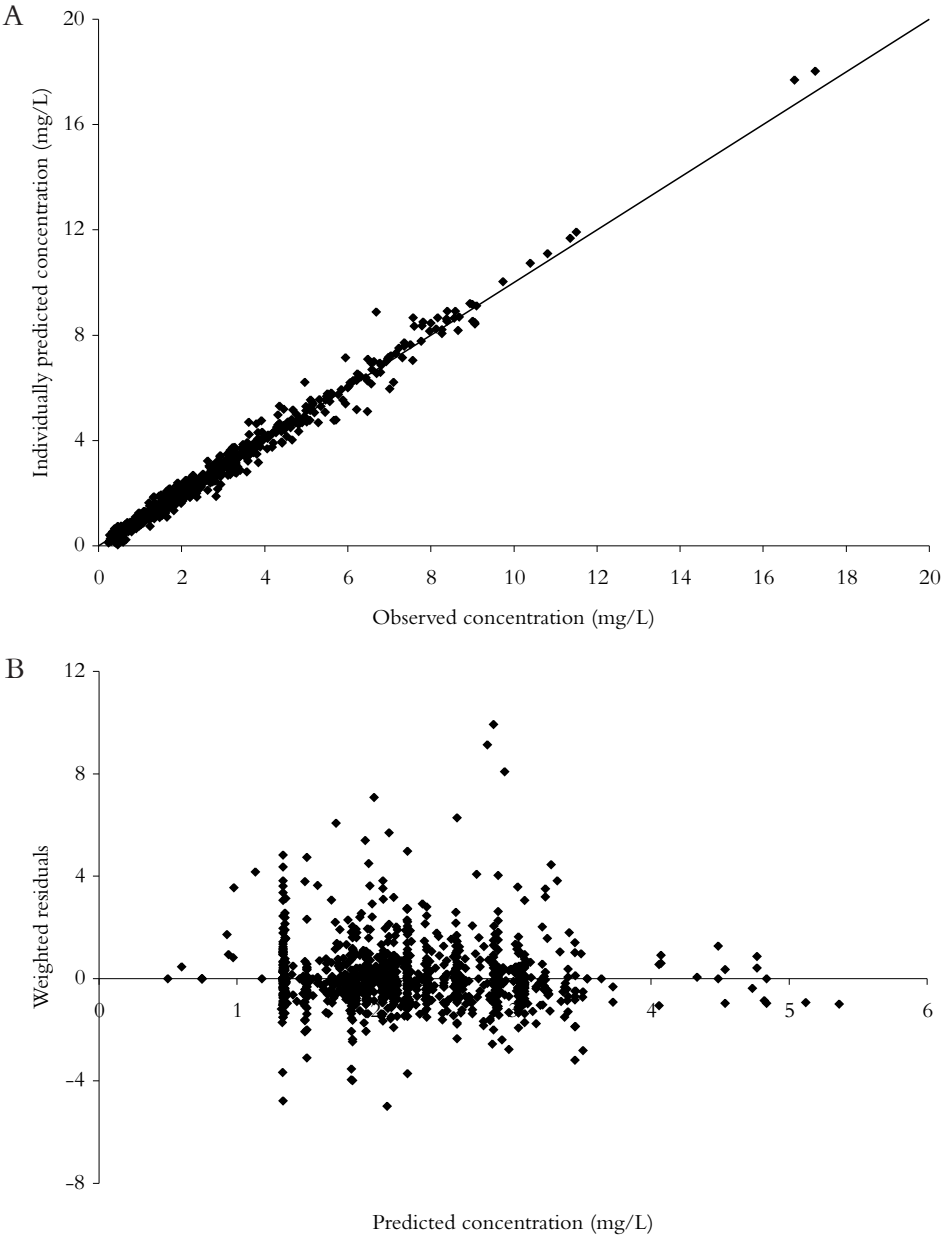
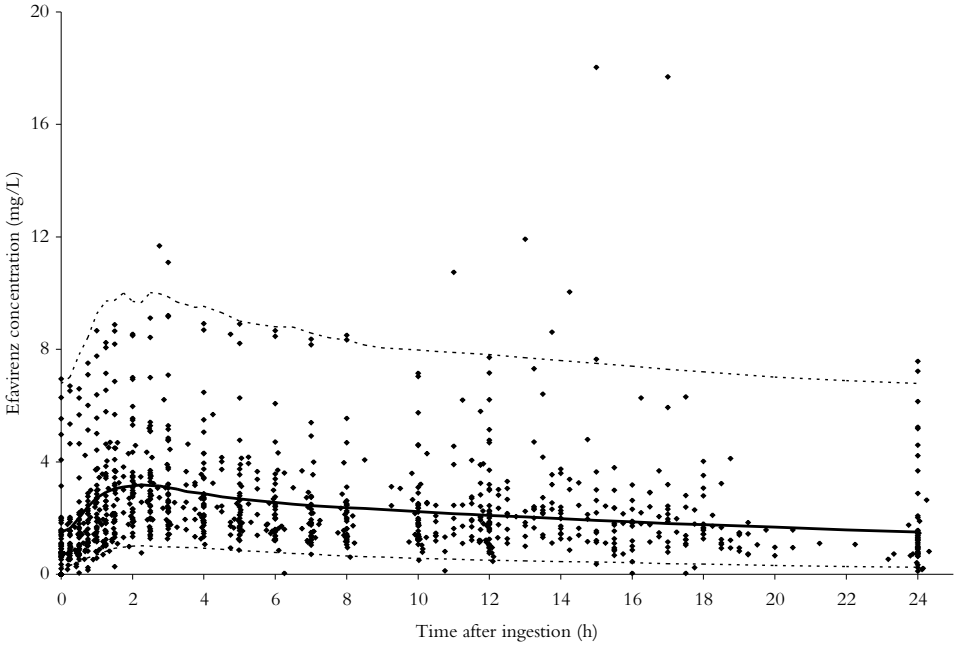


Figure 5. Solid dots represent observed efavirenz concentrations, the solid line represents the median value of simulated data, the dotted lines represent the 90% prediction intervals of simulated data.



DISCUSSION

In case of the antiretroviral drug efavirenz it is important to understand the pharmacokinetic profile and the influencing covariables, as they determine the plasma concentration of efavirenz. Optimal plasma concentrations are of tremendous importance to maintain optimal viral suppression and to avoid toxicity.[4,6] Dosage reduction of efavirenz can resolve psychiatric disorders caused by high concentrations of efavirenz.[8] TDM can be executed with Bayesian estimates from the population pharmacokinetic model. The population pharmacokinetic approach is increasingly recognised as a valuable tool in support of TDM. The major advantages of this technique include the ability to describe more complex pharmacokinetic models, the ability to quantify interindividual variability and the proper identification of the influence of patient characteristics on this variability. The pharmacokinetics of efavirenz were adequately described with the developed population pharmacokinetic model. The absorption of efavirenz is fast, however with a slow onset and therefore, zero or first-order absorption models did not

describe this phase sufficiently. Models with lag-time or transition compartment both show a slow onset, however with lag-time the plasma concentration of efavirenz will decrease till the absorption occurs. In the observed data a clear but minimal increase was observed in the early absorption phase and therefore, the absorption phase was best described with three transition compartments.
The final model gave estimates for the two volumes of distribution of 189 L and 233 L, respectively. Also in literature high values for volume of distribution were found.[10,12] For clearance 11.7 L/h was estimated, which was consistent with literature.[3,10,12,19]
The difference between the interindividual variability of clearance (40%) and the interoccasion variability of clearance (22%) in the final model is noteworthy. It indicates that high variations in clearance occur between patients but not within patients, which may support the usefulness of TDM for efavirenz. All parameters were estimated with acceptable precision as the relative standard error (RSE) varied between 4% and 39%. The accuracy was high with a small residual variability of 0.138 mg/L (additive) and 8.85% (proportional).
In many patients covariate information was missing (1-34%). It is preferred to model the missing patient characteristics,[20] however covariate information was missing non-randomly.
The inclusion of relevant covariables in the model has been performed according to a stepwise procedure. Asian race and baseline TBR had a significant and relevant relation with relative bioavailabiliy. However, the decrease of OFV and the reduction in interindividual variabilities in the final model with regard to the basic model were very limited. Therefore, the predicting effects of Asian race and raised bilirubin at baseline should be interpreted with caution. Because the clinical relevance of these covariate effects on the relative bioavailability of efavirenz may be marginal it is not reasonable to give a patient with these covariates a priori a decreased starting dose. However, with knowledge about race and baseline bilirubin HIV-1-infected patients at risk for complications may be given extra attention.
A baseline value of TBR >1.5 x upper limit of normal increased bioavailabiliy of efavirenz with 57%. The quantity of total bilirubin in the blood comprises two fractions, namely conjugated and nonconjugated bilirubin. An increase in nonconjugated bilirubin only may reflect a defective processing of the nonconjugated bilirubin, which does not by definition means a hepatic dysfunction.
In this study, patients from the Asian race appeared to have higher bioavailabiliy than patients from other races. Efavirenz is primarily metabolised by the isoenzyme

CYP3A4, which is expressed predominantly in the liver and gastrointestinal tract.[21,22] Inhibition of CYP3A4 has been shown to lead to an increase in the bioavailability of many drugs metabolised by this enzyme.[23,24] Although CYP3A4 is involved in the metabolism of a variety of drugs and responsible for substantial variability of pharmacokinetics, no polymorphism has been demonstrated convincingly.[21,25-27] Ethnic differences in pharmacokinetics, however, have been reported for several drugs.[22,24,28]

The expression of CYP3A in the small intestine can account for the significant presystemic metabolism of a number of drugs and subsequently affect the bioavailability.[24,29] Therefore, the possible explanation for a higher bioavailability of efavirenz in Asian patients might be that Asian subjects have lower intestinal CYP3A4 functionality leading to less CYP3A4-dependent gut wall metabolism and thus a higher efavirenz concentration. Alternatively, a lower CYP3A4 activity may have arisen from environmental or dietary influences.[21,28]

Also intestinal P-glycoprotein (P-gp) might have an effect on the bioavailability of efavirenz. Several studies have demonstrated that P-gp plays a significant role in drug absorption and disposition.[30-32] The genetic polymorphisms of P-gp may represent a major source of individual variability in the pharmacokinetics of drugs.

The effect of food intake and compliance could not be taken into account. The absorption of efavirenz is affected by the administration of meals with a high-fat content resulting in a mean increase in AUC of 50% in a small study of healthy volunteers. Meals with a moderate fat content do not influence the absorption of efavirenz appreciable.[1,3]

Also compliance might have an influence on the concentrations of efavirenz. Non-compliance may cause two effects on the plasma concentrations. Plasma levels can become higher than expected if more medication is taken or medication is taken later than reported. Also incomplete or absent autoinduction caused by missed doses may result in higher plasma concentrations. However, concentrations may become lower than expected if a dose is missed.

The presence of a relationship between plasma concentration and efficacy and/or toxicity suggests that the use of plasma concentrations to optimise efavirenz containing therapy might be useful. Bayesian estimation of individual pharmacokinetics of efavirenz based on the developed model may be applied, with which maximal and minimal plasma concentrations can be assessed from a randomly timed blood sample. Subsequently, appropriate TDM can be performed and consequently toxicities and/or virologic failure may be prevented.

In conclusion, a model for the pharmacokinetics of efavirenz was developed. To develop this model, a patient population that reflects the "real life" situation has been used with concentration-time points over the complete dosing interval. Patients characteristics involved in the interindividual variability of efavirenz were identified. In the near future, an individual dosing strategy based on patient characteristics and population pharmacokinetics may lead to a further optimisation of efavirenz containing therapy

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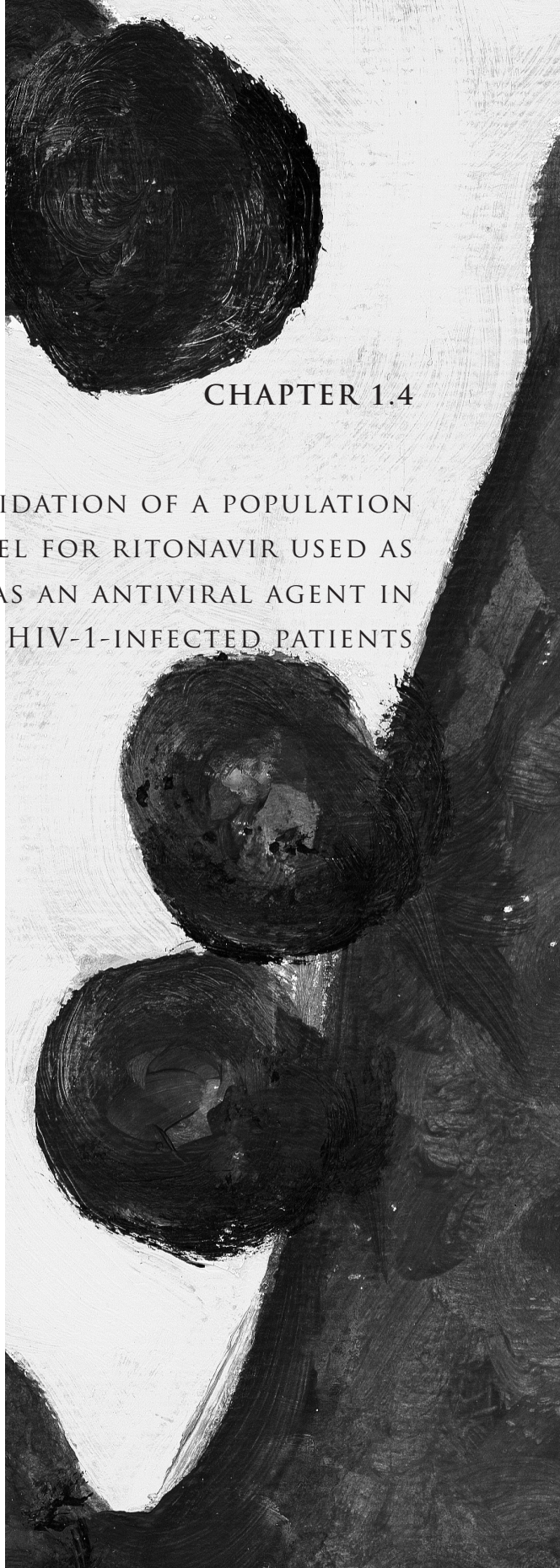

CHAPTER 1.4

DEVELOPMENT AND VALIDATION OF A POPULATION PHARMACOKINETIC MODEL FOR RITONAVIR USED AS A BOOSTER OR AS AN ANTIVIRAL AGENT IN HIV-1-INFECTED PATIENTS

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ABSTRACT

The aim of this study was to develop and validate a population pharmacokinetic model of ritonavir, used as an antiviral agent or as a booster, in a large patient population and to identify factors influencing its pharmacokinetics.

Ambulatory HIV-1-infected patients from the outpatient clinic of the Slotervaart Hospital, Amsterdam, the Netherlands, who were being treated with a ritonavir containing regimen were included. During regular visits, blood samples were collected for the determination of ritonavir plasma concentrations and several clinical chemistry parameters. Furthermore, complete pharmacokinetic curves were available in some patients. Using non-linear mixed effect modelling (NONMEM), pharmacokinetic parameters and interindividual, interoccasion and residual variability were estimated. In addition, the influence of several factors (e.g. patient characteristics, co-medication) on the pharmacokinetics of ritonavir was explored.

From 186 patients 505 ritonavir plasma concentrations at a single time point and 55 full pharmacokinetic profiles were available, resulting in a database of 1228 plasma ritonavir concentrations. In total 62% of the patients used ritonavir as a booster of their protease inhibitor containing antiretroviral regimen. First-order absorption in combination with one-compartment disposition best described the pharmacokinetics of ritonavir. Clearance, volume of distribution and absorption rate constant were 10.5 L/h (95% prediction interval (95% PI) 9.38-11.7), 96.6 L (95% PI 67.2-121) and 0.871 h^{-1} (95% PI 0.429-1.47), respectively, with 38.3%, 80.0% and 169% interindividual variability, respectively. The interoccasion variability in the apparent bioavailability was 59.1%. The concomitant use of lopinavir resulted in a 2.7-fold increase in the clearance of ritonavir (p-value <0.001). No patients characteristics influenced the pharmacokinetics of ritonavir.

In conclusion, the pharmacokinetic parameters of ritonavir were adequately described by our population pharmacokinetic model. Concomitant use of the protease inhibitor lopinavir strongly influenced the pharmacokinetics of ritonavir. The model has been validated and can be used for further investigation of the interaction between ritonavir and other protease inhibitors.

INTRODUCTION

Ritonavir is a potent human immunodeficiency virus (HIV) protease inhibitor and shows effective antiretroviral activity. However, the use of ritonavir in therapeutic doses is limited by neurological and gastrointestinal toxicity.[1] Both

antiviral activity and side effects have been correlated with plasma ritonavir concentrations.[1-3]

Initial, ritonavir was used for its antiviral effect (twice daily 600 mg) in combination with other antiretroviral drugs.[3] However, it was soon recognised that ritonavir in a low dose improves the pharmacokinetic profile of the different co-administered protease inhibitors by raising their concentrations in plasma, increasing their elimination half-lifes and reducing the influence of food on their gastrointestinal absorption. Ritonavir has revolutionised antiretroviral therapy and a large increase in the use of low-dose ritonavir in combination with a variety of protease inhibitors has occurred since its introduction.[4]

Potent inhibition of cytochrome P450 (CYP) 3A4-mediated metabolism in the gut wall and liver by ritonavir results in the desirable drug-drug interactions with other protease inhibitors.[4-9] Ritonavir also inhibits CYP2D6-mediated metabolism, and to a lesser extent CYP2C9, CYP2C19 and CYP1A2.[10,11] In addition, ritonavir may induce the activity of CYP1A2 and glucuronosyl transferase, and possibly CYP2C9 and CYP2C19.[12,13] Ritonavir is also an inhibitor of the drug transporters P-glycoprotein (P-gp) and/or the multidrug resistance-associated protein (MRP1). This might result in increased absorption, decreased elimination and improved retention into viral sanctuary sites of other protease inhibitors.[14,15]

The maintenance of high plasma concentrations of protease inhibitors is associated with a more potent and durable suppression of viral replication and with a delay in the development of resistance.[16] Thus, the large increase in the plasma concentrations of other protease inhibitors when co-administered with ritonavir forms the basis of rational dual protease inhibitor regimens.

Few data on the pharmacokinetics of ritonavir are available.[13,17-21] Furthermore, most studies [17-19] have been executed with HIV-negative subjects and/or before steady-state pharmacokinetic conditions were reached.

Because of several potential drug-drug interactions with ritonavir and variability in the expression of CYP enzymes, P-gp and MRP1, the pharmacokinetics of ritonavir may be complex. Knowledge about its variability may be of help in understanding differences in antiretroviral activity and side effects, and in gaining more insight into the interaction between ritonavir and other protease inhibitors. Therefore, the aim of this study was to characterise the population pharmacokinetics of ritonavir, both used as therapeutic antiretroviral drug or as booster, in a large and representative patient population, in which various dosages were being used. Furthermore, patient characteristics and other factors influencing the pharmacokinetics of ritonavir were investigated.

METHODS

Patients

Subjects were ambulatory HIV-1-infected patients from the outpatient clinic of the Slotervaart Hospital, Amsterdam, the Netherlands. Data were collected during regular outpatient visits, between January 1999 and June 2003. Each visit of the patient to the clinic was considered as an occasion. All patients were using ritonavir as part of their antiretroviral regimen and had at least one plasma ritonavir concentration available for analysis. Patients received ritonavir as a booster or as therapeutic drug. When patients had a ritonavir plasma concentration below 0.01 mg/L, they were excluded from pharmacokinetic analysis because of questionable adherence to therapy.[22] In addition to the random samples, full pharmacokinetic profiles (12–15 time points) were available from 55 patients, which were collected as part of several studies performed in our hospital.[5,23–26] The rationale for pooled analysis is to increase the power of the study. Combining plasma concentration–time points from several studies with random samples resulted in a large robust data set. When data from studies are used separately to develop pharmacokinetic models, parameters may be estimated with less precision. Furthermore, a small data set would contain less variability in patient characteristics and factors contributing to the interindividual variability may be hard to detect. Study protocols were approved by the institutional committee on medical ethics and informed consent was obtained from all patients. Single blood samples were obtained during regular follow-up of HIV-infected patients in our hospital according to local treatment guidelines and ethical approval.

Sampling and bioanalysis

At each visit to the clinic, a blood sample was obtained for the determination of plasma ritonavir concentration. Within the therapeutic drug monitoring (TDM)-program in our hospital, a strict protocol is utilised in which plasma concentrations of antiretroviral drugs are routinely and frequently monitored. As a consequence, patients are conversant with the principle of the recording time of ingestion of the last dose. Additionally, sampling times are recorded electronically at the Department of Clinical Chemistry. From this information, time after ingestion was estimated. All samples were collected at steady-state, at least two weeks after initiation of a ritonavir containing regimen.

Plasma concentrations of ritonavir were determined using an isocratic reversed-phase ion-pair, high-performance liquid chromatographic (HPLC) assay with ultraviolet detection (UV) at 239 nm.[27] This method was validated over the

range 0.05-25 mg/L using 600 μL of plasma. The assay was precise and accurate with between-day and within-day variation of quality control samples of ritonavir in plasma ranging from 0.7% to 7.6%. The mean accuracy was 104.0%.

Covariates

To identify possible relationships between the pharmacokinetics of ritonavir and patient characteristics, data on the following variables were collected at baseline: gender, race, alanine aminotransferase (ALAT, in U/L), aspartate aminotransferase (ASAT, in U/L), alkaline phosphatase (AP, in U/L), γ-glutamyltransferase (GGT, in U/L), total bilirubin (TBR, in μmol/L), CD4 cells (in 10^6/L), CD8 cells (in 10^6/L) and HIV viral load (in number of copies/mL). Patients were considered to have a chronic hepatitis B infection when hepatitis surface antigen (HbsAg) could be detected at baseline. When anti-hepatitis C antibodies (anti-HCV) were present at baseline, patients were considered to have a chronic hepatitis C infection. In addition, during treatment with ritonavir data on the following covariates were collected: age (in years), body weight (in kg), serum creatinine (in μmol/L). The effects of concomitant use of lopinavir, saquinavir and indinavir were also investigated. CD4 cells, CD8 cells, viral load, age, weight and serum creatinine were examined as continuous variables. Gender, race, hepatitis B and hepatitis C infection were examined as dichotomous variables. The values of ALAT, ASAT, AP, GGT and TBR were transformed to dichotomous variables by using 1.5 times the upper limit of normal for ASAT, ALAT, AP, GGT and TBR as cut-off values. Not all variables were available from all patients.

Population pharmacokinetic analyses

The non-linear mixed effect modelling software program NONMEM (version V, level 1.1, GloboMax LLC, Hanover MD, USA), using a Fortran compiler (Compaq Visual Fortran Version 6.5, Compaq Computer Corporation, Houston, TX, USA), was used to perform the analyses. The first-order conditional estimation (FOCE) procedure was used throughout. The INTERACTION option was used to account for interaction between the interindividual, intraindividual and residual error. The adequacy of the developed structural models was evaluated using both statistical and graphical methods. The minimal value of the objective function (OFV) provided by NONMEM was used for the comparisons of the nested models. Discrimination between these hierarchical models was based on the OFV using the log-likelihood ratio test.[28] A p-value of 0.05, representing a decrease in OFV of 3.84 was considered statistically significant (chi-square distribution,

degrees of freedom (df)=1).
Standard errors for all parameters were approximated using the COVARIANCE option of NONMEM. Individual Bayesian pharmacokinetic estimates of the pharmacokinetic parameters were obtained using the POSTHOC option.[28]

Basic pharmacokinetic model
Zero-order and first-order absorption models with and without absorption lag-time were tested. To describe the distribution kinetics of ritonavir, single and multiple compartment models with linear and non-linear elimination were investigated.
Population pharmacokinetic parameters such as clearance, volume of distribution and absorption rate constant were estimated. Interindividual and interoccasion variability in the pharmacokinetic parameters and in the apparent bioavailability were estimated from an exponential error model, according to Karlsson and Sheiner.[29] For instance, variability in clearance was determined from the equation:

$$CL/F_{ij} = \theta_1 \star \exp(\eta_i + \kappa_j)$$

in which CL/F_{ij} represents the clearance of the i^{th} individual on the j^{th} occasion, θ_1 is the typical value of clearance, η_i is the interindividual random effect with a mean of 0 and variance ω^2, and κ_j is the interoccasion random effect with a mean of 0 and variance π^2. Residual variability was modelled with a combined additive and proportional error model. Subpopulations were estimated using the $MIX function in the control stream.

Covariate pharmacokinetic model
To identify factors influencing the pharmacokinetics of ritonavir, covariates were introduced separately into the basic model. Covariates were also incorporated into the model to determine the influence of missing data and to avoid bias. For instance, the influence of a dichotomous covariate X on clearance with missing data of X for some individuals was modelled using the equation:

$$TVCL = \theta_1 \star \theta_2^{X \star (1-MIS)} \star \theta_3^{MIS}$$

in which TVCL is the typical value of clearance in the population, MIS is equal to 1 for records with missing data and 0 for all other records, θ_1 is the typical value of an individual with X=0 (no missing data), θ_2 is the relative difference in clearance for individuals with X=1 (no missing data) and θ_3 is the relative difference in clearance for individuals with missing data.
A covariate was considered statistically significant when the inclusion was

associated with a decrease in OFV associated with a p-value of <0.05 (log-likelihood ratio test). Clinical relevance was assumed when the typical value of the pharmacokinetic parameter of interest changed at least 10% in the range of the covariate observed in the population in order to prevent the detection of an irrelevant albeit significant relationship.
All significant and relevant covariates were included in an intermediate model. Finally, a stepwise backward elimination procedure was carried out. A covariate was retained in the model when the influence of this parameter was statistically significant ($p<0.05$) and clinically relevant (10% change in pharmacokinetic parameter).

Statistical refinement

The validity of the interindividual and interoccasion variability model was assessed by evaluating correlations between individual random effects (η) and interoccasion random effects (κ) for all of the pharmacokinetic parameters.[30] When a substantial correlation was present, covariance between these parameters was included in the model.

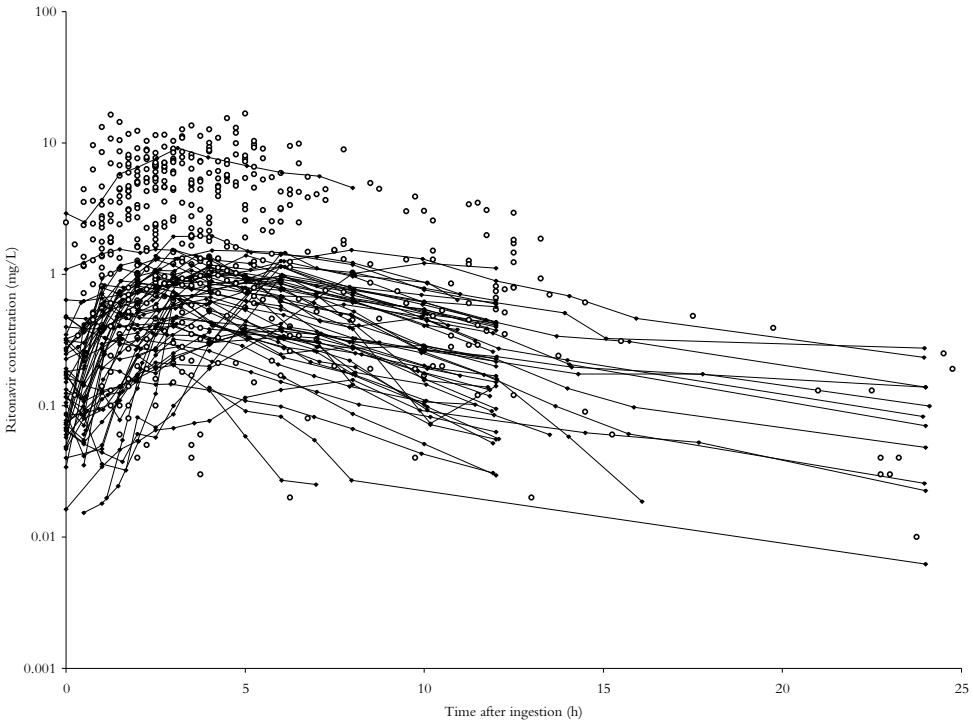
Model validation

The bootstrap resampling technique was applied as an internal validation. Bootstrap replicates were generated by sampling randomly approximately 65% from the original data set with replacement.[31] The final model was fitted to the replicate data sets using the bootstrap option in the software package Wings for NONMEM (by N. Holford, version 222, May 2001, Auckland, New Zealand) and parameter estimates for each of the replicate data sets were obtained. The precision of the model was evaluated by visual inspection of the distribution of the model parameters. Furthermore, the median parameter values and 95% prediction intervals of the bootstrap replicates were compared with the estimates of the original data set.

RESULTS

From 186 patients, 55 full pharmacokinetic profiles and 505 plasma concentrations at a single time point were available, resulting in a database of 1228 plasma ritonavir concentrations. A total of 115 patients received 100 mg ritonavir once a day or 100 mg, 133 mg or 200 mg ritonavir twice a day as a booster. A total of 71 patients received ritonavir as an antiviral drug in a dosage of 300 mg, 400 mg, 500 mg, 600 mg or 750 mg twice daily. When the full profiles were not taken into

Figure 1. Concentration-time data for ritonavir. Open circles represent plasma concentrations at a single time point, dots connected with lines represent full pharmacokinetic profiles.



account, average 3 to 4 samples (over a follow-up of 7 to 12 months) per patient (ranging from 1 to 15, i.e. follow-up up to 28 months) were available. Figure 1 shows all the concentration-time data for ritonavir. The patient population was predominantly male and Caucasian. Demographics and other patient characteristics were not available from 0-37% of the patients (depending on the covariable). In most cases covariates were missing non-randomly. Thus when one covariate was missing, there was a high probability all covariates were missing for that patient. This limited the opportunities to use joint-modelling or multiple imputations as techniques for dealing with missing data.[32] The characteristics of the patients studied are presented in table 1.

The population pharmacokinetics of ritonavir were best described by a one-compartment model with first-order absorption and elimination. Models with non-linear Michaelis Menten elimination were investigated but proved to be less satisfactory than linear models with first-order elimination. Zero-order absorption

Table 1. Characteristics of the 186 patients studied.

Parameter		Median	IQR	Missing (n,%)
Regimen				
Ritonavir (therapeutic (n,%))	71 (38.2)			
Ritonavir (booster (n,%))	115 (61.8)			
Indinavir / Ritonavir (therapeutic (n,%))	9 (4.8)			
Indinavir / Ritonavir (booster (n,%))	40 (21.5)			
Saquinavir / Ritonavir (therapeutic (n,%))	39 (21.0)			
Saquinavir / Ritonavir (booster (n,%))	39 (21.0)			
Lopinavir / Ritonavir (booster (n,%))	36 (19.4)			
Age (years)		39.4	35.0 - 46.0	0 (0)
Gender M/F (n,%)	146/23 (78.5/12.4)			17 (9.1)
Weight (kg)		71.5	63.0 - 79.8	32 (17.2)
Race				29 (15.6)
Caucasian (n,%)	120 (64.5)			
Black (n,%)	19 (10.2)			
Asian (n,%)	8 (4.3)			
Latino (n,%)	10 (5.4)			
Clinical chemistry				
Baseline ASAT (U/L)		32	27 - 46	44 (23.7)
Baseline ALAT (U/L)		40	28 - 52	44 (23.7)
Baseline GGT (U/L)		30	20 - 61	69 (37.1)
Baseline AP (U/L)		76	62 - 93	45 (24.2)
Baseline TBR (μmol/L)		11	9 - 15	55 (29.6)
Clinical immunology at baseline				
CD4 cell count (10^6/L)		240	110 - 380	49 (26.3)
CD8 cell count (10^6/L)		960	580 - 1360	49 (26.3)
Molecular biology at baseline				
Plasma $\log_{10}$ HIV-1 RNA (copies/mL)		4.86	3.48 - 5.47	40 (21.5)
HBV / no HBV (n,%)	7/138 (3.8/74.2)			41 (22.0)
HCV / no HCV (n,%)	17/121 (9.1/65.1)			47 (25.3)

M=male, F=female, ASAT=aspartate aminotransferase, ALAT=alanine aminotransferase, GGT=gamma-glutamyltransferase, AP=alkaline phosphatase, TBR=total bilirubin, HBV=hepatitis B infection, HCV=hepatitis C infection, IQR=interquartile range.

and two-compartmental models were studied but also turned out to be inadequate. However, the addition of an absorption lag-time (0.778 h), significantly improved the fit (ΔOFV=-150, $p<0.001$). The residual error in ritonavir pharmacokinetics incorporated both an additive and a proportional component. The magnitude of the residual error was not constant across all individuals, which may influence parameter estimates. Therefore, several models were investigated to allow for interindividual varying residual error. Ultimately, inclusion of two different populations with different magnitudes of residual variability proved to be the most optimal model (ΔOFV=-48, $p<0.001$). As a result, 64.8% of the population were associated

with a relative small additive error of 0.0600 mg/L, whereas the remainder was associated with a larger additive error of 0.199 mg/L. The proportional error for both populations was 15.4%. Including interoccasion variability in the apparent bioavailability in the model resulted in further optimisation of the model.
The different covariates and the effects of indinavir, lopinavir and saquinavir on the pharmacokinetics of ritonavir were introduced separately in the model, using a univariate procedure. Only the introduction of lopinavir resulted in a statistically significant increase in goodness-of-fit, ΔOFV=-69.2 ($p<0.001$) and a significant effect on the clearance of ritonavir. No other covariates were significantly related to the pharmacokinetics of ritonavir. The magnitude of increase in clearance was 272% during concomitant use of lopinavir. The following equation describes the final model for clearance:

$$CL/F = 10.5 \star 2.72^{LPV}$$

in which LPV is 1 for individuals using lopinavir in their antiretroviral regimen and 0 for all others.
A correlation between the individual random effects of volume of distribution and absorption rate constant (η_V and η_{ka}) was observed and covariance between these parameters was added to the model. The correlation coefficient was 0.868 ($p=0.001$). In the final model the estimate of clearance was 10.5 L/h with an

Table 2. Parameter estimates from the population pharmacokinetic model and the results of the bootstrap analysis.

			Bootstrap analysis	
Parameter	Estimate	RSE (%)	Median	95% PI
CL/F (L/h)	10.5	5.55	10.4	9.38 – 11.7
θ_{LPV}	2.72	11.7	2.72	2.20 – 3.67
V/F (L)	96.6	10.7	92.6	67.2 – 121
k_a (h^{-1})	0.871	23.1	0.775	0.429 – 1.47
Lag-time (h)	0.778	4.91	0.768	0.388 – 0.867
IIV CL/F (%)	38.3	23.0	38.3	29.4 – 47.6
IIV V/F (%)	80.0	31.6	77.3	51.1 – 117
IIV k_a (%)	169	25.8	163	113 – 209
IOV F (%)	59.1	15.6	58.5	48.4 – 67.6
Correlation η_V-η_{ka}	0.868	34.7	0.876	0.718 – 1.00
Fraction in P1 (%)	64.8	18.5	63.5	37.2 – 84.1
Additive error P1 (mg/L)	0.0600	13.5	0.0575	0.0353 – 0.101
Additive error P2 (mg/L)	0.199	15.2	0.202	0.0731 – 0.276
Proportional error (%)	15.4	23.8	14.6	10.1 – 25.5

F=apparent bioavailability, CL/F=oral clearance, LPV=lopinavir, V/F=volume of distribution, k_a=absorption rate constant, IIV=interindividual variability, IOV=interoccasion variability, P=population, RSE=residual standard error, PI=prediction interval.

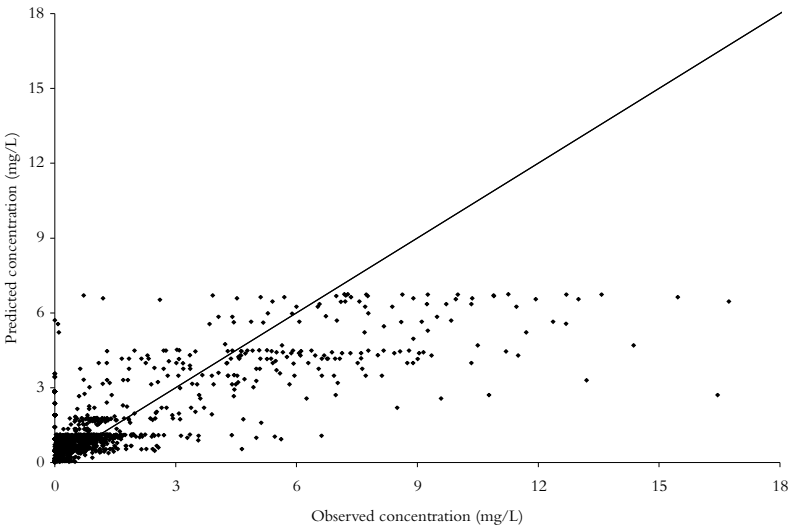
interindividual variability (IIV) of 38.3%. The estimates of volume of distribution and absorption rate constant were 96.6 L (IIV=80.0%) and 0.871 h^{-1} (IIV=169%), respectively. The calculated value for half-life from these estimates was 6.4 h. The results of the final pharmacokinetic model are summarised in table 2.

Figure 2 shows the model predicted plasma concentrations from the final model versus the observed concentrations of ritonavir, the weighted residuals versus time and the absolute weighted residuals versus predicted plasma concentrations.

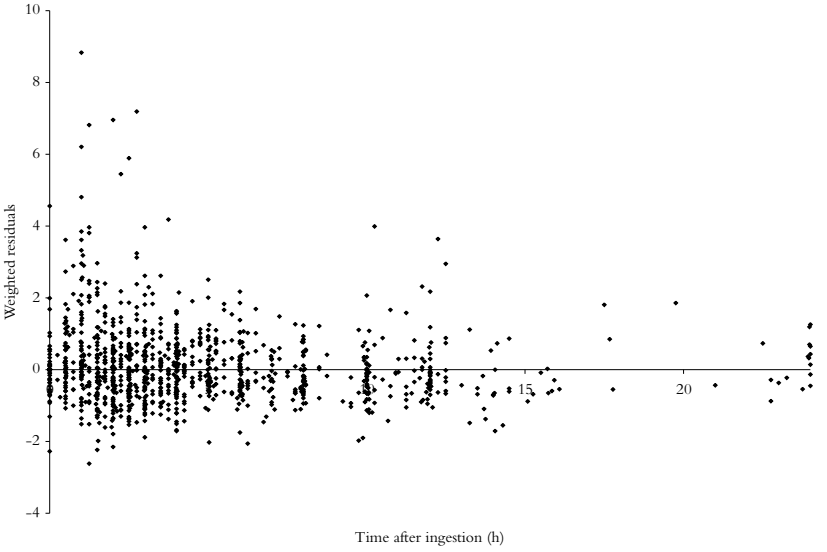
From the original data set more than 1000 replicate bootstrap data sets were generated and used for the evaluation of the precision of the parameter estimates. Unsuccessful terminations due to boundaries were run again with enlarged borders. Abnormal and unsuccessful terminations were excluded from the bootstrap calculations. Successful minimizations and unsuccessful termination due to rounding errors with number of significance above 2 were included in the bootstrap calculations. However, less than 5% of the bootstrap runs were unsuccessful. Table 2 lists the results of the 1000 included bootstraps, presented as medians and 95% prediction intervals, and the parameter estimates of the final model with the corresponding relative standard error. Similar median bootstrap

Figure 2. Model predicted concentrations versus observed concentrations of ritonavir (A), weighted residuals versus time (B) and absolute weighted residuals versus predicted concentrations of ritonavir (C) using the final model.

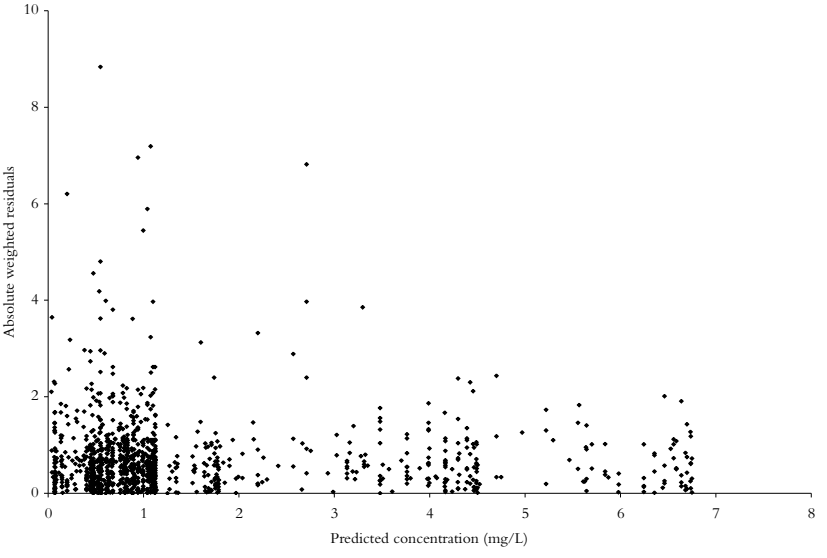
A



B


Weighted residuals
10
8
6
4
2
0
-2
-4
15
20
Time after ingestion (h)

C


Absolute weighted residuals
10
8
6
4
2
0
0
1
2
3
4
5
6
7
8
Predicted concentration (mg/L)

values to the parameter estimates of the original data set indicated acceptable precision.

DISCUSSION

Despite the widespread use of ritonavir, few data are available on its pharmacokinetics in clinical practice. The aim of this study was to characterise the population pharmacokinetics of ritonavir and to identify any covariates. The model development started with a careful data check. The influence of outliers was studied extensively during this phase of model development. The combination of the described cut-off of 0.01 mg/L with an additive residual error model as described proved to be the most efficient way to minimise the influence of possible non-adherence on the results of this study.

The pharmacokinetics of ritonavir were adequately described by a population pharmacokinetic model consisting of one compartment with first-order absorption with a lag-time and first-order elimination. This structural model was similar to that of Sale et al.[18] However, Hsu et al.[17] described a pharmacokinetic model consisting of one compartment with first-order absorption and Michaelis-Menten saturable metabolism. In this study samples were collected between days 1 and 17. Thus only at the end of the study would subjects be at steady-state, which may have influenced the characterisation of the pharmacokinetics of ritonavir.

Our estimation for volume of distribution fell within the wide range (28-123 L) of values found in previous studies.[7,17-19] In addition, the estimates of clearance and half-life were similar to those found previously.[7,13,17,19] Because both clearance (CL/F) and volume of distribution (V/F) depend on bioavailability (F), these pharmacokinetic parameters may be correlated. Large interindividual and interoccasion variability in clearance, volume of distribution, absorption rate constant and apparent bioavailability was observed (38.3%, 80.0%, 169% and 59.1%, respectively). Differences in protein binding, absorption, enzyme induction or variability in expression of CYP enzymes, P-gp or MRP1 may contribute to this variability, as may environmental factors and dietary habits. It has been reported that dose may be determinant of the pharmacokinetics of ritonavir.[17] However, in the present work dosage was found to be a statistically non-significant and clinically non-relevant covariate. Finally, all plasma samples were obtained after at least 14 days of treatment with ritonavir, suggesting that patients were at steady-state and thus that autoinduction of metabolism was no longer contributing to interoccasion variability.

Since data were obtained from different studies, it was anticipated that the residual

error may not be constant across all subjects. Therefore, the individual contribution to the residual error was accounted for by including an interindividual variability term in the residual error. However, successful termination was not achieved. Therefore, two populations were introduced in the additive error, the reason for which could not be explained. Nevertheless, this approach to the residual variability resulted in less biased parameter estimates and improved goodness-of-fit of the model.

The concomitant use of lopinavir resulted in a 2.72-fold increase in the clearance of ritonavir, but saquinavir and indinavir were apparently without effect. It is known that trough plasma concentrations of ritonavir are significantly higher in patients receiving combinations containing saquinavir or indinavir than combinations with lopinavir or amprenavir.[20,21] We had no patients on the combination amprenavir-ritonavir in our database, and thus the results of these studies [20,21] may be compatible with our data. The development of the current population pharmacokinetic model was undertaken for subsequent investigation of the pharmacokinetics of dual protease inhibitor regimens. Therefore, model validation was of particular importance. In the current study, the bootstrap resampling technique was performed as an internal validation. The 1000 replicate datasets yielded median model parameters that were comparable with the estimates of the original dataset, indicating the high precision of the developed model.

In conclusion, a model for the population pharmacokinetics of ritonavir was developed and validated. To this end, a large patient population was used, incorporating concentration-time points over the complete dosing interval. Except for the concomitant use of lopinavir, no patients characteristics influenced ritonavir pharmacokinetics. The model will be integrated into other population models to investigate the pharmacokinetics of other protease inhibitors used in combination with ritonavir, which may lead to a further optimisation of ritonavir containing antiretroviral therapy.

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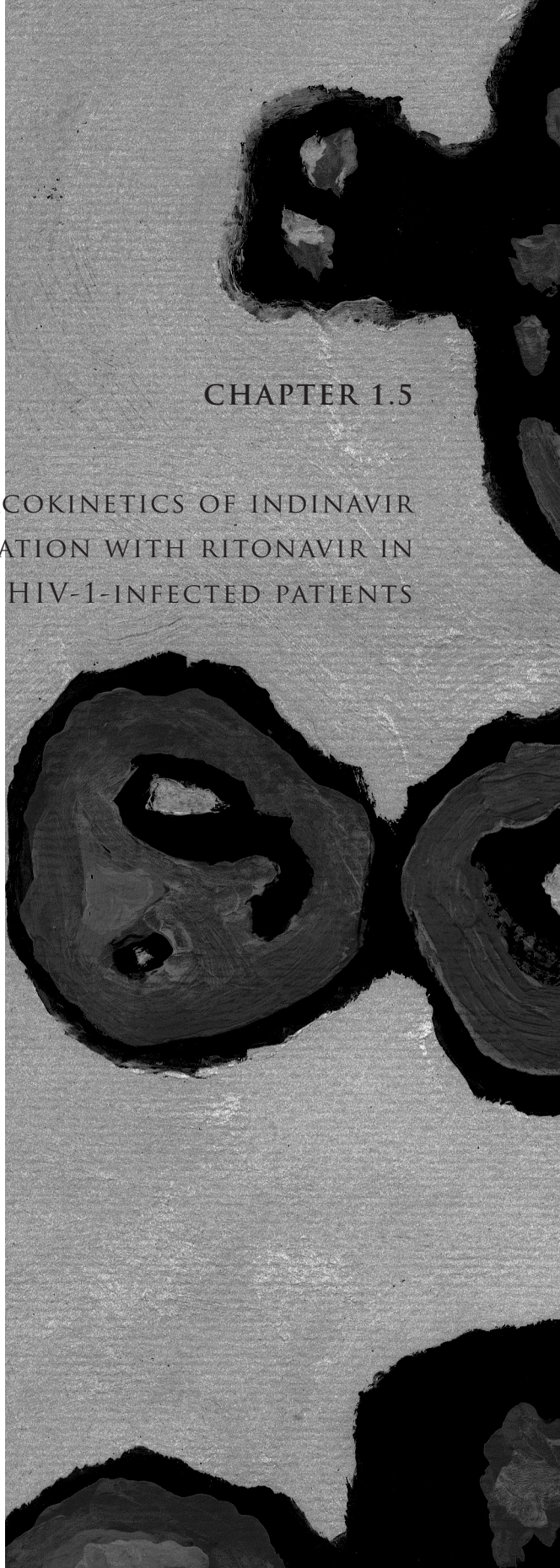

CHAPTER 1.5

POPULATION PHARMACOKINETICS OF INDINAVIR ALONE AND IN COMBINATION WITH RITONAVIR IN HIV-1-INFECTED PATIENTS

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ABSTRACT

The aim of the study was to characterise the population pharmacokinetics of indinavir, the relationship between the pharmacokinetics of indinavir and ritonavir, and to identify patient characteristics influencing the pharmacokinetics of indinavir with or without ritonavir.

HIV-1-infected patients using an indinavir containing regimen were included. During regular visits, 102 blood samples were collected for the determination of the indinavir and ritonavir plasma concentrations. From 45 patients full pharmacokinetic curves were available. Concentrations of indinavir and ritonavir were quantitatively assessed by liquid chromatography coupled with electrospray tandem mass spectrometry (LC-MS/MS).

Pharmacokinetic modelling was performed using non-linear mixed effect modelling (NONMEM). Disposition of indinavir was described by a single-compartment model with first-order absorption and elimination. Clearance, volume of distribution and the absorption rate constant were determined. Interindividual (IIV), interoccasion (IOV) and residual variability were calculated. The influence of the ritonavir pharmacokinetics on indinavir disposition was studied. Patient characteristics influencing the pharmacokinetic parameters of indinavir were explored.

In total 853 samples could be used for analysis. Clearance, volume of distribution and the absorption rate constant were 46.8 L/h (24.2% IIV), 82.3 L (24.6% IIV) and 2.62 h^{-1}, respectively. An absorption lag-time of 0.485 h was identified for patients who concomitantly used ritonavir. Concomitant use of ritonavir decreased the clearance of indinavir with 64.6%. This decrease was independent of the ritonavir dose (100-400 mg), concentration or exposure. Co-administration of ritonavir did not increase the bioavailability of indinavir. Co-administration of efavirenz or nevirapine increased the clearance of indinavir with 41%, also in the presence of ritonavir. Female patients had a 48% higher apparent bioavailability.

Ritonavir used concomitantly with indinavir delayed the absorption by an absorption lag-time of about 30 minutes. Furthermore, ritonavir reduced the clearance of indinavir in a ritonavir exposure independent way. This may indicate that 100 mg of ritonavir is sufficient for maximal inhibition of indinavir metabolism. The developed model may be used for further indinavir treatment optimisation of HIV-1-infected patients.

INTRODUCTION

Indinavir is a potent human immunodeficiency virus (HIV) protease inhibitor.[1-3] The oral bioavailability of indinavir when dosed without ritonavir is decreased when administered together with food and, therefore, the drug should be taken on an empty stomach.[4] However, when co-administered with ritonavir the pharmacokinetic profile of indinavir improves.[5,6] This desirable drug-drug interaction is caused by potent inhibition of cytochrome P450 (CYP) 3A4-mediated metabolism in the liver by ritonavir, resulting in a decreased elimination rate of indinavir.[7] In addition, inhibition of drug-transporting cellular efflux proteins such as P-glycoprotein (P-gp) by ritonavir might improve the bioavailability of indinavir.[8] However, the inhibiting potential of ritonavir on this transporter has not been established irrefutably.[9]

Both toxicity and efficacy have been related to indinavir plasma concentrations.[10-12] Therefore, therapeutic drug monitoring (TDM) of indinavir has demonstrated to be a valuable tool in the treatment with this drug.[13]

The aim of this study was to characterise the population pharmacokinetic parameters of indinavir, and, in particular, to model the interaction between ritonavir and indinavir, in a representative HIV-1-infected patient population, in which various dosage regimens were being used. In addition, patient characteristics involved in the variability of the pharmacokinetic parameters were investigated.

METHODS

Patients

Included patients were ambulatory HIV-1-infected patients from the outpatient clinic of the Slotervaart Hospital, Amsterdam, The Netherlands. All patients were using indinavir as part of their antiretroviral regimen and had at least one indinavir plasma concentration available for analysis. Patients received either indinavir alone, or indinavir in combination with ritonavir. Data were collected during regular outpatient clinic visits at random time points. At each visit to the clinic, a blood sample was obtained for the determination of an indinavir and ritonavir plasma concentration, according to local treatment guidelines and with approval of the hospital ethics committee. Each visit of the patient to the clinic was considered as an occasion. In addition to the random samples, full pharmacokinetic profiles (8-12 time points per patient) were available from 45 patients, which were collected as part of several studies performed in our hospital and in the Academic Medical Centre, Amsterdam, The Netherlands. Full details of these studies have been

presented elsewhere.[5,14–16] Study protocols were approved by the institutional ethics committees and informed consent was obtained from all patients.

Sampling and bioanalysis

Within the TDM-program of the Slotervaart Hospital, a strict protocol was followed in which plasma concentrations of antiretroviral drugs were routinely and frequently monitored during each visit of the outpatient clinic. As a consequence patients were conversant with the principle of recording time of ingestion of the last dose. Additionally, sampling times were recorded electronically at the department of clinical chemistry. From this information, time after ingestion was extracted.

Full pharmacokinetic curves were determined during an administration interval. All concentrations were collected at steady-state, at least two weeks after initiation of an indinavir containing regimen.

Plasma concentrations of indinavir and ritonavir were quantitatively assessed using a validated liquid chromatography coupled with electrospray tandem mass spectrometry (LC-MS/MS). This method was validated over the range 0.01-10 mg/L and 0.05-10 mg/L, respectively, using 100 μL of plasma. Recoveries of indinavir and ritonavir from human plasma were 105.4% and 91.7%, respectively. Within- and between-day precisions were always less than 9.4% for all quality control samples covering the complete calibration curve.[17]

Population pharmacokinetic analyses

The non-linear mixed effect modelling software program NONMEM (Version V, level 1.1, GloboMax LLC, Hanover MD, USA) using a Fortran compiler (Compaq Visual Fortran Version 6.5, Compaq Computer Corporation, Houston, TX, USA), was used to perform all analyses. The first-order conditional estimate method (FOCE) procedure with interaction between interindividual, intraindividual and residual variability was used throughout. The adequacy of the developed structural models was evaluated using both statistical and graphical methods. The minimal value of the objective function (OFV) provided by NONMEM was used as goodness-of-fit characteristic to discriminate between hierarchical models using the likelihood ratio test.[18] A p-value of 0.05, representing a decrease in OFV of 3.84 points, was considered statistically significant (chi-square distribution, df=1). Standard errors for all parameters were approximated using the COVARIANCE option of NONMEM. Individual Bayesian estimates of the pharmacokinetic parameters were obtained using the POSTHOC option.[18] The

program PDx-Pop (version 1.1, release 4, Globomax LLC, Hanover MD, USA) was used as interface for conducting the population pharmacokinetic analyses with NONMEM and for graphical model diagnostics. In addition, the S-plus (MathSoft, Inc, Seattle, USA) based model-building aid Xpose 3.0 was used for graphical model diagnosis.[19]

Basic pharmacokinetic model

First-order absorption models with and without absorption lag-time were tested. Also different numbers of transition compartments instead of an absorption lag-time were tested to describe the absorption process. To describe the distribution kinetics of indinavir, single and multiple compartment models with linear and non-linear elimination were investigated.

Since ritonavir is used as a kinetic booster in indinavir containing regimens, the effect of ritonavir on the pharmacokinetics of indinavir was incorporated in the basic model. This modelling process consisted of two phases. In the first phase, a previously developed and validated population pharmacokinetic model of ritonavir, that also included data of patients treated with indinavir/ritonavir, was used to obtain individual Bayesian estimates of the ritonavir pharmacokinetic parameters.[20] In short, this model used first-order absorption in combination with one-compartment disposition and first-order elimination to describe the pharmacokinetics of ritonavir. Clearance, volume of distribution, absorption rate constant were 10.5 L/h, 96.6 L and 0.871 h^{-1}, respectively, with 38.3%, 80.0% and 169% interindividual variability, respectively. The interoccasion variability in the apparent bioavailability was 59.1%. The validation of the model indicated adequate and precise estimation of the pharmacokinetic parameters.[20] The ritonavir exposure over a dosing interval (AUC) was calculated by dividing dose by clearance.

In the second phase, the influence of the ritonavir exposure on the pharmacokinetics of indinavir was studied. Ritonavir can interact with indinavir on the absorption and the elimination process, mainly due to inhibition of CYP3A4 in the gut wall and the liver and inhibition of P-gp.

Various models were tested to study the effect of ritonavir on the clearance of indinavir. The first model described the mechanism of the interaction assuming a time-dependent effect of ritonavir on the pharmacokinetics of indinavir. In this model the concentration of ritonavir was directly related to the elimination of indinavir (1). In the second model, a direct relationship between the clearance of indinavir and the total exposure, expressed as AUC, of ritonavir was assumed (2).

These relationships were coded as follows:

$$CL/F_{ij} = \theta_1 \star (1 - ((E_{max}^{\gamma} \star C_{ij}(t)^{\gamma}) / (C_{50}^{\gamma} + C_{ij}(t)^{\gamma})) \quad (1)$$

$$CL/F_{ij} = \theta_1 \star (1 - ((E_{max}^{\gamma} \star AUC_{ij}^{\gamma}) / (AUC_{50}^{\gamma} + AUC_{ij}^{\gamma})) \quad (2)$$

in which CL/F_{ij} represents the indinavir clearance of the i^{th} individual on the j^{th} occasion, θ_1 is the typical value of clearance, E_{max} is the maximum inhibitory effect of ritonavir, $C_{ij}(t)$ is the concentration of the i^{th} individual on the j^{th} occasion at time t, C_{50} is the concentration of ritonavir that is associated with half-maximal inhibition of the clearance of indinavir, AUC_{ij} is the AUC of the i^{th} individual on the j^{th} occasion, AUC_{50} is the AUC of ritonavir that is associated with half-maximal inhibition of the clearance of indinavir, and γ is a constant to be estimated.

The effect of ritonavir on the apparent bioavailability was also assessed.

Population pharmacokinetic parameters such as clearance, volume of distribution and the absorption rate constant were estimated. Interindividual and interoccasion variability in the pharmacokinetic parameters were estimated with an exponential error model, according to Karlsson and Sheiner.[21] For instance, variability in clearance was estimated using:

$$CL/F_{ij} = \theta_1 \star \exp(\eta_i + \kappa_j)$$

in which CL/F_{ij} represents the clearance of the i^{th} individual on the j^{th} occasion, θ_1 is the population value of clearance, η_i is the interindividual random effect with mean 0 and variance ω^2 and κ_j is the interoccasion random effect with mean 0 and variance π^2. Residual variability was modelled with a combined additive and proportional error model.

Covariate pharmacokinetic model

To identify possible relationships between the pharmacokinetics of indinavir and patient characteristics, the following covariates were collected at baseline: age, weight, gender, race, alanine aminotransferase (ALAT, in U/L), aspartate aminotransferase (ASAT, in U/L), alkaline phosphatase (AP, in U/L), γ-glutamyltransferase (GGT, in U/L), total bilirubin (TBR, in μmol/L) and serum creatinine (CR, in μmol/L). Patients were considered to have a chronic hepatitis B infections when hepatitis surface antigen (HbsAg) could be detected at baseline. When anti-hepatitis C antibodies (anti-HCV) were present at baseline, patients were considered to have a chronic hepatitis C infection. Concomitant use of the CYP3A4-inducing drugs efavirenz and nevirapine was determined during treatment. Age and weight were examined as continuous variables. Gender, race, hepatitis B infection and hepatitis C infection were examined as dichotomous variables. The values of ALAT, ASAT, AP, GGT, TBR and CR were transformed

to dichotomous variables by using 1.5 times the upper limit of normal as cut-off value. Some covariates were missing in a small subset of patients. In order to avoid bias, a covariate was included in the model indicating the missing data. For instance, the influence of a dichotomous covariate X on clearance with missing data of X for some individuals was modelled as:

$$TVCL = \theta_1 \star \theta_2^{X \star (1-MIS)} \star \theta_3^{MIS}$$

in which TVCL is the typical value of clearance in the population, MIS is 1 for records with missing data and 0 for all other records, θ_1 is the typical value of an individual with X=0 (no missing data) and θ_2 is the relative difference in clearance for individuals with X=1 (no missing data) and θ_3 is the relative difference in clearance for individuals with missing data.

The inclusion of a covariate relationship in a pharmacokinetic model was based on a combination of the statistical significance and the clinical importance of the relationship. A stepwise forward inclusion and backward elimination procedure was carried out for the detection of covariate relations. A covariate was considered statistically significant in the forward inclusion process when the inclusion was associated with a decrease in the minimal value of the objective function associated with a p-value of <0.05 (ΔOFV=3.84 points, log-likelihood ratio test). In the backward elimination procedure a p-value of 0.01 combined with a clinical relevant effect was required. Clinical relevance was considered when the typical value of the pharmacokinetic parameter of interest changed at least 10% in the range of the covariate, as observed in the population to prevent the detection of an irrelevant, albeit significant, relationship.

Statistical refinement

The validity of the interindividual variability model was checked by evaluating correlations between individual random effects (η) and interoccasion random effects (κ) for all of the pharmacokinetic parameters.[22] When a substantial correlation was present or suspected, covariance between these parameters was included in the model.

Model validation

The bootstrap resampling technique was applied as an internal validation for the final model.[23] Bootstrap replicates were generated by randomly sampling approximately 65% of the original data set with replacement. The final model was fitted to the replicate data set using the bootstrap option in the software package Wings for NONMEM (by N. Holford, version 406, May 2004, Auckland,

Table 1. Baseline characteristics of included patients (n=147, 443 occasions).

Parameter	No. of occ. (n, (%))		No. of pts. (n, (%))		Median	IQR	No. of patients >1.5 x ULN	Missing (n,(%))
Regimen								
3x600 mg IDV	1	(0.2)						
3x800 mg IDV	112	(25.3)						
3x1000 mg IDV	26	(5.9)						
3x1200 mg IDV	4	(0.9)						
2x1200 mg IDV	7	(1.6)						
2x1400 mg IDV	5	(1.1)						
2x200 mg IDV + 2x100 mg RTV	1	(0.2)						
2x600 mg IDV + 2x100 mg RTV	18	(4.1)						
2x800 mg IDV + 2x100 mg RTV	201	(45.4)						
2x1000 mg IDV + 2x100 mg RTV	20	(4.5)						
2x1200 mg IDV + 2x100 mg RTV	2	(0.5)						
2x800 mg IDV + 2x400 mg RTV	6	(1.4)						
2x400 mg IDV + 2x400 mg RTV	37	(8.4)						
1x800 mg IDV + 1x100 mg RTV	3	(0.7)						
concomitant NNRTI	35	(7.9)						0
Gender M/F			138/9	(93.9/6.1)				0
Age (years)					40.3	34.9 – 47.1		0
Weight (kg)					73.0	65.0 – 80.0		29 (19.7)
Race								0
Caucasian			121	(82.3)				
Black			14	(9.5)				
Asian			7	(4.8)				
Latino			5	(3.4)				
Clinical chemistry								
Baseline ASAT (U/L)					31.7	24.4 – 43.5	21	4 (2.7)
Baseline ALAT (U/L)					34.0	22.0 – 48.1	20	4 (2.7)
Baseline GGT (U/L)					37.8	22.0 – 62.8	27	7 (4.8)
Baseline AP (U/L)					78.8	65.0 – 92.6	4	7 (4.8)
Baseline TBR (µmol/L)					18.0	12.3 – 27.0	40	5 (3.4)
Baseline CR (µmol/L)					81.0	72.0 – 91.0	1	4 (2.7)
Clinical immunology at baseline								
CD4 cell count (10^6/L)					380	220 – 575		8 (5.4)
CD8 cell count (10^6/L)					1060	725 – 1565		12 (8.2)
Molecular biology at baseline								
Plasma $\log_{10}$ HIV-1 RNA (copies/mL)					2.30	2.30 – 3.67		6 (4.1)
HBV / no HBV			5/133	(3.4/90.5)				9 (6.1)
HCV / no HCV			8/130	(5.4/88.4)				9 (6.1)

IDV=indinavir, RTV=ritonavir, M=male, F=female, ASAT=aspartate aminotransferase, ALAT=alanine aminotransferase, GGT=gamma-glutamyltransferase, AP=alkaline phosphatase, TBR=total bilirubin, CR=creatinine, HBV=hepatitis B infection, HCV=hepatitis C infection, IQR=interquartile range.

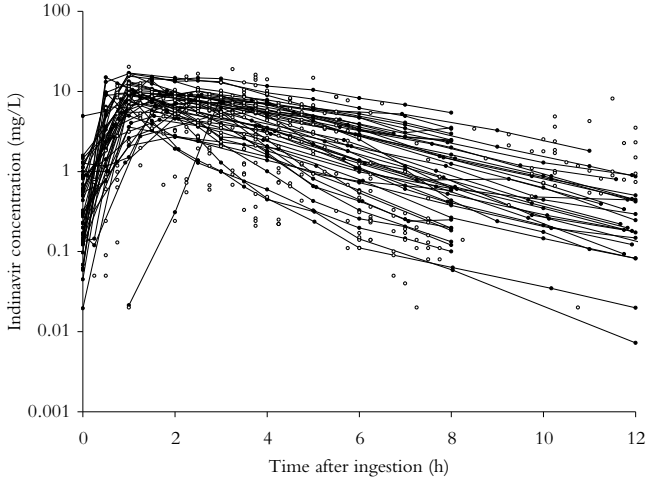
New Zealand). Parameter estimates for the replicate data set were obtained in this way.[23] The precision of the model was evaluated by visual inspection of distribution of model parameters. Furthermore, the median parameter values and 95% prediction intervals of the bootstrap replicates were compared with the estimates of the original data set.

RESULTS

Patients

From 45 patients full pharmacokinetic curves were available. In addition, from 102 patients indinavir plasma concentrations at a single time point were available. In total 853 samples could be used for analysis. Most patients received 800 mg indinavir orally either three times daily (112 occasions) or twice daily in combination with 100 mg ritonavir (201 occasions) or 400 mg indinavir and 400 mg ritonavir twice daily (37 occasions). Alternative regimens and patient characteristics of the data set are presented in table 1. The patient population was predominantly male and Caucasian. From 0-19.7% of the patients (depending on the covariable), characteristics were not available. Not taking the data from the full curves into account, a mean of 2 to 3 samples per patient (ranging from 1 to 18, follow-up 0 to 64 months) was used in this study. Figure 1 shows the concentration-time data of indinavir.

Figure 1. Concentration-time data of indinavir. Open circles represent concentrations at single time points, dots connected with lines represent full pharmacokinetic profiles.



Population pharmacokinetics

The population pharmacokinetics of indinavir were best described with a one-compartment model with first-order absorption and elimination. Both zero-order and first-order models with and without peripheral compartments were tested. However, the use of a zero-order absorption model or inclusion of a peripheral compartment did not increase the goodness-of-fit.

Several models with and without absorption lag-time, as well as models with several numbers of transition compartments to describe the absorption phase [24] were evaluated. It appeared that concomitant use of ritonavir slowed the absorption of indinavir by introducing an absorption lag-time. Therefore, this lag-time (0.485 h) was included in the model for patients who concomitantly used ritonavir (ΔOFV=-79.5, $p<0.001$).

The influence of the ritonavir exposure on the pharmacokinetics of indinavir was modelled in two steps. First, Bayesian estimates of the ritonavir pharmacokinetic parameters were obtained by use of the earlier developed population pharmacokinetic model for ritonavir.[20] Thereafter, the influence of the ritonavir exposure on the pharmacokinetics of indinavir was studied using several models. At first, a direct time-dependent relationship between ritonavir and the clearance of indinavir was tested. However, an increase in the clearance of indinavir during the dose-interval due to elimination of ritonavir over time could not be demonstrated. Thereafter, a continuous relationship between exposure of ritonavir, expressed as AUC, and clearance of indinavir was tested. The OFV of this model decreased substantially in comparison to the model without the effect of ritonavir. However, AUC_{50} was estimated very small and E_{max} was estimated at 0.638, indicating full inhibition of indinavir at very low exposures of ritonavir. Therefore, in the final model the effect of ritonavir was modelled as a dichotomous variable (ritonavir present or not). This model resulted in a decrease in OFV of 137.4 points ($p<0.001$) compared to the model without this influence.

No relationship between the exposure of ritonavir and the apparent bioavailability could be demonstrated (ΔOFV=-2.4, $p=0.121$).

In view of statistical refinement, a correlation between the individual random effects of clearance and volume of distribution (η_{CL} and η_V) of indinavir was observed and covariance between these parameters was added to the model. The correlation coefficient was 0.729.

The results of the basic pharmacokinetic model are summarised in table 2. The estimate of clearance was 49.3 L/h with an interindividual variability (IIV) and interoccasion variability of 34.8% and 21.2%, respectively. The estimate of volume

Table 2. Parameter estimates of the final pharmacokinetic model of indinavir and the results of bootstrap analysis.

	Basic model		Final model		Bootstrap analysis	
	Est	RSE (%)	Est	RSE (%)	Median	95% PI
CL/F (L/h)	49.3	6.19	46.8	5.75	46.6	41.5 – 55.3
$\theta_{ritonavir}$★	0.362	7.27	0.354	6.07	0.357	0.314 – 0.406
$\theta_{concomitant\ NNRTI}$★	-	-	1.41	4.78	1.41	1.27 – 1.56
V/F (L)	77.2	5.03	82.3	4.70	81.8	74.4 – 102
k_a (h^{-1})	2.64	16.4	2.62	16.0	2.59	1.90 – 3.93
Lag-time (h)#	0.483	2.40	0.485	1.79	0.485	0.431 – 0.545
IIV CL/F (%)	34.8	24.1	24.2	44.5	24.4	11.2 – 52.3
IIV V/F (%)	28.5	50.6	24.6	52.3	24.0	10.6 – 77.7
Correlation IIV CL/F-V/F	0.729	44.4	0.629	84.8	0.757	-1 – 1
IOV CL/F (%)	21.2	40.6	20.9	37.0	20.5	11.5 – 32.6
IOV F (%)	22.8	48.3	23.1	50.7	22.2	7.74 – 56.5
θ_{female}★	-	-	1.48	16.7	1.46	0.882 – 2.04
Additive error (mg/L)	0.0491	17.0	0.0491	16.7	0.0492	0.0306 – 0.0782
Proportional error (%)	35.0	6.06	35.3	6.18	34.8	25.0 – 39.4

★: relative change in pharmacokinetic parameter in the presence of the covariate, #:only estimated when indinavir and ritonavir were combined. CL/F=clearance, V/F=volume of distribution, F=apparent bioavailability, k_a=absorption rate constant, IIV=interindividual variability, IOV=interoccasion variability, Est=parameter estimate, RSE=relative standard error, PI=prediction interval.

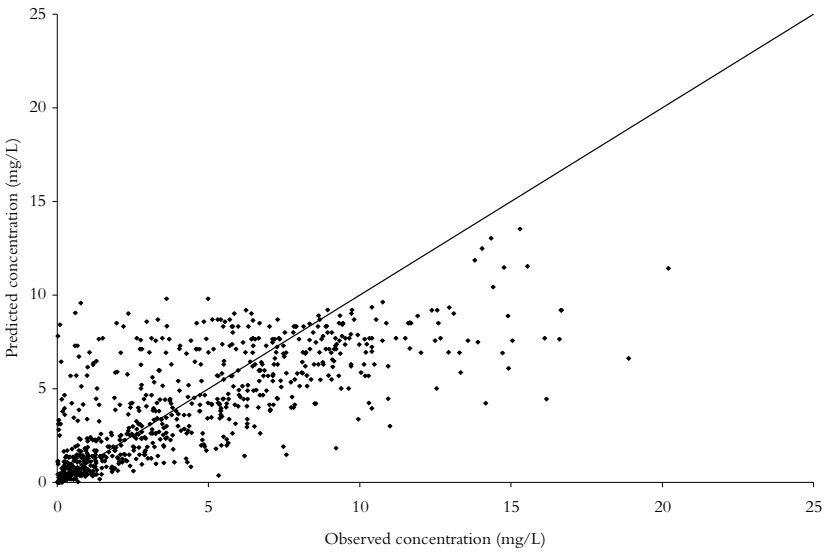
of distribution and absorption rate constant were 77.2 L (IIV=28.5%) and 2.64 h^{-1}, respectively. The relative change in clearance of indinavir caused by the kinetic enhancer ritonavir was 0.362.

The different covariates were introduced separately into the basic model on clearance, volume of distribution and apparent bioavailability, using a univariate procedure. Gender, baseline TBR and concomitant use of efavirenz or nevirapine had a statistically significant relation with clearance and apparent bioavailability. None of the covariates had a significant relation with volume of distribution.

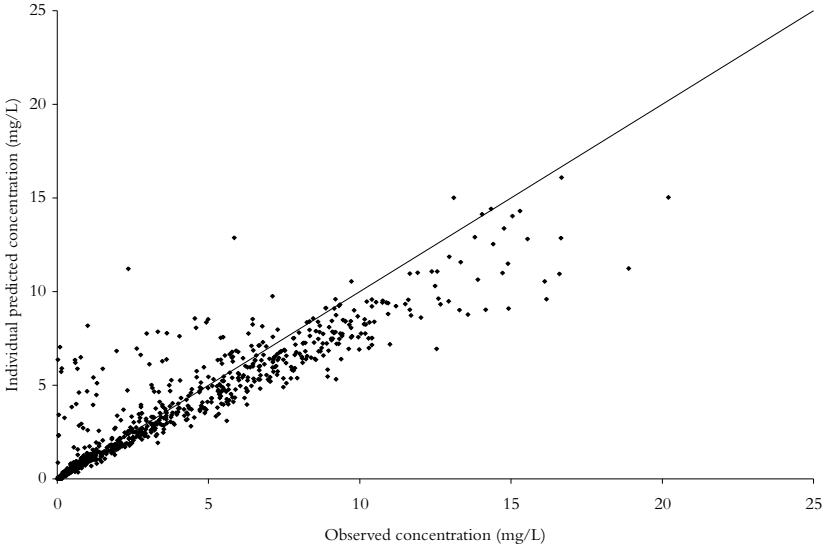
From the backwards elimination, it appeared that concomitant use of efavirenz or nevirapine (ΔOFV=-34.8, change in clearance=41%) and gender (ΔOFV=-8.6, change in apparent bioavailability=48%) had statistically significant and clinically relevant influence on the pharmacokinetics of indinavir. The influences of efavirenz and nevirapine on the clearance of indinavir were similar and inclusion of separate effects for each drug did not improve goodness-of-fit. Furthermore, this reduction was independent of the concomitant use of ritonavir. The results of the final model are summarised in table 2. The model predicted and individual predicted concentrations versus observed concentrations of indinavir using the final model are presented in figure 2. The model based predictions were symmetrically distributed around the line of identity, indicating that the model

Figure 2. Population (panel A) and individual (panel B) predicted concentrations versus observed concentrations of indinavir, and weighted residuals versus time after ingestion (panel C) using the final model.

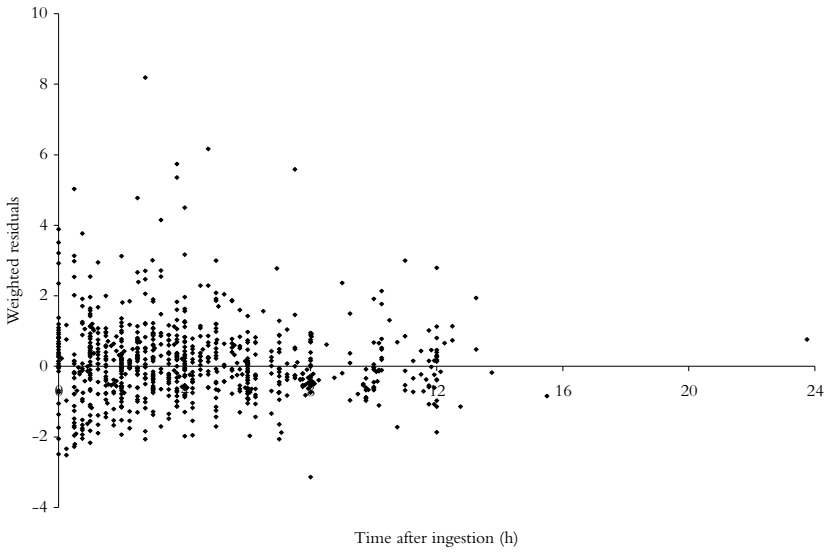
A



B



C



adequately describes the pharmacokinetic profile of indinavir. Figure 2C shows the plot of the weighted residuals versus the time after ingestion.

The following equation describes the final model for clearance and apparent bioavailability:

$$CL = 46.8 \star 0.354^{RTV} \star 1.41^{NNRTI}$$

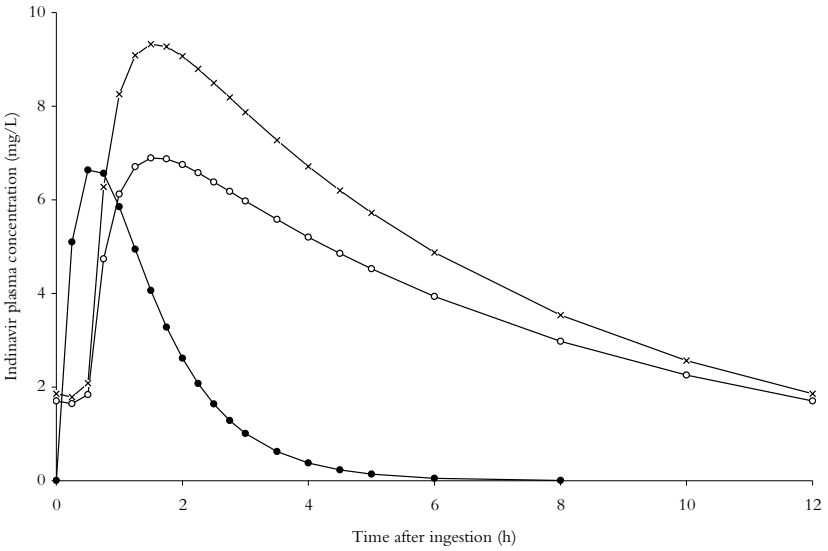
$$F = 1 \star 1.48^{SEX}$$

in which RTV is 1 for concomitant ritonavir use and 0 for all others, NNRTI is 1 for concomitant use of efavirenz or nevirapine use and 0 for all others, and SEX is 0 for males and 1 for female patients. In figure 3 typical concentration-time data of the three mainly used indinavir containing regimens are shown (3 times daily 800 mg indinavir, twice daily 800 mg indinavir with twice daily 100 mg ritonavir, twice daily 400 mg indinavir with twice daily 400 mg ritonavir).

Model validation

From the original data set 1000 replicate bootstrap data sets were generated and used for the evaluation of the precision of the parameter estimates. In addition to the parameter estimates of the basic and final model, table 2 list the results of the bootstrap procedure, presented as medians and 95% prediction intervals. Median values of the bootstrap analysis were close to the parameter estimates of the original data set and all parameters could be estimated with acceptable precision.

Figure 3. Concentration-time points of three indinavir containing regimen. Solid dots represent 3 times daily 800 mg indinavir, open circles represent 2 times daily 400 mg indinavir in combination with 400 mg ritonavir, crosses represent 2 times daily 800 mg indinavir with 100 mg ritonavir.



DISCUSSION

A population pharmacokinetic model was developed that characterised the interaction between indinavir and ritonavir with a dichotomous effect of ritonavir exposure on the clearance of indinavir. As can be observed from the results of the bootstrap analysis and figure 2, the developed model enabled adequate description of the pharmacokinetics of indinavir. Our estimates of clearance (46.8 L/h) and volume of distribution (82.3 L) fell within the wide range (25.6-110 L/h for clearance and 30-195 L for volume of distribution) of values found in previous studies.[25-29]

To describe the relationship between ritonavir exposure and indinavir pharmacokinetics we initiated the investigations with a direct time-dependant influence of ritonavir on the clearance of indinavir. However, no time dependency was observed in the relationship, nor a time dissociated relation between ritonavir exposure and clearance of indinavir could be demonstrated. Finally, it appeared that the boosting effect of ritonavir was independent of dosage (100 mg - 400 mg). Concomitant use of ritonavir caused a decrease in clearance of 63.8%, resulting

in an increase in elimination half-life from 1.2 h to 3.4 h. In contrast with the results of Saah et al.,[30] this indicates that 100 mg of ritonavir may be sufficient for maximal inhibition of indinavir metabolism.

Also the absorption phase was influenced by ritonavir. Ritonavir may have caused delayed gastric passage,[31] since only for patients using indinavir in combination with ritonavir a lag-time could be estimated (0.483 h). As also observed in other studies,[5,30] the maximum concentration of indinavir occurred later in regimens with ritonavir compared to regimens without, while the absorption rate was similar for patient with and without ritonavir.

No relationship between the exposure of ritonavir and the apparent bioavailability could be demonstrated, indicating that this effect may be of minor relevance for ritonavir boosting of indinavir.

In patients who concomitantly used the NNRTIs efavirenz and nevirapine, the clearance of indinavir increased with 41% to 66.0 L/h. This interaction has been previously characterized in other studies.[32-34] Aarnoutse et al.[34] showed that the addition of efavirenz to indinavir resulted in significant reductions in indinavir AUC (25%), C_{min} (50%) and C_{max} (17%). Murphy et al.[32] demonstrated that nevirapine significantly reduced indinavir AUC (27.4%), C_{min} (47.5%), and to a lesser extent C_{max} (11%). In our study we could not demonstrate a difference in the interaction between efavirenz or nevirapine with indinavir since no additional increase in the goodness-of-fit was gained with the inclusion of separate factors for either drug. By inclusion of nevirapine and efavirenz as determinants for the clearance of indinavir a marked reduction in interindividual variability was observed from 35% to 24%.

It appeared that gender had a significant effect on the pharmacokinetics of indinavir. Female patients had 48% higher bioavailability compared to male patients, suggesting that females reach higher plasma levels. This has previously been shown by Csajka et al.[29] Investigations using midazolam as a probe of intestinal CYP3A4 and verapamil, a mixed CYP3A and P-gp substrate, showed higher bioavailability in women compared to men.[35] No other tested covariables showed influence on the pharmacokinetics of indinavir.

The presence of a relationship between plasma concentration and efficacy and/or toxicity suggests that the use of plasma concentrations to optimise indinavir containing therapy is useful.[13] Bayesian estimation of individual pharmacokinetics of indinavir based on the developed model may be applied, with which maximal and minimal plasma concentrations can be assessed from a randomly timed blood sample. Subsequently, appropriate therapeutic drug monitoring can be performed

and consequently toxicity and/or virologic failure may be prevented.
In conclusion, a model for the pharmacokinetics of indinavir was developed and validated. To develop this model, a large patient population has been used, incorporating concentration-time points over the complete dosing interval. Concomitant use of ritonavir delayed the absorption phase by an absorption lag-time of approximately 30 minutes and highly affected the elimination phase of indinavir. The influence of ritonavir on clearance of indinavir was independent of dosage and disposition, indicating that 100 mg of ritonavir is sufficient for maximal inhibition of CYP3A4-mediated metabolism of indinavir. Concomitant use of NNRTIs increased the clearance of indinavir and female patients showed increased bioavailability.

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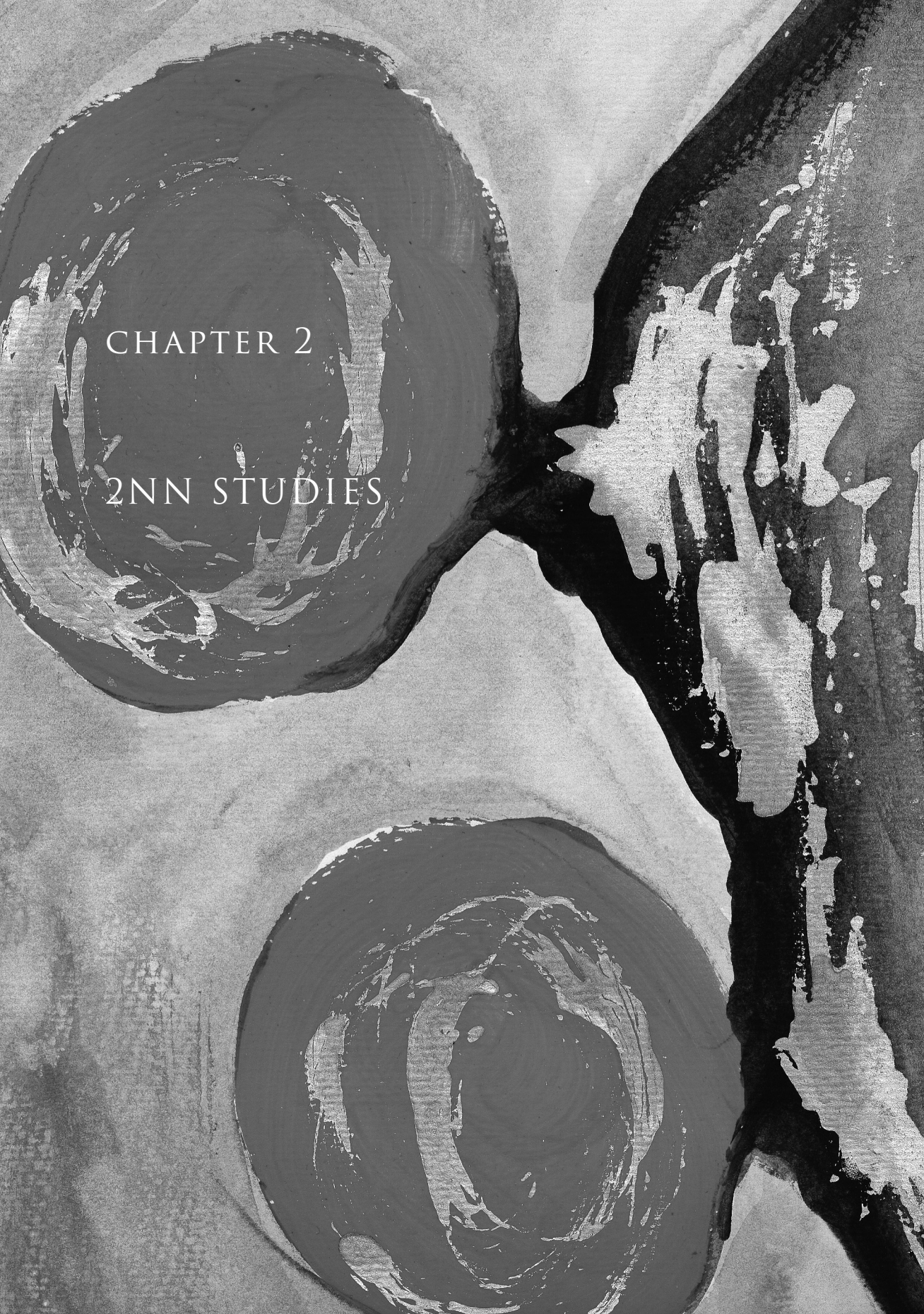
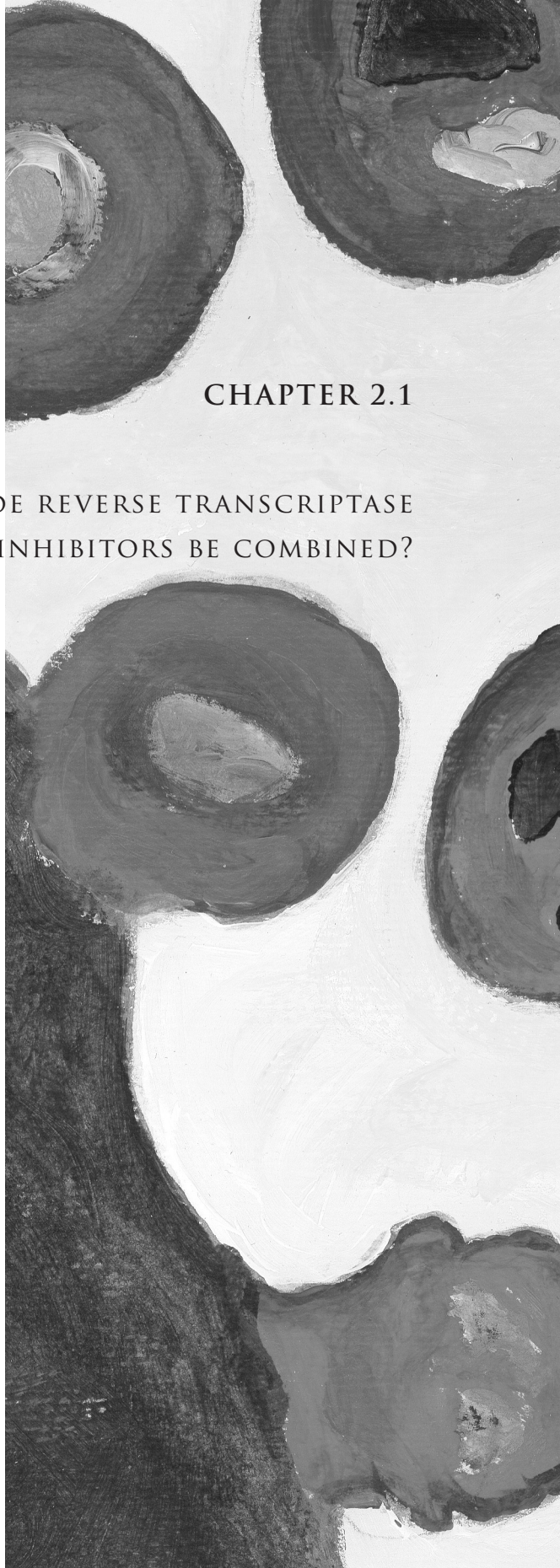


CHAPTER 2

2NN STUDIES



CHAPTER 2.1

SHOULD NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS BE COMBINED?

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Jos H. Beijnen

Drugs in R&D
in press

ABSTRACT

In the treatment of HIV-infected patients, an urgent need exists for more conveniently dosed and better tolerated regimens with improved virological and immunological efficacy. Based on preclinical studies, the combination of two non-nucleoside reverse transcriptase inhibitors (NNRTIs) was considered to fulfil this.

Several clinical studies, however, have shown different results with regards to mechanism of action, pharmacokinetics, efficacy and toxicity of dual NNRTI regimens. Combinations of two NNRTIs have shown additive or synergistic inhibitory effects on the HIV-1 reverse transcriptase activity and the viral replication of HIV-1 *in vitro*, although also antagonistic effects have been described. When nevirapine and efavirenz are administered in combination, the exposure to efavirenz was decreased due to induction of the metabolism by nevirapine. When compared to single NNRTI regimens, dual NNRTI regimens showed similar, but not superior results with regards to virological and immunological success in treatment-naive and pre-treated HIV-1-infected patients. However, NNRTI-associated adverse events, such as clinical hepatitis, elevated liver enzymes, rash, central nervous system toxicity and psychiatric disorders, occurred more frequently when two NNRTIs are dosed concomitantly.

In conclusion, regimens with both nevirapine and efavirenz seem to result in similar antiviral and immunological efficacy with increased incidence of adverse events, compared to single NNRTI regimens. The combination of two NNRTIs is therefore less desirable than other available and effective treatment options.

INTRODUCTION

The primary objective of antiretroviral therapy is to achieve maximal and durable suppression of human immunodeficiency virus (HIV) replication, restore immunological function, improve patient quality of life and reduce HIV-related morbidity and mortality. Combination therapy against HIV has been shown to cause a significant reduction on morbidity and mortality.[1] Highly active antiretroviral therapy (HAART) usually consists of two nucleoside reverse transcriptase inhibitors (NRTIs) and one or two protease inhibitors (PIs) or a non-nucleoside reverse transcriptase inhibitor (NNRTI).[2] Several therapeutic options have been considered as a result of the search for more conveniently dosed and better-tolerated regimens with more virological and immunological effect. A number of these alternative regimens have been investigated in clinical trials.

Among others four-drug regimens containing drugs from three drug classes,[3-6] triple NRTI regimens [7-9] and NRTI-sparing regimens [10,11] were explored.
Another strategy consists of the administration of two NNRTIs with a backbone of NRTIs or PIs. With a regimen without PIs, this class of antiretroviral agents can be spared as second-line treatment options. Furthermore, these regimens lack the typically PI-associated side effects as lipodystrophy and other metabolic disturbances, such as dyslipidaemia and diabetes mellitus.[12,13] An NRTI-sparing regimen might reduce mitochondrial toxicity, such as elevated lactate levels, polyneuropathy and lipoatrophy, which are common NRTI-associated adverse events. Besides, when two NNRTIs are combined an additive or synergistic antiviral effect might occur.[14,15] A further potential benefit could be the increased total NNRTI exposure, and subsequently more pressure on viral replication. However, nevirapine and efavirenz have some overlapping toxicity such as elevated liver enzymes.[16-18] In addition, nearly universal cross-resistance has been found to develop rapidly after initiation of NNRTI therapy.[19] Three NNRTIs are currently approved in the United States: nevirapine, efavirenz and delavirdine, while delavirdine is not approved in Europe.
The aim of the current review was to give an overview of virological, pharmacokinetic and clinical studies of double NNRTI regimens, with the ultimate goal to advise on use of dual NNRTI regimens in HIV-1-infected patients.

Mechanism of action

The NNRTIs inhibit non-competitively the HIV-1 reverse transcriptase by binding directly and reversibly to the reverse transcriptase enzyme and thereby interfering with viral RNA to DNA-directed polymerase activities.[16] Currently approved NNRTIs are characterised by the rapid development of cross-resistance when used as monotherapy.[18,20] The mutations Y181C and K103N are the most frequently observed mutations among treatment failures in patients using nevirapine, efavirenz and delavirdine.[21] However, the combination of two potent NNRTIs may be able to prevent development of this NNRTI-associated resistance, or to overcome NNRTI-resistant HIV-strains. Quan et al. reported that the combination of nevirapine and calanolide A, an NNRTI in development, might have a rationale for combination therapy since it possessed an additive to weakly synergistic inhibitory effect on viral replication of HIV-1 *in vitro*.[15] Also the NNRTIs in development, DPC083, DPC961 and DPC963, acted additively with efavirenz against the HIV-1 reverse transcriptase activity and the replication of HIV-1.[22] On the other hand, an *in vitro* study of Gu et al. showed that the

combination of nevirapine and delavirdine was mutually antagonistic with regard to inhibition of HIV-1 reverse transcriptase polymerase activity.[23] Contrasting results have been reported about the combination of nevirapine and efavirenz. Kollmann et al.[14] showed results indicating that this combination is synergistic to additive. King et al.,[22] however, demonstrated antagonistical effects of this combination.
These variable *in vitro* outcomes suggest that antiretroviral combination regimens containing multiple NNRTIs should be given thorough consideration before being used in HIV-infected patients. Because of the antagonistic inhibitory effect, the combination of nevirapine and delavirdine is not preferred, the NNRTIs in development on the contrary, might be combined with efavirenz. The efficacy of the combination of nevirapine and efavirenz has not been assessed unambiguously *in vitro*.

Pharmacokinetics
The primary route of metabolism of nevirapine, efavirenz and delavirdine is via cytochrome P450 (CYP) 3A4.[16,24,25] Nevirapine and efavirenz are also converted to inactive metabolites by CYP2B6.[16,25] Delavirdine is a potent inhibitor of CYP3A4, which results in autoinhibition.[24] At clinically relevant concentrations, efavirenz is an inhibitor of CYP3A4, CYP2C9 and CYP2C19. The effect of efavirenz on CYP3A4 appears to be mixed, as efavirenz has also been shown to be an inducer of this isoenzyme. Nevirapine induces both CYP3A4 and CYP2B6 metabolism. Furthermore, nevirapine and efavirenz both show autoinduction.[24,26-28]
Since nevirapine, efavirenz and delavirdine induce and/or inhibit several CYP enzymes, it is difficult to predict the net effect of these processes in combination therapy. The DONUT study [29] and the 2NN-PK substudy [30] showed that the concomitant use of nevirapine plus efavirenz only affects clearance of efavirenz. Compared to patients receiving efavirenz alone (once daily 600 mg), patients with both nevirapine and efavirenz (once daily 400 mg and 600 mg, respectively) showed a significant lower area under the plasma concentration-time curve over 24 h (AUC_{0-24h}; 54.8 versus 38.8 h⋆mg/L, p=0.001), maximum plasma concentration (C_{max}; 3.63 versus 3.36 mg/L, p=0.048) and minimum plasma concentration (C_{min}; 1.55 versus 0.96 mg/L, p=0.001) of efavirenz.[29] From the pharmacokinetic substudy of the large randomised clinical trial 2NN, it was concluded that concomitant use of nevirapine increased clearance of efavirenz with 43% from 8.82 L/h to 12.6 L/h at steady-state.[30] Due to the increased dose of efavirenz in the double NNRTI arm of the 2NN study (800 mg versus 600

mg in the single NNRTI arm), the increased clearance of efavirenz resulted in comparable exposure to efavirenz in the efavirenz plus nevirapine arm (once daily 800 mg plus 400 mg) and the efavirenz-only arm (once daily 600 mg): 1.84 and 1.86 mg/L for C_{min}, 2.87 and 2.67 mg/L for C_{max} and 58.3 and 56.3 mg/L*h for AUC_{0-24h}, respectively.[30]

When nevirapine is used concomitantly, the efavirenz dose has to be increased to 800 mg, to reach efavirenz plasma concentrations, which are comparable with levels from 600 mg efavirenz once daily. However, in the combination studies of Olivieri et al.[31] and Jordan et al.[32] the standard dose of efavirenz (600 mg once daily) was administered. Unfortunately, no pharmacokinetic data are available from these studies. Nevertheless, in these studies adequate virological and immunological results have been observed, indicating that an increased dose of efavirenz might be unnecessary. From these results it is difficult to conclude which is the optimal dosage of efavirenz when it is combined with nevirapine. Several studies in HIV-1-infected patients have shown that treatment failure and toxicity are associated with low and high efavirenz plasma concentrations.[33,34] However, when efavirenz is concomitantly used with nevirapine, these observed target plasma concentrations for efavirenz might not be predictive of treatment failure and toxicity. The increased dose of 800 mg efavirenz in combination with nevirapine resulted in similar efavirenz plasma levels compared to 600 mg efavirenz when dosed alone, however, the increased dose of 800 mg efavirenz might increase the risk for toxicity. In table 1 the clinical studies with two NNRTIs containing treatments are summarised.

Efficacy

The retrospective chart review of Olivieri et al. showed that nevirapine plus efavirenz based salvage therapy in 13 pre-treated HIV-infected patients resulted in remarkably high efficacy. Median baseline viral load was 33,900 copies/mL (range 3,100-750,000 copies/mL). After a median follow-up of 11 months (range 3-18 months) 11 of 13 patients had undetectable viral loads (<50 copies/mL). Considering previous treatment experience, 9 of 10 of NNRTI-naive patients (90%) and 2 of 3 of NNRTI-experienced patients (67%) had viral loads <50 copies/mL.[31] Jordan et al. demonstrated comparable results with dual NNRTI therapy of nevirapine and efavirenz. Enrolled subjects were patients who requested a simple, once daily initial regimen, and patients already on therapy who requested a switch to a simpler, more tolerable regimen. After 12 months of therapy 11 of 12 treatment-naive subjects (92%) and 8 of 9 treatment-experienced subjects

Table 1. Characteristics of clinical studies with antiretroviral regimens containing efavirenz and nevirapine.

Study design	Dosage	Patients	Time of follow-up	Results	Reference
PK study	400mg NVP qd + 600mg EFV qd	19 on treatment	43 days	EFV-exposure ↓ , EFV-CL ↑ 2 pts AE	Veldkamp et al.[29]
PK study	400mg NVP qd + 800mg EFV qd	123 tr-naive	48 weeks	EFV-CL ↑	Kappelhoff et al.[30]
ROC study	400mg NVP qd + 600mg EFV qd	13 (10 PI-exp, 3 PI+NNRTI-exp)	11 months (range 3-18)	9/10 PI-exp pts + 2/3 PI+NNRTI-exp pts VL<50 copies/mL 3/13 pts AE	Olivieri et al.[31]
ROC study	400mg NVP qd + 600mg EFV qd	26 (15 tr-naive, 11 tr-exp)	12 months	11/12 tr-naive pts + 8/9 tr-exp pts VL <400 copies/mL, mean↑ CD4+ tr-naive and tr-exp: 438 and 367 cells/mm^3 5/26 pts AE	Jordan et al.[32]
PP study (switch)	200mg NVP bid + 800mg EFV qd	21 tr-exp	48 weeks	↑ CD4+: 9, ↓ VL: 0.34 log 9/21 pts AE	Gey et al.[35]
PP study	200mg NVP bid + 800mg EFV qd	30 (3 tr-naive, 16 successful PI-based HAART, 11 failed PI-based HAART)	6 months	3/3 tr-naive + 16/16 PI-exp pts <50 copies/mL, 6/8 failed PI-exp pts >50 copies/mL or rebound 9/30 pts AE	Arranz-Caso et al.[36]
RCT	400mg NVP qd + 800mg EFV qd	209 tr-naive	48 weeks	131/209 pts VL <50 copies/mL, median↑ CD4+: 160 cells/mm^3 51/209 pts clinical AE,	Van Leth et al.[37]

PK=pharmacokinetic, ROC=retrospective observational cohort, PP=prospective pilot, RCT=randomised clinical trial, qd=once daily, bid=twice daily, tr=treatment, exp=experienced, NVP=nevirapine, EFV=efavirenz, CL=clearance, pts=patients, AE=adverse events, VL=viral load.

(89%) had viral loads of <400 copies/mL. Both groups had an excellent immune response as well. After one year of treatment, the mean increase in CD4+ cells/mm^3 was 438 and 367 among treatment-naive and treatment-experienced subjects, respectively.[32]

In the study of Gey et al. 21 antiretroviral therapy-experienced patients who required a change in therapy because of symptoms of mitochondrial toxicity and/or lipoatrophy, showed encouraging results after 48 weeks with an NRTI-sparing regimen, consisting of saquinavir (1000 mg twice daily), ritonavir (100 mg twice daily), efavirenz (800 mg once daily) and nevirapine (200 mg twice daily). All patients remained clinically stable and showed no or no new opportunistic infections or AIDS-defining events. Comparing baseline versus week 48, CD4+ cell count slightly increased (434 versus 443 cells/mm^3) and viral load dropped (417 versus 191 copies/mL).[35]

In the study of Arranz-Caso et al. patients were treated with nevirapine (200 mg twice daily), efavirenz (800 mg once daily) and didanosine (250 or 400 mg once daily adjusted to body weight). After 6 months of follow-up, all (3) treatment-naive patients and all (16) PI-experienced patients with undetectable viral load for at least 6 months, had <50 HIV-1 RNA copies/mL. In the group with patients with failing PI-based regimens, 6 of 8 patients on treatment did not achieve undetectable levels of HIV or had early rebound.[36]

In the 2NN study, the only comparative randomised study with a large number of patients and statistical power, patients were randomly assigned to either nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, or a combination of nevirapine 400 mg plus efavirenz 800 mg once daily. In total, 1216 treatment-naive patients were included in the four treatment arms, of which 209 were assigned nevirapine plus efavirenz.[37] With 62.7% of the patients with a viral load <50 copies/mL at week 48, the percentage in this dual NNRTI treatment arm was remarkably lower than in the studies of Olivieri et al.[31] and Jordan et al.[32] However, the antiretroviral success was comparable with the other 2NN treatment arms with only one NNRTI (70.0% for nevirapine once daily, 65.4% for nevirapine twice daily, and 70.0% for efavirenz arm). Also the median CD4+ cells/mm^3 increase was lower in the 2NN study compared to the study of Jordan et al.,[32] albeit comparable to the other treatment arms in this 2NN study (150 versus 160-170 cells/mm^3 in the single NNRTI arms).

Since both nevirapine and efavirenz are potent antiretroviral drugs it is not unexpected that a combination of both drugs results in immunological and virological success in treatment-naive or NNRTI-naive patients, comparable to

the NNRTIs given alone with appropriate NRTI backbone. However, it is of special interest whether a dual NNRTI regimen has the potency to overcome development of NNRTI-associated resistance due to a possible additive or synergistic effect, or is able to lead to successful antiretroviral treatment in NNRTI-experienced patients. The observation of the two patients with virological success from the three NNRTI-experienced patients from the study of Olivieri et al. goes into that direction although obviously the number of patients is too small to draw conclusions.

Toxicity

In the DONUT study the combination of nevirapine 400 mg once daily with efavirenz 600 mg once daily was well tolerated in all (19) except 2 patients. One patient experienced nausea and general malaise when nevirapine was introduced, and withdrew from the study. Another patient reported peripheral neuropathy after nevirapine was added to the regimen, without withdrawal.[29] In this study, patients used the combination of nevirapine and efavirenz for only 4 weeks and therefore, the long-term tolerability could not be assessed in this study.

In the study of Olivieri et al. elevated liver function test results (>3 x upper limit of normal), liver toxicity, anaemia and central nervous system disturbances were recorded. Due to the relatively low incidence of adverse events and the fact that none of the patients requested discontinuation of therapy because of these adverse events, Olivieri et al. concluded that dual NNRTI based regimen might be an appealing alternative in heavily pre-treated PI-experienced patients.[31]

Also in the study of Jordan et al. the combination of nevirapine and efavirenz showed no increase in NNRTI-associated adverse events compared to single NNRTI regimens. Treatment-limiting adverse events were intractable insomnia, rash and central nervous system toxicity and occurred in 3/15 treatment-naive (20%) and in 2/11 treatment-experienced (18%) subjects.[32]

However, in the study of Gey et al., in which patients were switched to an NRTI-sparing regimen with nevirapine and efavirenz because of unacceptable NRTI-related side effects, frequent discontinuation due to adverse events were reported. Out of 21 patients, 6 discontinued due to rash and 3 due to nausea, saquinavir intolerance and non-adherence.[35]

Van Leth et al. concluded from the large randomised 2NN study that in the dual NNRTI arm more patients suffered from grade 3 or 4 clinical adverse events, including clinical hepatitis, rash, central nervous system toxicity and psychiatric disorders, compared to the nevirapine or efavirenz-only arms (24.4% for the

nevirapine plus efavirenz arm versus 15.0% for the nevirapine once daily arm, 20.4% for the nevirapine twice daily and 18.0% for the efavirenz arm).[37]
Resembling the results of the 2NN study, the pilot study of Arranz-Caso et al. reported a percentage of severe adverse events of 30%, which is higher than that reported with regimens of treatment that include only one NNRTI.[36] Two patients developed clinical hepatitis, two had central nervous system disturbances, two showed gastric intolerance, and three had a severe skin rash.
Despite of the relative good tolerability of concomitant use of nevirapine and efavirenz in the relative small studies of Veldkamp et al.,[29] Jordan et al.[32] and Olivieri et al.,[31] the results from the large comparative 2NN study [37] and also from the pilot studies of Gey et al.[35] and Arranz-Caso et al.[36] indicate that adverse events occur more frequently in dual NNRTI regimens.

DISCUSSION AND CONCLUSION

The investigated studies with concomitant use of nevirapine and efavirenz showed different results with regards to *in vitro* activity, pharmacokinetics, efficacy and toxicity. The variation may be due to differences in study design, patient population, dosage and time of follow-up, which might make a comparison between the studies insincere. Several studies were retrospective with only a limited number of well-motivated patients and scarce information about the extent of the pre-treatment and the reason for treatment failure.[31,32] Other studies were very small prospective pilot studies.[35,36] When the pre-treatment is not exactly known, interpretation of results is complicated. Bias might be introduced with small numbers of intensively selected patient. On the contrary, the 2NN study was a randomised trial with 1216 treatment-naive patients of which 209 were assigned nevirapine plus efavirenz, which provided results from which clear conclusions can be drawn.[37]
The additive to synergistic inhibitory effect on HIV-1 replication of two concomitantly used NNRTI in *in vitro* studies only is a weak rationale for combination therapy. Besides, two drugs with the same mechanism of action and the same resistance pattern, in case of nevirapine and efavirenz, is theoretically not the most convincing combination. Despite of the encouraging results in the small studies of Olivieri et al.[31] and Jordan et al.,[32] regimens with nevirapine plus efavirenz seems to have no additional efficacy in treatment-naive patients and to cause more adverse events than each drug separately. Whether combinations of two NNRTIs may be beneficial in single NNRTI pre-treated patients remains controversial, as well as dual NNRTI regimens with next generation NNRTIs, which have other resistance patterns.

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CHAPTER 2.2

SIMPLE AND RAPID METHOD FOR THE SIMULTANEOUS DETERMINATION OF THE NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS EFAVIRENZ AND NEVIRAPINE IN HUMAN PLASMA USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Efavirenz and nevirapine are non-nucleoside reverse transcriptase inhibitors for the treatment of HIV-1-infected individuals. A simple and rapid high-performance liquid chromatographic method for the simultaneous quantification of efavirenz and nevirapine in human plasma suitable for therapeutic drug monitoring is described. Sample pre-treatment consisted of protein precipitation with acetonitrile and subsequently dilution with distilled water. The drugs were separated from endogenous compounds by isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection at 275 nm. The method was validated over the therapeutically relevant concentration range of 0.05 mg/L to 15.0 mg/L and 0.25 mg/L to 15.0 mg/L for efavirenz and nevirapine, respectively using a volume of 100 μL of plasma. The calibration curves were linear over this concentration range. Carbamazepine was used as internal standard. The assay proved to be accurate (accuracies varied between -12.7% and 8.5%) and precise (intra- and inter-assay precisions were less then 5.9%). The tested batches of control human plasma and frequently co-administered drugs did not interfere with the described methodology. Efavirenz and nevirapine were stable under various relevant storage conditions. This validated assay is suited for use in pharmacokinetic studies with efavirenz and nevirapine and can readily be implemented in the setting of a hospital laboratory for the monitoring of efavirenz and nevirapine concentrations.

INTRODUCTION

Efavirenz and nevirapine are non-nucleoside reverse transcriptase inhibitors (NNRTIs). NNRTIs are one of the three classes of drugs currently used to treat human immunodeficiency virus (HIV) infection. These drugs inhibit non-competitively the human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. NNRTIs bind directly and reversibly to the catalytic site of the reverse transcriptase enzyme and therefore, interfere with viral RNA to DNA-directed polymerase activities.[1,2]

Relationships between plasma drug concentrations of NNRTIs and efficacy and toxicity have been identified.[3,4] The plasma concentration of the NNRTIs depends on the pharmacokinetic parameters, which can be influenced by patient characteristics (i.e. co-morbidity, demographics). Large interpatient pharmacokinetic variability suggest a role for therapeutic drug monitoring to individualise antiretroviral therapy, when target values of the NNRTIs have been established.

There is an urgent need for a simple and rapid assay for routine measurement of NNRTI concentrations in plasma and large-scale pharmacokinetic studies. Ideally, micro-volumes of plasma are required for this assay, which enables studies in special populations like HIV-1-infected children. Moreover, sample pre-treatment should be minimal. Obviously, a combined assay of these extensively used compounds strongly reduces workload for routine measurements. Current assays, however, lack these requirements.

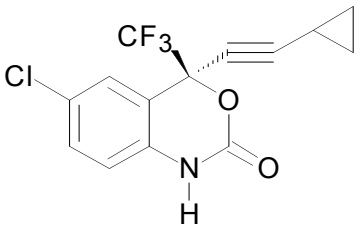
Recently, a few methods for the simultaneous determination of NNRTIs have been published.[5-9] Commonly, reversed-phase high-performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection or mass spectrometry (MS) is used. The methods of Rezk et al.[6] and Simon et al.[8] use 500 μL sample, and Aymard et al.[7] and Titier et al.[9] use 1 mL samples for the determination of the plasma levels. Besides, all methods use a complicated time-consuming sample pre-treatment consisting of solid phase or liquid-liquid extraction combined with evaporation of the extract to dryness, which is reconstituted afterwards. Furthermore, complicated instrument set-up is necessary consisting of a combination of two HPLC systems or gradient elution.[5-8] We here report the development and validation, according to current FDA guidelines, of a simple and rapid, isocratic HPLC assay with UV detection for the simultaneous, quantitative determination of efavirenz and nevirapine in human plasma. Only 100 μL of sample was used combined with a very rapid and simple sample pre-treatment. The proposed technique implies protein precipitation, isocratic elution on a reversed-phase HPLC-system, and UV detection at a single wavelength. The usefulness of the method is demonstrated by the analysis of plasma samples of treated HIV-1-infected patients.

EXPERIMENTAL

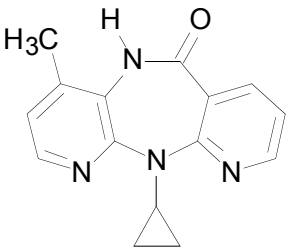
Equipment and supplies

The HPLC system consisted of a P1000 solvent delivery pump (Thermo Separation Products, Fremont, CA, USA), an SCM1000 degasser (Thermo Separation Products), an AS3000 automatic sample injection device (Thermo Separation Products), and a UV1000 wavelength detector (Thermo Separation Products). The analytical column was a Zorbax Extend C18 (150 x 2.1 mm I.D., 5 μm particle size; Agilent Technologies, Amstelveen, The Netherlands) protected by a ChromGuard HPLC pre-column (10 x 3.0 mm I.D. reversed phase; Varian, Middelburg, The Netherlands) and a precolumn microfilter frit, 0.5 μM, C-425X (Upchurch Scientific, Oak Harbor, WA, USA). Analytical runs were processed

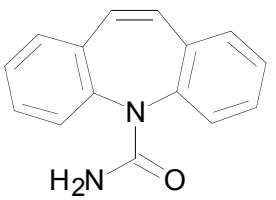
Figure 1. The chemical structures of efavirenz, nevirapine and the internal standard carbamazepine.



Efavirenz



Nevirapine



Carbamazepine

by Chromquest on Windows (version 2.51, ThermoQuest Corporation, San Jose, USA).

Chemicals

Efavirenz was kindly provided by Merck Sharp & Dohme, Haarlem, The Netherlands and nevirapine by Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA. Carbamazepine (BUFA Pharmaceutical Products, Uitgeest, The Netherlands) was used as the internal standard for this assay. Chemical structures of efavirenz, nevirapine and carbamazepine are shown in figure 1. Acetonitrile and methanol (both HPLC supra-gradient) were purchased from Biosolve (Amsterdam, The Netherlands). Dimethylsulfoxide (DMSO) was purchased from Merck (Darmstadt, Germany) and triethylamine was obtained from Merck (Schuchardt, Hohenbrunn, Germany). Distilled water originated from Aqua B. Braun (Melsungen, Germany). Blank, drug-free plasma was obtained from the Central Laboratory of Blood Transfusion Service (Amsterdam, The Netherlands). Drugs for interference analysis were obtained from the hospital pharmacy (Slotervaart Hospital, Amsterdam, The Netherlands), either as solutions for injection or after dissolving solid reference material in 50% methanol (final concentration 500 mg/L).

Preparation of standards

Amounts of 5 mg of the analytes were accurately weighted and transferred to separate volumetric flasks of 5.0 mL. Efavirenz and nevirapine were dissolved in methanol and DMSO, respectively, to give final concentrations of 1 mg/mL. Independently prepared stock solutions with separate weighting were made for the preparation of the calibration (CAL) standards and quality control (QC) samples. The stock solutions of both efavirenz and nevirapine were used for preparing a combined CAL-working solution. This CAL-working solution was diluted with methanol to acquire CAL-working solutions at several concentration levels. Calibration concentrations of 0.05 mg/L to 15.0 mg/L efavirenz and nevirapine were prepared in blank human plasma. QC samples in the concentrations of 0.25, 1.0, 5.0 and 12.5 mg/L were prepared independently in a similar way. CAL and QC samples contained 5% of organic solvent.

Internal standard (IS) preparation

Carbamazepine (30 mg) was dissolved in acetonitrile to achieve a final concentration of 30 mg/L (IS-solution).

Sample pre-treatment

In an Eppendorf tube of 1.5 mL, 200 μL IS-solution was added to 100 μL of plasma. The samples were vortexed for 2 seconds and then mixed for 15 minutes on a shaking device. Afterwards the tubes were centrifuged for 10 minutes at 10,500 g, and subsequently 200 μL of the clear supernatant was transferred to another tube and mixed with 200 μL of distilled water. The tubes were mixed on a vortex for 10 seconds and then centrifuged for 10 minutes at 10,500 g. 200 μL of the clear supernatant was transferred to an autosampler vial.

Chromatography

The chromatographic analysis was performed at ambient temperature on a Zorbax Extend C18 analytical column with a mobile phase composed of 25 mM triethylamine in water-acetonitril (65:35, v/v) pH: 11.7. Absorbance was measured at 275 nm. The flow-rate was maintained at 0.2 mL/min. Aliquots of 25 μL were injected.

VALIDATION PROCEDURES

All validations were performed according to the recently published FDA guidelines for validation of bioanalytical assays.[10]

Response functions

Calibration curves were constructed by least-squares linear regression analysis without weighting and by using $1/x$ and $1/x^2$ (x=concentration) as weighting factors. In order to establish the best quantification method (with IS or without IS) and the best weighting factor, back-calculated calibration concentrations were determined. The model with the lowest total bias and the most constant bias across the concentration range was used for further analysis and quantification.

Accuracy and precision

Accuracy and precision were determined by analysing QC samples with analyte concentrations at the lower limit of quantification (LLQ) and in the low, mid and high concentration ranges of the calibration curves. In addition, QC samples with analyte concentrations 2.5 times higher than the upper limit of quantification (ULQ) were analysed after 5-fold dilution of the samples. Each QC sample was analysed in a minimum of 5 replicates together with a calibration curve, independently prepared from the control samples, in 3 analytical runs. The accuracy was defined as percent difference between the mean concentration and the nominal concentration. The coefficient of variation was used to report the precisions.

The intra- and inter-assay accuracies for the LLQ concentration should be within ±20% and for all other concentrations within ±15%. The precisions should be less than 20% for the LLQ and less than 15% for all other concentrations.[10,11]

Specificity and selectivity

Six different batches of control drug-free plasma were tested to determine whether endogenous matrix constituents co-eluted with the analytes or the internal standard (IS). Double blanks (without IS), blanks (with IS) and spiked samples (with efavirenz and nevirapine) at the LLQ were prepared, processed according to the described procedures and analysed.

To investigate the potential interference of co-medication with the quantification of the analytes, the co-medicated drugs were added to double blank samples at therapeutic drug concentrations. The samples were then processed and assayed according to the described method. The following drugs, frequently used by HIV-infected individuals, were investigated for interference: abacavir, acetaminophen, amprenavir, caffeine, delavirdine, didanosine, fluconazole, folic acid, ganciclovir, indinavir, itraconazole, lamivudine, lopinavir, methadone, nelfinavir, oxazepam, pyrazinamide, pyrimethamine, ranitidine, rifampicine, ritonavir, saquinavir,

stavudine, sulfamethoxazole, tenofovir, trimethoprim, zalcitabine, zidovudine and zidovudine-glucuronide. The peak areas of compounds co-eluting with one of the analytes should be less than 20% of the peak areas of the analyte at the LLQ. For compounds co-eluting with the internal standard the peak area should be less than 5% of the IS area.[10]

Recovery

Extraction recoveries were determined by comparing the peak area of the QC samples in the low, mid and high concentrations with unprocessed solutions of corresponding concentrations in 6-fold.

Stability

Several stability tests were performed to verify the stability of the drugs during all handling procedures. Samples were assayed at two concentrations (at low and high concentrations) in triplicate.

The samples were kept for 24 hours at ambient temperatures, 7 days at 4°C and subjected to three freeze-thaw cycles. The concentration of the drugs after each storage period was related to the concentration of freshly prepared samples in the same analytical run. In addition, long term stability of efavirenz and nevirapine in plasma was investigated after 6 and 12 months of storage at -20°C, respectively.

Also a stability test was performed to verify the stability of the drugs in the autosampler vials pending analysis. The samples were left at ambient temperatures in the autosampler for 5 days prior to analysis.

Furthermore, stability of the IS-solution was investigated over 24 hours and 30 days at ambient temperatures. The stability of the stock solutions of efavirenz and nevirapine was determined at 24 hours at ambient temperatures, and 24 and 36 months, respectively, at -20°C.

Analytes were considered stable if the concentrations deviated less than ±20% from the concentrations of freshly prepared samples or from nominal concentrations, with coefficients of variance less than 20%. For stability of stock solutions the deviation should be less than ±5% and the C.V. values less than 5%.[10]

Analysis of patient samples

Plasma samples of several HIV-infected individuals on an efavirenz or nevirapine containing regimen were analysed with the currently reported method to assess applicability of the method.

RESULTS

Chromatography and detection

The starting point for the development was the previously described HPLC-UV method for efavirenz.[12] Using this system, however, nevirapine eluted without relevant retention. Because of this very short retention time, the amount of organic modifier in the mobile phase was decreased. Under these conditions efavirenz did not elute within 60 minutes and the chromatography of nevirapine was unacceptable. Thereupon, the pH of the mobile phase was increased to 8.5 and higher. As a consequence, a column specially designed for stable use with high pH mobile phases up to 11.5 (Zorbax Extend), was used. Phosphate, ammonia and triethylamine buffers combined with different percentages of modifiers (both acetonitrile and methanol) were tested as mobile phase. No elution of efavirenz was obtained with methanol as modifier. The influence of the pH on the retention times of efavirenz and nevirapine was established. With an increase of the pH of the eluent, the capacity factor of efavirenz decreased. Furthermore, at constant pH, the increase of molarity of triethylamine reduced the retention time of efavirenz. A mobile phase consisting of 25 mM triethylamine in water-acetonitrile (65:35, v/v) with a pH of 11.7 yielded the best separation of efavirenz and nevirapine from endogenous plasma compounds with short retention times and was therefore chosen.

The UV spectra of efavirenz and nevirapine in 30% acetonitrile and in eluent were recorded. For nevirapine the maximal absorbance was measured at 282 nm. With efavirenz a pH-shift was seen. In 30% acetonitrile and in eluent the maximal absorbance was measured at 248 nm and 269 nm, respectively. Therefore, UV detection at 275 nm was chosen for the detection of both efavirenz and nevirapine.

An internal standard was used to improve robustness. Carbamazepine was selected as internal standard because the drug is structurally allied to nevirapine. Besides, carbamazepine showed proper elution characteristics and was detectable at the same wavelength as efavirenz and nevirapine.

Retention times of nevirapine, carbamazepine and efavirenz were 2.8, 5.0 and 7.8 minutes, respectively. Total run time was 10 minutes. Representative selected chromatograms of control human plasma and spiked quality controls with internal standard are shown in figure 2.

Figure 2. Selected chromatograms of efavirenz (retention time 7.8 min) and nevirapine (retention time 2.8 min) from control human plasma (A), blank (B) and two quality controls (0.25 mg/L and 5.0 mg/L) with internal standard (retention time 5.0 min) (C,D).

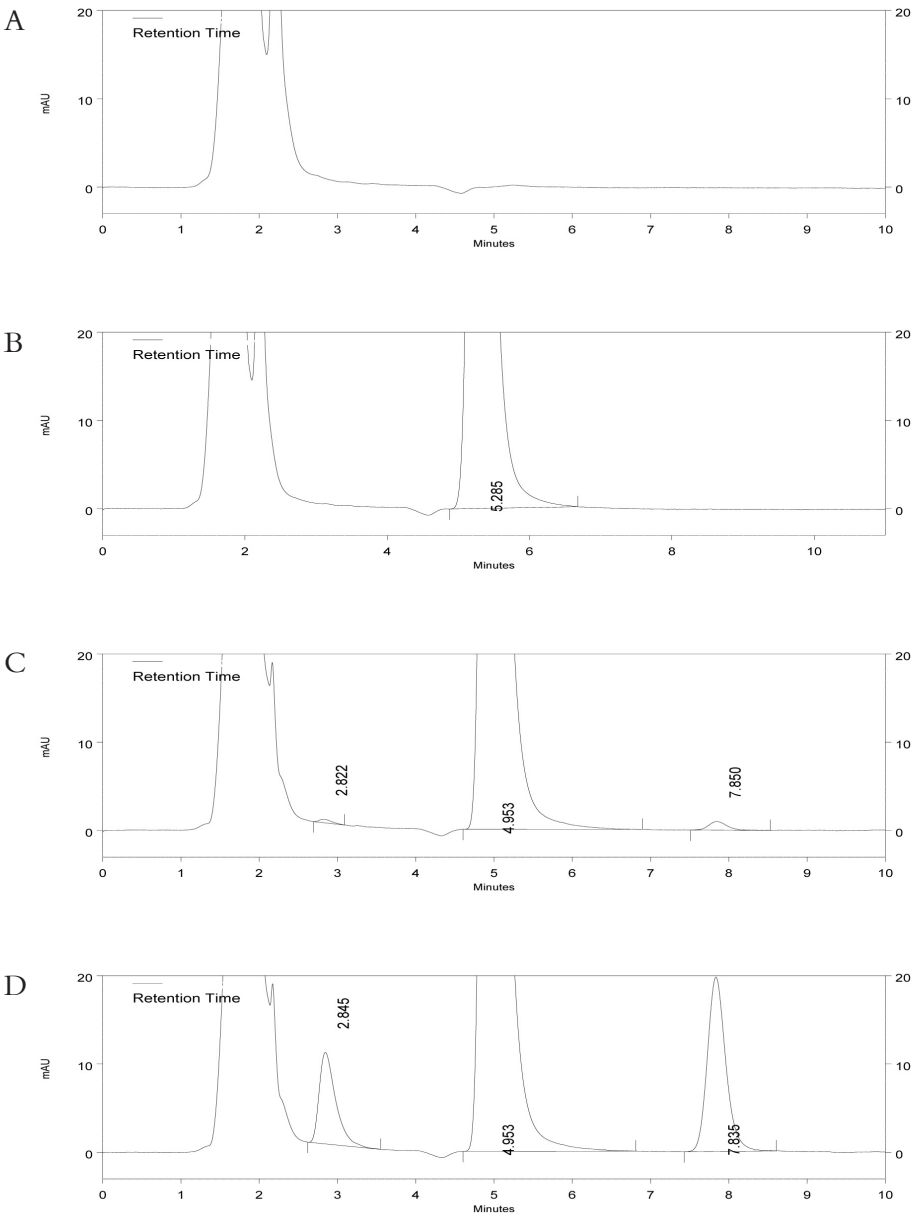


Table 1. Calibration concentrations back calculated from the responses.

	Efavirenz concentration (mg/L)									
Run	0.0517	0.1034	0.2585	0.5169	1.034	2.585	5.169	7.754	10.34	15.51
1	0.0476	0.1065	0.2515	0.4668	1.011	2.605	5.147	7.852	10.39	15.86
	0.0572	0.0987	0.2495	0.5143	1.074	2.619	5.196	7.932	10.58	15.89
2	0.0561	0.1025	0.2496	0.5193	1.030	2.667	5.199	7.967	10.40	15.66
	0.0487	0.1050	0.2352	0.4805	1.025	2.575	5.247	8.006	10.60	15.77
3	0.0527	0.1052	0.2544	0.4806	1.005	2.580	5.173	7.885	10.42	15.44
	0.0509	0.1022	0.2603	0.5184	1.056	2.628	5.119	7.986	10.27	15.83
Mean	0.0522	0.1034	0.2501	0.4967	1.034	2.612	5.180	7.938	10.44	15.74
SD	0.00	0.00	0.01	0.02	0.03	0.03	0.04	0.06	0.13	0.17
DEV (%)	0.99	-0.03	-3.24	-3.92	-0.02	1.08	0.22	2.38	1.01	1.51
CV (%)	7.45	2.73	3.33	4.69	2.56	1.30	0.86	0.75	1.20	1.07

SD=standard deviation, DEV=deviation, CV=coefficient of variation.

	Nevirapine concentration (mg/L)							
Run	0.2528	0.5056	1.011	2.528	5.056	7.584	10.11	15.17
1	0.2723	0.4685	0.9752	2.461	5.006	7.689	10.28	15.77
	0.2505	0.4887	0.9835	2.563	5.057	7.651	10.29	15.59
2	0.2603	0.4897	0.9507	2.462	5.062	7.763	10.53	15.59
	0.2690	0.4547	0.9594	2.456	5.120	7.826	10.45	15.65
3	0.2396	0.4661	0.9219	2.444	5.002	7.767	10.35	15.60
	0.2882	0.4872	0.9739	2.523	4.973	7.955	10.43	16.03
Mean	0.2633	0.4758	0.9608	2.485	5.037	7.775	10.39	15.71
SD	0.02	0.01	0.02	0.05	0.05	0.11	0.10	0.17
DEV (%)	4.16	-5.89	-4.98	-1.71	-0.38	2.52	2.73	3.54
CV (%)	6.51	3.09	2.33	1.90	1.06	1.39	0.97	1.11

SD=standard deviation, DEV=deviation, CV=coefficient of variation.

Response functions

Details of the calibration curves are shown in table 1. The lowest total bias and most constant bias across the concentration range were obtained using a weighting factor of $1/x^2$. For each calibration curve the calibration concentrations were back calculated from the response. The deviation for all concentrations from the nominal concentration for all concentrations was between -3.92% and 2.38% for efavirenz and between -5.89% and 4.16% for nevirapine. The C.V. values ranged from 0.75% to 7.45% for efavirenz and from 0.97% to 6.51% for nevirapine.

Accuracy and precision

The assay performance data for the determination of efavirenz and nevirapine in plasma are presented in table 2. The intra- and inter-assay accuracies for all tested efavirenz concentrations were between -14.2% and -10.4% for the LLQ and between -5.0% and 8.0% for all other concentrations. Precisions were less than 8.0% for all tested concentrations. The intra-and inter-assay accuracies for all tested nevirapine concentrations were between 6.5% and 12.5% for the LLQ and

Table 2. Assay performance data.

Efavirenz concentration (mg/L)						
		val llq efv	qc low efv	qc mid	qc high	>ULQ
Run	Replicate	0.1020	0.2551	5.102	12.76	24.90
1	1	0.0912	0.2333	5.068	12.93	26.92
	2	0.0952	0.2728	5.163	13.12	27.31
	3	0.0839	0.2486	5.127	13.30	26.17
	4	0.0930	0.2571	5.223	13.19	27.37
	5	0.0782	0.2637	5.304	13.39	26.68
2	1	0.0935	0.2567	5.095	12.98	26.59
	2	0.0890	0.2605	5.244	13.15	26.68
	3	0.0900	0.2419	5.251	12.81	26.68
	4	0.0910	0.2524	5.178	13.04	26.28
	5	0.0936	0.2408	5.328	13.06	27.47
3	1	0.0855	0.2389	5.317	12.84	26.74
	2	0.0855	0.2378	5.217	12.97	26.92
	3	0.0971	0.2412	5.306	12.77	25.92
	4	0.0821	0.2471	5.295	12.86	26.02
	5	0.0873	0.2472	5.262	12.80	26.62
Mean		0.0891	0.2493	5.225	13.01	26.69
Intra-assay accuracy 1 (%)		-13.5	0.0	1.5	3.4	8.0
Intra-assay accuracy 2 (%)		-10.4	-1.8	2.3	2.0	7.4
Intra-assay accuracy 3 (%)		-14.2	-5.0	3.5	0.7	6.2
Inter-assay accuracy (%)		-12.7	-2.3	2.4	2.0	7.2
mean intra-assay precision		5.6	3.8	1.4	1.0	1.7
Intra-assay precision 1 (%)		8.0	5.9	1.8	1.4	1.8
Intra-assay precision 2 (%)		2.3	3.5	1.7	1.0	1.6
Intra-assay precision 3 (%)		6.5	1.8	0.8	0.6	1.7
Inter-assay precision (%)		5.9	4.4	1.6	1.4	1.7

Nevirapine concentration (mg/L)						
		val llq nvp	qc low nvp	qc mid	qc high	>ULQ
Run	Replicate	0.2490	0.9960	4.980	12.45	25.51
1	1	0.2382	0.9841	5.114	12.83	26.19
	2	0.2812	0.9795	5.157	12.74	25.54
	3	0.2700	0.9782	4.850	12.66	25.16
	4	0.2574	0.9898	5.084	13.31	26.37
	5	0.2797	1.0070	4.839	13.22	26.00
2	1	0.2819	0.9739	5.181	12.86	25.98
	2	0.2916	0.9947	5.270	13.09	26.04
	3	0.2808	0.9749	5.280	12.78	26.11
	4	0.2751	0.9686	5.123	13.16	25.99
	5	0.2709	0.9991	5.357	13.03	26.78
3	1	0.2742	1.0164	5.329	13.13	26.09
	2	0.2531	1.0250	5.252	13.23	26.63
	3	0.2735	1.0349	5.344	12.86	25.30
	4	0.2564	1.0231	5.339	13.26	25.83
	5	0.2693	1.0505	5.265	13.14	26.40
Mean		0.2702	1.0000	5.186	13.02	26.03
Intra-assay accuracy 1 (%)		6.5	-0.8	0.6	4.0	1.3
Intra-assay accuracy 2 (%)		12.5	-1.4	5.3	4.3	2.0
Intra-assay accuracy 3 (%)		6.5	3.4	6.5	5.4	2.4
Inter-assay accuracy (%)		8.5	0.4	4.1	4.6	2.0
mean intra-assay precision		4.4	1.3	1.9	1.6	1.3
Intra-assay precision 1 (%)		6.7	1.2	3.0	2.3	1.9
Intra-assay precision 2 (%)		2.8	1.4	1.7	1.2	0.2
Intra-assay precision 3 (%)		3.7	1.3	0.8	1.2	1.7
Inter-assay precision (%)		5.1	2.5	3.2	1.6	1.7

val=validation, llq=lower limit of quantification, qc=quality control, ulq=upper limit of quantification, efv=efavirenz, nvp=nevirapine.

Table 3. Stability data of efavirenz and nevirapine.

Storage condition	Concentration (mg/L)	Recovery (%)	RSD (%)	n
Efavirenz				
plasma				
24 h at ambient temperatures	0.2551	99.5	0.3	3
	12.76	100.4	-1.3	3
7 days at 4°C	0.2551	87.1	-7.2	3
	12.76	99.8	-1.0	3
3 freeze-thaw cycles	0.2551	97.5	1.7	3
	12.76	100.0	-0.4	3
6 months at -20°C	0.2468	107.1	12.9	2
	0.9870	103.2	2.3	2
	3.948	98.8	5.3	2
	7.896	95.0	-2.2	2
final extract				
5 days at ambient temperatures	0.2551	92.1	-1.8	3
	12.76	99.9	-1.0	3
stock solutions				
24 h at ambient temperatures	996.00	103.8	3.8	3
24 months at -20°C	996.00	102.3	2.3	3
Nevirapine				
plasma				
24 h at ambient temperatures	0.9960	99.7	1.2	3
	14.45	89.4	0.1	3
7 days at 4°C	0.9960	99.9	-3.0	3
	14.45	90.8	0.4	3
3 freeze-thaw cycles	0.9960	105.8	1.3	3
	14.45	92.1	0.2	3
12 months at -20°C	0.5056	104.6	4.6	2
	1.011	106.6	6.6	2
	2.528	102.3	2.3	2
	5.056	93.3	-6.7	2
	7.584	97.2	-2.7	2
	10.11	100.9	0.9	2
	15.17	96.8	-3.2	2
final extract				
5 days at ambient temperatures	0.9960	106.6	3.6	3
	14.45	91.3	1.0	3
stock solutions				
24 h at ambient temperatures	1020.4	100.5	0.5	3
36 months at -20°C	1020.4	104.3	4.3	3

RSD=relative standard deviation, n=number of replicates.

between -1.4% and 6.5% for all other concentrations. Precisions were less than 6.7% for all tested concentrations.

Specificity and selectivity

Blank plasma from 6 different individuals showed no interfering endogenous compounds. Potentially co-administered drugs or metabolites tested had retention times that were different from the analytes or were not detected with the described analytical method.

Recovery

The total recoveries for efavirenz were 85.0%, 96.6% and 95.8% for the low, mid and high concentration ranges, respectively. The C.V. values ranged from 0.8% to 4.1%. For nevirapine the total recoveries were 86.2%, 92.2% and 93.9% for the 3 concentrations respectively. The C.V. values ranged from 0.6% to 2.7%.

Stability

Data on stability of samples and stock solutions are presented in table 3. Under all conditions tested efavirenz and nevirapine were stable. Deviations were between -7.2% and 12.9% in plasma, between -1.8% and 3.6% in the final extract and between 0.5% and 3.8% in stock solutions.

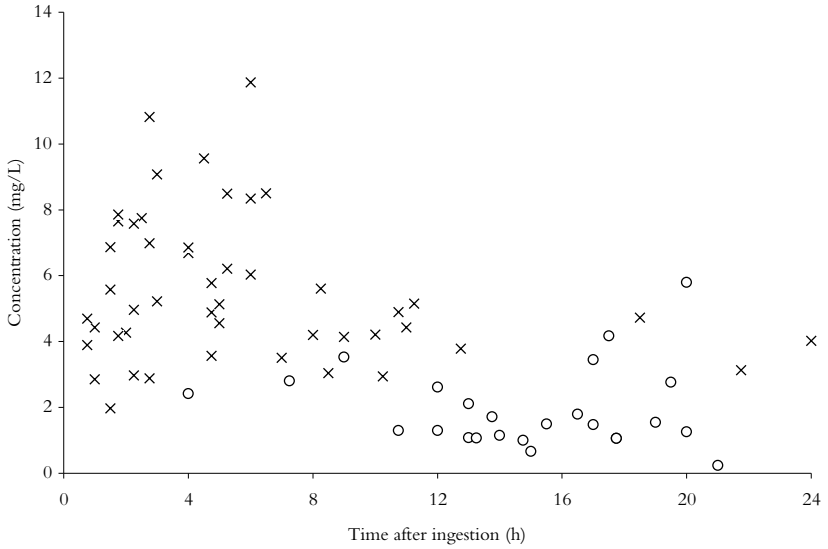
Analysis of patient data

The applicability of the assay for pharmacokinetic research in HIV-1-infected individuals was demonstrated by measuring 73 patient samples of efavirenz (600 mg once daily) or nevirapine (200 mg twice daily). In this population we found plasma concentrations between 0.24 mg/L and 5.8 mg/L for efavirenz and between 1.97 mg/L and 11.87 mg/L for nevirapine. Concentration versus time data of these samples are shown in figure 3.

DISCUSSION AND CONCLUSIONS

In conclusion, a simple and rapid assay was developed and validated, according to FDA guidelines, for the simultaneous determination of efavirenz and nevirapine in human plasma. The applicability of the assay for pharmacokinetic research in HIV-1-infected individuals is demonstrated with the analysis of plasma samples from HIV-1-infected patients. A small aliquot of plasma and simple and easily available instrumentation are used. The assay proved to be accurate and precise and is currently used for therapeutic drug monitoring and pharmacokinetic research in our institute.

Figure 3. Concentration versus time data of efavirenz and nevirapine in plasma samples from HIV-1-infected patients. Open circles represent efavirenz concentrations, crosses represent nevirapine concentrations.



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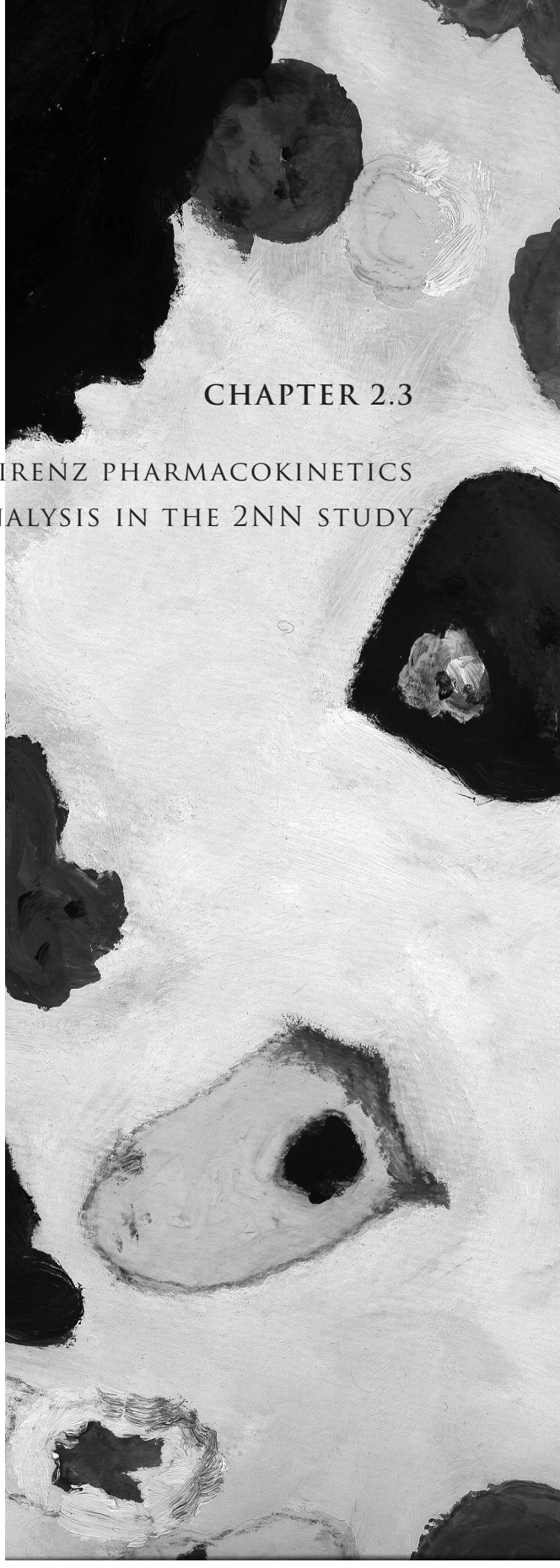

CHAPTER 2.3

NEVIRAPINE AND EFAVIRENZ PHARMACOKINETICS AND COVARIATE ANALYSIS IN THE 2NN STUDY

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ABSTRACT

Aim of this 2NN pharmacokinetic substudy was to investigate the population pharmacokinetics of nevirapine and efavirenz.

Treatment-naive HIV-1-infected patients received nevirapine (once daily or twice daily), efavirenz or the combination with lamivudine and stavudine. On day 3, weeks 1, 2, 4, 24 and 48, blood samples were collected. Using non-linear mixed effects modelling pharmacokinetics of nevirapine and efavirenz and factors involved in the interindividual variability were investigated.

Clearance of nevirapine in the induction phase (<14 days) and at steady-state (>28 days) were 2.02 L/h and 2.81 L/h, respectively. Volume of distribution and absorption rate constant were 77.0 L and 1.66 h^{-1}, respectively. Clearance of nevirapine was lower in females (13.8%) and in patients with hepatitis B (19.5%). Patients from South America and Western countries had higher clearance of nevirapine compared to Thai and South Africans.

Clearances of efavirenz in the induction phase and at steady-state were 7.95 L/h and 8.82 L/h, respectively. Volume of distribution and absorption rate constant were 418 L and 0.287 h^{-1}, respectively. Concomitant use of nevirapine increased clearance of efavirenz (43%). Patients from Thailand had a lower clearance than the rest of the population.

The population pharmacokinetics of nevirapine and efavirenz were assessed in the 2NN trial. For both drugs an induction phase was distinguished from the steady-state phase. Gender, hepatitis B, and geographical region were involved in the variability of the pharmacokinetics of nevirapine. Region and concomitantly used nevirapine were determinants of the pharmacokinetics of efavirenz.

INTRODUCTION

Nevirapine and efavirenz are non-nucleoside reverse transcriptase inhibitors and have demonstrated potency, safety and convenience in several clinical trials.[1-4] As a result, both drugs are frequently used as the basis of highly active antiretroviral therapy (HAART) for the management of both treatment-naive and treatment-experienced patients. However, there have been no large-scale, randomised clinical trials to compare nevirapine and efavirenz. Apart from two small studies [5,6] the value of combining these non-nucleoside reverse transcriptase inhibitors had also not been studied in humans. The double non-nucleoside study (2NN) is the first large-scale, international, multicentre, open-label, randomised study to compare the efficacy and safety of nevirapine, efavirenz and the combination of

these drugs, each in combination with background therapy including stavudine and lamivudine. More details of the trial have been reported separately.[7] Except from the DONUT study,[8] no pharmacokinetic studies are available about the concomitant use of efavirenz and nevirapine.

The aim of the present 2NN pharmacokinetic (2NN-PK) substudy was to investigate pharmacokinetic parameters of nevirapine and efavirenz, and their determinants in a large and diverse global population.

Both drugs are metabolised in the liver, predominantly by the cytochrome P450 (CYP) 3A4 and 2B6 isoenzymes. Since the autoinduction of CYP3A4 is completed in 14 days, however the increase in clearance caused by induction of CYP2B6 isoenzymes occurs between 14 and 28 days,[9,10] the clearance of nevirapine and efavirenz will change during this induction phase. Therefore, in addition to pharmacokinetics of nevirapine and efavirenz at steady-state, we present pharmacokinetics during the induction phase of both antiretroviral agents.

METHODS

Patients

HIV-1-infected patients were enrolled between February 2000 and June 2001 from several study sites in Europe, South Africa, Canada, United States, Argentina, Brazil, Australia and Thailand. The main eligibility criterion was HIV-1 RNA >5000 copies/mL at screening, without prior antiretroviral therapy. Full details have been reported recently by van Leth et al.[7] Upon inclusion, patients were randomly assigned to either nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, or a combination of nevirapine 400 mg plus efavirenz 800 mg once daily. Nevirapine was given as a 200 mg once daily dose for the first 2 weeks. All patients received also stavudine 40 mg twice daily (30 mg twice daily if weight was less than 60 kg) and lamivudine 150 mg twice daily. From the 1216 patients included in the 2NN study, patients with apparent poor adherence to the study treatment regimen, defined as less than 95% compliance with study drugs, were excluded for the 2NN-PK substudy. These subjects were identified based on the reported treatment interruption.

Sampling and bioanalysis

Plasma samples for evaluation of study drug concentrations were collected at day 3, weeks 1, 2, 4, 24, and 48. The time of ingestion of the last dose and the sampling time were recorded. Time after ingestion was extracted from this information.

The concentrations of nevirapine and efavirenz were quantitatively assessed by a

validated high-performance liquid chromatographic (HPLC) with a ultraviolet detection (UV) method in a Good Laboratory Practice (GLP) licensed laboratory. Briefly, sample pre-treatment consisted of protein precipitation with acetonitrile. Subsequently, nevirapine and efavirenz were separated from endogenous compounds by isocratic, reversed phase, high performance liquid chromatography. Absorbance was measured at 275 nm. The method was validated over the range of 0.25-15.0 mg/L and 0.05-15.0 mg/L for nevirapine and efavirenz, respectively. Samples with concentrations above the upper limit of quantification were reanalysed after dilution. The assay proved to be accurate and precise. The average accuracy at three different concentrations ranged from -12.7% to 8.5%. Within- and between-day precisions were less that 5.9% for all quality control samples.[11]

Covariates

To identify possible relationships between the pharmacokinetics of nevirapine and efavirenz and patient characteristics, the following covariates were collected at baseline: gender, age, weight, body mass index, geographical region, and treatment arm. Patients were considered to have a chronic hepatitis B infection when the hepatitis surface antigen (HbsAg) could be detected at baseline. When anti-hepatitis C antibodies were present at baseline, patients were considered to have a chronic hepatitis C infection. The effect of a combined use of nevirapine and efavirenz on the pharmacokinetics of both drugs was also investigated. Age, weight, and body mass index were examined as continuous variables. Gender, region, treatment arm, hepatitis B co-infection and hepatitis C co-infection were examined as dichotomous variables.

Population pharmacokinetic analysis

The non-linear mixed effect modelling software program NONMEM (level 1.1, GloboMax LLC, Hanover MD, USA) was used to perform the analyses. The first-order conditional estimate method (FOCE) with interaction between the interindividual and residual error was used throughout the study. The adequacy of the developed structural models was evaluated using both statistical and graphical methods. The minimal value of the objective function (OFV) provide by NONMEM was used for the comparisons of nested models. Discrimination between these models was based on the OFV using the likelihood ratio test.[12] A p-value of 0.05, representing a decrease in OFV of 3.84, was considered statistically significant (chi-square distribution, df=1). Standard errors for all parameters were

approximated using the COVARIANCE option of NONMEM. Individual Bayesian pharmacokinetic estimates of the pharmacokinetic parameters were obtained using the POSTHOC option.[12]

Basic pharmacokinetic models

For nevirapine, a previously developed population pharmacokinetic model that was built with data from a cohort of 173 HIV-1-infected patients was used as starting point for model development.[13] This pharmacokinetic model consisted of a single-compartment with first-order absorption and elimination. The pharmacokinetic model of efavirenz was initially built with data from 172 HIV-1-infected patients and comprised a central and a peripheral compartment with first-order elimination. The fast absorption with slow onset was best described with a chain of three transition compartments between the absorption and the central compartment.[14]

Population pharmacokinetic parameters including clearance, volume of distribution and absorption rate constant were estimated. Since clearance of nevirapine and efavirenz increased in time due to autoinduction,[10] special attention was paid to the pharmacokinetics of nevirapine and efavirenz during the first few weeks of antiretroviral therapy. The induction phase of both drugs was modelled using the same equation as used for the covariate analysis:

$$TVCL = \theta_1 \star \theta_2^{IND}$$

In which, TVCL is the typical value of clearance in the population; θ_1 is the typical value of clearance of an individual during steady-state (with IND=0); and θ_2 is the relative difference in clearance for individuals in the induction phase (with IND=1).

Interindividual variability in the pharmacokinetic parameters were estimated with an exponential error model. For instance, variability in clearance was estimated using:

$$CL/F_i = \theta_1 \star \exp(\eta_i)$$

In which, CL/F_i represents the apparent clearance of the i^{th} individual; θ_1 is the typical value of clearance; η_i is the interindividual random effect with a mean of 0 and a variance of ω^2. Residual variability was modelled with a combined additive and proportional error model.

Covariate pharmacokinetic models

To identify factors influencing the pharmacokinetics of nevirapine and efavirenz, covariates were introduced in the basic models. For instance, the influence of a

dichotomous covariate X on clearance was modelled as:

$$TVCL = \theta_1 \star \theta_2^X$$

In which, TVCL is the typical value of clearance in the population; θ_1 is the typical value of an individual with X=0; and θ_2 is the relative difference in clearance for individuals with X=1.

Efficient screenings of covariates in the population models of nevirapine and efavirenz were carried out using the Wald's approximation to the likelihood ratio test statistic in conjunction with Schwartz's Bayesian criterion.[15] This Wald Approximation Method (WAM) is constructed to compare submodels (i.e. one or more covariates removed or set to zero) to the full model (i.e. all covariate parameters in the model).

The inclusion of a covariate relationship in a pharmacokinetic model is ideally based on a combination of the scientific plausibility, the clinical importance and the statistical significance of the relationship. A covariate was considered statistically significant when the inclusion was associated with a decrease in the minimal value of the objective function associated with a p-value of <0.05 (log-likelihood ratio test). Clinical relevance was considered when the typical value of the pharmacokinetic parameter of interest changed at least 10% in the range of the covariate, as observed in the population to prevent the detection of an irrelevant, albeit significant, relationship. A covariate was retained in the model when the influence of this parameter was statistically significant and clinically relevant.

Statistical refinement

The validity of the interindividual variability model was checked by evaluating the correlations between individual random effects (η) for all of the pharmacokinetic parameters.[16] When a substantial correlation was present, covariance between these parameters was included in the model.

Model validation

The bootstrap resampling technique was applied as an internal validation for the final models.[17] Bootstrap replicates were generated by randomly sampling approximately 65% of the original data sets with replacement. The final models were fitted to the replicate data sets using the bootstrap option in the software package Wings for NONMEM (by N. Holford, version 406, May 2004, Auckland, New Zealand). Parameter estimates for each of the replicate data sets were obtained in this way.[17] The precision of the models was evaluated by visual inspection of distribution of model parameters. Furthermore, the median parameter values

and 95% prediction intervals of the bootstrap replicates were compared with the estimates of the original data sets.

Wings for NONMEM was also used to perform a randomisation test by randomly permuting a covariate in the original data set to validate the significant and relevant covariates. The purpose of the procedure is to confirm the significance of a covariate in the original data set and to permutate a covariate value randomly to each subject in order to create a new randomised data set. The model is fitted to the new permutated data set and the actual significance level can be estimated by repeating this procedure (n>1000).[18]

Since Bayesian estimates of minimum concentration (C_{min}), maximum concentration (C_{max}), and area under the concentration-time curve (AUC) will be calculated using the developed pharmacokinetic models for the investigation of relationships between pharmacokinetic parameters and viral efficacy and adverse events, the posterior predictive check technique was also applied.[19] This method was used to assess whether simulated data have the same characteristics as the original data. Index data sets were created by selecting trough concentrations from the concentration data of the original data sets. A nevirapine concentration was considered a trough concentration when the sample was taken 10 or 20 hours after ingestion of a twice daily dose or a once daily dose, respectively. Due to the long half-life of efavirenz a concentration was considered a trough concentration when the sample was taken 16 hours after ingestion. The median trough concentrations were determined. Subsequently, 1000 data sets were simulated based on the index data sets and the final pharmacokinetic parameters of the nevirapine and efavirenz models. The median in the index data set was compared with the 90% prediction intervals of the median values, calculated in all 1000 simulated data sets.[19] Also the median value and interquartile ranges of Bayesian estimated trough concentrations were calculated for all patients, and compared with the actual troughs and the results of the posterior predictive check. Bayesian estimates of the trough concentrations were made using the POSTHOC option of NONMEM, taking both the data from the individual patient and the population into account. Trough concentrations were defined as the concentrations at t=0 h, which is the time of drug intake.

RESULTS

Patients

In total, 3127 nevirapine and 1728 efavirenz plasma concentrations from 1091 naive HIV-1-infected patients were included in this 2NN-PK substudy. However, data were excluded due to unknown time of sampling, or unknown regimen.

Table 1. Analysed plasma samples of included patients.

	NVP qd	NVP bid	EFV	NVP+EFV	Total
No. of patients in study	205	373	378	135	1091
No. of patients in analysis	205	373	376	123	1077
Day 3	184	329	325	0	838
Week 1	181	348	332	0	861
Week 2	175	342	329	0	846
Week 4	167	322	1	0	490
Week 24	147	290	304	123/123	987
Week 48	141	273	279	0	693

NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, EFV=efavirenz 600 mg once daily, NVP+EFV=nevirapine 400 mg plus efavirenz 800 mg once daily.

Finally, a total of 3024 nevirapine and 1694 efavirenz plasma samples from 1077 patients were included, analysed and used to investigate the pharmacokinetics of nevirapine and efavirenz in the 2NN-PK substudy. An overview of the analysed plasma samples is shown in table 1. In figure 1 and 2 the concentration-time data of nevirapine and efavirenz are illustrated.

The study included a diverse global population, many of whom are usually underrepresented in clinical trials. A total of 17.8% of the patients were from Thailand, 35.7% from South Africa, 21.3% from South America and 25.6%

Figure 1. Concentration-time data of nevirapine.

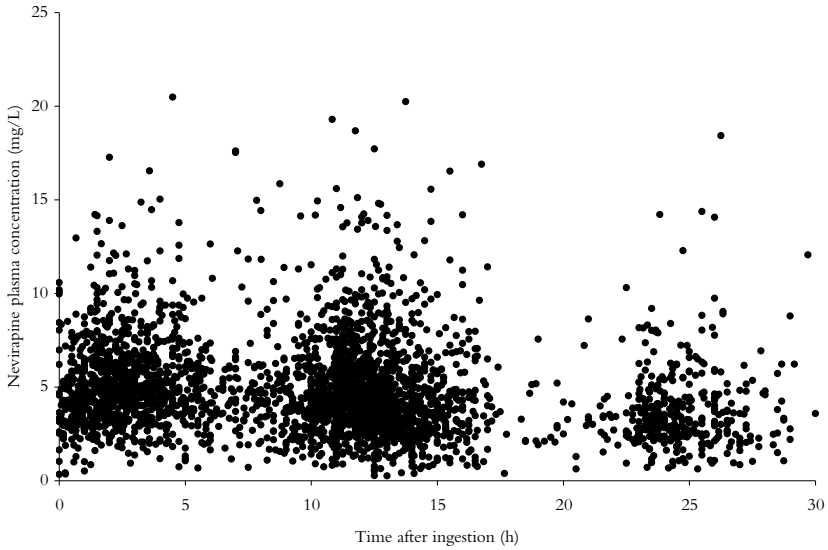
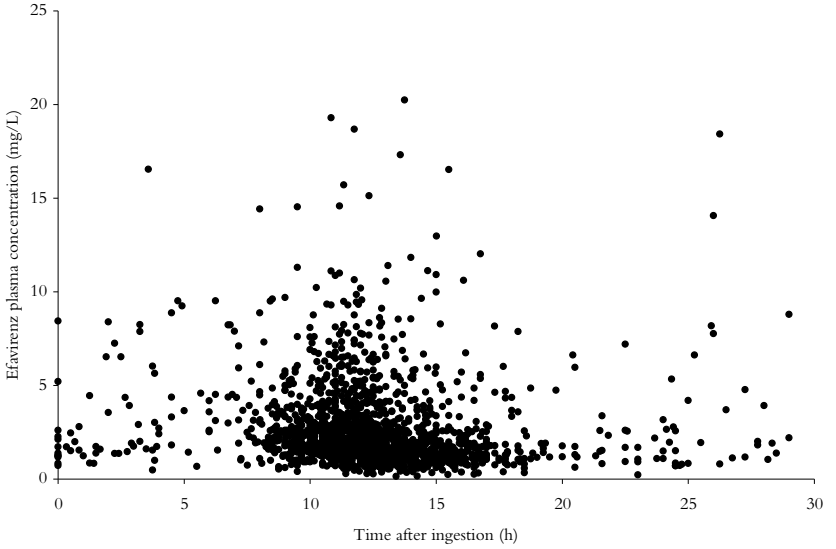


Figure 2. Concentration-time data of efavirenz.



from the Western countries (Australia, Canada, Europe and United States). Furthermore, a substantial proportion of the patients was female (36.3%). From literature it is known that co-medication can have a significant influence on the pharmacokinetics of nevirapine and efavirenz.[20] Unfortunately, no data on co-medication was available for covariate analysis. Characteristics at baseline from included patients are shown in table 2.

Table 2. Baseline characteristics of included patients.

	NVP qd	NVP bid	EFV	NVP+EFV	Total (%)
No. of patients	205	373	376	123	1077
Gender M/F	129/76	228/145	240/136	89/34	686/391 (63.7/36.3)
Age (years)★	35.0 ±8.5	36.0 ±9.2	35.9 ±8.9	35.5 ±8.8	35.7 ±8.9
Weight (kg)★	65.1 ±12.7	68.1 ±14.5	66.7 ±13.3	67.1 ±12.0	66.9 ±13.5
BMI (kg/m^2)★	19.3 ±3.3	20.2 ±3.9	19.7 ±3.5	19.7 ±3.1	19.8 ±3.6
Region					
Thailand	49	43	69	26	187 (17.4)
South Africa	66	139	134	46	385 (35.7)
South America	38	87	80	24	229 (21.3)
Western countries	52	104	93	27	276 (25.6)
HBV	15	16	14	9	54 (5.0)
HCV	21	34	32	9	96 (8.9)

★ mean value ± standard deviation. NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, EFV=efavirenz 600 mg once daily, NVP+EFV=nevirapine 400 mg plus efavirenz 800 mg once daily, M=male, F=female, BMI=body mass index, HBV=hepatitis B co-infection, HCV=hepatitis C co-infection.

Population pharmacokinetics

Nevirapine

In agreement with the earlier developed pharmacokinetic model for nevirapine [13] the pharmacokinetics of nevirapine in the 2NN study were best described with a one-compartment model with first-order absorption and elimination. Since blood samples were collected at day 3 and weeks 1, 2, 4, 24, 48 after start of the regimen, the data contained information about both the induction phase and steady-state.

In a first attempt, the possibility to estimate a separate value for clearance for each time point was investigated. It appeared that the clearance of nevirapine was not statistically different between day 3 and week 2 as it was from week 4 and onwards. Therefore, in the final model clearance was estimated for the first period (until week 2) and for the second period (week 4 onwards) as 2.02 L/h and 2.81 L/h, respectively. The introduction of an induction phase in the model significantly improved the model (ΔOFV=-399 points, $p<0.001$).

The population pharmacokinetic estimate of volume of distribution was 77.0 L. Due to the irregular absorption phase of nevirapine,[13,21] and only single

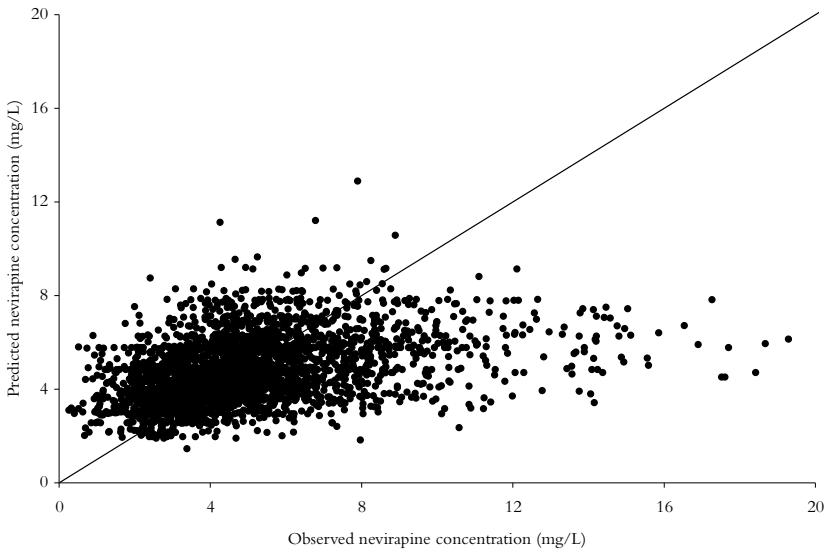
Table 3. Parameter estimates of the final pharmacokinetic model of nevirapine and the results of the randomisation test and bootstrap analyses.

NVP model				Bootstrap analyses	
	Estimate	RSE (%)	p-value#	Median	95% PI
CL/F ≤ week 2 (L/h)	2.02	4.99		2.01	1.95 – 2.08
CL/F ≥ week 4 (L/h)	2.81	2.60		2.80	2.67 – 2.96
$\theta_{\text{Female gender}}$★	0.862	2.99	<0.001	0.863	0.813 – 0.910
$\theta_{\text{Hepatitis B infection}}$★	0.805	7.11	<0.001	0.808	0.705 – 0.931
θ_{Thailand}★	1	-		1	-
$\theta_{\text{South Africa}}$★	1	-		1	-
$\theta_{\text{South America}}$★	1.11	3.29	0.001	1.11	1.04 – 1.19
$\theta_{\text{Western countries}}$★	1.28	3.84	<0.001	1.28	1.18 – 1.38
V/F (L)	77.0	2.79		77.1	73.1 – 81.3
k_a (h^{-1})§	1.66	-		1.66	-
Additive error (mg/L)	0.388	29.1		0.393	0.150 – 0.604
Proportional error (%)	27.3	3.99		27.2	0.250 – 0.292
IIV CL/F (%)	33.8	7.44		33.6	0.312 – 0.362
IIV V/F (%)	41.5	17.9		41.4	0.335 – 0.483
Correlation IIVs	0.183	35.1		0.240	0.0810 – 0.400

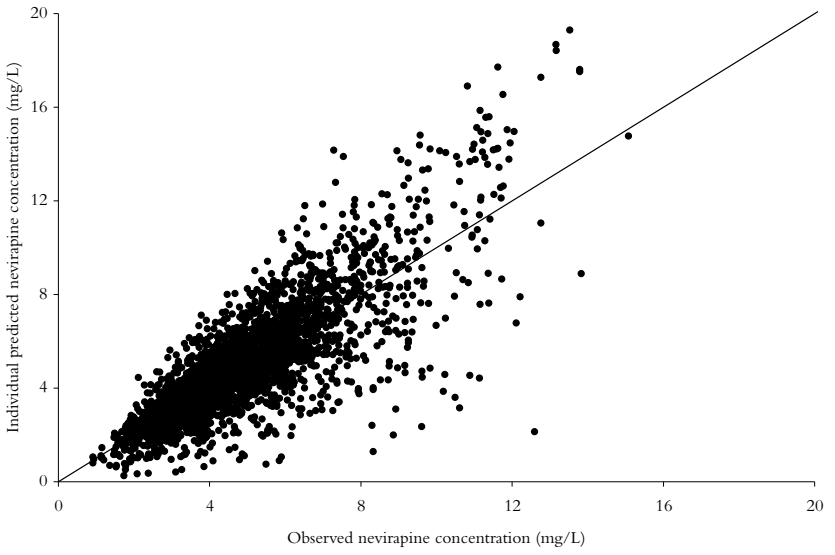
★ relative change in CL/F, § fixed from previous model, # non-parametric permutation randomisation test. NVP=nevirapine, CL/F=clearance, V/F=volume of distribution, k_a=absorption rate constant, IIV=interindividual variability, RSE=relative standard error, PI=prediction interval.

Figure 3. Model population (panel A) and individual (panel B) predicted concentrations versus observed concentrations of nevirapine using the final model.

A



B



concentration-time points instead of complete pharmacokinetic curves, it proved to be impossible to characterise the absorption phase. In previous studies it has been demonstrated that both fast and slow absorbers exist. Furthermore, both high interpatient and intrapatient variability in the absorption process was found. According to Wade et al.,[22] it is a good alternative to fix the estimate of the absorption rate constant at 1.66 h^{-1}, according to the information available from the earlier developed pharmacokinetic model of nevirapine.[13] In addition, a sensitivity analysis has been performed. With this analysis it was confirmed that 1.66 h^{-1} was a reasonable value for absorption rate constant, because fixing this parameter at other values resulted in the same outcomes. The residual error in nevirapine pharmacokinetics incorporated an additive and a proportional component of 0.388 mg/L and 27.3%, respectively.

In view of statistical refinement, a correlation between the individual random effects of clearance and volume of distribution (η_{CL} and η_{V}) of nevirapine was observed and covariance between these parameters was added to the model. The correlation coefficient was 0.240.

After the screening of covariates using the Wald Approximation Method,[15] gender (ΔOFV=-49.7, change in CL (ΔCL)=13.8%), hepatitis B co-infection (ΔOFV=-16.3, ΔCL=19.5%), and geographical region (ΔOFV=-88.9, ΔCL=11% and 28%) had a statistically significant increase in goodness-of-fit and a clinically relevant effect on clearance of nevirapine. Neither the concomitant use of efavirenz nor the dose or the frequency of administration of nevirapine showed a relation with clearance of nevirapine. The results of the final model are summarised in table 3. The model population and individual predicted concentrations versus observed concentrations of nevirapine using the final model are presented in figure 3.

Efavirenz

The pharmacokinetics of efavirenz were best described with a one-compartment model showing first-order absorption and elimination. Also, for efavirenz the induction phase was investigated in more detail and resulted in an significant improve in the model (ΔOFV=-31.1, $p<0.001$). It appeared that clearance of efavirenz was unchanged up to week 2 (7.95 L/h) and after week 24 (8.82 L/h). Volume of distribution and absorption rate constant were estimated at 418 L and 0.287 h^{-1}, respectively. The residual error in efavirenz pharmacokinetics incorporated an additive and a proportional component, 0.216 mg/L and 24.2%, respectively.

Considering statistical refinement, a correlation between individual random

effects of clearance and volume of distribution (η_{CL} and η_V) was observed in the efavirenz model. Covariance between these parameters was added to the model. Using the Wald Approximation Method, geographical region (ΔOFV=-42.5, ΔCL=53% and 76%) and concomitant use of nevirapine (ΔOFV=-25.6, ΔCL=43%) seemed to have a significant and relevant relation with clearance of efavirenz. The results of the final efavirenz model are summarised in table 4. The model population and individual predicted concentrations versus observed concentrations of efavirenz using the final model are presented in figure 4.

Table 4. Parameter estimates of the final pharmacokinetic model of efavirenz and the results of the randomisation test and bootstrap analyses.

EFV model				Bootstrap analyses	
	Estimate	RSE (%)	p-value#	Median	95% PI
CL/F ≤ week 2 (L/h)	7.95	19.2		7.94	7.02 – 8.79
CL/F ≥ week 4 (L/h)	8.82	6.98		8.79	7.55 – 10.0
$\theta_{\text{Concomitant use of NVP}}$★	1.43	7.69	<0.001	1.42	1.20 – 1.65
θ_{Thailand}★	1	-		1	-
$\theta_{\text{South Africa}}$★	1.53	8.10	<0.001	1.54	1.30 – 1.83
$\theta_{\text{South America}}$★	1.53	8.10	<0.001	1.54	1.30 – 1.83
$\theta_{\text{Western countries}}$★	1.76	8.98	0.037	1.78	1.48 – 2.12
V/F (L)	418	7.58		399	319 – 477
k_a (h^{-1})	0.287	29.3		0.277	0.127 – 0.584
Additive error (mg/L)	0.216	21.6		0.215	0.120 – 0.368
Proportional error (%)	24.2	6.16		24.0	19.2 – 27.1
IIV CL/F (%)	60.2	9.31		59.9	54.0 – 65.3
IIV V/F (%)	54.1	28.5		50.0	34.1 – 69.0
Correlation IIVs	-0.122	89.9		-0.168	-0.456 – 0.0670

★ relative change in CL/F, # non-parametric permutation randomisation test.
EFV=efavirenz, NVP=nevirapine, CL/F=clearance, V/F=volume of distribution, k_a=absorption rate constant, IIV=interindividual variability, RSE=relative standard error, PI=prediction interval.

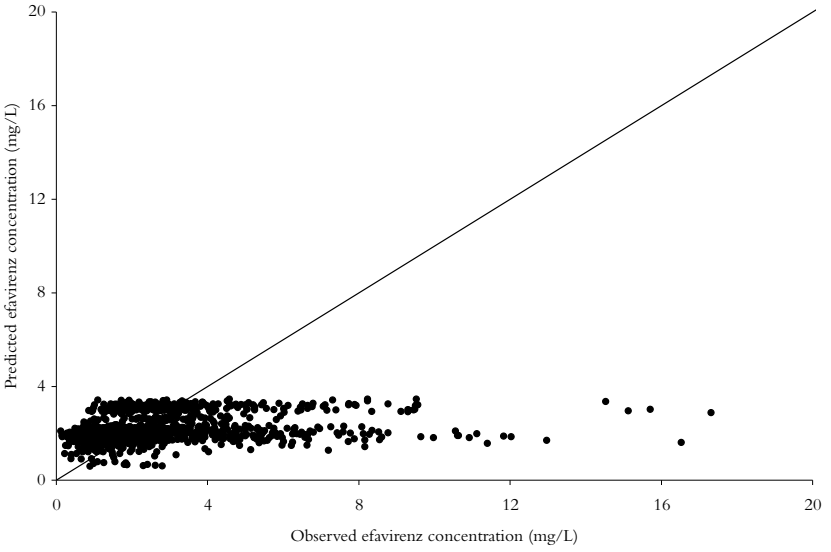
Model validation

From the original data sets 1000 replicate bootstrap data sets were generated and used for the evaluation of the precision of the parameter estimates. In addition to the parameter estimates of the final models, tables 3 and 4 list the results of the bootstrap procedures, presented as medians and 95% prediction intervals. Median values of the bootstrap procedures were very similar to the parameter estimates of the original data sets.

Furthermore, 1000 replicate randomisation data sets from the original data sets

Figure 4. Model population (panel A) and individual (panel B) predicted concentrations versus observed concentrations of efavirenz using the final model.

A



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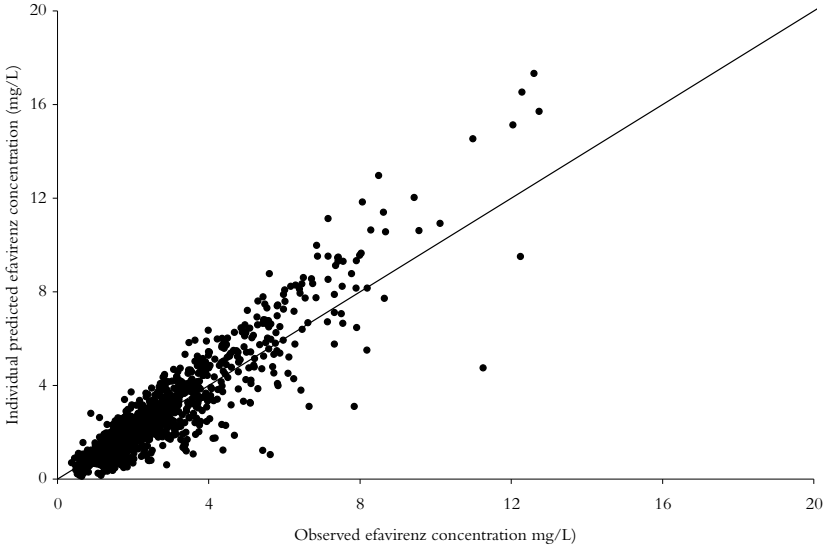

were generated for each covariate of interest to evaluate the level of significance. In tables 3 and 4, the p-values of the randomisation tests are also shown. As can be seen, all included covariates were statistically significant.

The median values and interquartile ranges of observed trough concentrations of nevirapine and efavirenz as well as the results of the posterior predictive check are shown in table 5. The results of the posterior predictive check showed that the observed median of the trough concentration was well included in the 90% prediction interval from the simulated data sets. The results of the estimated trough concentration from all patients, also listed in table 5, showed also similar ranges, indicating that both models adequately simulate data having the same characteristics as the original data.

Table 5. Median values and interquartile ranges of observed and Bayesian estimated C_{min} of nevirapine and efavirenz and the results of the posterior predictive check of the C_{min} at steady-state.

	n	Observed C_{min} (IQR) (mg/L)	PPC C_{min} (90% PI) (mg/L)	Bayesian estimated C_{min} (IQR)★ (mg/L)
NVP qd	75	3.87 (2.77 – 5.70)	3.66 (2.63 – 4.73)	3.73 (2.71 – 5.51)
NVP bid	424	4.71 (3.60 – 6.39)	4.85 (4.32 – 5.41)	4.68 (3.88 – 6.23)
NVP★★	20	3.95 (2.49 – 7.87)	3.61 (2.26 – 5.33)	3.53 (2.46 – 5.95)
EFV	48	1.44 (1.02 – 2.02)	1.50 (1.07 – 2.06)	1.37 (1.10 – 2.26)
EFV★★	12	0.975 (0.803 – 1.57)	1.29 (0.686 – 2.13)	0.948 (0.838 – 1.49)

★ of all patients. NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, EFV=efavirenz 600 mg once daily, ★★=nevirapine 400 mg plus efavirenz 800 mg once daily, C_{min}=minimum concentration, IQR=interquartile range, PPC=posterior predictive check, PI=prediction interval.

DISCUSSION

The goal of the study was to investigate the population pharmacokinetic parameters of nevirapine and efavirenz and factors involved in a large and diverse global population.

The structural model of nevirapine adequately described the data in correspondence with the results of Zhou et al.[21] and De Maat et al.,[13] from which the latter was used as starting point for model development. Furthermore, the estimates of the pharmacokinetic parameters were comparable to the results of these studies.

The structural model of efavirenz, which consisted of one-compartment with first-order absorption, was similar to the model presented by Pfister el al.,[23] however differed from the model that was used as basic consideration. The data set contained to few concentration-time points between 0 and 5 hours to model

the fast absorption phase with slow onset as we described earlier in an intensively sampled population.[14] The statistical and graphical methods did not support the implementation of a peripheral compartment and a one-compartment model appeared to describe the data adequately. Estimated pharmacokinetic parameters were in correspondence with results from earlier published studies.[23,24]

Because both data sets contained concentration-time data from patients before reaching steady-state, an induction phase was introduced in the pharmacokinetic models. Autoinduction is a known and important phenomenon for both nevirapine and efavirenz. Therefore, it was regarded as crucial to properly characterise this process for both drugs. Since sampling was only performed at some discrete and previously defined time points, extensive data on the time course of autoinduction was not available. In a first attempt, it was investigated whether it was possible to estimate separate values for clearance at each time point. Finally, it appeared that the clearance of nevirapine was similar between day 3 and week 2 (2.02 L/h) and from week 4 onwards (2.81 L/h). This increase in clearance of approximately 40% means that when a dose adjustment to 200 mg once daily during the first two weeks would not be applied, plasma levels of nevirapine with substantial more risk for adverse events would be achieved. From our data it can be observed that no induction was noticeable between day 3 and week 2 and between week 4 and week 48 and that the induction of the enzyme system metabolising nevirapine reached completeness between week 2 and week 4. Whether the first phase of induction has taken place before day 3 is a lingering question. From previous literature it is known that nevirapine is primarily metabolised by the CYP3A4 and CYP2B6 isoenzymes.[25] It is known that a small but significant part of the induction phase of CYP3A4 occurs during the first fourteen days and that between day 14 and 28 the clearance of nevirapine increases with a substantial increase in the formation of the CYP2B6 metabolite, 3-hydroxynevirapine.[9] Our results are remarkable consistent with these published results.

Between day 3 and week 2 the clearance of efavirenz was 7.95 L/h, and 8.82 L/h afterwards. The difference in clearance of efavirenz is only 10% and, as contrasted with nevirapine, dose adjustment is unnecessary. As found for nevirapine, there was no change in the induction of efavirenz between day 3 and week 2, and thereafter the completion of the autoinduction of the CYP isoenzymes was observed.

Extensive data are available on the pharmacokinetics of both nevirapine and efavirenz.[13,14,21,23,24] These studies, however, have been executed with a limited number of mainly male Caucasian individuals. In a small cohort with little variability in patient characteristics, significant and relevant covariates will

scarcely be found. Key features of the 2NN-PK substudy, were its size and its wide variety of patients. The 2NN-PK substudy included a large diverse global population, with a substantial portion of female patients (36.3%) and only 25.6% patients from Western countries (Australia, Canada, Europe and United States). The ethnicity of included patients was not documented, since no permission was granted for this by the ethical review committee in several countries, and only the country of residence was recorded. Unfortunately, no data on co-medication was available, which might explain a part of the residual error in both pharmacokinetic models.

Clearance of nevirapine was related to gender, geographical region and hepatitis B co-infection. The pharmacokinetics of efavirenz were influenced by geographical region, and concomitant use of nevirapine. Hepatitis B co-infection led to a decrease in clearance of nevirapine of 19.5%. More than 80% of the patients with a hepatitis B co-infection had elevated liver enzymes as well, indicating a hepatic dysfunction, which might result in a smaller capacity of the liver to metabolise nevirapine to its inactive metabolites. Female patients showed a 13.8% lower clearance of nevirapine than did men. Since nor body weight nor BMI had an influence on the clearance of nevirapine, which is in contrast with the study of De Maat et al.,[13] the effect of gender can not solely be explained by body size. Compared to men, women may have a smaller capacity of the liver, resulting in a lower clearance.[26] Gender and hepatitis B co-infection also influenced the clearance of efavirenz, however only significant and relevant in the univariate analysis.

Patients from Western countries and South America showed a 28% and 11% increase in clearance of nevirapine, respectively, with regard to patients from Thailand and South Africa. With efavirenz, the clearance in patients from Thailand was higher than in patients from South Africa and South America (53%) and Western countries (76%). Differences in pharmacokinetics due to ethnicity have been reported for several drugs that are metabolised by CYP3A [27-30] and CYP2B6.[29] The presence of the variant CYP3A4*1B allele and the CYP2B6*9 genotype, of which the frequency appears to differ between races, has been suggested to be associated with altered activity of CYP3A4 and CYP2B6, respectively.[29-31] Besides genetic factors, environmental factors (including shared cultural and dietary habits) could be responsible for differences in pharmacokinetics.

The metabolism of nevirapine and efavirenz is an auto inducible enzymatic process. Since both drugs also induce and inhibit several CYP enzymes, it is hard to predict what will be the absolute effect on the metabolism of each drug. However, this

study shows that the concomitant use of nevirapine plus efavirenz affects only the clearance of efavirenz, most likely due to induction of CYP enzyme activity. Because of increased clearance of efavirenz, which was already observed in the DONUT study,[8] the dose of efavirenz was increased to 800 mg in the treatment arm in which nevirapine and efavirenz were combined.

In conclusion, pharmacokinetic models of nevirapine and efavirenz during both induction phase and steady-state were developed. Patient characteristics gender, hepatitis B co-infection, and geographical region were involved in the interindividual variability of nevirapine. The concomitant use of nevirapine and geographical region influenced the pharmacokinetics of efavirenz. These pharmacokinetic models will greatly aid to further investigate relationships between pharmacokinetic parameters, viral efficacy, and adverse events.

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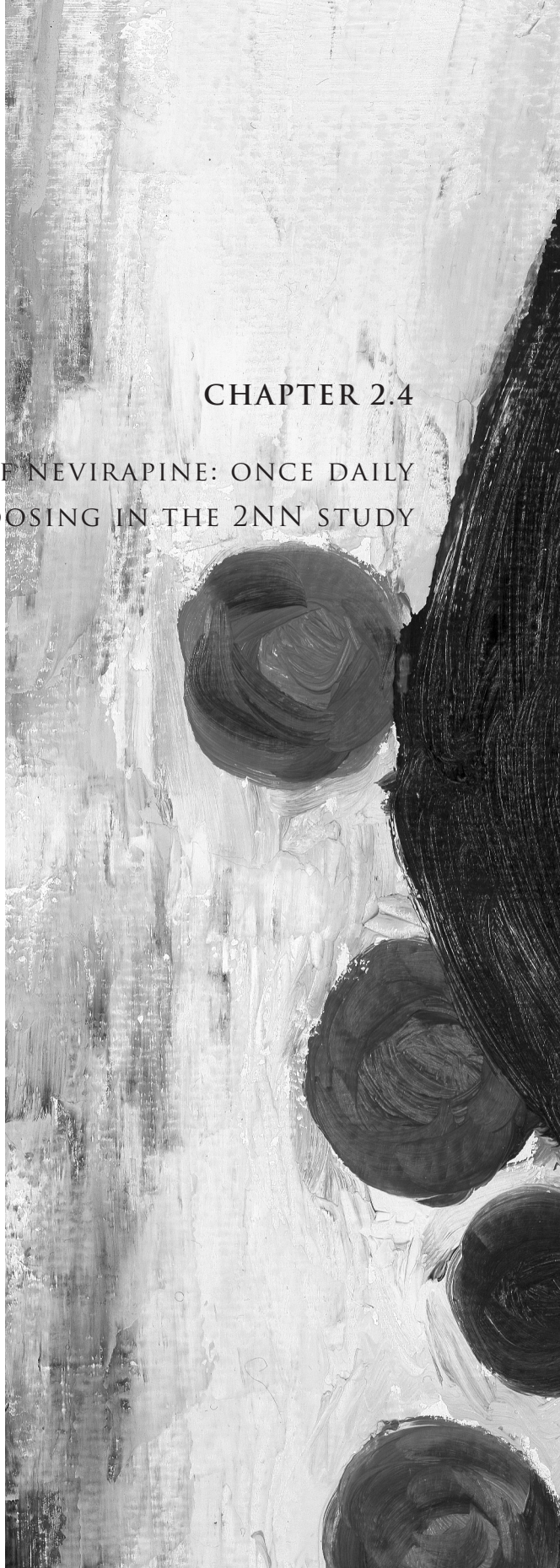

CHAPTER 2.4

PHARMACOKINETICS OF NEVIRAPINE: ONCE DAILY VERSUS TWICE DAILY DOSING IN THE 2NN STUDY

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ABSTRACT

As part of the large international, randomised 2NN trial the pharmacokinetics of nevirapine in a once daily 400 mg and twice daily 200 mg dosing regimen were investigated.

Following a two-week nevirapine 200 mg once daily lead-in regimen, treatment-naive HIV-1-infected patients were randomised to receive nevirapine 400 mg once daily or 200 mg twice daily, in combination with lamivudine and stavudine. Blood samples were collected during the induction phase (day 3, weeks 1 and 2) and during steady-state (weeks 4, 24 and 48). Concentrations of nevirapine were quantitatively assessed by a validated high-performance liquid chromatography assay. Non-linear mixed effects modelling (NONMEM) investigated whether clearance or volume of distribution differed between once *versus* twice daily dosing. For each patient Bayesian estimates of the area under the plasma concentration-time curve over 24 hours (AUC_{24h}), minimum and maximum plasma concentrations (C_{min} and C_{max}) and full pharmacokinetic profiles of nevirapine were generated using the population pharmacokinetic parameters from the developed model. These pharmacokinetic parameters were compared for patients in the once daily (n=205) and twice daily (n=373) treatment arms.

In total, from 578 patients, 2899 nevirapine plasma concentrations were available. Dosage and dosing frequency did not influence clearance or volume of distribution of nevirapine, indicating linear pharmacokinetic behaviour of nevirapine whether given as a single daily dose or as divided doses over 24 hours. At steady-state, the C_{min} was lower and the C_{max} was higher in the once daily arm compared to the twice daily arm. However, compared to total variability in plasma nevirapine levels for the two treatments, these differences were minor. Total exposure, measured as AUC_{24h} was comparable for both regimens.

These data show that the daily exposure to nevirapine, as measured by the AUC_{24h} was similar for the 400 mg once daily and the 200 mg twice daily dosing regimen. The C_{min} and C_{max} of nevirapine are lower and higher, respectively, for the once daily regimen as compared to the twice daily regimen. Whether this may lead to a higher risk for toxicity or virological failure needs further assessment.

INTRODUCTION

Nevirapine is a human immunodeficiency virus type 1 (HIV-1) specific non-nucleoside reverse transcriptase inhibitor (NNRTI) which binds directly to the viral reverse transcriptase of HIV-1 to block polymerase activity by causing disruption

of the enzyme's catalytic site.[1] Combination antiretroviral therapy with nevirapine in a twice daily dosing regimen has been proven safe and effective in HIV-1-infected individuals.[1-5] As a result, nevirapine is frequently used as part of highly active antiretroviral therapy (HAART) for the management of both treatment-naive and treatment-experienced patients. The substantial benefits conferred by HAART, however, require strict patient adherence to the prescribed medication since poor compliance will lead to virologic treatment failure.[6] Compliance might be increased with a more conveniently dosed regimen. An advance in simplifying antiretroviral therapy is the use of once daily dosed regimens. The long plasma half-life of nevirapine of approximately 25-30 h after multiple dosing,[1] may justify once daily dosing. The 2NN study was the first large international randomised trial to compare the efficacy and safety of nevirapine and/or efavirenz, together with stavudine and lamivudine. The study incorporated a treatment group where nevirapine was dosed once daily and a treatment group where nevirapine was dosed twice daily. This design offered the unique opportunity to investigate, in a randomised fashion, in a large population and over a long period the exposure to nevirapine in a once daily and twice daily dosed regimen. The outcome of this pharmacokinetic analysis may provide pivotal information about the therapeutic window of nevirapine, and can be instrumental in the discussion about once daily *versus* twice daily nevirapine dosing.

METHODS

Patients

HIV-1-infected patients were enrolled between February 2000 and June 2001 from 65 study sites in Europe, South Africa, Canada, United States, Argentina, Brazil, Australia and Thailand. The main eligibility criterion was HIV-1 RNA >5000 copies/mL at screening, without prior antiretroviral therapy. Full details have been reported recently by van Leth et al.[7] Upon inclusion, patients were randomly assigned to four study arms: nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, and nevirapine 400 mg plus efavirenz 800 mg once daily. For this analysis only patients included in the nevirapine 400 mg once daily and nevirapine 200 mg twice daily arm were considered. Nevirapine was given as a 200 mg once daily dose for the first 2 weeks in both treatment arms. All patients received also stavudine 40 mg twice daily (30 mg twice daily if weight was less than 60 kg) and lamivudine 150 mg twice daily. Patients with apparent poor adherence to the study treatment regimen, defined as less than 95% compliance with study drugs, were excluded from this substudy.

These subjects were identified based records of drug interruptions and treatment interruption.[7]
Among others, the following patient characteristics were collected at baseline: gender, age, weight, body mass index, geographical region, CD4 cell count and viral load. Patients were considered to have a chronic hepatitis B infection when the hepatitis surface antigen (HbsAg) could be detected at baseline. When anti-hepatitis C antibodies were present at baseline, patients were considered to have a chronic hepatitis C infection.

Sampling and bioanalysis
Plasma samples for evaluation of nevirapine plasma concentrations were collected at day 3, weeks 1, 2, 4, 24, and 48. When blood samples were drawn, the time of ingestion of the last dose and the sampling time were recorded. Time after ingestion was extracted from this information.
The concentration of nevirapine was quantitatively assessed by a validated high-performance liquid chromatographic with ultraviolet detection method for the simultaneous determination of nevirapine and efavirenz, in a Good Laboratory Practice (GLP) licensed laboratory. Briefly, sample pre-treatment consisted of protein precipitation with acetonitrile. Subsequently, nevirapine was separated from endogenous compounds by isocratic, reversed phase, high performance liquid chromatography. Absorbance was measured at 275 nm. The method was validated over the range of 0.25-15.0 mg/L. Samples with concentrations above the upper limit of quantification were reanalysed after dilution. The assay proved to be accurate and precise. The average accuracy at three different concentrations ranged from -12.7% to 8.5%. Within- and between-day precisions were less that 5.9% for all quality control samples.[8]

Pharmacokinetic analysis
The pharmacokinetic model for nevirapine, which has been previously developed in the scope of the 2NN pharmacokinetic (2NN-PK) substudy [9] with the non-linear mixed effect modelling (NONMEM) software program (level 1.1, GloboMax LLC, Hanover MD, USA) was used to perform the pharmacokinetic analysis. The model consisted of a single compartment with first-order absorption and elimination. Clearance of nevirapine in the induction phase (through the visit at day 14 days) and at steady-state (from the visit at 28 days or later) were 2.02 L/h and 2.81 L/h, respectively. Volume of distribution and absorption rate constant were 77.0 L and 1.66 h^{-1}, respectively. Clearance was lower in females

(13.8%) and in patients with hepatitis B co-infection (19.5%). Patients from South America and Western countries had higher clearance (11% and 28%, respectively) compared to Thai and South Africans.

To investigate the influences of the dosage and dosing frequency (once daily *versus* twice daily), the treatment arm was tested as covariate on the pharmacokinetic parameters. Both statistical and graphical methods were used for the comparisons of nested models. Discrimination between these models was based on the objective function (OFV) using the likelihood ratio test.[10] A p-value of 0.01, representing a decrease in OFV of 6.6, was considered statistically significant (chi-square distribution, df=1). Clinical relevance was considered when the typical value of the pharmacokinetic parameter of interest changed at least 10% between the treatment arms to prevent the detection of an irrelevant, albeit statistically significant, relationship.

Random plasma concentration-time points of nevirapine and the population pharmacokinetic model were used to obtain individual Bayesian estimates of minimum concentrations (C_{min}), maximum concentrations (C_{max}, estimated as the concentration 2 h after intake), area under the plasma concentration-time curve over 24 hours (AUC_{24h}) and full pharmacokinetic profiles in the induction phase (until week 4) and at steady-state (from week 4 and onwards) using the POSTHOC option in NONMEM.[10] These pharmacokinetic parameters were used to investigate the differences in pharmacokinetics of once daily and twice daily dosed nevirapine.

RESULTS

Patients

In total 1216 patients were included in the 2NN study. In the once daily arm and in the twice daily arm 220 and 387 patients were randomised, respectively. There were 208 and 378 patients, respectively, who started treatment as allocated; the remainder of the patients were not evaluated due to never starting treatment, patient's request and lost to follow-up. Furthermore, unknown time of sampling, or unknown dosage resulted in exclusion of 3 and 5 patients from the once daily and the twice daily treatment arms, respectively. In total 2899 nevirapine plasma concentrations were analysed. In table 1 an overview of the baseline characteristic of included patients is shown.

Table 1. Baseline characteristics of included patients.

	NVP qd	NVP bid	Total
No. of patients in study	205	373	578
Gender			
Male	129 (62.9%)	228 (61.1%)	357 (61.8%)
Female	76 (37.1%)	145 (38.9%)	221 (38.2%)
Age (years)★	35.0 ±8.5	36.0 ±9.2	35.7 ±8.9
Weight (kg)★	65.1 ±12.7	68.1 ±14.5	66.9 ±13.5
BMI (kg/m^2)★	19.3 ±3.3	20.2 ±3.9	19.8 ±3.6
Region			
Thailand	49 (23.9%)	43 (11.5%)	92 (15.9%)
South Africa	66 (32.1%)	139 (37.3%)	205 (35.5%)
South America	38 (18.5%)	87 (23.3%)	125 (21.6%)
Western countries	52 (23.4%)	104 (27.9%)	156 (27.0%)
HBV	15 (7.3%)	16 (4.3%)	31 (5.4%)
HCV	21 (10.2%)	34 (9.1%)	55 (9.5%)
CD4 cell count ($10^6/L$)★	235 ±184	209 ±181	218 ±182
HIV-1 RNA (log_{10} copies/mL)★	4.82 ±0.70	4.83 ±0.75	4.83 ±0.73

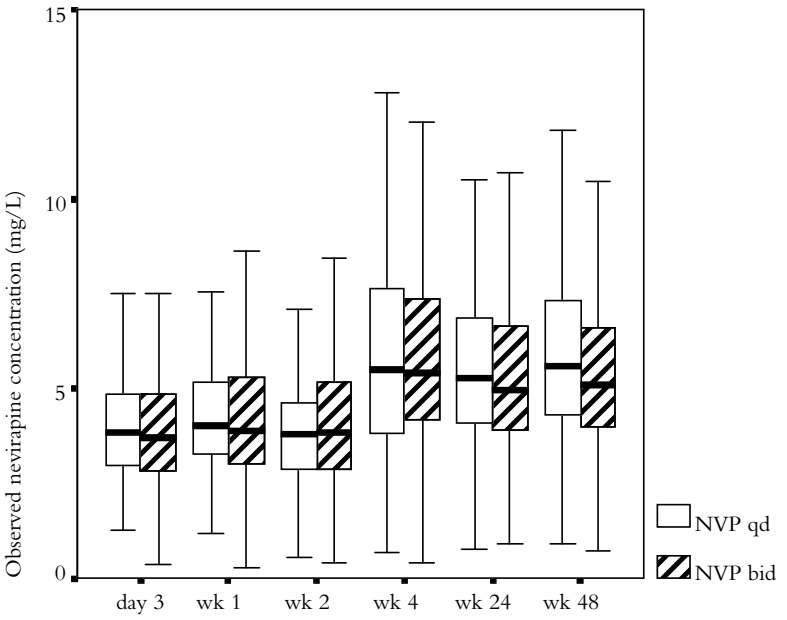
★: mean value ± standard deviation. NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, BMI=body mass index, HBV=hepatitis B co-infection, HCV=hepatitis C co-infection.

Pharmacokinetic analysis

In figure 1 boxplots are shown to compare observed nevirapine concentrations between study arms for each time point. As can be seen, during both the induction phase (until week 4) and at steady-state (from week 4 and onwards), the range of the concentrations was very similar for the once daily and twice daily treatment arms. The comparability within the first 2 weeks demonstrated that the treatment groups were pharmacokinetically matched. Except from the difference in concentrations between the induction phase (< day 14 visit) and at steady-state (> day 28 visit), no time effect on the plasma concentrations within each period was observed. The plasma levels were higher during steady-state compared to the induction phase, but the variability in each of the two periods was comparable between once daily and twice daily dosed nevirapine. During the induction phase (once daily 200 mg nevirapine in both arms) the lowest and highest observed plasma concentrations were 0.26 mg/L and 14.16 mg/L. At steady-state, all concentrations were between 0.68 mg/L and 16.90 mg/L for the once daily 400 mg nevirapine arm, and between 0.41 mg/L and 20.49 mg/L for the twice daily 200 mg nevirapine arm, respectively.

The influence of dosage/dosing frequency on the pharmacokinetic parameters

Figure 1. Boxplots of observed nevirapine concentrations during day 3, week 1, 2, 4, 24 and 48.



was investigated with the earlier developed pharmacokinetic model.[9] It appeared that the dosing scheme had no statistically significant nor a clinically relevant effect on the clearance (ΔOFV=-4.3, p=0.04, change in CL=2%) nor on volume of distribution (ΔOFV=0, change in V=0%). This indicates that the pharmacokinetics of nevirapine were linear whether given as a single daily dose or as divided doses over 24 hours at steady-state.

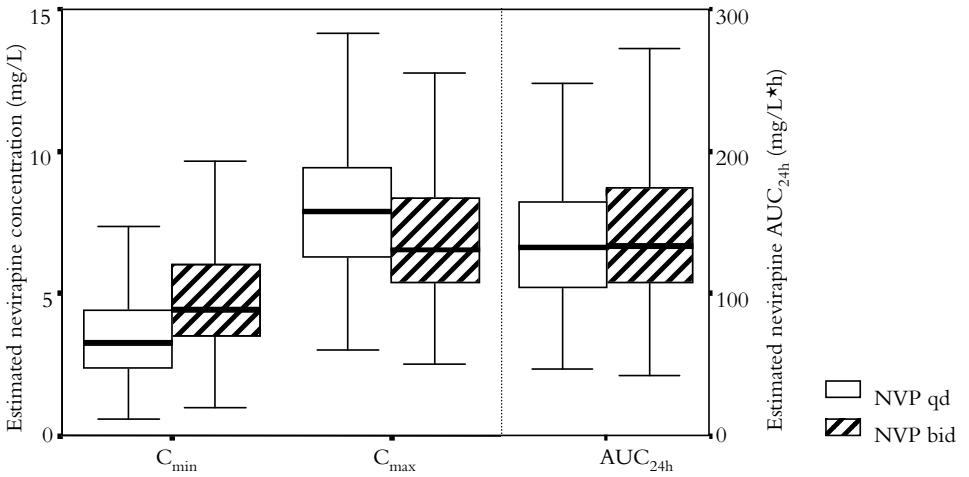
Using this pharmacokinetic model, the pharmacokinetic parameters C_{min}, C_{max} and AUC_{24h} and full pharmacokinetic profiles of nevirapine were determined with NONMEM. In table 2 these parameters during the induction phase and at steady-state are listed. As expected for randomly assigned treatment groups utilising the same nevirapine dose regimen during the first two weeks of treatment, no differences in pharmacokinetic parameters were detected between both treatment arms during the induction period. On the other hand, at steady-state, C_{min} was lower and C_{max} was higher in the nevirapine once daily arm compared to the twice daily arm, while the total exposures, expressed as AUC_{24h}, were comparable in both arms (figure 2). In figure 3, simulated full pharmacokinetic profiles of both nevirapine treatment arms are presented. Both figure 2 and 3 indicate that

Table 2. Median values and interquartile ranges (IQR) of Bayesian estimated pharmacokinetic parameters of nevirapine during the induction phase and at steady-state.

	Induction phase*				Steady state			
	NVP qd		NVP bid		NVP qd		NVP bid	
	median	IQR	median	IQR	median	IQR	median	IQR
C_{min} (mg/L)	2.92	2.26 – 3-72	2.68	2.11 – 3.63	3.26	2.38 – 4.40	4.44	3.50 – 6.02
C_{max} (mg/L)	5.28	4.40 – 6.15	5.03	4.19 – 6.04	7.88	6.28 – 9.45	6.55	5.41 – 8.36
AUC_{24h} (mg/L*h)	101	82.6 – 123	92.5	75.1 – 120	133	104 – 164	133	108 – 174

*: all patients in both arms received nevirapine 200 mg once daily during the induction phase. NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, C_{min}=minimum concentration, C_{max}=maximum concentration, AUC_{24h}=area under the concentration-time curve over 24 hours.

Figure 2. Boxplots of estimated nevirapine exposure at steady-state, expressed as minimum concentration (C_{min}), maximum concentration (C_{max}) and area under the concentration-time curve (AUC_{24h}).



compared to the variability in plasma concentrations, the effect of once daily or twice daily dosing on the pharmacokinetics is minor.

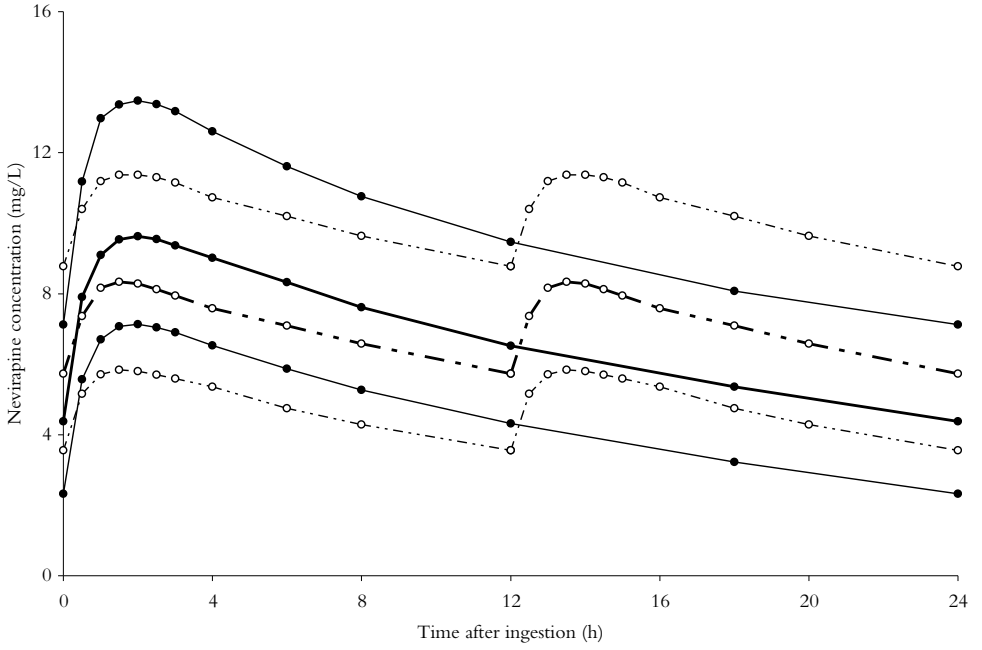
DISCUSSION

The importance of patient adherence to the prescribed antiretroviral regimen is underscored by a study reporting association between adherence and virologic response to therapy.[6] Pill burden and dosing frequency are important factors that determine adherence.[11] However, several other factors, such as food-restrictions and the patient's belief in the effectiveness of the medication are also involved.[12]

Investigations of adherence do not unequivocally indicate that once daily dosing is superior to twice daily, however, reduced daily dosing may add to the overall convenience of HAART and may improve adherence.[13] To maintain a safe and effective plasma concentration, the pharmacokinetic profile of a drug needs to be considered. Therefore, the differences in pharmacokinetics of nevirapine between once daily and twice daily dosed regimens were investigated in a large population over a long period. The design of the large international, randomised 2NN study allows us to address the question of whether nevirapine 400 mg once a day is similar to the approved dose of 200 mg twice daily.[7] Findings from the current analysis will also pertain to the potential for clinical efficacy and safety of using 400 mg once daily, compared to 200 mg twice daily. These questions are addressed in other substudies of 2NN.[14,15]

This substudy demonstrated that the estimated pharmacokinetic parameters of exposure, clearance and volume of distribution, and the empirically-determined measurement of daily exposure to nevirapine, AUC_{24h}, were not different between the 400 mg once daily and 200 mg twice daily dosing regimen. However, the

Figure 3. Simulated median, 25th and 75th percentiles concentration-time data of nevirapine at steady-state. Solid dots represent nevirapine 400 mg once daily and open circles represent nevirapine 200 mg twice daily.



C_{min} and C_{max} were significantly lower and higher, respectively, for the once daily regimen as compared to the twice daily regimen. These differences were expected, since no differences between clearance and volume of distribution were demonstrated and the same total dose was administered.

Although for efficacy several target plasma concentrations of nevirapine have been identified,[16–18] the clinical relevance of a lower C_{min} in the once daily nevirapine treatment arm is unclear. A study exploring the association of exposure to nevirapine with virological response in antiretroviral-naive HIV-1-infected patients yielded a target C_{min} of 3.4 mg/L.[16] De Vries-Sluijs et al.[17] have demonstrated that nevirapine drug concentrations <3.0 mg/L were associated with virological failure. Bonora et al.[18] have shown that patients with C_{min} of nevirapine above 5.5 mg/L showed a significant probability of achieving more rapidly viral suppression. These different associations should be interpreted with caution since these analyses had different definitions of virologic failure or success. Besides, the substudy of 2NN investigating the relation between C_{min} and virologic failure did not identify a concentration-response relation for nevirapine.[15] With respect to adherence, the included patient populations may have differed considerably between the studies from De Vries-Sluijs et al., Veldkamp et al. and the present 2NN study. All patients included in the present analyses had an adequate adherence to their allocated treatment, while De Vries-Sluijs et al.[17] included patients with plasma concentrations below the lower limit of quantitation (<0.052 mg/L). In the study of Veldkamp et al.[16] a lower median plasma concentration has been reported, compared to the median of samples from the twice daily arm in the present 2NN study (3.79 mg/L versus 5.11 mg/L).

In the 2NN study, all patients reached plasma concentrations far above the concentration required to inhibit 50% viral replication *in vitro* (the IC_{50} for nevirapine is 10.6 μg/L[19,20]) and after 48 weeks of treatment, the once daily nevirapine arm did not show more virological failure compared to the twice daily nevirapine arm.[7] This suggests that, despite differences in C_{min} of nevirapine, once daily may be as effective as twice daily dosing.

Although some analyses have suggested that transaminase elevations and rash are related to plasma concentrations,[21,22] the question of the relationship of toxicity and nevirapine plasma concentrations remains unanswered.[23,24] From the substudy of 2NN investigating the relationship between adverse events and plasma concentrations it appeared that pharmacokinetic parameters of nevirapine did not have a relationship to adverse events in the 2NN trial. Also no clear cut-off value above which patients are at increased risk for adverse events during nevirapine

containing antiretroviral therapy has been identified.[14] Until more data are available, dose adjustments in case of high plasma concentrations should be guided by the clinical condition of the patient.[25] Therefore, the clinical relevance of a higher C_{max} might be unclear. However, patients assigned nevirapine once daily had a higher frequency of hepatic laboratory abnormalities than those assigned twice daily administration,[7] although a relation between pharmacokinetics and adverse events could not be demonstrated in a previous analysis within the 2NN study.[14]

To consider a simpler dosing regimen a risk-benefit assessment might be needed. A once daily regimen might facilitate better adherence, however, higher C_{max} might result in increased toxicity, decreasing the utility of once daily dosing. Further, perhaps one of the greatest concerns with a once daily regimen is the possibility of subtherapeutic drug levels if doses are missed or delayed, which may be of less importance for the twice daily scheme.

This substudy of the large randomised 2NN trial demonstrated that total drug exposure, expressed as AUC_{24h}, was comparable in once daily and twice daily dosed nevirapine. Compared to the variability in plasma concentrations of nevirapine, the differences in C_{min} and C_{max} between both treatment arms are minor. In addition, the lower C_{min} in the once daily arm did not result in lower efficacy in adherent naive patients. However, once daily dosed nevirapine might be less forgiving in missing a dose. The higher C_{max} in the once daily arm might result in increased risk for adverse events. Therefore, we conclude that the 200 mg twice daily may be preferred to 400 mg once daily. However, assessment of efficacy-pharmacokinetic and safety-pharmacokinetic analyses will be important to help in determining whether the once daily regimen may also be appropriate.

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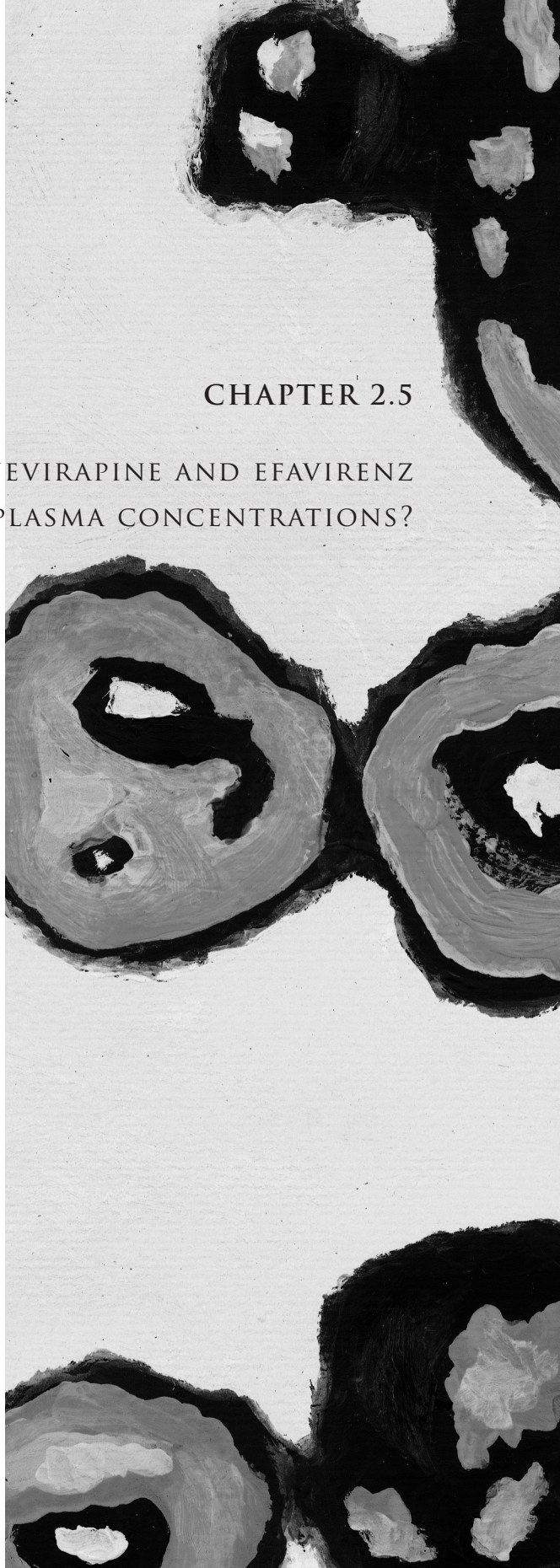

CHAPTER 2.5

ARE ADVERSE EVENTS OF NEVIRAPINE AND EFAVIRENZ RELATED WITH PLASMA CONCENTRATIONS?

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ABSTRACT

The objective of this study was to investigate the relationships between adverse events and plasma concentrations of nevirapine and efavirenz as part of the large international randomised 2NN study.

Treatment-naive HIV-1-infected patients received nevirapine (once or twice daily), efavirenz or the combination of both drugs, in combination with lamivudine and stavudine. On day 3, weeks 1, 2, 4, 24 and 48, blood samples were collected. Concentrations of nevirapine and efavirenz were quantitatively assessed by a validated high-performance liquid chromatography assay. Individual Bayesian estimates of the area under the plasma concentration-time curve over 24 hours (AUC_{24h}), minimum and maximum plasma concentrations (C_{min} and C_{max}) of nevirapine and efavirenz, as measures for drug exposure, were generated, using a previously developed population pharmacokinetic model. Pharmacokinetic parameters were compared for patients with and without central nervous system (CNS) and psychiatric adverse events, hepatic events, liver enzyme elevations and rash. Furthermore, it was investigated whether a clear cut-off for a pharmacokinetic parameter could be identified above which the incidence of adverse events was clearly increased. Adverse events were also related with demographic parameters and baseline characteristics.

In total, from 1077 patients, nevirapine (3024 samples) and efavirenz (1694 samples) plasma concentrations and adverse event data (825 observations) were available. For all patients C_{min}, C_{max} and AUC_{24h} were determined. When corrected for known covariates of gender, CD4 cell count at baseline, region, hepatitis co-infection and possible interactions between these, no significant associations between adverse events and any tested exposure parameter of nevirapine was observed. Also no target C_{min} value, above which patients were at increased risk for adverse events, could be established. On the other hand, geographical region, hepatitis co-infection, CD4 cell count and gender were found to be significantly related with the incidence of CNS and psychiatric adverse events, hepatic events, liver enzyme elevations and rash during the treatment with nevirapine.

The occurrence of elevated liver enzymes during the first 6 weeks in the efavirenz containing arm was significantly ($p=0.036$) correlated to the exposure of efavirenz (C_{min}). Only hepatitis co-infection impacted on liver enzyme elevations during the first 6 weeks of treatment. With an efavirenz C_{min} above 2.18 mg/L during the induction phase, patients were 4.4 (1.3-15.5) times more at risk for elevated liver enzymes. No other correlations between adverse events and efavirenz pharmacokinetics or patients characteristics could be identified.

Pharmacokinetic parameters of nevirapine did not have a relationship to adverse events in the 2NN trial when corrected for known covariates. The value of periodical drug monitoring of nevirapine as a way to prevent toxicity is therefore limited. Treating physicians should instead focus on factors which are more predictive of adverse events (gender, CD4 count and hepatitis co-infection). High efavirenz C_{min} levels resulted in elevated liver enzyme values during the first 6 weeks of treatment. Regular measurement of efavirenz levels and liver enzymes at the start of therapy may therefore be advised.

INTRODUCTION

Nevirapine and efavirenz are non-nucleoside reverse transcriptase inhibitors which disrupt the human immunodeficiency virus (HIV) replication within host cells by inhibition of the HIV reverse transcriptase enzyme. Both drugs have demonstrated potency, safety and convenience in several clinical trials.[1-4] As a result, nevirapine and efavirenz are frequently used as the basis of highly active antiretroviral therapy (HAART) for management of both treatment-naive and treatment-experienced patients. The double non-nucleoside study (2NN) was the first large-scale, randomised study to compare the efficacy and safety of nevirapine, efavirenz and the combination of both drugs, each in combination with stavudine and lamivudine. The trial outcome has been reported recently.[5]

In the 2NN study, Van Leth et al.[5] have shown that central nervous system (CNS) and psychiatric adverse events, hepatic events, liver enzyme elevations and rash occurred during antiretroviral therapy with nevirapine and/or efavirenz with frequencies similar to those described previously.[1,3,6,7] The relationship between exposure to nevirapine or efavirenz and the occurrence of adverse events has been the subject of several studies. However, most studies were underpowered, and contradicting outcomes have been reported. Indications exist that transaminase elevations and rash are related to plasma concentrations of nevirapine,[8,9] however, this has not been found in other studies.[10,11] Efavirenz plasma concentrations have been related to the occurrence of CNS toxicity, including dizziness, abnormal dreams and insomnia.[12-14] However, other studies could not confirm this association.[15]

The large international 2NN study in 1216 patients, on the other hand, allowed us to investigate thoroughly whether pharmacokinetic parameters of nevirapine and efavirenz are related to CNS or psychiatric adverse events, hepatic laboratory abnormalities, hepatic events or rash. The current analyses might help to determine if periodic drug monitoring could predict or lessen the risk of adverse events.

MATERIALS and METHODS

Patients

HIV-1-infected patients were included between February 2000 and June 2001 from 65 study sites in Europe, South Africa, Canada, United States, Argentina, Brazil, Australia and Thailand. The main eligibility criterion was HIV-1 RNA >5000 copies/mL at screening, without prior antiretroviral therapy. Full details have been reported by van Leth et al.[5] Upon inclusion, patients were randomly assigned to either nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, or a combination of nevirapine 400 mg plus efavirenz 800 mg once daily. In all nevirapine containing treatment arms, nevirapine was given as 200 mg once daily for the first 2 weeks. All patients received also stavudine 40 mg twice daily (30 mg twice daily if weight was less than 60 kg) and lamivudine 150 mg twice daily. From the 1216 patients included in the 2NN study, patients with apparent poor adherence to the study treatment regimen, which was defined as less than 95% compliance, were excluded from this substudy. These subjects were identified based on the reported treatment interruption.

Sampling and bioanalysis

Subjects were evaluated for clinical and laboratory adverse events at screening (week -6), at the initiation of therapy (day 0), and at weeks 2, 4, 8, 12, 24, 36 and 48. Plasma samples for evaluation of study drug concentrations were collected at day 3, weeks 1, 2, 4, 24, and 48. When blood samples were drawn, the time of ingestion of the last dose and the sampling time were recorded. Time after ingestion was extracted from this information.

The concentrations of nevirapine and efavirenz were quantitatively assessed by a validated high-performance liquid chromatographic (HPLC) with ultraviolet detection (UV) method in a licensed laboratory, conducted under good laboratory practice (GLP). Briefly, sample pre-treatment consisted of protein precipitation with acetonitrile. Subsequently, nevirapine and efavirenz were separated from endogenous compounds by isocratic, reversed phase, high performance liquid chromatography. Absorbance was measured at 275 nm. The method was validated over the range of 0.25-15.0 mg/L and 0.05-15.0 mg/L for nevirapine and efavirenz, respectively. Samples with concentrations above the upper limit of quantification were re-analysed after dilution. The assay proved to be accurate and precise. The average accuracy at three different concentrations ranged from -12.7% to 8.5%. Within- and between-day precisions were less than 5.9% for all quality control samples.[16]

Pharmacokinetic analysis

The pharmacokinetic models for nevirapine and efavirenz, which have been previously developed within the scope of the 2NN pharmacokinetic (2NN-PK) substudy [17] with the non-linear mixed effect modelling (NONMEM) software program (level 1.1, GloboMax LLC, Hanover MD, USA), were used to perform the pharmacokinetic analysis. Both pharmacokinetic models for nevirapine and efavirenz consisted of a single central compartment with first-order absorption and elimination. Gender, hepatitis B, and geographical region were involved in the variability of the pharmacokinetics of nevirapine. Geographical region and concomitantly used nevirapine were determinants of the pharmacokinetics of efavirenz.[17] Random plasma concentration-time points of nevirapine and efavirenz, and the population pharmacokinetic models were used to acquire individual Bayesian estimates of minimum concentrations (C_{min}), maximum concentrations (C_{max}, estimated at t=2 h) and area under the plasma concentration-time curve over 24 hours (AUC_{24h}) in the induction phase (day 3 till week 2) and at steady-state (from week 4) using the POSTHOC option in NONMEM.[18]

Adverse events

All laboratory tests were done at local or regional laboratories by standardised techniques and assays. The Virtual Central Laboratory (VCL, Zeist, The Netherlands) selected these laboratories, performed quality assurance, and normalised all results.[5]

Clinical adverse events were coded using the Meddra coding system. The events that were reported in more than 1% of the patients enrolled in the 2NN study could be categorised as hepatobiliary, cutaneous, CNS/psychiatric, diarrhoea, vomiting and pyrexia. CNS and psychiatric adverse events included dizziness, concentration impairment, anxiety, depression and sleep associated disorders, such as insomnia and abnormal dreams. The toxicity grading scale of the AIDS Clinical Trial Group was used for the reporting of all adverse events.[19] In addition, rashes were divided into four functional groups, which were developed as part of the nevirapine rash management, in consultation with leading experts on drug-induced skin reactions (I: erythema and pruritis; IIA: diffuse, no constitutional findings; IIB: urticarial rash; III: rash with constitutional findings or with elevated liver function tests, including Stevens-Johnson syndrome with <10% exfoliation; IV: Stevens-Johnson syndrome with exfoliation >10% and toxic epidermal necrolysis (TEN)).

Laboratory abnormalities were classified as hepatic or non-hepatic. Hepatic

abnormalities included an increase (equal or greater than 5 times the upper limit of normal) for alanine aminotransferase (ALAT) or aspartate aminotransferase (ASAT). Rises in γ-glutamyl transferase in the absence of other liver abnormalities were excluded because these reflect only enzyme induction caused by the use of nevirapine, efavirenz or both.

Investigators were to report all adverse events on case report forms. Blinded reviews of all clinical or laboratory findings that might have represented clinical hepatic events, rash events or CNS events were conducted by study team medical reviewers, in order to assure consistency of classification with definitions of these adverse events. In addition, the reviewers confirmed severity, seriousness, dates of onset and outcomes of these events.

If an adverse event occurred more than once in the same patient, the event with the greatest severity was used. Otherwise, the event with the earliest onset was used. This classification of adverse events was different from the classification used in the reporting of the 2NN study. In the latter, there was no blinded review of the events by the study team and adverse events were categorised as reported by the treating physician. To relate adverse events to pharmacokinetic parameters, both the pharmacokinetic parameters and adverse events were divided into two periods. Pharmacokinetic parameters were split up into induction phase (till week 2) and steady-state (from week 4). Adverse events were divided into those occurring during the first 6 weeks or in the period thereafter, because rash, hepatic events or elevated liver enzymes predominantly occur within this time period.[7,20]

Statistical analysis

Statistical calculations were performed with Statistical Product and Service Solutions (SPSS) for Windows, version 11.0.1 (SPSS Inc., Chicago, IL). In previous studies it was shown that gender, CD4 cell count at baseline, hepatitis B/C co-infection and ethnicity might cause differences in exposure to nevirapine or efavirenz and adverse events.[17,21,22] Therefore, associations between patients with or without selected adverse events and pharmacokinetics were tested both with univariable and with multivariable logistic regression models. Multivariable analysis was conducted to correct for these possibly confounding covariables. Interaction terms between possible confounders were included when $p<0.2$. We were specifically interested whether the effect of geographical region or baseline CD4 cell count differed by sex. Since ethnicity was not recorded, geographical region was used instead. As a measure of drug exposure the C_{min}, C_{max} and AUC_{24h} in the induction phase and at steady-state for each patient were used.

Furthermore, a cut-off for C_{min} levels was investigated, to determine whether such a cut-off could be correlated with an increased incidence of adverse events. For nevirapine, patients were divided into two groups: above or below the 75th percentile of C_{min}. Since to date no conclusively confirmed evidence is present for a relationship between plasma concentrations of nevirapine and adverse events, the limit of 75th percentile was arbitrarily chosen. C_{min} was chosen as the basis of comparison, because in drugs with long half-life, such as nevirapine and efavirenz, C_{min} is strongly correlated C_{max} and AUC. Furthermore, an actual C_{max} is difficult to determine because it is not possible to predict when this exact point is reached in a particular patient on a certain day.

For efavirenz, patients were divided in above or below the from literature known upper limit of the C_{min} of 4.0 mg/L.[12,14] Also the 75th percentile of the C_{min} was investigated as target value. Thereupon, logistic regression was performed using the incidence of adverse events divided into two groups (adverse event or not) as the dependent variable and the exposure to nevirapine or efavirenz and potential covariates, including gender, geographical region, hepatitis co-infection and CD4 cell count at baseline and the interaction terms of interest.

RESULTS

Patients

In total 1216 patients were included in the 2NN study. Of these, 1091 patients had at least one pharmacokinetic sample taken while remaining on their allocated treatment. For this substudy, 3127 nevirapine and 1728 efavirenz plasma concentrations were available. Two patients in the efavirenz arm and 12 patients in the nevirapine plus efavirenz arm were excluded due to unknown sampling time or unknown regimen. The division of the remaining 1077 patients over the four treatment arms was: 205 (19.0%), 373 (34.6%), 376 (34.9%) and 123 (11.4%) patients in the nevirapine once daily arm, nevirapine twice daily arm, efavirenz arm and nevirapine plus efavirenz arm, respectively. After 6 weeks, still 177 (18.7%), 335 (35.4%), 313 (33.1%) and 122 (12.9%) patients were on treatment in the different study arms. Finally, in total 3024 nevirapine and 1694 efavirenz plasma samples were included and used to calculate Bayesian estimated pharmacokinetic parameters of nevirapine and efavirenz and to investigate the relationships between drug exposure and adverse events. Demographics and baseline characteristics for all included patients in this substudy are shown in table 1.

Table 1. Baseline characteristics of included patients.

	NVP qd	NVP bid	EFV	NVP+EFV	Total (%)
No. of patients in study	205	373	378	135	1091
No. of patients in analysis	205	373	376	123	1077
Gender M/F	129/76	228/145	240/136	89/34	686/391 (63.7/36.3)
Age (years)★	35.0 ±8.5	36.0 ±9.2	35.9 ±8.9	35.5 ±8.8	35.7 ±8.9
Weight (kg)★	65.1 ±12.7	68.1 ±14.5	66.7 ±13.3	67.1 ±12.0	66.9 ±13.5
BMI (kg/m^2)★	19.3 ±3.3	20.2 ±3.9	19.7 ±3.5	19.7 ±3.1	19.8 ±3.6
Region					
Thailand	49	43	69	26	187 (17.4)
South Africa	66	139	134	46	385 (35.7)
South America	38	87	80	24	229 (21.3)
Western countries	52	104	93	27	276 (25.6)
HBV	15	16	14	9	54 (5.0)
HCV	21	34	32	9	96 (8.9)
CD4 cell count ($10^6/L$)★	235 ±184	209 ±181	224 ±177	209 ±170	219 ±179
HIV-1 RNA (log_{10} copies/mL)★	4.82 ±0.70	4.83 ±0.75	4.86 ±0.73	4.90 ±0.68	4.85 ±0.73

★: mean value ± standard deviation. NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, EFV=efavirenz 600 mg once daily, NVP+EFV=nevirapine 400 mg plus efavirenz 800 mg once daily, M=male, F=female, BMI=body mass index, HBV=hepatitis B co-infection, HCV=hepatitis C co-infection.

Pharmacokinetic analysis

With the earlier developed pharmacokinetic models of nevirapine and efavirenz,[17] the pharmacokinetic parameters C_{min}, C_{max} and AUC_{24h} were determined with the POSTHOC option of NONMEM. The population pharmacokinetic parameters are presented in table 2.

The mean values and 95% confidence intervals of the steady-state Bayesian estimated pharmacokinetic parameters of nevirapine and efavirenz for each treatment arm are presented in table 3.

Adverse events

Adverse events were divided into grades for CNS and psychiatric events (grade 1 to 4) and rash (grade I to IV). Hepatic events and liver enzyme elevations were scored as present or not. In table 4 the incidence of adverse events in each treatment arm is given. In total 825 adverse events were observed. Most of the adverse events occurred during the first 6 weeks of treatment, as expected. CNS and psychiatric events occurred more frequently in the efavirenz containing treatment arms (55.9% and 54.5% for efavirenz and efavirenz plus nevirapine arm, respectively, versus 19.0% and 20.4% in the once daily and twice daily nevirapine arms, respectively). Patients treated with nevirapine experienced more hepatic events and elevated liver enzymes compared to patients in the efavirenz-only arm (8.8% and 5.6% for hepatic events in the nevirapine containing arms versus 2.1% in the efavirenz arm, and 15.1% and 9.4% for liver enzyme elevations in

Table 2. Population pharmacokinetic parameters of nevirapine and efavirenz.[17]

	Nevirapine		Efavirenz	
	Estimate	RSE (%)	Estimate	RSE (%)
CL/F ≤ week 2 (L/h)	2.02	4.99	7.95	19.2
CL/F ≥ week 4 (L/h)	2.81	2.60	8.82	6.98
$\theta_{\text{Female gender}}$★	0.862	2.99	-	-
$\theta_{\text{Hepatitis B infection}}$★	0.805	7.11	-	-
$\theta_{\text{Concomitant use of NVP}}$★	-	-	1.43	7.69
θ_{Thailand}★	1	-	1	-
$\theta_{\text{South Africa}}$★	1	-	1.53	8.10
$\theta_{\text{South America}}$★	1.11	3.29	1.53	8.10
$\theta_{\text{Western countries}}$★	1.28	3.84	1.76	8.98
V/F (L)	77.0	2.79	418	7.58
k_a (h^{-1})	1.66§	-	0.287	29.3
Additive error (mg/L)	0.388	29.1	0.216	21.6
Proportional error (%)	27.3	3.99	24.2	6.16
IIV CL/F (%)	33.8	7.44	60.2	9.31
IIV V/F (%)	41.5	17.9	54.1	28.5
Correlation IIVs	0.183	35.1	-0.122	89.9

★ relative change in CL/F, § fixed from previous model. NVP=nevirapine, CL/F=clearance, V/F=volume of distribution, k_a=absorption rate constant, IIV=interindividual variability, RSE=relative standard error.

Table 3. Mean values and standard deviations of Bayesian estimated pharmacokinetic parameters of nevirapine and efavirenz at steady-state.

	NVP qd	NVP bid	NVP★★	EFV	EFV★★
C_{min} (mg/L)	3.63 ±1.82	4.95 ±2.08	3.66 ±2.02	1.86 ±1.79	1.84 ±2.70
C_{max} (mg/L)	8.01 ±2.58	7.03 ±2.29	7.83 ±2.55	2.67 ±1.78	2.87 ±2.67
AUC_{24h} (mg/L★h)	139 ±54.4	145 ±52.1	135 ±55.2	56.3 ±43.1	58.3 ±64.9

NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, EFV=efavirenz 600 mg once daily, NVP★★/EFV★★, in the double non-nucleoside treatment group=nevirapine 400 mg once daily plus efavirenz 800 mg once daily C_{min}=minimum concentration, C_{max}=maximum concentration, $AUC_{0\text{-}24h}$=area under the concentration-time curve over 24 hours, each calculated for steady state conditions.

the nevirapine containing arms versus 5.3% in the efavirenz arm). Patients treated with both nevirapine and efavirenz experienced the highest frequency of adverse events.

Table 4. Incidence of adverse events divided per treatment arm.

	NVP qd			NVP bid			EFV			NPV/EFV			Total
	Total	<wk6	>wk6	Total	<wk6	>wk6	Total	<wk6	>wk6	Total	<wk6	>wk6	
No. of pts in analysis	205	205	177	373	373	335	376	376	313	123	123	122	1077
CNS	39 (19.0%)	22 (10.7%)	17 (9.6%)	76 (20.4%)	45 (12.1%)	31 (9.3%)	210 (55.9%)	172 (45.7%)	38 (12.1%)	67 (54.5%)	53 (43.1%)	14 (11.5%)	391
grade 1	31 (15.1%)	18 (8.8%)	13 (7.3%)	46 (12.3%)	32 (8.6%)	14 (4.2%)	131 (34.8%)	116 (30.9%)	15 (4.8%)	41 (33.3%)	34 (27.6%)	7 (5.7%)	249
grade 2	8 (3.9%)	4 (2.0%)	4 (2.3%)	23 (6.2%)	11 (2.9%)	12 (3.6%)	62 (16.5%)	48 (12.8%)	14 (4.5%)	20 (16.3%)	15 (12.2%)	5 (4.1%)	113
grade 3	0	0	0	4 (1.1%)	1 (0.3%)	3 (0.9%)	14 (3.7%)	6 (1.6%)	8 (2.6%)	6 (4.9%)	4 (3.3%)	2 (1.6%)	24
grade 4	0	0	0	3 (0.8%)	1 (0.3%)	2 (0.6%)	3 (0.8%)	2 (0.5%)	1 (0.3%)	0	0	0	6
Hepatic events	18 (8.8%)	15 (7.3%)	3 (1.7%)	21 (5.6%)	19 (5.1%)	2 (0.6%)	8 (2.1%)	5 (1.3%)	3 (1.0%)	5 (4.1%)	2 (1.6%)	3 (2.5%)	52
LEE	31 (15.1%)	23 (11.2%)	8 (4.5%)	35 (9.4%)	23 (6.2%)	12 (3.6%)	20 (5.3%)	11 (2.9%)	9 (2.9%)	9 (7.3%)	3 (2.4%)	6 (4.9%)	95
Rash	60 (29.3%)	53 (25.9%)	7 (4.0%)	89 (23.9%)	66 (17.7%)	23 (6.9%)	87 (23.1%)	70 (18.6%)	17 (5.4%)	51 (41.5%)	43 (35.0%)	8 (6.6%)	287
grade I	18 (8.8%)	15 (7.3%)	3 (1.7%)	21 (5.6%)	12 (3.2%)	9 (2.7%)	22 (5.9%)	18 (4.8%)	4 (1.3%)	13 (10.6%)	9 (7.3%)	4 (3.3%)	74
grade IIA	16 (7.8%)	12 (5.9%)	4 (2.3%)	38 (10.2%)	29 (7.8%)	9 (2.7%)	44 (11.7%)	37 (9.8%)	7 (2.2%)	25 (20.3%)	22 (17.9%)	3 (2.5%)	123
grade IIB	4 (2.0%)	4 (2.0%)	0	7 (1.9%)	6 (1.6%)	1 (0.3%)	12 (3.2%)	8 (2.1%)	4 (1.3%)	11 (8.9%)	10 (8.1%)	1 (0.8%)	34
grade III	22 (10.7%)	22 (10.7%)	0	23 (6.2%)	19 (5.1%)	4 (1.1%)	9 (2.4%)	8 (2.1%)	1 (0.3%)	2 (1.6%)	2 (1.6%)	0	56
grade IV	0	0	0	0	0	0	0	0	0	0	0	0	0
Total AE	148	113	35	221	153	68	325	258	67	132	101	31	825
Total no. of pts#	99 (48.3%)	76 (37.1%)	31 (17.5%)	166 (44.5%)	118 (31.6%)	66 (19.7%)	252 (67.0%)	221 (58.8%)	93 (29.7%)	89 (72.4%)	77 (62.6%)	37 (30.3%)	606

#: number with at least one AE. NVP qd=nevirapine 400 mg once daily, NVP bid=nevirapine 200 mg twice daily, EFV=efavirenz 600 mg once daily, NVP/EFV=nevirapine 400 mg plus efavirenz 800 mg once daily, CNS=central nervous system and psychiatric adverse events, LEE=liver enzyme elevations, AE=adverse event.

Pharmacokinetic-pharmacodynamic relationships

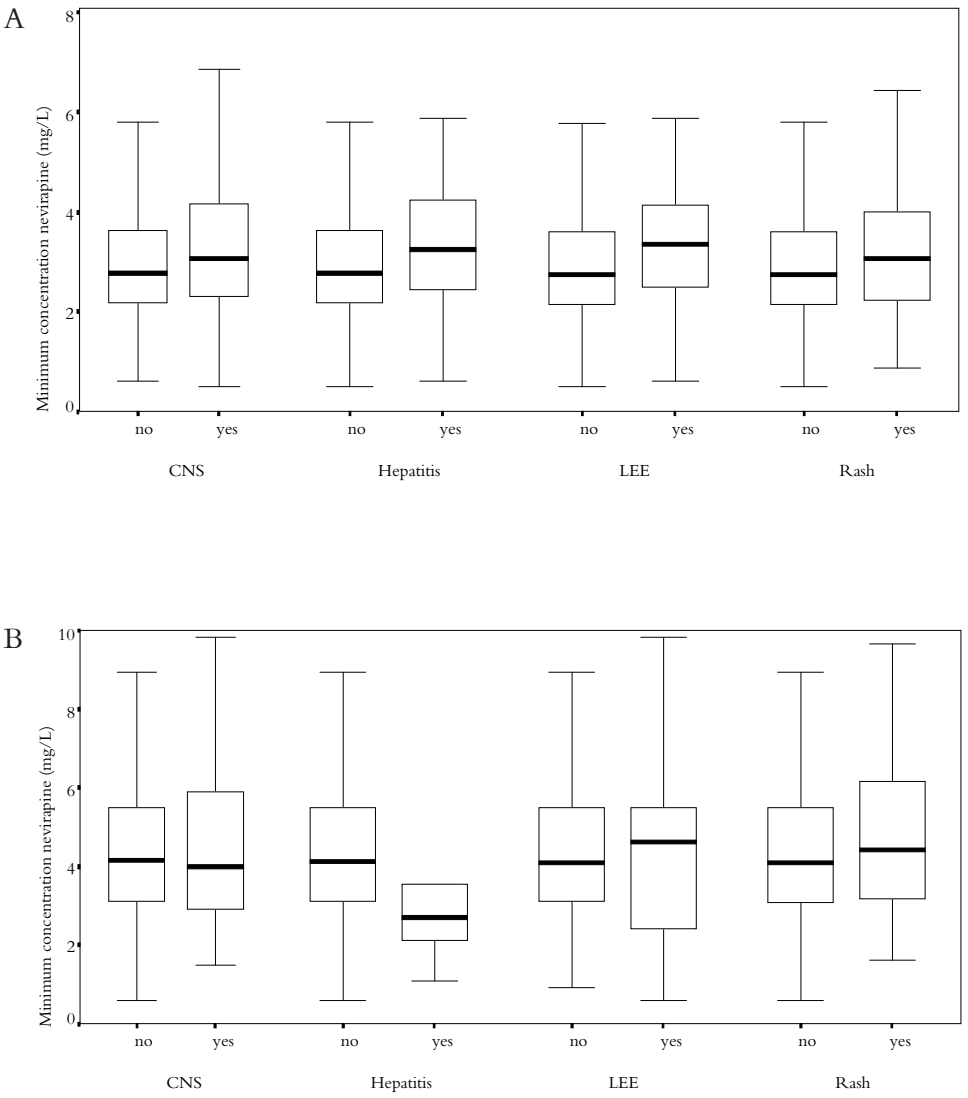
Adverse events occurred most frequently in the treatment arm with nevirapine plus efavirenz (table 4). However, the exposure to nevirapine and efavirenz in this arm did not differ from the exposure in the treatment arms with only nevirapine or efavirenz (table 3). Consequently, the higher incidence of adverse events in the combination arm cannot be explained solely by plasma concentrations. However, an additive or synergistic pharmacokinetic-pharmacodynamic effect of nevirapine and efavirenz cannot be excluded. Therefore, data from the nevirapine plus efavirenz treatment group were not included in the analysis for the relation between plasma concentrations and adverse events.

Nevirapine and adverse events

Adverse events, except for CNS and psychiatric events, occurred more frequently in the first 6 weeks of treatment, than later. During the first 6 weeks of treatment CNS and psychiatric events, hepatic events, elevated liver enzymes and rash occurred in 10.7%, 7.3%, 11.2% and 25.9% of the patients in the nevirapine once daily arm and in 12.1%, 5.1%, 6.2% and 17.7% of the patients in the nevirapine twice daily arm, respectively. Patients with adverse events during this period had non-significantly higher nevirapine plasma concentrations. There were no associations between nevirapine concentrations and adverse events with onset through week 6. When adverse events were divided into two periods with a cut-off at week 4, also no associations between nevirapine exposure and adverse events could be identified. However, treatment in Thailand was significantly associated with emergent CNS and psychiatric events ($p=0.005$, OR=2.4 (95% CI=1.3-4.5)), liver enzyme elevations ($p=0.004$, OR=3.0 (95% CI=1.4-6.4)) and rash ($p<0.001$, OR=2.8 (95% CI=1.6-4.7)) with onset during the first 6 weeks of treatment with nevirapine. Hepatitis co-infection was significantly related with hepatic events ($p=0.040$, OR=34.9 (95% CI=1.2-1029.4)) during the first 6 weeks. High CD4 cell count at baseline was significantly associated with developing liver enzyme elevation ($p<0.001$, OR=1.004 (95% CI=1.002-1.005)) during the first 6 weeks of treatment. Female gender appeared to be related with elevated liver enzymes occurring ($p=0.009$, OR=2.5 (95% CI=1.3-4.8)) during the first 6 weeks. The interaction between gender and CD4 cell count was related with hepatic events ($p=0.056$, OR=1.004 (95% CI=1.000-1.007)) and rash ($p=0.013$, OR=1.003 (95% CI=1.001-1.005)).

After 6 weeks of treatment, for patients receiving nevirapine once daily, CNS and psychiatric events, hepatic events, liver enzyme elevations and rash first had

Figure 1. Boxplots of minimum concentrations of nevirapine for adverse events with onset through week 6 (A) and after week 6 (B). CNS=central nervous system and psychiatric adverse events, LEE=liver enzyme elevations.



onset in 9.6%, 1.7%, 4.5% and 4.0% of patients. For patients receiving nevirapine twice daily, the frequencies were 9.3%, 0.6%, 3.6% and 6.9%, respectively. No associations between pharmacokinetic parameters and adverse events with onset after 6 weeks of treatment were observed. Only hepatitis co-infection was significantly associated with developing liver enzyme elevations after 6 weeks of treatment ($p<0.001$, OR=13.9 (95% CI=4.0-47.8)). Rash was influenced by the interaction between gender and CD4 cell count ($p=0.062$, OR=0.995 (95% CI=0.990-1.000)). The associations between adverse events and C_{min} of nevirapine are listed in table 5.

There were no apparent associations of adverse events and nevirapine C_{min} (figure 1). Similar boxplot analyses of C_{max} and AUC_{24h} also revealed no correlations between these concentration parameters and adverse events. Patients with a C_{min} of nevirapine above 5.50 mg/L (75^{th} percentile) at steady-state were not at increased risk for adverse events. Odds ratios for experiencing an adverse event during treatment with nevirapine are presented in table 6.

When adverse events were divided into two periods with a cut-off at week 4, no correlations between nevirapine exposure and adverse events could be identified.

Efavirenz and adverse events

During the first 6 weeks of treatment CNS and psychiatric events, hepatic events, liver enzyme elevations and rash occurred in 45.7%, 1.3%, 2.9% and 18.6% of the patients in the efavirenz-only arm, respectively. In this period, liver enzyme elevations were significantly related to an increased exposure (C_{min}) to efavirenz ($p=0.036$, OR=1.5 (95% CI=1.0-2.2)). CNS and psychiatric events occurred more frequently during the first six weeks of treatment than after six weeks (45.7% versus 12.1%, table 4). The first six weeks also was when most of the other adverse events emerged; after 6 weeks 12.1%, 1.0%, 2.9% and 5.4% of the patients showed CNS and psychiatric events, hepatic events, liver enzyme elevations and rash. As demonstrated in figure 2, efavirenz C_{min} was not related to the occurrence of adverse events, except for a possible association with emergent liver enzyme elevations. Treatment in Thailand appeared to influence the incidence of CNS and psychiatric events ($p<0.001$, OR=5.8 (95% CI=2.5-13.6)). Hepatitis co-infection could be identified as risk factor for liver enzyme elevations ($p=0.023$, OR=5.8 (95% CI=1.3-26.4)) during the first 6 weeks. The associations between adverse events and C_{min} of efavirenz are listed in table 5.

A C_{min} of efavirenz above 2.18 mg/L (75^{th} percentile) during the induction phase was associated with liver enzyme elevations ($p=0.021$, OR=4.41 (95% CI=1.3-

Table 5. Mean value and standard deviation of Bayesian estimated C_{min} of nevirapine and efavirenz correlated with adverse events.

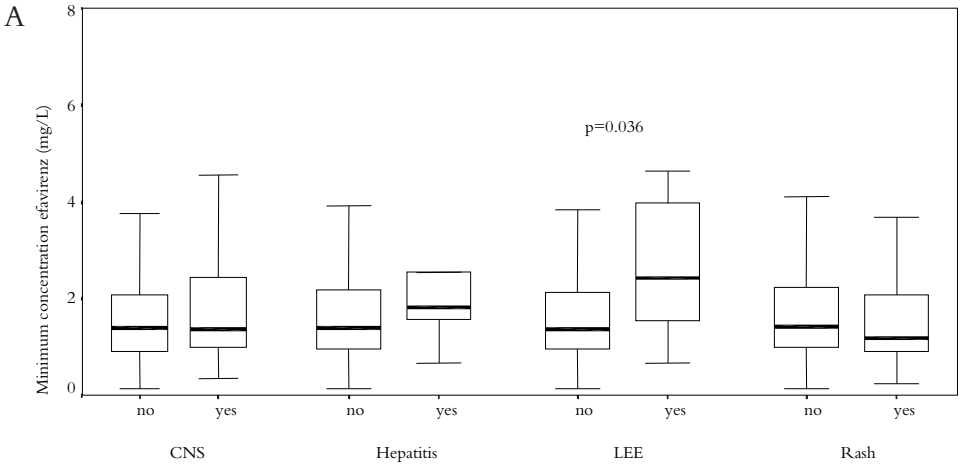
Nevirapine		Onset <= 6 weeks				Onset > 6 weeks			
Event type	AE	n	C_{min} (mg/L)	OR (95% CI) p-value*	OR (95% CI) p-value**	n	C_{min}	OR (95%CI) p-value*	OR (95% CI) p-value**
CNS	no	511 (88.4%)	2.98 ±1.17	1.236 (1.019 – 1.499)	1.137 (0.899 – 1.438)	465 (90.6%)	4.50 ±2.11	0.963 (0.831 – 1.116)	0.921 (0.787 – 1.077)
	yes	67 (11.6%)	3.32 ±1.45	0.032	0.283	48 (9.4%)	4.35 ±1.89	0.616	0.302
Hepatic events	no	544 (94.1%)	3.00 ±1.21	1.221 (0.943 – 1.579)	1.217 (0.887 – 1.671)	508 (99.0%)	4.50 ±2.08	0.839 (0.504 – 1.396)	0.183 (0.027 – 1.235)
	yes	34 (5.9%)	3.33 ±1.25	0.129	0.224	5 (1.0%)	3.86 ±3.46	0.499	0.081
LEE#	no	521 (90.1%)	2.98 ±1.20	1.215 (0.963 – 1.534)	1.076 (0.819 – 1.415)	493 (96.1%)	4.50 ±2.09	0.949 (0.757 – 1.190)	0.938 (0.734 – 1.199)
	yes	46 (8.0%)	3.28 ±1.08	0.100	0.598	20 (3.9%)	4.28 ±2.28	0.650	0.610
Rash	no	459 (79.4%)	2.98 ±1.23	1.136 (0.968 – 1.333)	1.035 (0.865 – 1.237)	483 (94.2%)	4.46 ±2.06	1.120 (0.956 – 1.311)	0.890 (0.632 – 1.255)
	yes	119 (20.6%)	3.18 ±1.15	0.118	0.709	30 (5.8%)	5.01 ±2.52	0.161	0.507

#: no LEE data available from 11 patients. *: for univariable logistic regression, **: for multivariable logistic regression (corrected for gender, CD4 cell count at baseline, HBV/HCV at baseline, region (Thailand versus all others) and interaction terms with p<0.2). AE=adverse event, CNS=central nervous system and psychiatric adverse events, LEE=liver enzyme elevations.

Efavirenz		Onset <= 6 weeks				Onset > 6 weeks			
Event type	AE	n	C_{min} (mg/L)	OR (95% CI) p-value*	OR (95% CI) p-value**	n	C_{min}	OR (95% CI) p-value*	OR (95% CI) p-value**
CNS	no	203 (54.1%)	1.67 ±1.10	1.146 (0.965 – 1.360)	1.047 (0.870 – 1.261)	283 (89.8%)	1.83 ±1.65	1.087 (0.912 – 1.295)	1.057 (0.876 – 1.276)
	yes	172 (45.9%)	1.87 ±1.30	0.120	0.625	32 (10.2%)	2.14 ±2.78	0.351	0.561
Hepatic events	no	370 (98.7%)	1.75 ±1.19	1.302 (0.733 – 2.313)	1.425 (0.805 – 2.522)	313 (99.4%)	1.86 ±1.80	1.033 (0.504 – 2.115)	1.307 (0.570 – 2.997)
	yes	5 (1.3%)	2.25 ±1.49	0.369	0.224	2 (0.6%)	1.97 ±1.07	0.930	0.527
LEE#	no	358 (95.5%)	1.73 ±1.18	1.508 (1.066 – 2.132)	1.506 (1.027 – 2.208)	307 (97.5%)	1.88 ±1.81	1.207 (0.937 – 1.555)	0.737 (0.304 – 1.787)
	yes	12 (3.2%)	2.59 ±1.39	0.020	0.036	8 (2.5%)	1.29 ±0.860	0.145	0.499
Rash	no	303 (80.8%)	1.81 ±1.24	0.823 (0.643 – 1.054)	0.917 (0.631 – 1.333)	300 (95.2%)	1.83 ±1.74	1.180 (0.962 – 1.449)	0.948 (0.635 – 1.414)
	yes	72 (19.2%)	1.56 ±0.983	0.122	0.649	15 (4.8%)	2.60 ±2.57	0.113	0.792

#: no LEE data available from 5 patients. *: for univariable logistic regression, **: for multivariable logistic regression (corrected for gender, CD4 cell count at baseline, HBV/HCV at baseline, region (Thailand versus all others) and interaction terms with p<0.2). AE=adverse event, CNS=central nervous system and psychiatric adverse events, LEE=liver enzyme elevations.

Figure 2. Boxplots of minimum concentrations of efavirenz for adverse events with onset through week 6 (A) and after week 6 (B). CNS=central nervous system and psychiatric adverse events, LEE=liver enzyme elevations.



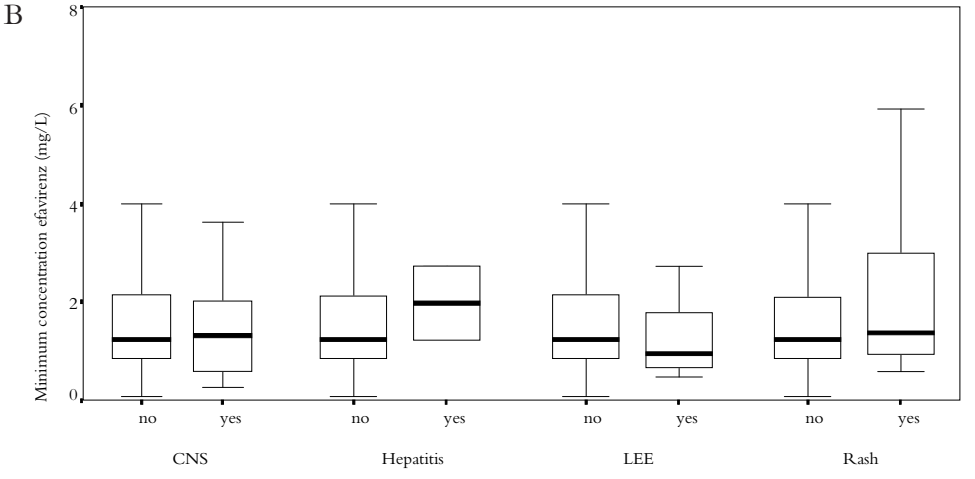


Table 6. Odds ratios and p-values for adverse events above specific plasma levels of nevirapine and efavirenz (corrected for gender, CD4 cell count at baseline, hepatitis co-infection at baseline, geographical region and interaction terms).

	>75th % of C_{min}-IND (3.67 mg/L)		>75th % of C_{min}-SS (5.50 mg/L)	
Nevirapine	OR (95% CI)	p-value	OR (95% CI)	p-value
CNS	1.890 (0.947 – 3.774)	0.071	0.909 (0.448 – 1.842)	0.791
Hepatic events	1.586 (0.716 – 3.514)	0.256	0.647 (0.063 – 6.685)	0.715
LEE	1.596 (0.802 – 3.173)	0.183	0.848 (0.269 – 2.675)	0.778
Rash	1.142 (0.702 – 1.856)	0.593	0.850 (0.174 – 4.152)	0.841
	>75th % of C_{min}-IND (2.18 mg/L)		>75th % C_{min}-SS (2.14 mg/L)	
Efavirenz	OR (95% CI)	p-value	OR (95% CI)	p-value
CNS	1.052 (0.625 – 1.770)	0.850	0.907 (0.373 – 2.202)	0.829
Hepatic events	2.558 (0.387 – 16.913)	0.330	6.609 (0.322 – 135.815)	0.221
LEE	4.413 (1.256 – 15.502)	0.021	1.559 (0.264 – 9.217)	0.625
Rash	0.845 (0.443 – 1.612)	0.610	0.395 (0.064 – 2.441)	0.317

CNS=central nervous system and psychiatric adverse events, LEE=liver enzyme elevations, C_{min}=minimum concentration, IND=induction phase, SS=steady state, OR=odds ratio, CI=confidence interval.

15.5)). A cut-off at 4.0 mg/L resulted in a similar outcome. Odds ratios are presented in table 6.

Discussion and conclusions

The 2NN study compared the efficacy, safety and tolerability of nevirapine given as 200 mg twice daily or 400 mg once daily, and efavirenz 600 mg once daily and the combination of nevirapine and efavirenz, with background stavudine and lamivudine therapy. The trial established comparability of nevirapine regimens and efavirenz in suppressing HIV-1 viral load. The primary aim of the current 2NN substudy was to investigate relationships between pharmacokinetic parameters of nevirapine and efavirenz and adverse events. Associations between toxicity and plasma concentration have been described in several studies.[8,9,12-14] Although, in other studies these relationships have not been confirmed.[10,11,15] The large, international, randomised 2NN study offers the opportunity in a large, well-powered study, to shed light onto these putative pharmacokinetic-adverse event relationships.

Van Leth et al.[5] have shown that in 2NN adverse events occurred during antiretroviral therapy with nevirapine and/or efavirenz with frequencies similar to those described previously.[1,3,6,7] In the pharmacokinetic substudy of 2NN has been shown that gender, geographical region and hepatitis B-co infection influenced

the pharmacokinetics of nevirapine. Clearance of efavirenz was influenced by concomitant used nevirapine and geographical region.[17]

Our present results show that there is no association between adverse events and exposure to nevirapine. Also no clear cut-off value of C_{min} level could be identified above which the incidence of adverse events was evidently increased. During the first 6 weeks of treatment it appeared that adverse events occurred significantly more often in females, patients with a higher CD4 cell count at baseline, patients from Thailand and patients with hepatitis co-infection. For the period after 6 weeks, positive hepatitis status at baseline was a risk factor for liver enzyme elevations.

Immune reactions to drugs can cause a variety of diseases involving the skin, liver, kidney and lungs. In many of these drug-related hypersensitivity reactions, drug-specific CD4+ and CD8+ T-cells recognise drugs and cause different antibody-mediated diseases, such as maculopapular exanthema, drug-induced hypersensitivity syndrome as well as the Stevens-Johnson syndrome.[23] Since these immune reactions mainly depend on the presence of an antigen and not on the quantity of it, it could possibly be expected that hepatic events, elevated liver enzymes and rash were not related to the extent of drug exposure, although other mechanisms may also be involved. Given that CD4 cells are involved in the immune reaction, the relation between CD4 cell count at baseline and rash and liver-associated adverse events might be expected. Genetic polymorphism of metabolising enzymes, such as cytochrome P450 enzymes and N-acetyltransferase, may contribute to the generation of hypersensitivity reactions.[23] Genetic variation in an ethnically diverse population and the influence of gender on pharmacokinetics have been reported for several drug metabolism enzymes.[24,25] This might explain differences in incidence of adverse events in gender and geographical region.

For efavirenz, a statistically significant association was found between elevated liver enzymes during the first 6 weeks and its plasma levels in the induction phase. Moreover, efavirenz C_{min} levels above 2.18 mg/L or 4.0 mg/L in the induction phase were related with these laboratory hepatic abnormalities. Since both outcomes were found as cut-off value, obviously no clear cut-off value for efavirenz C_{min} could be identified and only an association between hepatitis co-infection and liver enzyme elevations could be demonstrated. Marzolini et al.[12] and Joshi et al.[26] each demonstrated apparent associations between pharmacokinetic parameters and CNS and psychiatric events. In contrast, neither the current 2NN analyses nor Ribaudo et al.[15] found such associations.

This substudy of the large international randomised 2NN study demonstrated

that, nevirapine and efavirenz pharmacokinetic values were generally not related to adverse events. No clear cut-off values for C_{min} of nevirapine or efavirenz could be identified. The singular exception was a statistically significant association between the incidence of liver enzyme elevations occurring during the first 6 weeks and efavirenz exposure in the induction phase. There is no readily apparent reason for this association.

These findings in the 2NN analyses support the use of standard nevirapine and efavirenz doses. Since these standard doses resulted in plasma concentrations in ranges that did not appear to be correlated with important adverse events. It appears that therapeutic drug monitoring is generally unlikely to be helpful in avoiding these adverse events. However, drug monitoring may still be useful during the first weeks of efavirenz therapy and for specific situations such as, drug-drug interactions and evaluating non-adherence to antiretroviral regimens.[27,28]

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SUMMARY

Introduction

Nowadays, individuals infected with the human immunodeficiency virus (HIV) can be effectively treated with combination drug therapy, known as highly active antiretroviral therapy (HAART). HAART typically consists of three or four antiretroviral agents preferably from at least two different drug classes. The four classes of antiretroviral drugs currently available for the treatment of HIV include nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and fusion inhibitors (FIs). Although the treatment of HIV-infected individuals presents as a success story, improvement is still required. Problems such as toxicity, resistance, non-adherence and drug-drug interactions are frequently encountered in daily practice. The actual administered dose, drug-drug interactions and other determinants of pharmacokinetic variability, such as patient characteristics and co-infections, influence the plasma concentrations. Associations between plasma concentrations of antiretroviral drugs and treatment response (efficacy and adverse events) provide a clear rationale to carefully evaluate the pharmacokinetic characteristics of these drugs. In this thesis, pharmacological studies are presented, which aimed at improving the treatment of HIV-infected patients.

Clinical pharmacology & population pharmacokinetics

An important goal of pharmacological research in HIV-therapy is to define a therapeutic range for the investigated antiretroviral drug. This therapeutic window must anticipate optimal virological suppression with minimal toxicity. This desirable response depends on the concentration of the drug and the properties of the virus. To gain more knowledge about the pharmacokinetics of antiretroviral drugs, with the ambition to optimise the treatment for the individual patient, pharmacokinetics of selected PIs and NNRTIs were studied in representative patient populations. In addition, determinants of pharmacokinetic variability were assessed.

In **chapter 1.1** adequate dosages and plasma levels for optimal treatment to maintain maximally suppressive drug concentrations during a complete dosage interval, which represents a key defence against the emergence of resistance, were summarised. Low plasma concentrations of antiretroviral drugs may allow viral replication and the opportunity for mutant virus to be selected. On the other hand, for several drugs maximum concentrations in plasma have been shown to relate to toxicity. Therefore, the treatment of HIV-infected individuals is increasingly

monitored and optimised by use of therapeutic drug monitoring (TDM). In this chapter, practical guidelines for TDM of antiretroviral agents were provided, in which established target concentrations for the PIs amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir and the NNRTIs efavirenz and nevirapine were brought together. Detailed advices were provided on how to interpret the results of TDM and how to incorporate TDM in the treatment of HIV-infected patients.

Chapter 1.2 describes a case-report of a drug-drug interaction between nevirapine and paclitaxel, occurring in an HIV-1-infected patient with Kaposi´s sarcoma. Co-administration of these agents could theoretically led to a pharmacokinetic drug-drug interaction, since both drugs are metabolised via the same cytochrome P450 isoenzymes and have enzyme inducing and inhibiting potential. However, it appeared that plasma concentrations of paclitaxel and its hydroxylated metabolites were comparable for this HIV-1-infected patients and a control group. Also the concentrations of nevirapine when dosed concomitantly with paclitaxel were similar to those without paclitaxel. From this case, a pharmacokinetic drug-drug interaction between nevirapine and paclitaxel could not be demonstrated.

To optimise treatment of HIV-infected patients knowledge of pharmacokinetics of antiretroviral drugs is essential. **Chapter 1.3** describes the characterisation of the population pharmacokinetics of efavirenz in a representative patient population and the identification of patient characteristics influencing its pharmacokinetics. A rich data set with 40 full pharmacokinetic profiles and 315 efavirenz plasma concentrations at a single time point was available. Disposition of efavirenz was described by a two-compartment model and absorption was modelled using a chain of three transition compartments to describe the fast absorption with slow onset. Asian race and total bilirubin at baseline increased the apparent bioavailability of efavirenz with 56% and 57%, respectively. No significant correlations between patient characteristics and clearance nor volume of distribution were identified.

To gain more insight into the drug-drug interaction between ritonavir and other PIs, the development and validation of a population pharmacokinetic model for ritonavir used as a booster or as an antiretroviral agent in HIV-1-infected patients is described in **chapter 1.4**. From 186 patients full pharmacokinetic profiles and random concentration-time points were available for this analysis. First-order absorption in combination with one-compartment disposition best described the pharmacokinetics of ritonavir. The concomitant use of the PI lopinavir resulted in a 2.7-fold increase in the clearance of ritonavir. No other patient characteristics influenced the pharmacokinetics of ritonavir. The model was validated and used

for further investigation of interactions between ritonavir and other PIs.
In **chapter 1.5** the development of a population pharmacokinetic model of indinavir is described, in which the interaction between indinavir and ritonavir was characterised. In addition, relationships between patient characteristics and pharmacokinetics of indinavir were identified. Data of a representative population of HIV-1-infected patients (n=147) were available for this analysis. A one-compartment model with first-order absorption and elimination was applied. An absorption lag-time of indinavir was identified for patients who concomitantly used ritonavir. Ritonavir data were fitted to the previously developed model (chapter 1.4) to obtain individual Bayesian estimates of pharmacokinetic parameters. Afterwards, an integrated model for the description of the pharmacokinetics of indinavir and ritonavir was designed. The relationship between ritonavir and inhibition of the clearance of indinavir was independent of concentration or exposure of ritonavir. Therefore, it appeared that the boosting effect of ritonavir on the concentrations of indinavir was independent of dosage, indicating that 100 mg ritonavir was sufficient for maximal inhibition of indinavir metabolism.

2NN studies
The NNRTIs nevirapine and efavirenz have demonstrated potency, safety and convenience in separate clinical trials. As a result, these drugs are frequently used as part of HAART for the management of both treatment-naive and treatment-experienced HIV-1-infected patients. Nevertheless, until 2NN, a randomised comparison of antiviral potency, toxicity and tolerance between the two drugs as part of HAART regimens had not yet been performed. The 2NN study was designed as an open-label, randomised, international, multi-centre study conducted in the United States, Canada, Argentina, Brazil, Europe, South Africa, Australia and Thailand. The study comprised four arms and evaluated the efficacy of nevirapine and efavirenz in combination with stavudine and lamivudine. The primary efficacy measurement was comparison of nevirapine *versus* efavirenz. In addition, the study contained an arm with the combination of both NNRTIs and provided a comparison of nevirapine in a once daily dosing schedule with a twice daily schedule.
In the treatment of HIV-infected patients, an urgent need still exists for more conveniently dosed and better tolerated regimens with improved virological and immunological efficacy. Based on preclinical studies, the combination of two NNRTIs was considered a reasonable treatment option. In **chapter 2.1** an overview is given of published studies with dual NNRTI regimens. Clinical

studies have shown different results with regards to viral replication inhibiting properties, pharmacokinetics, efficacy and toxicity of dual NNRTI regimens. When compared to single NNRTI regimens, dual NNRTI regimens showed similar, but not superior results with regards to virological and immunological success in treatment-naive and pre-treated HIV-1-infected patients. NNRTI-associated adverse events, such as clinical hepatitis, elevated liver enzymes, rash, central nervous system side effects and psychiatric disorders, occurred more frequently when two NNRTIs were dosed concomitantly.

The availability of validated sensitive bioanalytical methods for antiretroviral drugs is a requirement for the pharmacological evaluation of these agents in HIV-infected patients. In **chapter 2.2** a simple and rapid method for the simultaneous determination of the NNRTIs efavirenz and nevirapine in human plasma using liquid chromatography is described. The sample pre-treatment, which consisted of protein precipitation, was simple and fast, and required only 100 μL plasma. The run time was 10.0 minutes. The method was validated over a concentration range of 0.05 mg/L to 15.0 mg/L for efavirenz and 0.25 mg/L to 15.0 mg/L for nevirapine. The method was precise and accurate with coefficients of variation less than 5.9% for both intra-day and inter-assay precisions, and with mean accuracies below ±12.7%. This validated assay was suited for use in the clinical pharmacokinetic studies within the 2NN study.

Chapter 2.3 describes the investigation of the population pharmacokinetics of nevirapine and efavirenz in the 2NN study. In this study, a large diverse global population, with a substantial portion of female patients (36.3%) and only 25.6% patients from Western countries, was included. The data of nevirapine and efavirenz were adequately described with a one-compartment model with first-order absorption and elimination. For both drugs an induction phase was distinguished from the steady-state phase. Clearance of nevirapine was lower in females (13.8%) and in patients with hepatitis B co-infection (19.5%). Also geographical region was involved in the variability of the pharmacokinetics of nevirapine. Patients from South America and Western countries had 11% and 28% higher clearance of nevirapine, respectively, compared to patients from Thailand and South Africa. Concomitantly used nevirapine was a determinant of the pharmacokinetics of efavirenz and increased the clearance of efavirenz with 43%. Patients from Thailand had a lower clearance than the rest of the population.

In addition, the pharmacokinetics of nevirapine in a once daily 400 mg and twice daily 200 mg dosing regimen were investigated. Results of this 2NN substudy are presented in **chapter 2.4**. In total, 2899 nevirapine plasma concentrations from 578

patients were available. Dosage and dosing frequency did not influence clearance or volume of distribution of nevirapine, indicating linear pharmacokinetic behaviour of nevirapine whether given as a single daily dose or as divided doses over 24 hours. The data showed that the daily exposure to nevirapine, measured as the area under the plasma concentration versus time curve over 24 hours (AUC_{24h}) was similar for the 400 mg once daily and the 200 mg twice daily dosing regimen. The minimum concentration (C_{min}) and maximum concentration (C_{max}) of nevirapine were lower and higher, respectively, for the once daily regimen as compared to the twice daily regimen. However, compared to total variability in plasma nevirapine levels for the two treatments, these differences were minor. Whether this may lead to a higher risk for toxicity or virological failure needs further assessment.

In **chapter 2.5**, the relationships between adverse events and plasma concentrations of nevirapine and efavirenz were investigated in the 2NN study. Pharmacokinetic parameters of nevirapine were not related to central nervous system and psychiatric adverse events, hepatic events, liver enzyme elevations and rash when corrected for known covariates (gender, geographical region, CD4 cell count at baseline and hepatitis co-infection). Also no clear cut-off value above which patients are at increased risk for adverse events could be identified. The value of periodical drug monitoring of nevirapine as a way to prevent toxicity is therefore limited. Treating physicians should instead focus on factors which may be more predictive of adverse events (gender, geographical region, CD4 count and hepatitis co-infection). During the first 6 weeks of treatment, high efavirenz C_{min} levels resulted in elevated liver enzyme values. No other relations could be identified for efavirenz in this period of treatment. Also at steady-state, no relationships between efavirenz exposure and adverse events were demonstrated. Since the standard doses of nevirapine and efavirenz resulted in plasma concentrations in ranges that did not appear to be correlated with important adverse events, it appears that TDM of these drugs is generally unlikely to be helpful in avoiding adverse events. However, drug monitoring may still be useful during the first weeks of efavirenz containing therapy and for specific situations such as, drug-drug interactions and evaluating non-adherence to antiretroviral regimens

Conclusions and future perspectives

In conclusion, more insights into the pharmacokinetics of selected antiretroviral drugs have been gained with the studies described in this thesis. High variability in pharmacokinetics has been observed, which can partially be related to patient

characteristics such as gender, race/geographical region, co-infections and co-medication. The variability in plasma concentrations of antiretroviral drugs might be diminished when dosages of antiretroviral drugs are tailored based on measurements of plasma concentrations. However, the value of TDM of NNRTIs as a way to prevent patients from toxicity might be limited, since no persuasive associations between exposure to nevirapine and efavirenz and adverse events were identified.

Hopefully, this thesis has contributed to the continuous process of optimisation of antiretroviral therapy and improvement of the response to HAART in HIV-1-infected patients.

SAMENVATTING

Inleiding

Tegenwoordig kunnen patiënten die geïnfecteerd zijn met het humane immunodeficiëntie virus (HIV) effectief behandeld worden met combinatietherapie, die ook wel "highly active antiretroviral therapy" (HAART) wordt genoemd. Kenmerkend van HAART is dat het bestaat uit drie of vier antiretrovirale geneesmiddelen met doorgaans verschillende aangrijpingspunten in de HIV-levenscyclus. De middelen die nu beschikbaar zijn voor de behandeling van een HIV-infectie zijn de nucleoside reverse transcriptase remmers (NRTI's), de non-nucleoside reverse transcriptase remmers (NNRTI's), de protease remmers (PI's) en de fusieremmers (FI's).

Hoewel de behandeling van HIV-geïnfecteerden succesvol lijkt te zijn, is er nog steeds grote behoefte aan verbetering. Problemen met betrekking tot bijwerkingen, resistentie, therapietrouw en geneesmiddelinteracties komen regelmatig voor in de dagelijkse praktijk. De werkelijk toegediende dosis, geneesmiddelinteracties en andere bepalende factoren van de farmacokinetische variabiliteit, zoals patiëntkarakteristieken en co-infecties, beïnvloeden de plasmaconcentraties. Aangezien er relaties bestaan tussen plasmaconcentraties van antiretrovirale geneesmiddelen en het effect van behandeling (effectiviteit en bijwerkingen), is er een goede reden voor onderzoek naar de farmacokinetiek van deze geneesmiddelen. In dit proefschrift wordt farmacologisch onderzoek gepresenteerd, dat tot doel heeft de behandeling van HIV-geïnfecteerde patiënten te verbeteren.

Klinische farmacologie & populatie farmacokinetiek

Een belangrijk doel van HIV-gerelateerd farmacologisch onderzoek is het definiëren van een therapeutische breedte van antiretrovirale geneesmiddelen. Dit therapeutische gebied moet voorzien in optimale therapeutische effectiviteit met minimale toxiciteit. Dit wenselijke effect is afhankelijk van de concentratie van het geneesmiddel en de eigenschappen van het virus. Om meer kennis te verkrijgen over de farmacokinetiek van antiretrovirale geneesmiddelen, met het doel de behandeling van de individuele patiënt te verbeteren, is de farmacokinetiek van een aantal PI's en NNRTI's in een representatieve patiëntenpopulatie onderzocht. Daarnaast is ook bepaald welke factoren van invloed zijn op de farmacokinetische variabiliteit.

In **hoofdstuk 1.1** zijn adequate plasmaspiegels voor optimale behandeling samengevat. Het bereiken van geneesmiddelconcentraties die hoog genoeg zijn om het virus te remmen gedurende het gehele dosisinterval, vervult een

sleutelrol bij het voorkomen van resistentie tegen antiretrovirale therapie. Indien plasmaconcentraties van antiretrovirale geneesmiddelen dalen en de virale replicatie toeneemt, kunnen virussen muteren en ongevoelig worden voor de antiretrovirale therapie. Anderzijds zijn er voor verscheidene geneesmiddelen relaties aangetoond tussen maximale plasmaconcentraties en bijwerkingen. De behandeling van HIV-geïnfecteerden wordt daarom in toenemende mate begeleid en geoptimaliseerd door het meten van plasmaconcentraties, hetgeen ook wel "therapeutic drug monitoring" (TDM) wordt genoemd. In dit hoofdstuk is een praktische leidraad gepresenteerd om TDM van antiretrovirale geneesmiddelen toe te passen. In deze leidraad zijn doelconcentraties bijeengebracht voor de PI's atazanavir, amprenavir, indinavir, lopinavir, nelfinavir, ritonavir en saquinavir en voor de NNRTI's efavirenz en nevirapine. Bovendien zijn gedetailleerde adviezen gegeven met betrekking tot de interpretatie van TDM-resultaten en de toepassing hiervan bij de behandeling van HIV-geïnfecteerden.

Hoofdstuk 1.2 beschrijft een casus over een geneesmiddelinteractie tussen nevirapine en paclitaxel in een HIV-geïnfecteerde patiënt met Kaposi's sarcoom. Theoretisch kan het gelijktijdig toedienen van nevirapine en paclitaxel leiden tot een farmacokinetische geneesmiddelinteractie, omdat beide middelen gemetaboliseerd worden via dezelfde cytochroom P450 isoenzymen. Het is echter gebleken dat de plasmaconcentraties van paclitaxel en de hydroxymetabolieten in de HIV-geïnfecteerde patiënt vergelijkbaar zijn met die in de controle groep. Gelijktijdig gebruik van paclitaxel beïnvloedde ook niet de concentratie van nevirapine. Aan de hand van deze casus kon de vermeende farmacokinetische geneesmiddelinteractie tussen nevirapine en paclitaxel niet worden aangetoond.

Om de behandeling van HIV-geïnfecteerde patiënten te optimaliseren is kennis van de farmacokinetiek van antiretrovirale geneesmiddelen essentieel. **Hoofdstuk 1.3** beschrijft de karakterisering van de farmacokinetiek van efavirenz in een representatieve patiëntenpopulatie. Bovendien zijn patiëntkarakteristieken geïdentificeerd die de farmacokinetiek van efavirenz beïnvloeden. Een rijke dataset met 40 volledige farmacokinetische curven en 315 plasmaconcentraties van efavirenz op een willekeurig tijdstip was beschikbaar. De farmacokinetiek van efavirenz is beschreven met een twee-compartimenten model met eerste orde eliminatie vanuit het centrale compartiment. Met behulp van een aaneenschakeling van transitiecompartimenten tussen het depot en het centrale compartiment is het trage begin van het snelle absorptieproces beschreven. Aziatisch ras en totaal bilirubine bij start van de behandeling bleken de biologische beschikbaarheid van efavirenz met 56% en 57% te verhogen. Er zijn geen significante correlaties

aangetoond tussen klaring of verdelingsvolume en patiëntkarakteristieken.
Om meer inzicht in de geneesmiddelinteractie tussen ritonavir en andere PI's te krijgen, is de ontwikkeling en validatie van een farmacokinetisch model voor ritonavir beschreven in **hoofdstuk 1.4**. In deze studie gebruikten HIV-geïnfecteerde patiënten ritonavir als "booster" of als een antiretroviraal geneesmiddel. Van 186 patiënten waren naast plasmaconcentraties op willekeurige tijdstippen ook volledige farmacokinetische curven beschikbaar. De farmacokinetiek van ritonavir is het best beschreven met behulp van een een-compartiment model met eerste orde absorptie. Gelijktijdig gebruik van lopinavir leidde tot een 2.7 maal verhoogde klaring van ritonavir. Andere patiëntkarakteristieken beïnvloedden de farmacokinetiek van ritonavir niet. Het model is vervolgens gevalideerd en gebruikt voor vervolgonderzoek op het gebied van geneesmiddelinteracties tussen ritonavir en andere PI's.
In **hoofdstuk 1.5** is de ontwikkeling van een farmacokinetisch model van indinavir beschreven, waarin de interactie tussen indinavir en ritonavir is opgenomen. Bovendien zijn relaties tussen patiëntkarakteristieken en de farmacokinetiek van indinavir geïdentificeerd in een representatieve populatie met HIV-geïnfecteerde patiënten. Gegevens van 147 HIV-geïnfecteerde patiënten waren beschikbaar voor deze analyse. Een een-compartiment model met eerste orde absorptie en eliminatie is toegepast. Patiënten die gelijktijdig ritonavir gebruikten, vertoonden een vertraagde absorptie van indinavir. Bayesiaanse schattingen voor de farmacokinetische parameters van ritonavir zijn berekend met behulp van het eerder ontwikkelde model (hoofdstuk 1.4). Vervolgens is een model ontworpen voor de beschrijving van de farmacokinetiek van indinavir in combinatie met ritonavir. Het bleek dat de relatie tussen de blootstelling aan ritonavir en de klaring van indinavir niet afhankelijk was van concentratie of tijd. Dit gaf aan dat 100 mg ritonavir voldoende was om het metabolisme van indinavir maximaal te remmen en de concentratie van indinavir te "boosten".

2NN studies
In verscheidene klinische studies is aangetoond dat de NNRTI's nevirapine en efavirenz krachtig, veilig en gebruiksvriendelijk zijn. Als resultaat hiervan worden deze geneesmiddelen regelmatig gebruikt als onderdeel van HAART om zowel therapie-naïeve en voorbehandelde HIV-geïnfecteerde patiënten te behandelen. Desondanks is, totdat de 2NN studie kwam, nooit eerder een gerandomiseerde studie uitgevoerd om nevirapine en efavirenz te vergelijken op het gebied van antivirale effectiviteit, bijwerkingen en verdraagbaarheid. De 2NN

studie is ontworpen als een open-label, gerandomiseerde, internationale, multicentrum studie in verschillende landen (Verenigde Staten, Canada, Argentinië, Brazilië, Europa, Zuid-Afrika, Australië en Thailand). De studie bestond uit vier behandelarmen, waarin de effectiviteit van nevirapine en efavirenz in combinatie met stavudine en lamivudine is geëvalueerd. Het primaire effectiviteitscriterium was de vergelijking van nevirapine versus efavirenz. Daarnaast bevatte de studie een arm met de combinatie nevirapine en efavirenz. Ook voorzag de studie in een vergelijking tussen eenmaal daags nevirapine en tweemaal daags nevirapine.
In de behandeling van HIV-geïnfecteerde patiënten is een grote behoefte naar gemakkelijker gedoseerde en beter getolereerde regimes met verbeterde virologische en immunologische effectiviteit. Op basis van preklinisch onderzoek, zou de combinatie van twee NNRTI's een potentiële behandeloptie kunnen zijn. In **hoofdstuk 2.1** is een overzicht gegeven van studies waarin twee NNRTI's gecombineerd worden. Diverse klinische studies hebben verschillende resultaten laten zien op gebied van het onderdrukken van de virale replicatie, farmacokinetiek, effectiviteit en bijwerkingen van antiretrovirale therapie met twee NNRTI's. Ten opzichte van enkelvoudige NNRTI regimes hebben regimes met twee NNRTI's vergelijkbare, maar niet superieure resultaten laten zien met betrekking tot virologisch en immunologisch succes in therapie-naïeve en voorbehandelde patiënten. NNRTI-geassocieerde bijwerkingen zoals klinische hepatitis, verhoogde leverenzymen, rash, bijwerkingen in het centraal zenuwstelsel en psychiatrische afwijkingen traden vaker op wanneer twee NNRTI's tegelijkertijd werden toegediend.
De basis van klinisch farmacologisch onderzoek wordt gevormd door de beschikbaarheid van gevalideerde en gevoelige bioanalytische bepalingsmethoden die het mogelijk maken om concentraties van antiretrovirale geneesmiddelen te bepalen. In **hoofdstuk 2.2** is een eenvoudige en snelle methode voor de simultane bepaling van de NNRTI's efavirenz en nevirapine in humaan plasma met behulp van vloeistofchromatografie (HPLC) beschreven. De monstervoorbewerking bestond uit een eiwitprecipitatie die snel en eenvoudig uitvoerbaar is en waarvoor slechts 100 μL plasma nodig is. De methode is gevalideerd over een concentratiebereik van 0.05 mg/L tot 15.0 mg/L voor efavirenz en van 0.25 mg/L tot 15.0 mg/L voor nevirapine. De methode was precies en nauwkeurig met variatiecoëfficiënten voor de precisie binnen en tussen de analytische runs van kleiner dan 5.9% en een gemiddelde nauwkeurigheid kleiner dan ±12.7%. Deze gevalideerde bioanalytische bepalingsmethode was geschikt voor gebruik in de 2NN studie.

Hoofdstuk 2.3 beschrijft het onderzoek van de populatie farmacokinetiek van nevirapine en efavirenz en de covariaat analyse in de 2NN studie. In deze studie was een grote diverse wereldpopulatie met een substantieel deel vrouwelijke patiënten (36.3%) en slechts 25.6% patiënten uit westerse landen geïncludeerd. De farmacokinetiek van zowel nevirapine als efavirenz is adequaat beschreven met behulp van een een-compartiment model met eerste orde absorptie en eliminatie. Voor beide geneesmiddelen kon een inductie fase van de steady-state worden onderscheiden. De klaring van nevirapine was lager in vrouwen (13.8%) en in patiënten met een hepatitis B co-infectie (19.5%). Ook de geografische regio was betrokken bij de variabiliteit in de farmacokinetiek van nevirapine. Patiënten uit Zuid-Amerika en Westerse landen hadden respectievelijk 11% en 28% hogere klaring van nevirapine in vergelijking met patiënten uit Thailand en Zuid-Afrika. Gelijktijdig gebruik van nevirapine beïnvloedde de farmacokinetiek van efavirenz en verhoogde de klaring van efavirenz met 43%. Patiënten uit Thailand hadden een lagere klaring dan de rest van de populatie.
Daarnaast is de farmacokinetiek van nevirapine in een eenmaal daags 400 mg regime en een tweemaal daags 200 mg regime onderzocht in **hoofdstuk 2.4**. In totaal waren van 578 patiënten 2899 nevirapine plasmaconcentraties beschikbaar voor dit onderzoek. Hoogte en frequentie van de dosering beïnvloedden de klaring en het verdelingsvolume van nevirapine niet. Dit geeft aan dat de kinetiek van nevirapine lineair was over deze dosis range. De data lieten zien dat de dagelijkse blootstelling aan nevirapine, uitgedrukt in "area under the concentration versus time curve" (AUC), gelijk was voor de eenmaal daags 400 mg dosering en de tweemaal daags 200 mg dosering. De minimum plasma concentratie en de maximum plasma concentratie van nevirapine waren lager respectievelijk hoger in het eenmaal daagse behandelschema in vergelijking met het tweemaal daagse schema. Deze effecten waren echter klein ten opzichte van de gehele variabiliteit in de plasmaconcentraties. Of deze verschillen zullen leiden tot een verhoogd risico op bijwerkingen en virologisch falen is onduidelijk.
In **hoofdstuk 2.5** is de relatie tussen bijwerkingen en plasma concentraties van nevirapine en efavirenz onderzocht in de 2NN studie. Farmacokinetische parameters van nevirapine waren niet gerelateerd aan bijwerkingen van het centraal zenuwstelsel, psychiatrische bijwerkingen, leveraandoeningen, verhoogde leverenzymen en rash wanneer gecorrigeerd was voor bekende covariaten (geslacht, geografische regio, CD4 cellen en hepatitis co-infectie). Ook een duidelijke grenswaarde waarboven patiënten een verhoogd risico hebben op bijwerkingen kon niet worden vastgesteld. De waarde van periodieke spiegelbepalingen van

nevirapine om bijwerkingen te voorkomen is daarom beperkt. Artsen zullen in plaats daarvan met name rekening moeten houden met factoren die een grotere invloed zouden kunnen hebben op bijwerkingen, zoals geslacht, geografische regio, CD4 cellen en hepatitis co-infectie. Tijdens de eerste 6 weken van behandeling resulteerden hoge minimum plasmaconcentraties van efavirenz in verhoogde leverenzymen. Andere relaties konden niet worden vastgesteld voor efavirenz in deze periode van behandeling. Ook op steady-state zijn geen relaties tussen efavirenzblootstelling en bijwerkingen aangetoond. Aangezien de standaard doseringen van nevirapine en efavirenz resulteerden in geneesmiddelspiegels die niet konden worden gecorreleerd aan belangrijke bijwerkingen, is het niet aannemelijk dat TDM van deze geneesmiddelen bijwerkingen zal voorkomen. Aan de andere kant kan TDM wel nuttig zijn gedurende de eerste 6 weken van behandeling met efavirenz bevattende antiretrovirale therapie, en in specifieke situaties zoals geneesmiddelinteracties en evaluatie van therapieontrouw.

Conclusies en toekomstperspectief

Concluderend kan gezegd worden dat meer inzicht in de farmacokinetiek van verschillende antiretrovirale geneesmiddelen is verkregen met de studies zoals die in dit proefschrift zijn beschreven. Grote variabiliteit in farmacokinetiek is geobserveerd, die deels verklaard kan worden door patiëntkarakteristieken zoals geslacht, ras/land van herkomst, co-infecties en co-medicatie. De variabiliteit in plasmaconcentraties van antiretrovirale geneesmiddelen kan verminderd worden door aan de hand van spiegelbepalingen in plasma de dosis van deze middelen aan te passen. De waarde van TDM van NNRTI's als een middel om bijwerkingen te voorkomen is echter beperkt. Voor nevirapine en efavirenz is namelijk geen overtuigende relatie gevonden tussen blootstelling en bijwerkingen.

Hopelijk heeft dit proefschrift bijgedragen aan het continue proces van optimalisering van de antiretrovirale therapie en het verbeteren van de respons op HAART in HIV-geïnfecteerde patiënten.

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Bregt
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CURRICULUM VITAE

Bregt Kappelhoff werd geboren op 11 augustus 1975 te Nieuwleusen. In 1993 behaalde zij het Gymnasium β diploma aan het Gymnasium Celeanum in Zwolle. Aansluitend begon zij met de studie Farmacie aan de Universiteit Utrecht. Tijdens de doctoraalopleiding deed zij onderzoek bij de Unit of Metabolic Medicine, Imperial College, School of Medicine at St Mary's Hospital in London. Het onderzoek was getiteld "Angiotensine I converting enzyme, matrix metalloproteases, calcium, and zinc in breast cyst fluid" en werd begeleid door Prof. Dr J.H. Thijssen en Prof. Dr M.J. Reed. In augustus 2000 behaalde ze haar apothekersdiploma. Vanaf september 2000 was ze werkzaam als projectapotheker in de apotheek van het Slotervaartziekenhuis/Antoni van Leeuwenhoek Ziekenhuis in Amsterdam. Het project betrof het verzorgen van de distributie van studiemedicatie bestemd voor studies in binnen- en buitenland. Tijdens dit project groeide de interesse voor wetenschappelijk onderzoek op het gebied van de antiretrovirale geneesmiddelen. Nadat het distributieproject gecertificeerd was, startte zij in september 2001 met het onderzoek dat beschreven is in dit proefschrift, onder leiding van promotor Prof. Dr J.H. Beijnen en copromotor Dr A.D.R. Huitema. Tegelijkertijd volgde zij de opleiding ter verkrijging van de aantekening "klinisch farmacoloog", onder auspiciën van de Nederlandse Vereniging voor Klinische Farmacologie en Biofarmacie (opleiders: Prof. Dr J.H.M. Schellens en Prof. Dr J.H. Beijnen). In maart 2005 zal zij in de apotheek van het Slotervaartziekenhuis/Antoni van Leeuwenhoek Ziekenhuis starten met de opleiding tot ziekenhuisapotheker.

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